A COMPREHENSIVE ANALYSIS OF EUKARYOTIC RIBOSOMAL PROTEIN uS9 (S16) FUNCTION IN TRANSLATION

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ABSTRACT

Ribosomal protein uS9 is a conserved protein of the small ribosomal subunit. The protein is located on the solvent side of the subunit head and has a long protruding C-terminal tail (CTT) that reaches the mRNA cleft. uS9/yRps16 contributes to the molecular environment of the ribosomal P-site and contacts initiator tRNA when base-paired to AUG codon in the P site. The last positively charged C terminal residue (Arg) of uS9 is invariably conserved across all kingdoms of life and is believed to enhance interaction with the negatively charged tRNA. To investigate the function of uS9/yRps16 and, in particular, the role of its C-terminally conserved region, we have obtained and characterized yeast *Saccharomyces cerevisiae* strains in which the wild type uS9/yRps16 gene has been replaced by the mutant uS9 variants. These mutants contain CTT deletions/extensions and/or substitution of the C-terminal Arg with the negatively charged Glu. In vivo, biochemical analysis of the uS9 mutants showed that uS9 CTT plays an important role in the initiation and elongation steps of protein synthesis. We have found that uS9 C-terminal residues (their exact location and nature) are critical for efficient recruitment of the eIF2•GTP•Met-tRNA$_i^{Met}$ ternary complex and for responding properly to an AUG codon in the P-site during scanning. We hypothesize that upon start codon recognition, the CTT of uS9 is important to hydrolyze GTP (from eIF2-GTP-Met-tRNAiMet complex) to GDP and Pi. The efficiency of GTP hydrolysis may serve as a measure of efficiency of initiation process and start codon recognition. To monitor the ability of the wild-type and uS9 mutant ribosomes to function in GTPase assay, we are using reconstituted in vitro translation initiation system with purified recombinant initiation factors (eIF1, 1A, 2, 5) and ribosomes.

INTRODUCTION

Fig. 1. Major steps of eukaryotic translation initiation
(from Dever & Lorsh. JBC, 2010)

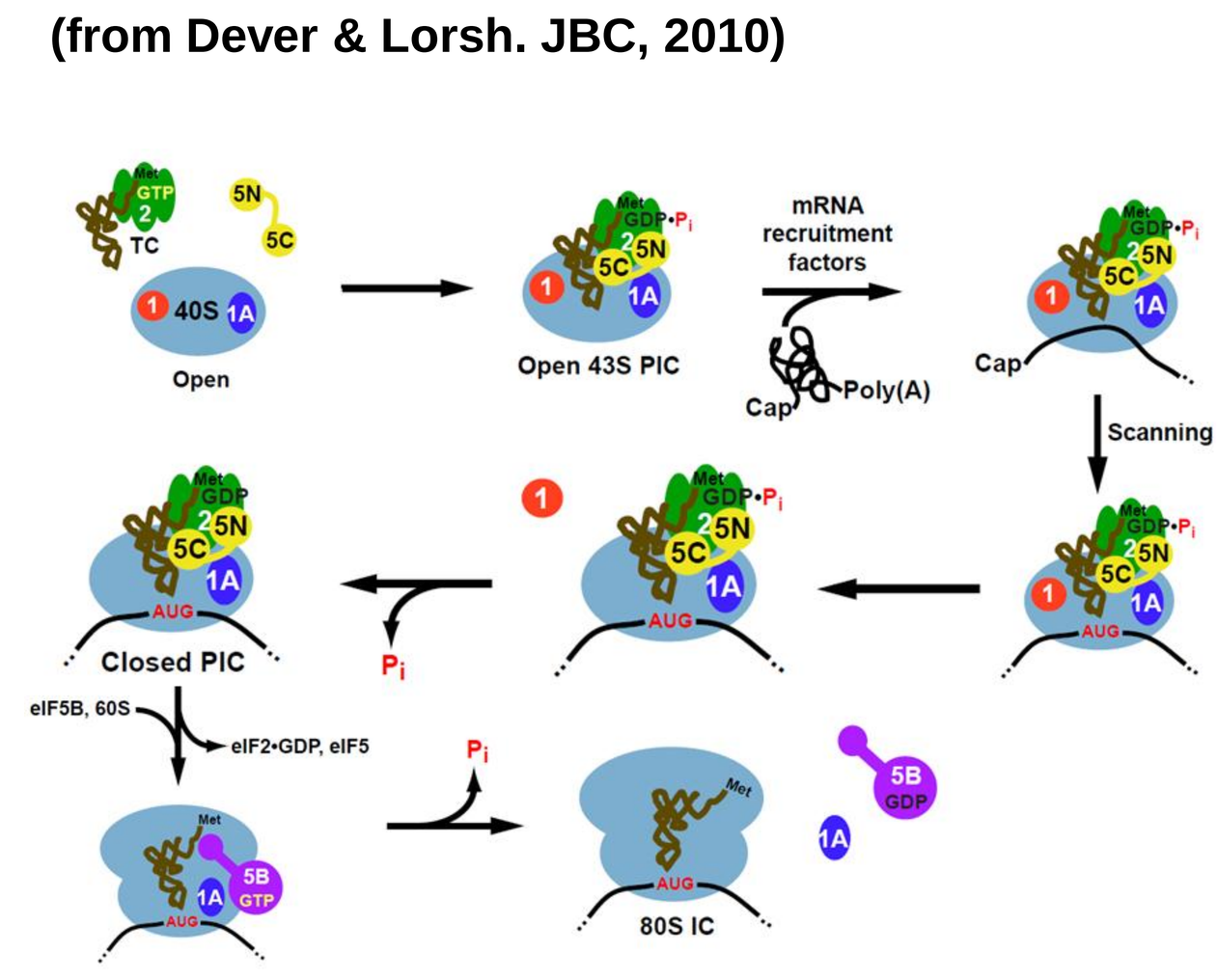
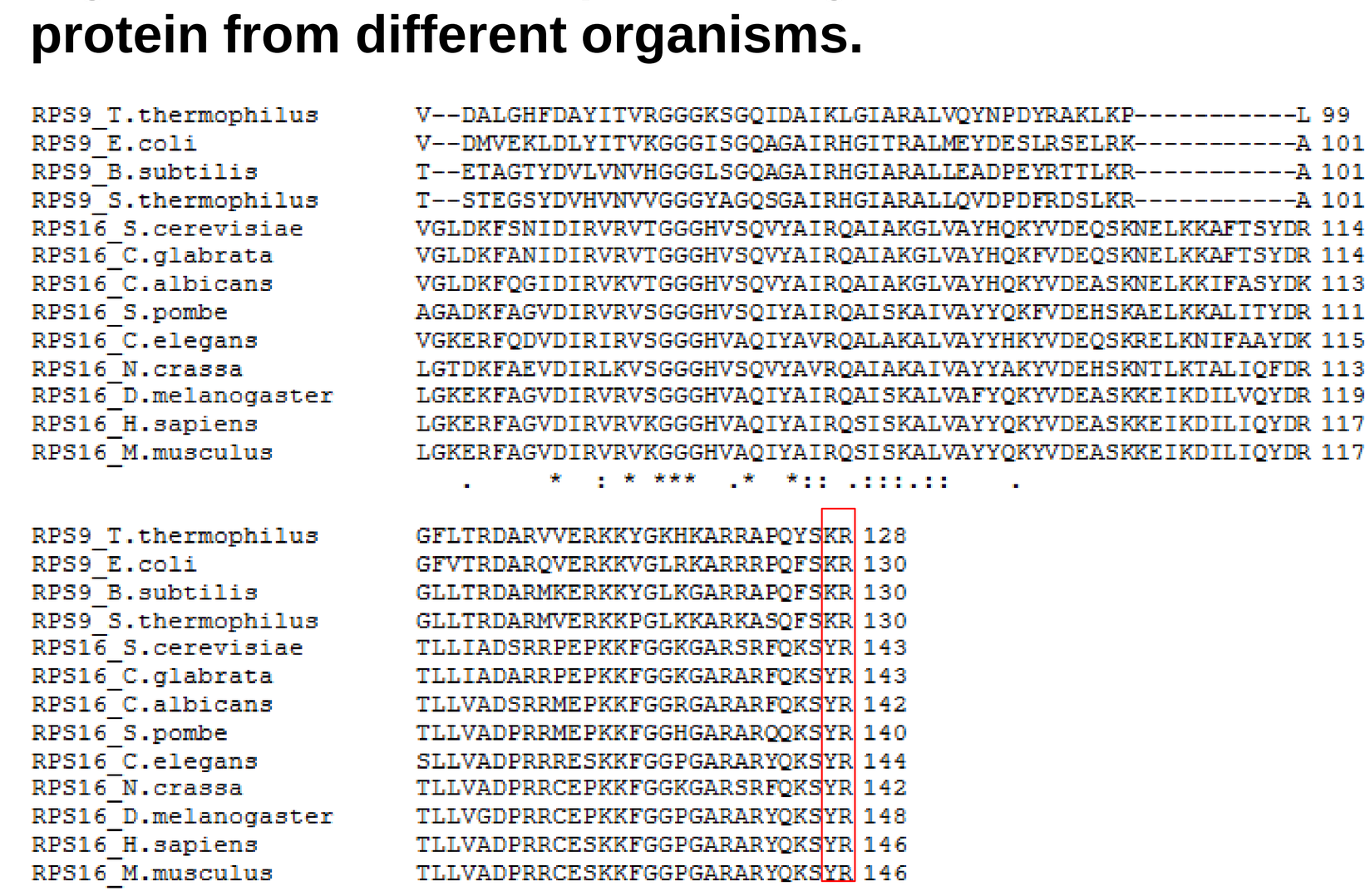


