

Protein domains responsible for evolutionary success of eukaryotic ribosomes



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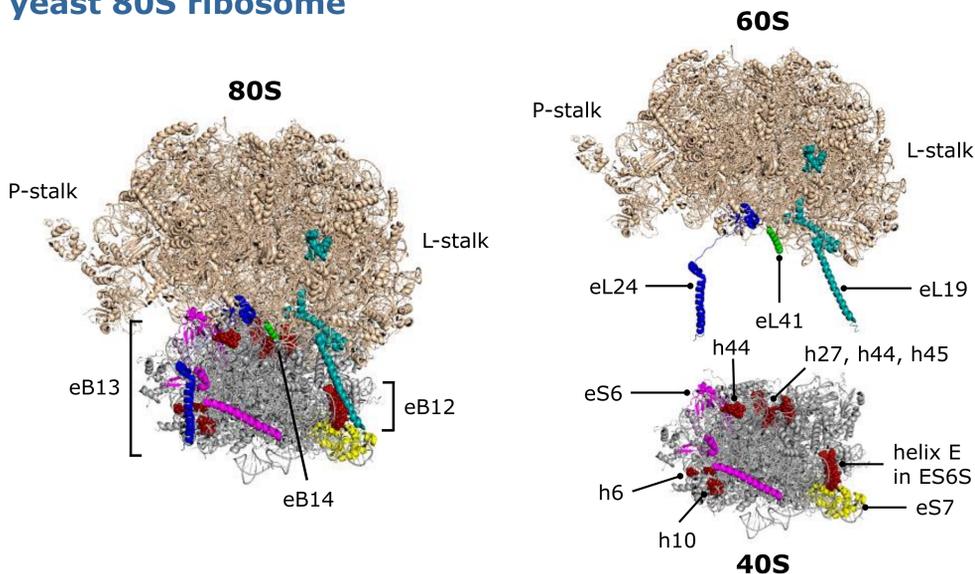
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Introduction

- Ribosomes share a common structural core, comprising of 33 conserved ribosomal proteins (r-proteins) and approximately 4400 RNA nucleotides.
- In addition to the universally conserved r-proteins, 35 r-proteins are shared between the archaeal and eukaryotic ribosomes. Many of these r-proteins have evolved eukaryote-specific extensions.
- This study focuses on three 60S r-proteins: eL19, eL24 and eL41.
- These r-proteins are present in both archaeal and eukaryotic ribosomes. Bacterial ribosome lacks the respective counterparts.
- The r-proteins, eL19 and eL24 contain the domains conserved in archaeal and eukaryotic ribosomes. In addition they have the eukaryote-specific additions that carry out specific functions. One of the function is the formation of the eukaryote-specific intersubunit bridges (eB12 and eB13, respectively).
- The eL41 is the smallest r-protein in the ribosomes. Interestingly, eL41 is present in all eukaryotic ribosomes and in some, but not all, archaeal ribosomes. R-protein eL41 forms the bridge eB14.

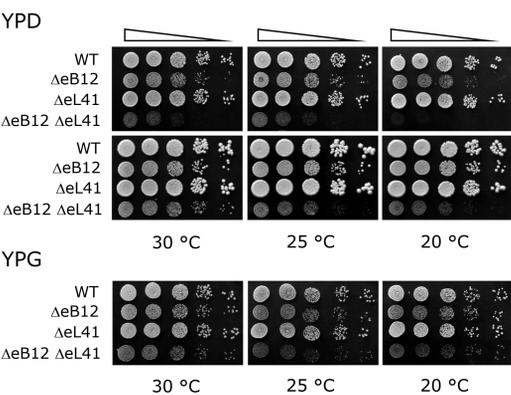
Aim of this study was to evaluate the essentiality and function of eukaryote-specific domains of r-proteins eL19, eL24 and eL41 in translation.

