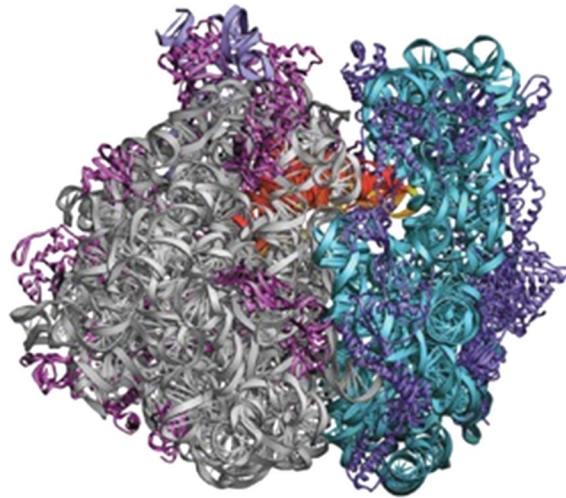


Role of uS9 C-terminal tail in translation initiation and elongation in *Saccharomyces cerevisiae*



Supriya Jindal

Center For Gene Regulation in Health and Disease,

Dept. of BGES, Cleveland State University

PhD Advisor: Dr. Anton A. Komar

Ribosomal Structural History

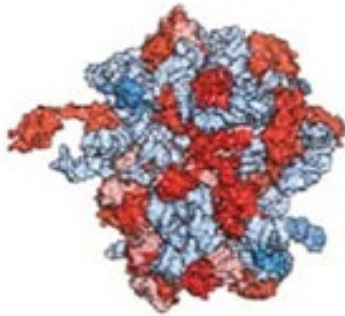
- **Late 1990s:** Crystal structure of 30S and 50S ribosomal subunits
Nobel Prize in 2009 (Yonath, Steitz, Ramakrishnan)
- **2010:** Eukaryotic 80S (yeast) ribosome structure elucidated at atomic resolution 4.1 Å (Ben Sham et. al)
- **2011:** Eukaryotic (yeast) ribosome structure elucidated at atomic resolution 3.0 Å (Ben Sham et. al)

Limited knowledge about mechanistic details of eukaryotic translation machinery

Combination of biochemical and structural approaches will help to learn more

Eukaryotic vs Prokaryotic Ribosome: one core two shells

Bacteria
(*T. thermophilus* or *E. coli*)



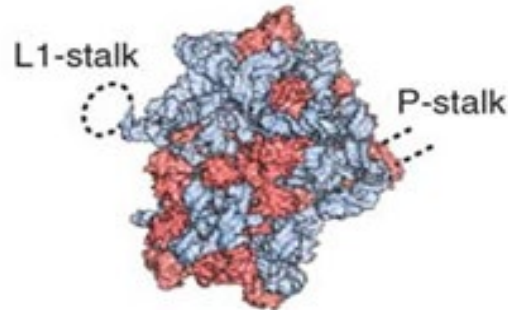
2.3 MDa

54 proteins
3 rRNA

Large subunit (50S):
33 proteins
23S rRNA—2,904 bases
5S rRNA—121 bases

Small subunit (30S):
21 proteins
16S rRNA—1,542 bases

The common core



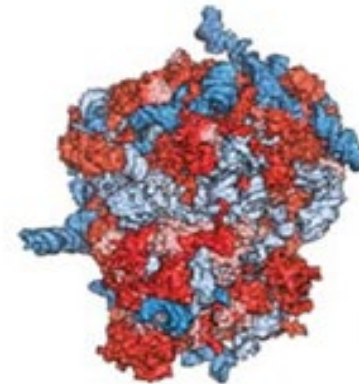
2.0 MDa

34 proteins
3 rRNA

Large subunit:
19 proteins
23S rRNA—2,843 bases
5S rRNA—121 bases

Small subunit:
15 proteins
16S rRNA—1,458 bases

Lower eukaryotes
(*S. cerevisiae*)



3.3 MDa

79 proteins
4 rRNA

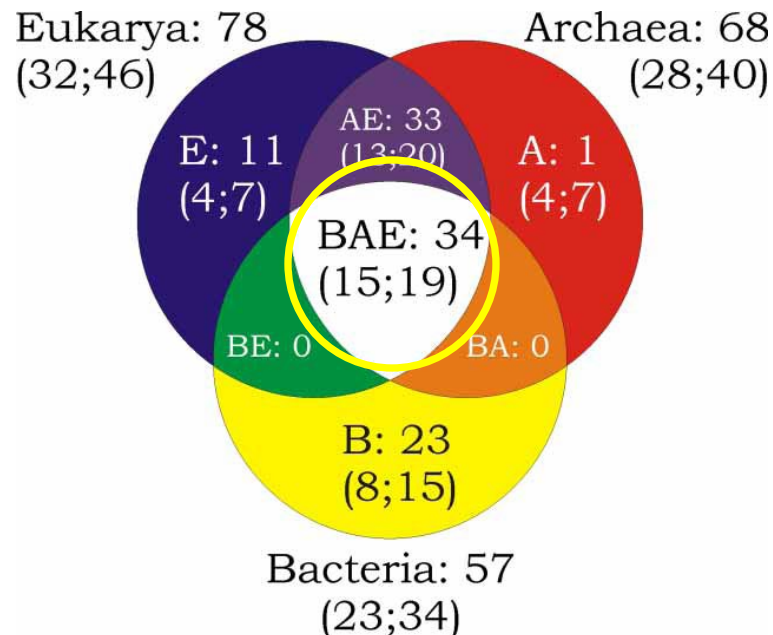
Large subunit (60S):
46 proteins
5.8S rRNA—158 bases
25S rRNA—3,396 bases
5S rRNA—121 bases