Fig. 2. C terminus sequence alignment of uS9 protein from different organisms.



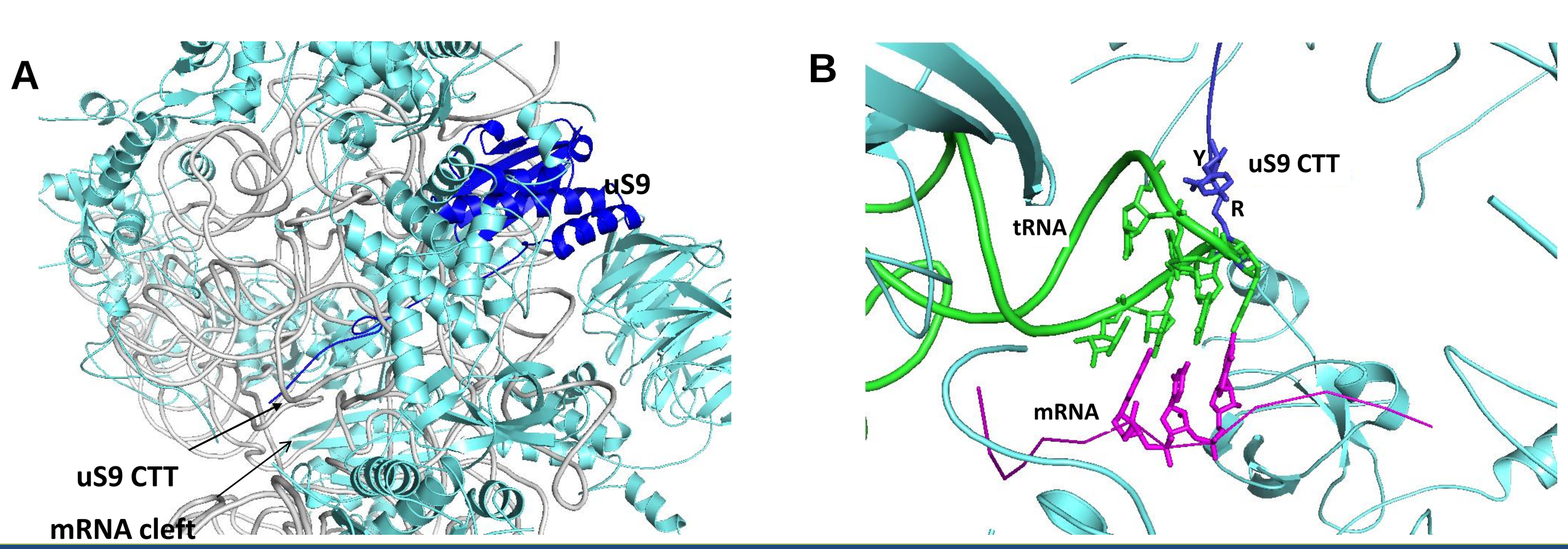
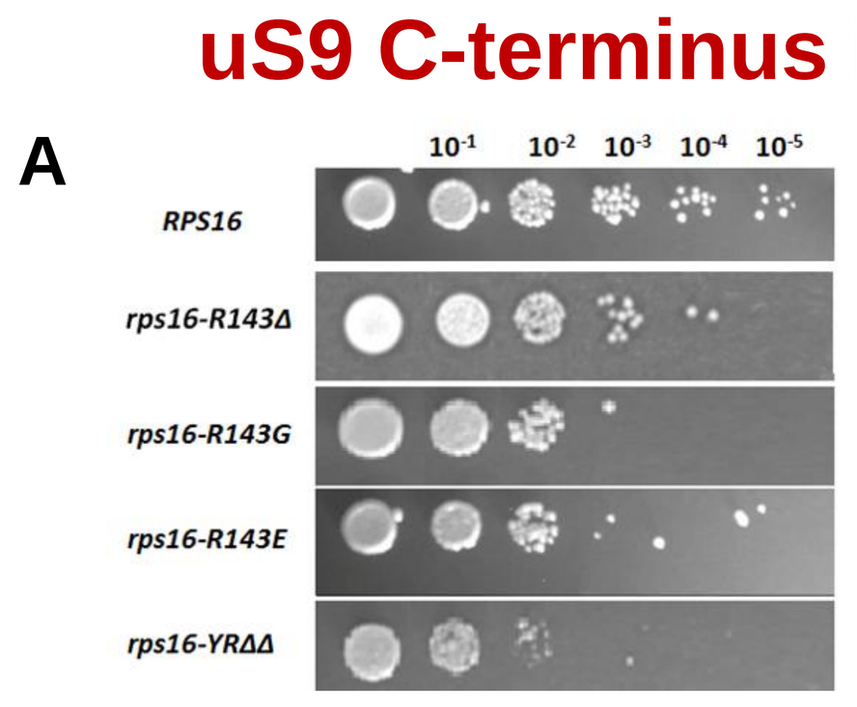


Fig. 3. Location of uS9 (A) Location of uS9 in the 40S head of yeast ribosome. **(B)** uS9 C-terminus interaction with Met-tRNAiMet in the rabbit 40S ribosomal subunit.