1. Location of r-proteins eL19, eL24 and eL41 in budding yeast 80S ribosome

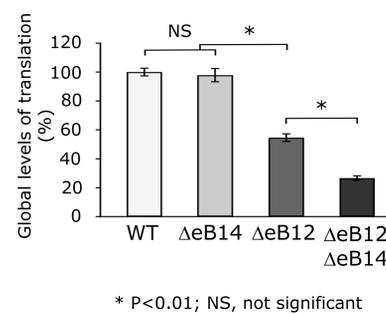


2. eL41 ensures the optimal cell growth when eB12 bridge is impaired

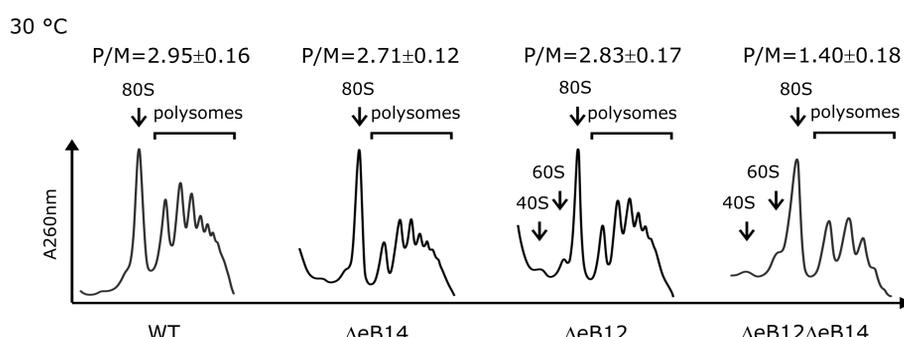
Serial dilutions spot-test assay



Translational efficiency analysis



Ribosome-polysome profile analysis

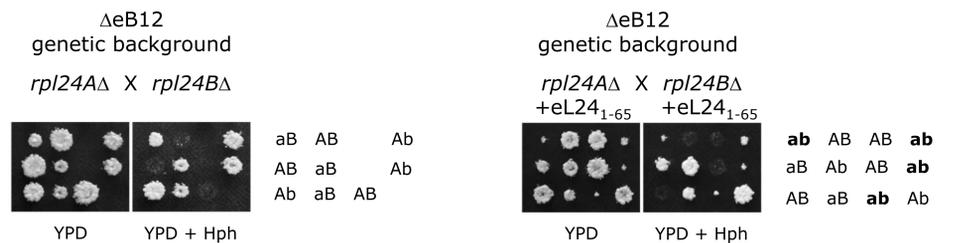


Conclusions

- The nonessential r-protein eL41 has an important role in stabilizing the structure of the 80S particle when other ribosomal bridges are defective.
- Although eL24 is a nonessential r-protein in wild type cells, its conserved N-terminal domain becomes essential when ribosome functionality is reduced due to the compromised eB12 bridge.
- The eukaryote-specific extensions of eL19 and eL24 contribute to the ribosome functionality, probably by affecting the initiation step of translation.
- The eukaryote-specific domains of eL19 and eL24 together with eL41 play an important role in formation of stable 80S ribosomes.
- The intersubunit bridges formed by eL19, eL24 and eL41 contribute to the efficient translation in eukaryotic cells.

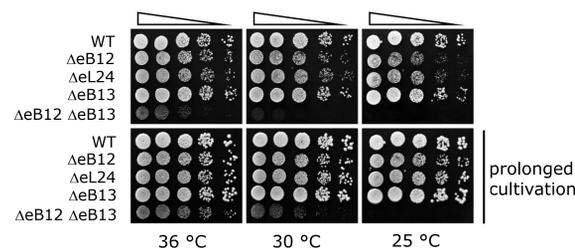
3. N-terminal domain of eL24 is essential when eB12 bridge is compromised

Construction of Archaea-like double bridge mutant (ΔeB12ΔeB13Δ)



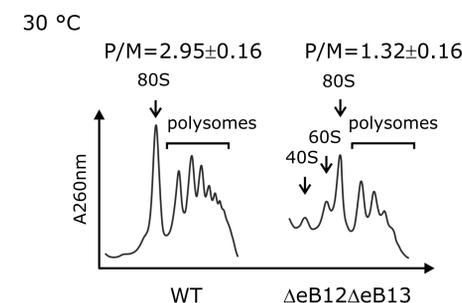
4. Loss of bridges eB12 and eB13 leads to reduced cell growth, cold sensitivity, and reduction of protein synthesis and polysome levels

Serial dilutions spot-test assay on YPD

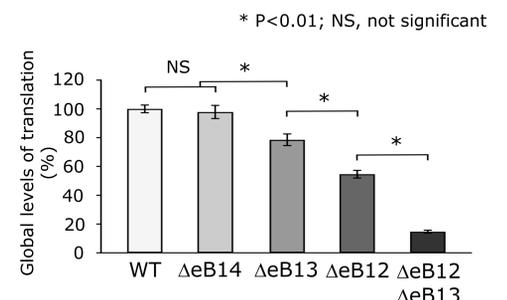


Strain	Generation time (YPD, 30 °C, min)
WT	85.4 ± 1.9
ΔeB12	128.7 ± 4.2
ΔeB13	99.1 ± 3.7
ΔeB14	85.6 ± 2.3
ΔeB12 ΔeB14	176.1 ± 2.0
ΔeB12 ΔeB13	224.2 ± 4.4
ΔeB12 ΔeB13 ΔeB14	366.7 ± 8.8

Ribosome-polysome profile analysis

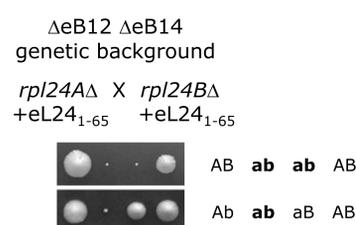


Translational efficiency analysis



5. Loss of three eukaryote-specific bridges leads to severely reduced ribosome functionality

Construction of Archaea-like triple bridge mutant (ΔeB12ΔeB13ΔeB14)



Ribosome-polysome profile analysis

