The Functional Role of eL19 and eB12 Intersubunit Bridge in the Eukaryotic Ribosome

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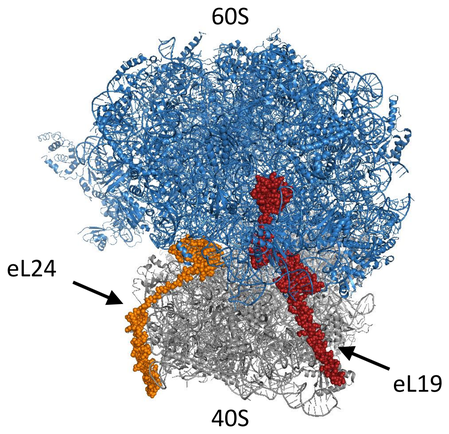
Introduction

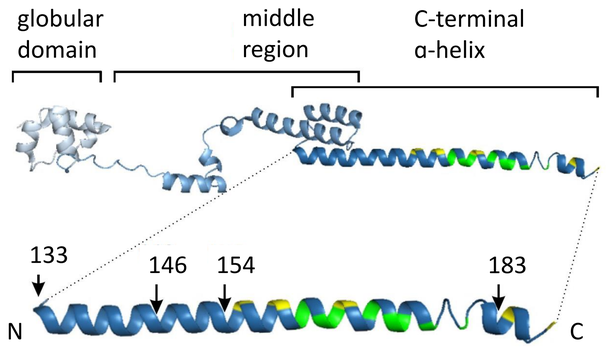
- Seventeen bridges are formed during association of yeast ribosome subunits [Ben-Shem *et al.,* 2011].
- Twelve intersubunit bridges are conserved among the all kingdoms.
- Five bridges are eukaryote-specific.
- Two eukaryote-specific bridges, eB12 and eB13, are created by the long protein α-helices extending from 60S subunit E- and A-site sides, respectively.
- eL24 (shown in orange), and consequently the eB13 bridge, is dispensable for cell viability [Steffen *et al.,* 2012].

- The main component of eB12 bridge – protein eL19 (shown in red) – is essential for cell growth and was shown to be an important pre-rRNA processing factor [Poll *et al*., 2009].
- eL19 has two functions – one in 60S biogenesis and a second in intersubunit bridge formation. It is not known which of these is essential for cell viability.

Aim of this study was to analyse the functional importance of the intersubunit bridge eB12.



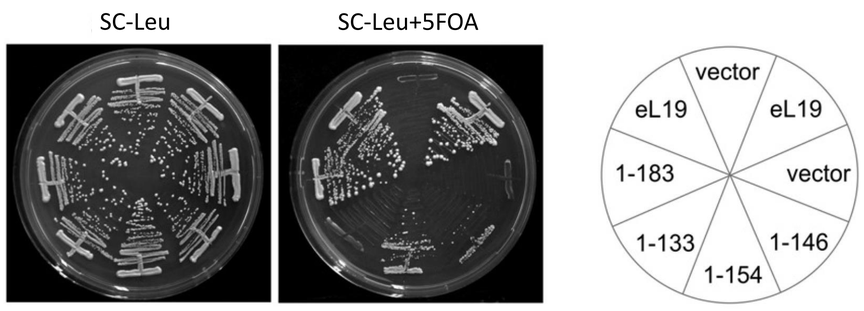
1. C-terminal deletions of eL19



➢ Structure of the C-terminal α–helix of the eL19. Residues forming contacts with ES6S of 18S rRNA and 40S subunit proteins (eS7, uS17) are colored green and yellow, respectively. Arrows indicate the positions of the last amino acids of the respective eL19 deletion alleles.