RESULTS

uS9 C-terminus is important for efficient translation initiation



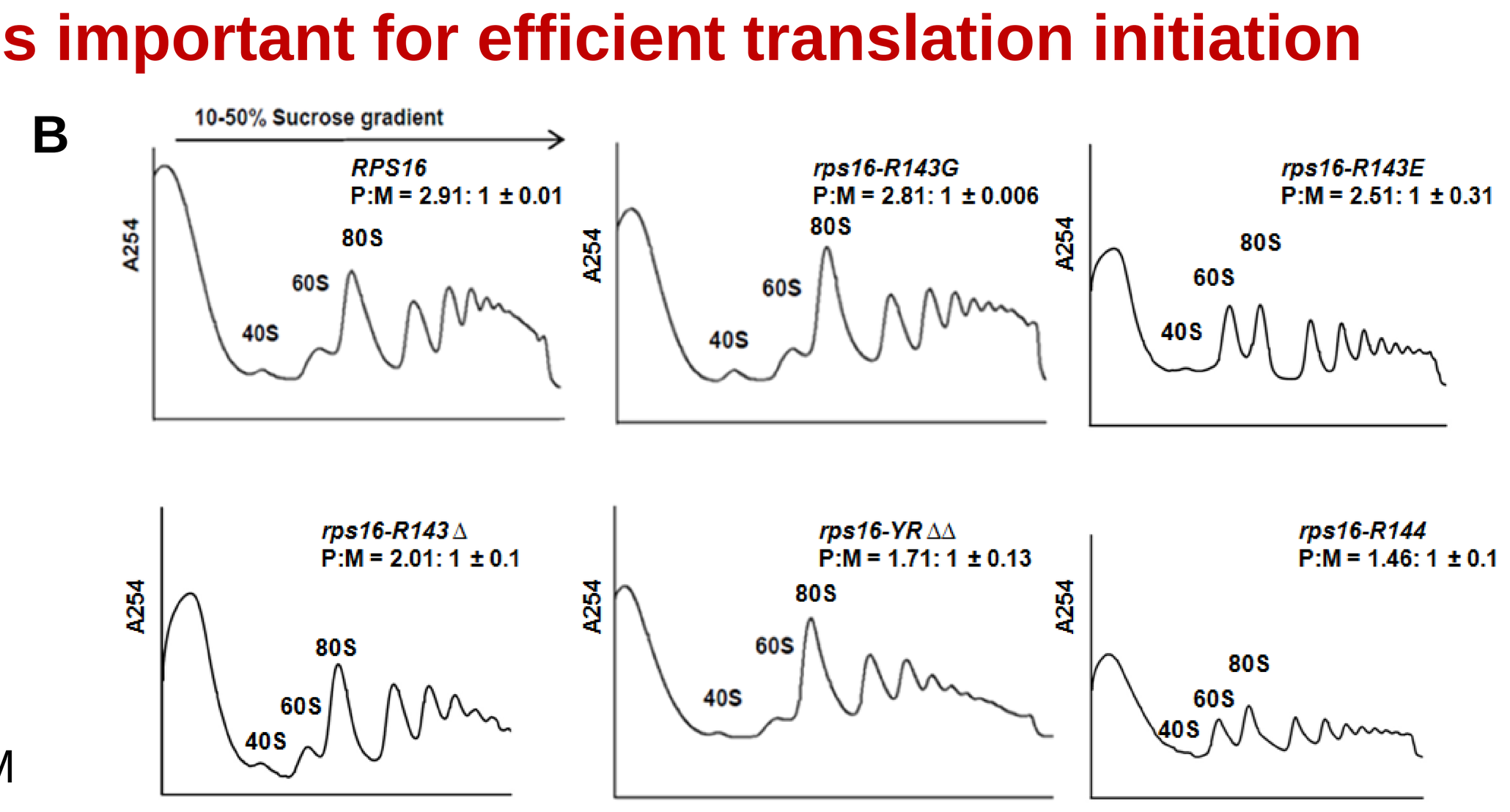


Fig. 4. Defective translation initiation of uS9 CTT mutants **(A)** slow growth phenotype **(B)** initiation defect (reduction of P:M ratio in polysome profiles).

uS9 C-terminus is important for efficient initiation.

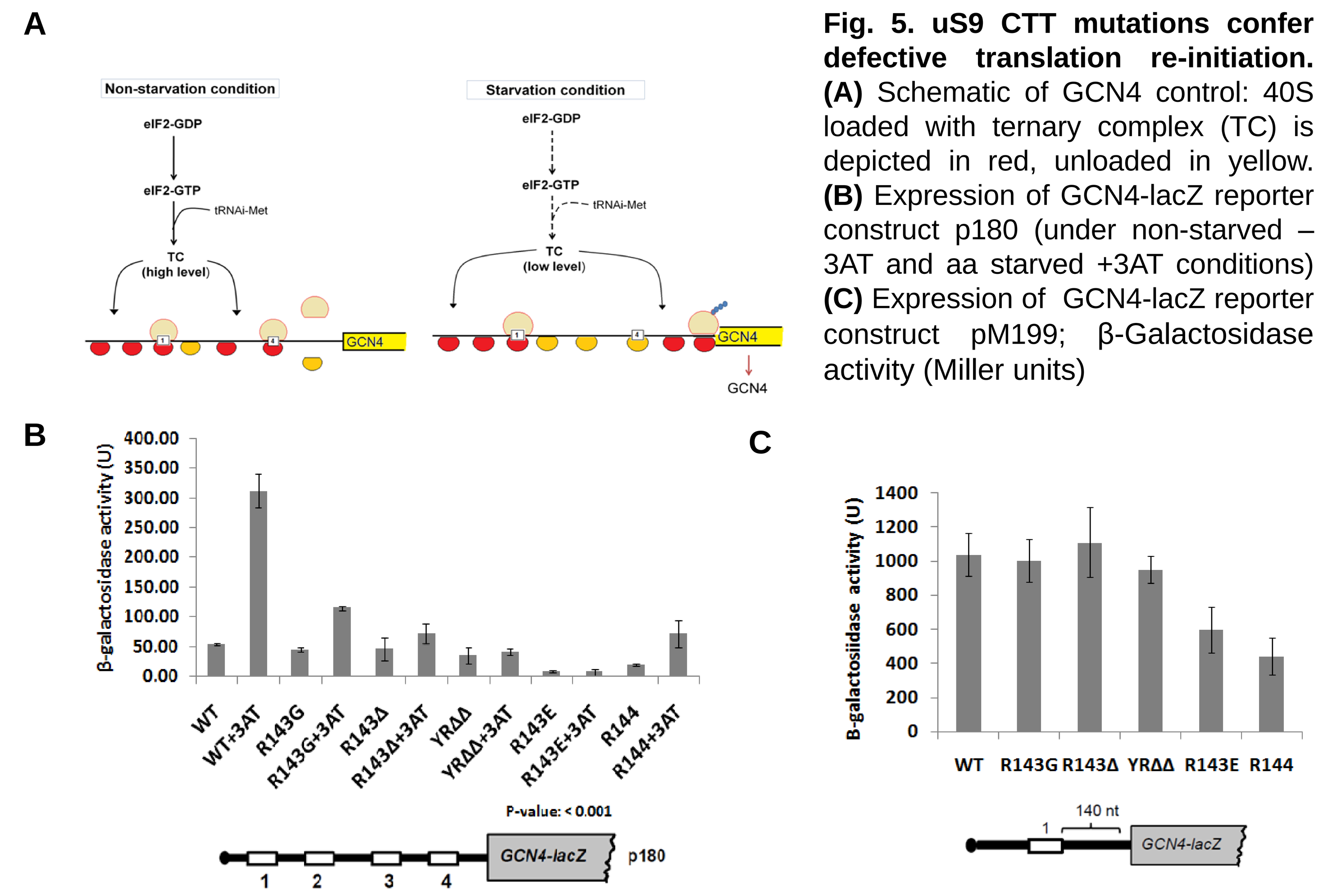
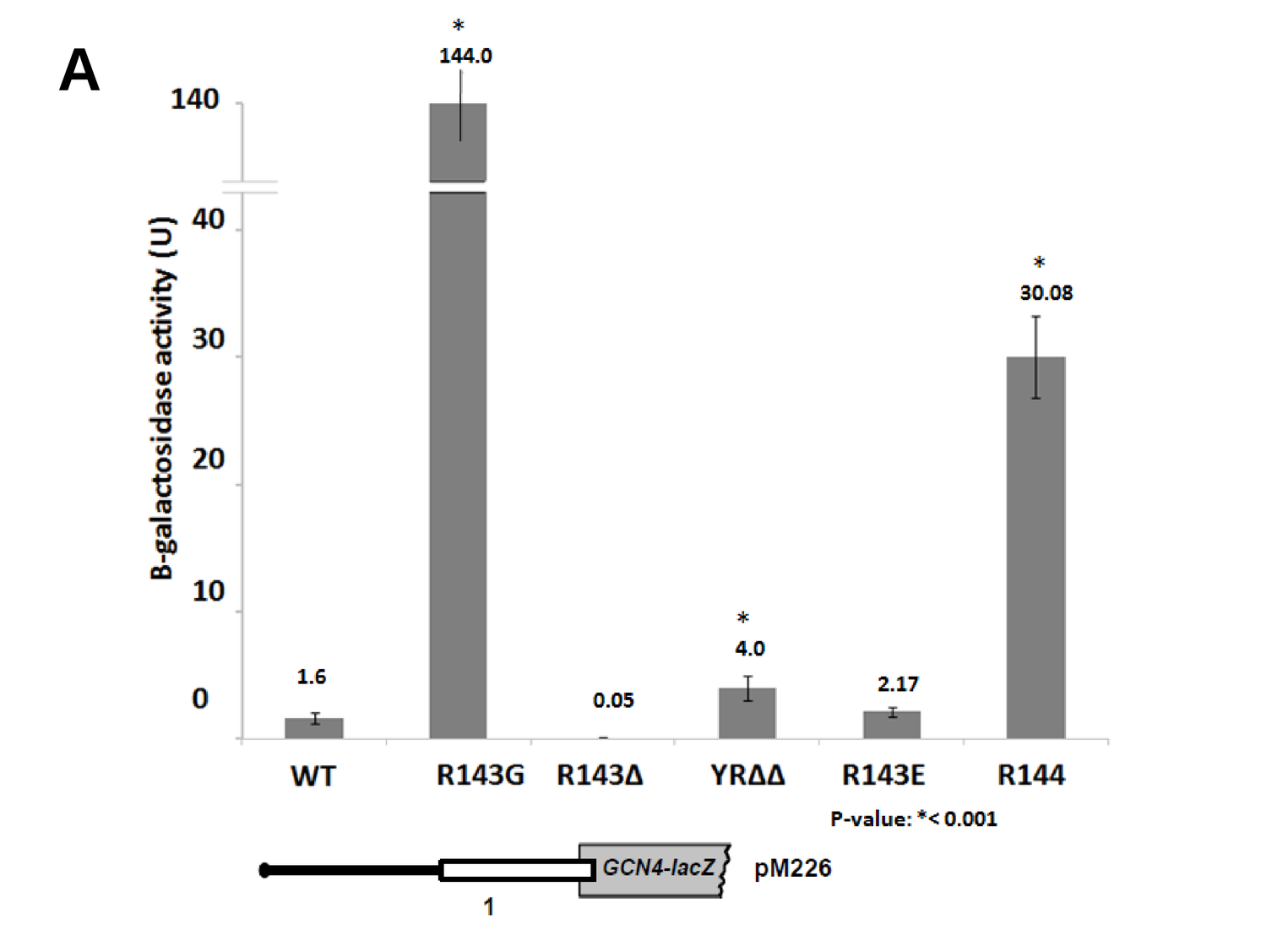