Prot-Prot Prot-rRNA

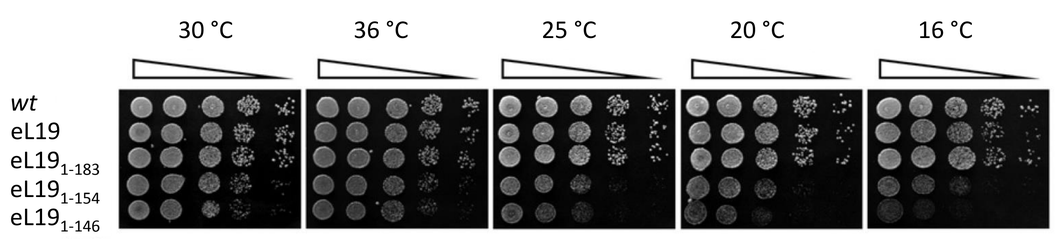
➢ Plasmid shuffling assay



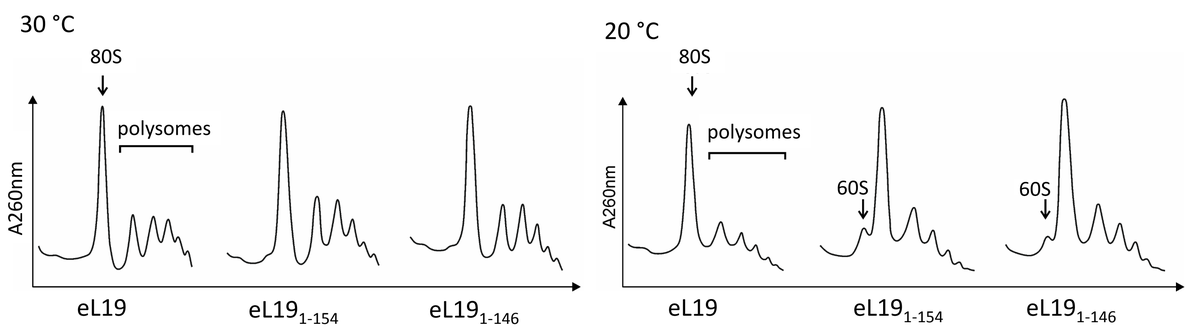
Deletion of the whole C-terminal α–helix of eL19 (mutant $eL19_{1\text{-}133}$) is lethal.

2. Phenotypic characterization of the eB12 bridge mutants

➢ Serial dilutions spot-test analysis



➢Ribosome-polysome profile analysis



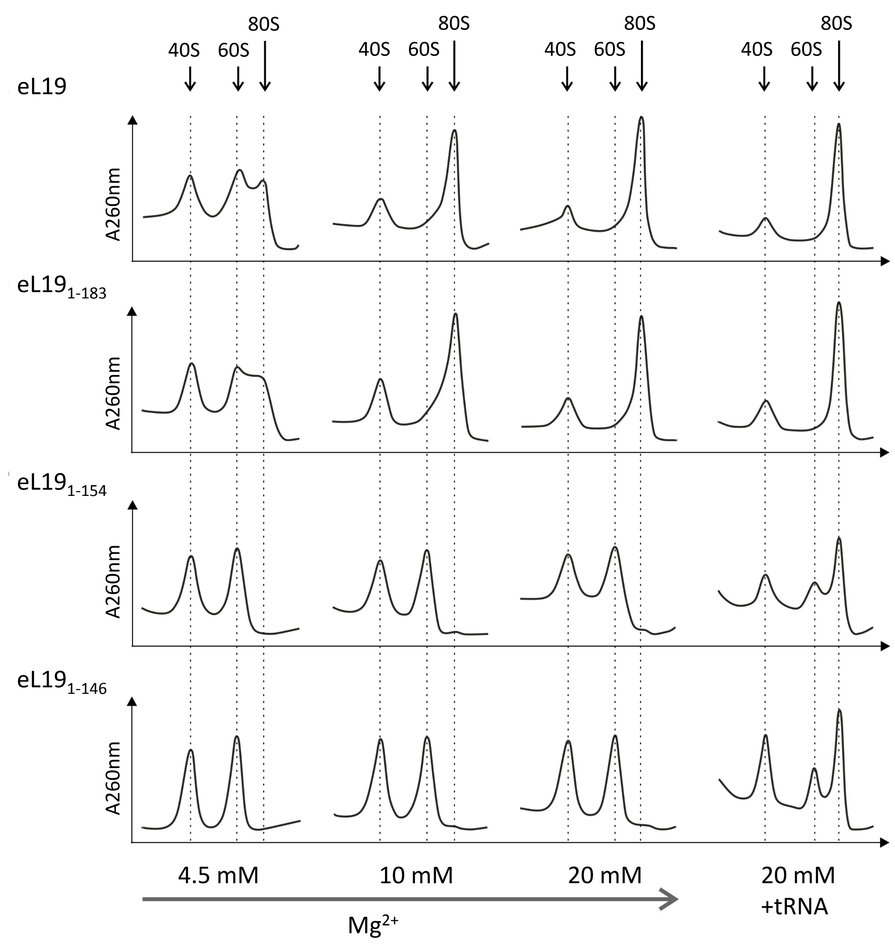
Mutants lacking eB12 bridge ($eL19_{1\text{-}154}$ and $eL19_{1\text{-}146}$) have slow-growth phenotype enhanced at lower temperatures. Ribosome-polysome profile analysis reveales increased levels of free 60S subunits at 20 °C.