Fig. 5. uS9 CTT mutations confer defective translation re-initiation. **(A)** Schematic of GCN4 control: 40S loaded with ternary complex (TC) is depicted in red, unloaded in yellow. **(B)** Expression of GCN4-lacZ reporter construct p180 (under non-starved – 3AT and aa starved +3AT conditions) **(C)** Expression of GCN4-lacZ reporter construct pM199; β-Galactosidase activity (Miller units)

AUG and UUG recognition is compromised in uS9 mutants



B

	β-gal activity (U) HIS4-lacZ expression					
	WT	R143G	R143Δ	YRΔΔ	R143E	R144
AUG	390.25	195.91	175.60	102.26	101.50	107.76
UUG	5.88	2.102	1.891	1.470	0.750	0.723

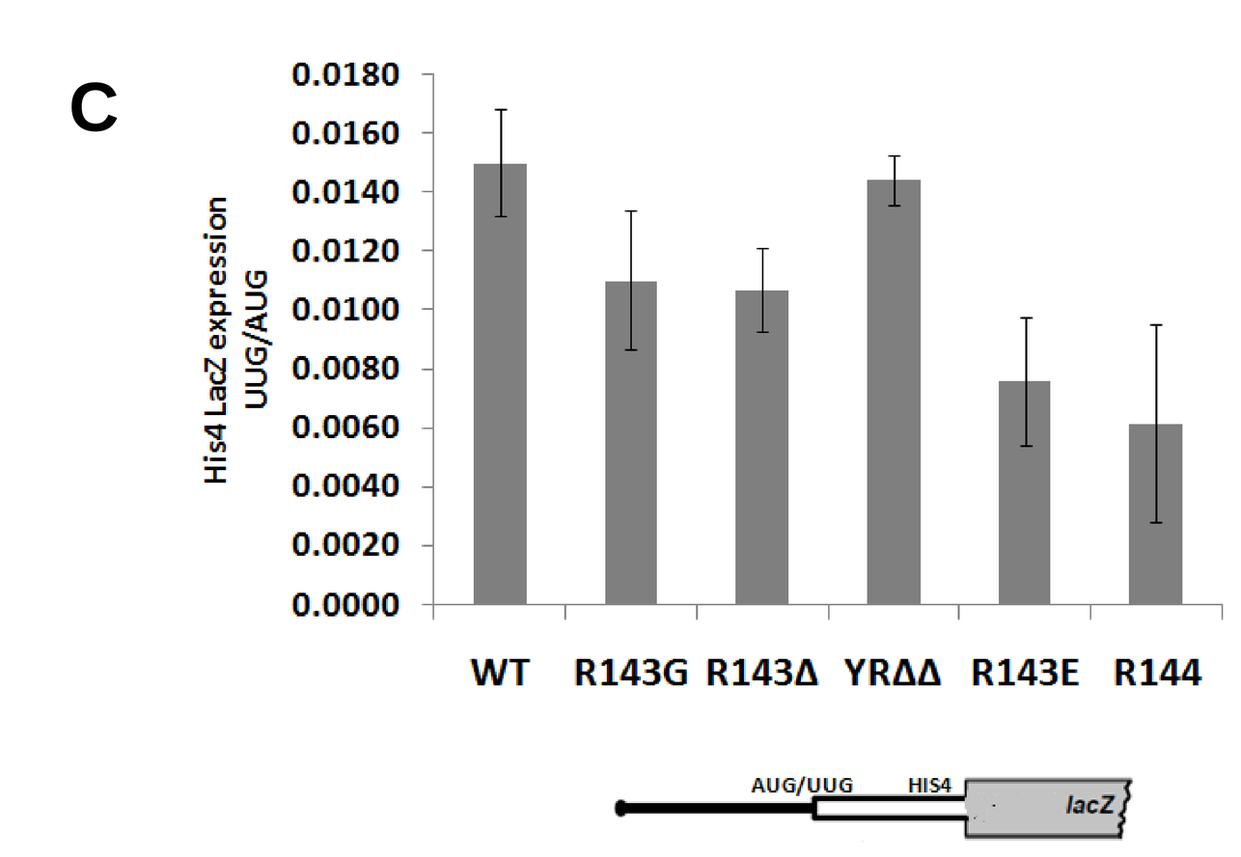
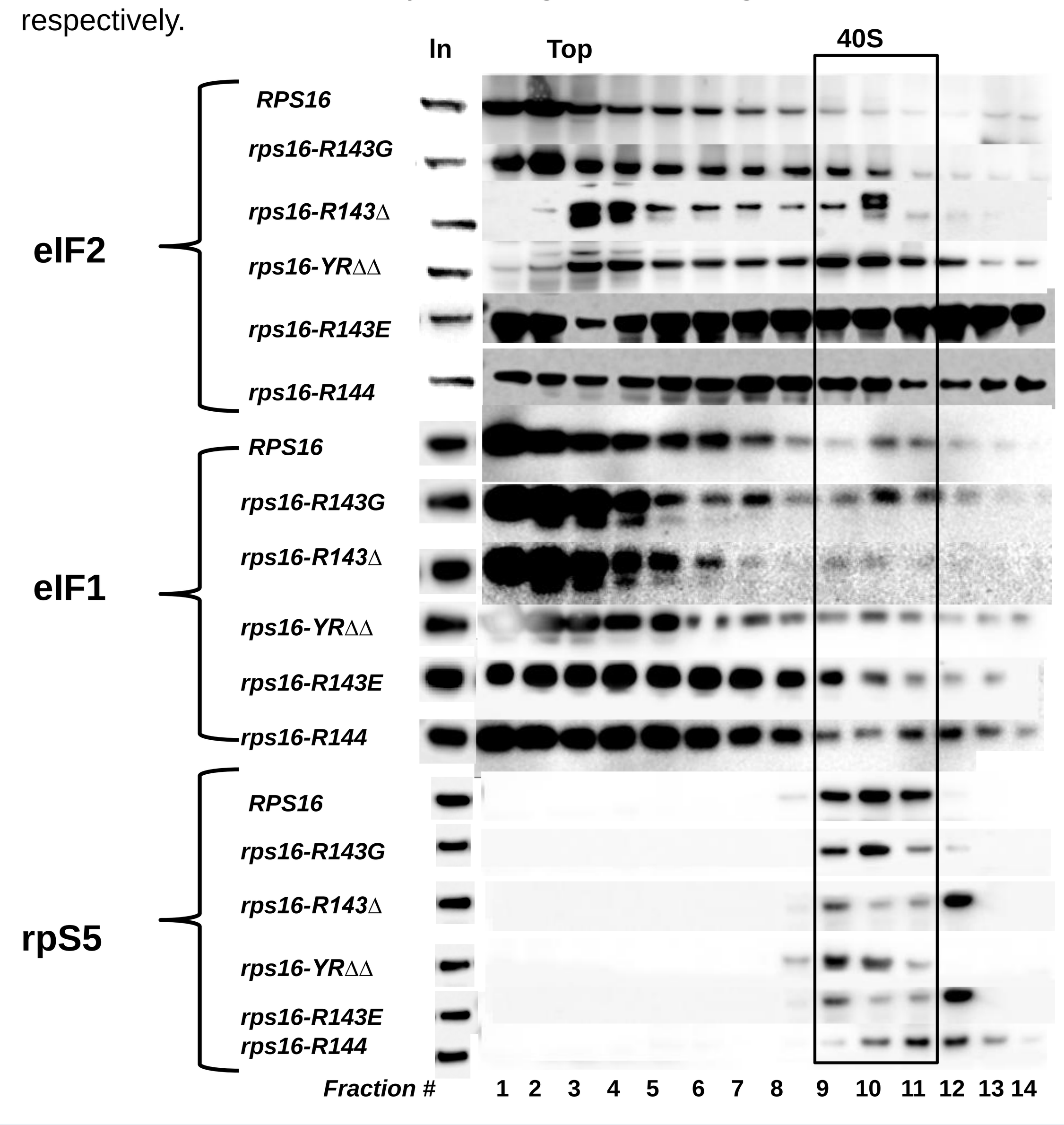


Fig. 6. uS9 CTT mutants display defective recognition of both cognate and non-cognate initiator codons. (A) Expression of *GCN4-lacZ* reporter construct demonstrating leaky scanning phenotype **(B) and (C)** Expression of *HIS4-lacZ* reporter constructs harboring AUG and UUG as initiator codons, β-Galactosidase activity (Miller units)

Altered association of eIFs with 40S subunits of uS9 mutants

Fig. 7. Association of initiation factors eIF2 and eIF1 with 40S ribosomal subunits in wt and uS9 mutants. Western blot analyses using antibodies against the initiation factors eIF2, eIF1 and rpS5 respectively.



Increased GAP function by *TIF5-G31R* rescues uS9 mutant phenotype

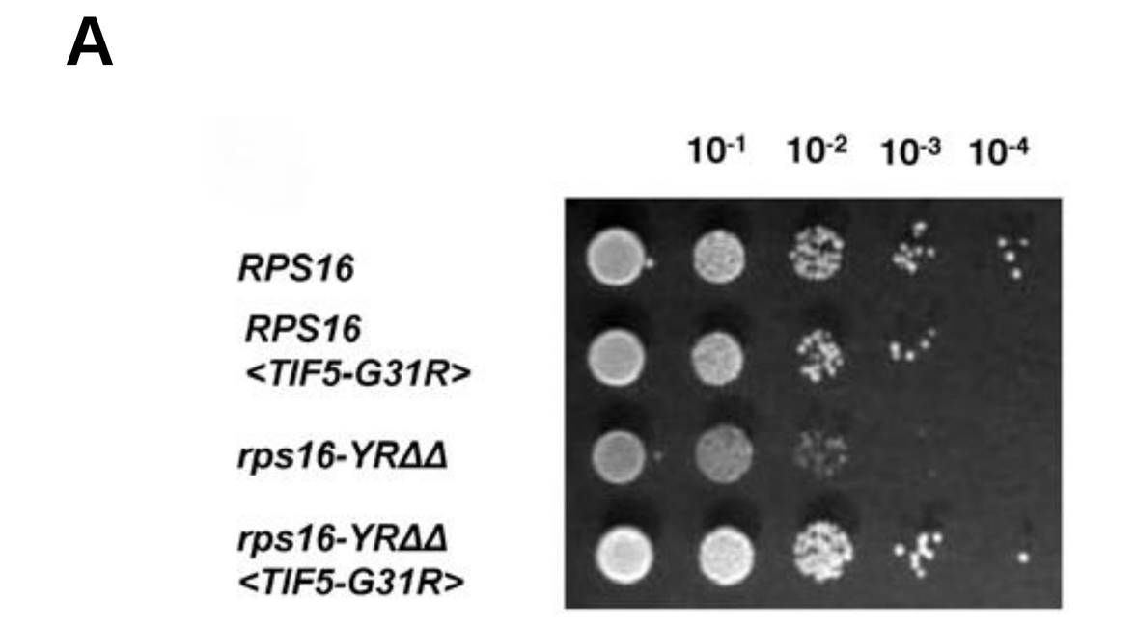
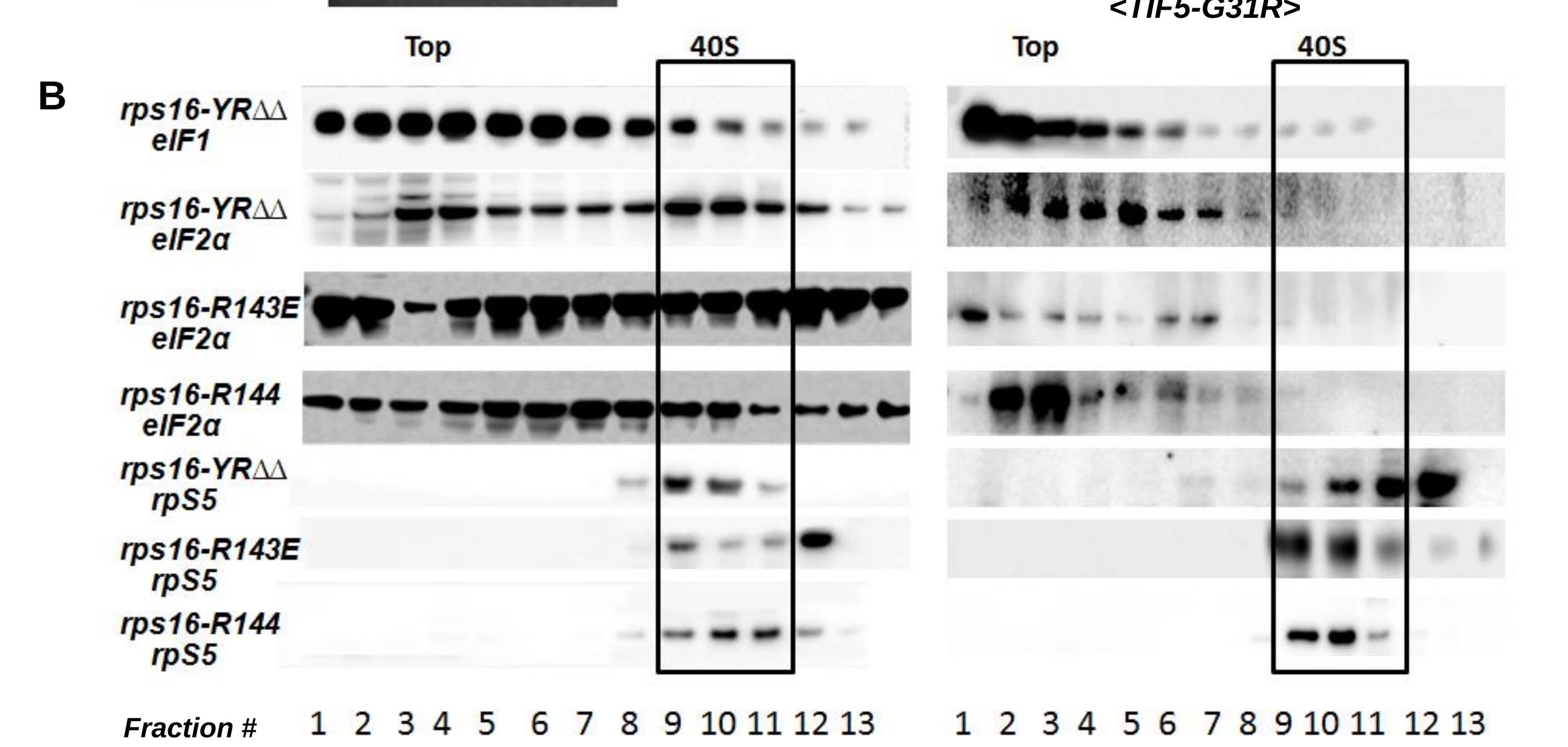
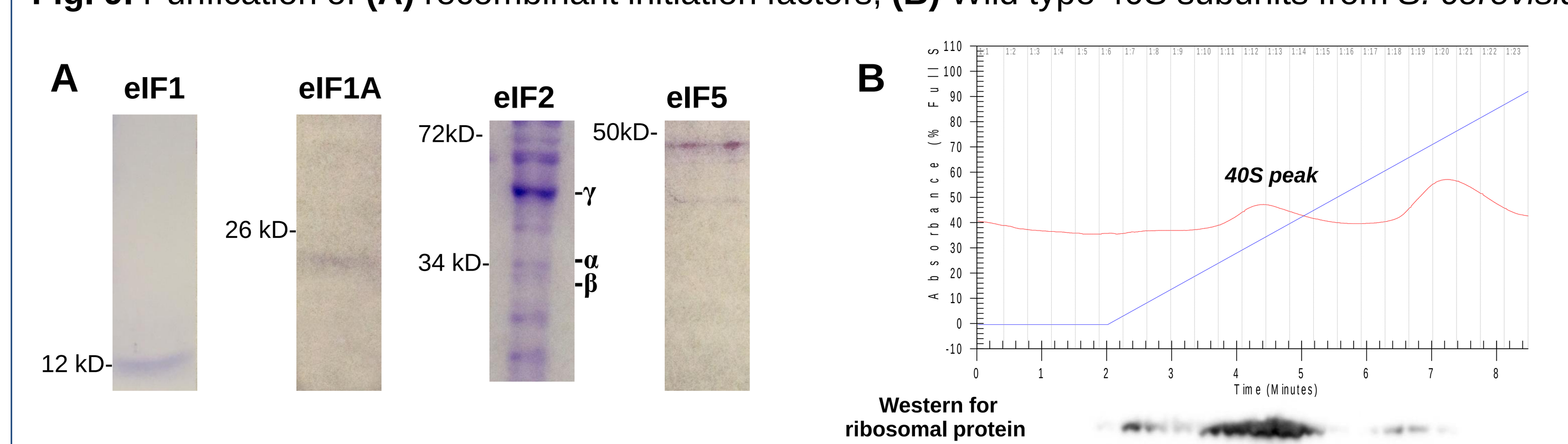