Conclusions

- ➢ Ribosomes lacking eB12 can support growth and are active in translation.
- ➢ eB12 bridge is essential for the subunit joining *in vitro*.
- ➢ Lack of eB12 induces the stress response and promotes aquired stress tolerance.
- ➢ Essential function of eL19, carried by the globular domain and middle region, is in ribosoome biogenesis.
- ➢ Secondary function of eL19, provided by the C-terminal domain, is eB12 bridge formation.

3. *In vitro* formation of 80S ribosomes

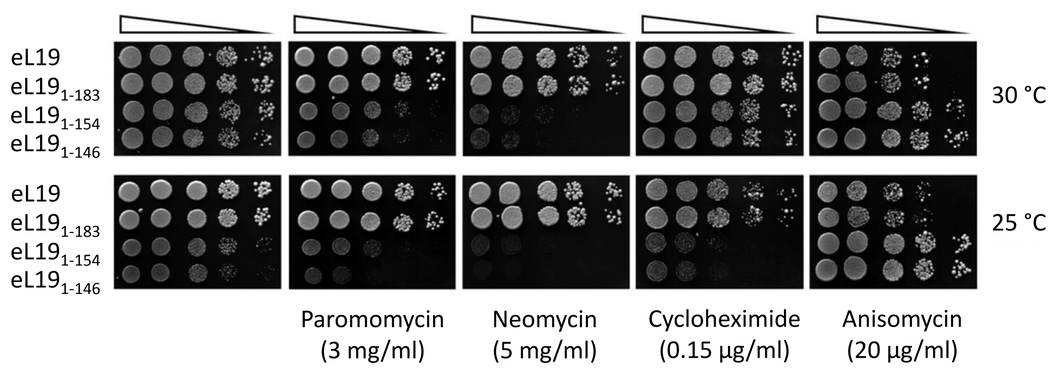
➢ *In vitro* reassociation of wild-type 40S subunits and wild-type or mutant 60S subunits at diferent Mg^{2+} concentrations



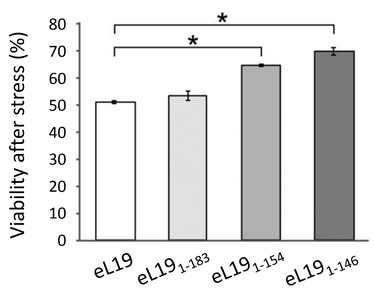
Mutants impaired in eB12 bridge formation ($eL19_{1\text{-}154}$ and $eL19_{1\text{-}146}$) fail to form 80S ribosomes, regardless of Mg^{2+} concentration.

4. Analysis of stress tolerance of the eB12 bridge mutants

➢ Serial dilutions spot-test analysis of drug resistance/sensitivity

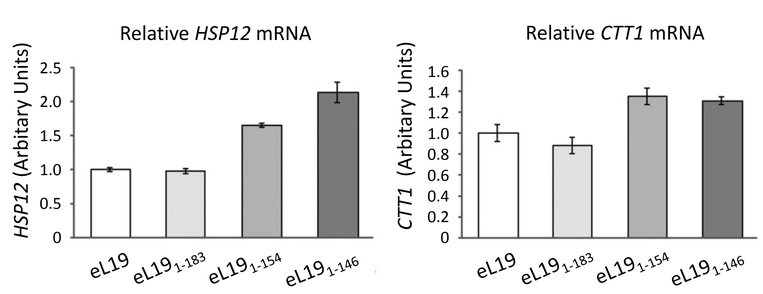


➢ Sensitivity to NaCl stress



(*P≤0.001)

➢ qPCR analysis of the *HSP12* and *CTT1* transcript levels



Mutants impaired in eB12 bridge formation display sensitivity to paromomycin, neomycin and cycloheximide and resistance to anisomycin.
Mutants lacking eB12 are less sensitiive to hyperosmotic stress than wild-type cells.

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