Fig. 8. Increased GAP function of eIF5 rescues slow growth phenotype and initiation defects of uS9 mutants. (A) *TIF5-G31R* allele complements the slow growth phenotype **(B)** *TIF5-G31R* allele rescues the enhanced accumulation of eIF1 and eIF2 on mutant 40S subunits



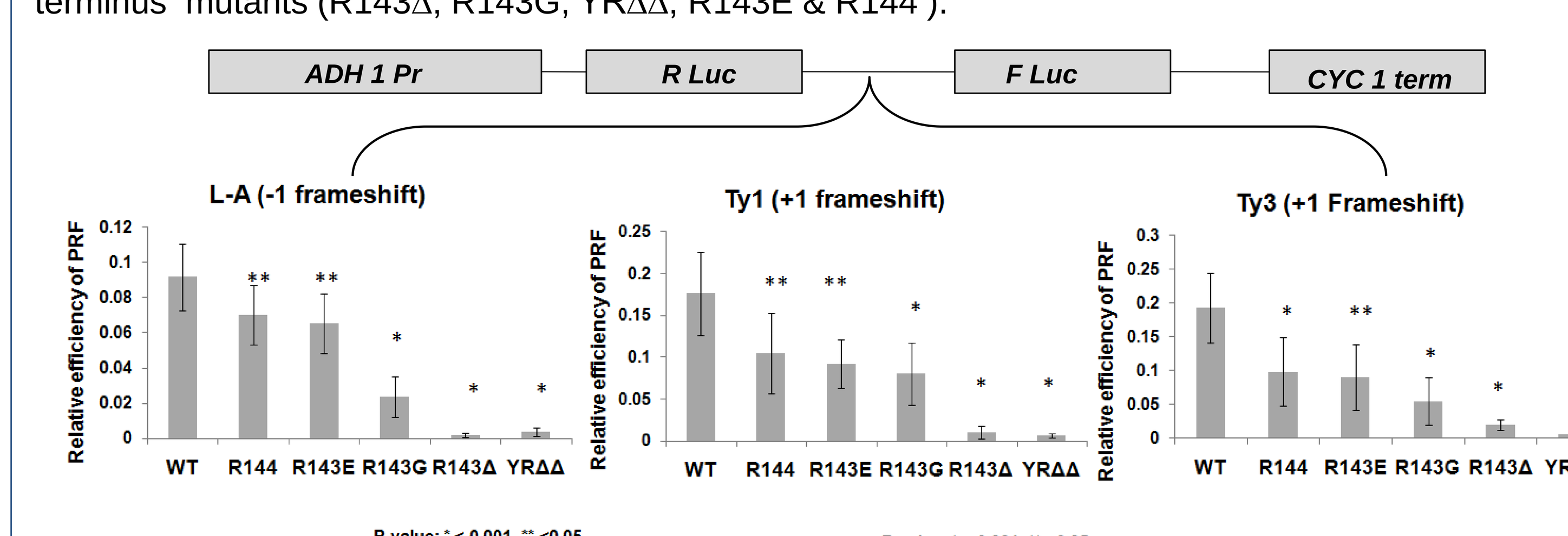
Purification of initiation factors and 40S subunits for GTPase assay

Fig. 9. Purification of **(A)** recombinant initiation factors, **(B)** Wild type 40S subunits from *S. cerevisiae*



Enhanced translation fidelity in uS9 mutants

Fig. 10. Programmed frame-shifting (PRF) efficiency. ***Top:*** A schematic of the dual-luciferase cassette subcloned into reporter vector (p416 ADH) used for measuring PRF efficiency. ***Bottom:*** Dual luciferase assays were performed and PRF efficiencies were calculated for *RPS16* (WT) and the C-terminus mutants (R143Δ, R143G, YRΔΔ, R143E & R144).



CONCLUSIONS

- uS9 C-terminal tail (CTT) is important for GTP hydrolysis or Pi release during translation initiation.
- uS9 seems to play an important role in elongation process.
- Deletion of the last two residues in the uS9 CTT causes reduced frame shifting.

FUTURE DIRECTIONS

- Biochemical assays to study translation termination defects in uS9 mutants
- In vitro assay to study GTP hydrolysis by uS9 mutants
- Association of elongation factors to 40S subunits of uS9 mutants

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