Small subunit (40S):
33 proteins
18S rRNA—1,800 bases

Role of Ribosomal proteins

- Clear from biochemical studies, ribosomal proteins are not involved in peptide formation , *per se*.

What is the role of ribosomal proteins especially universally conserved?



Role of Ribosomal proteins

Ribosome biogenesis: S3, S15 etc

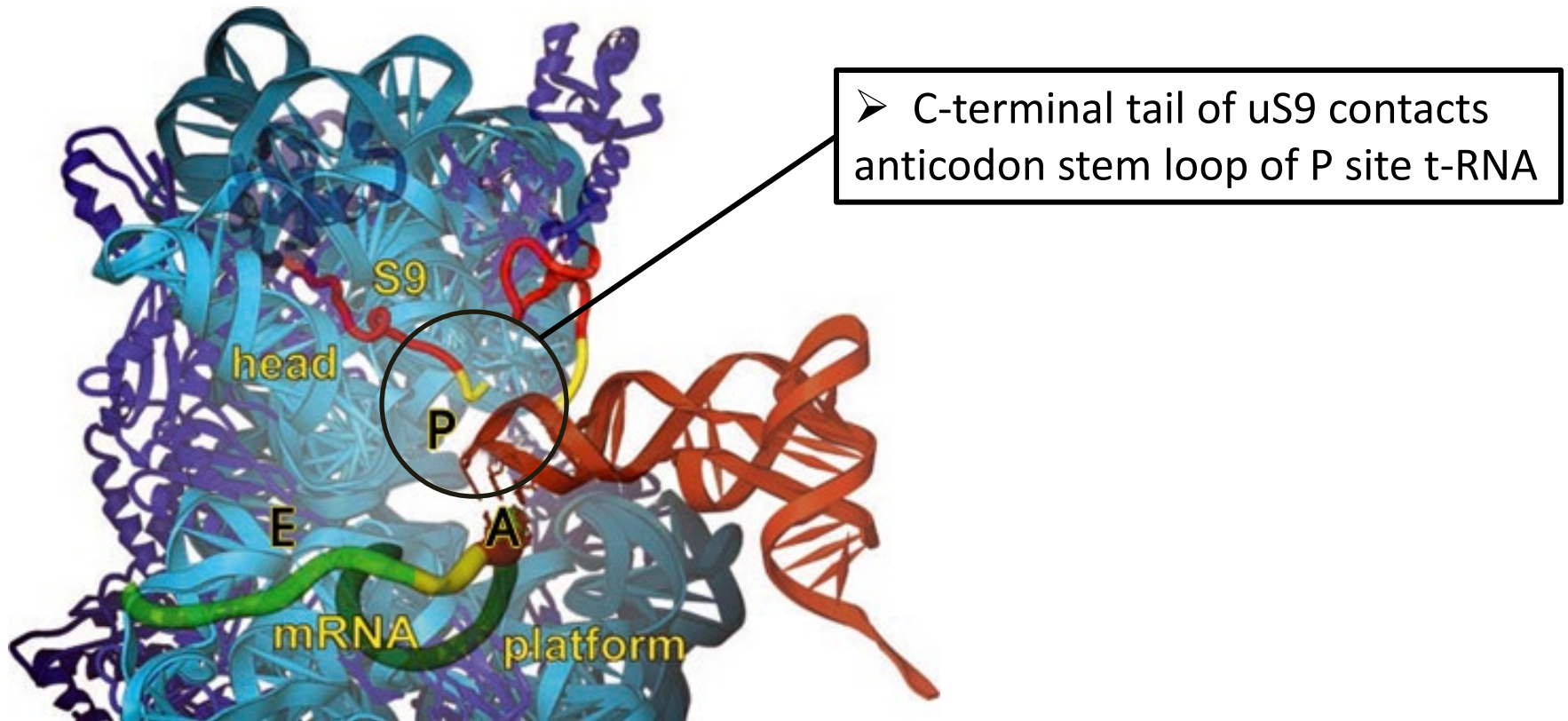
Ribosome independent function

Believed to be involved in recruitment of tRNA and translation factors

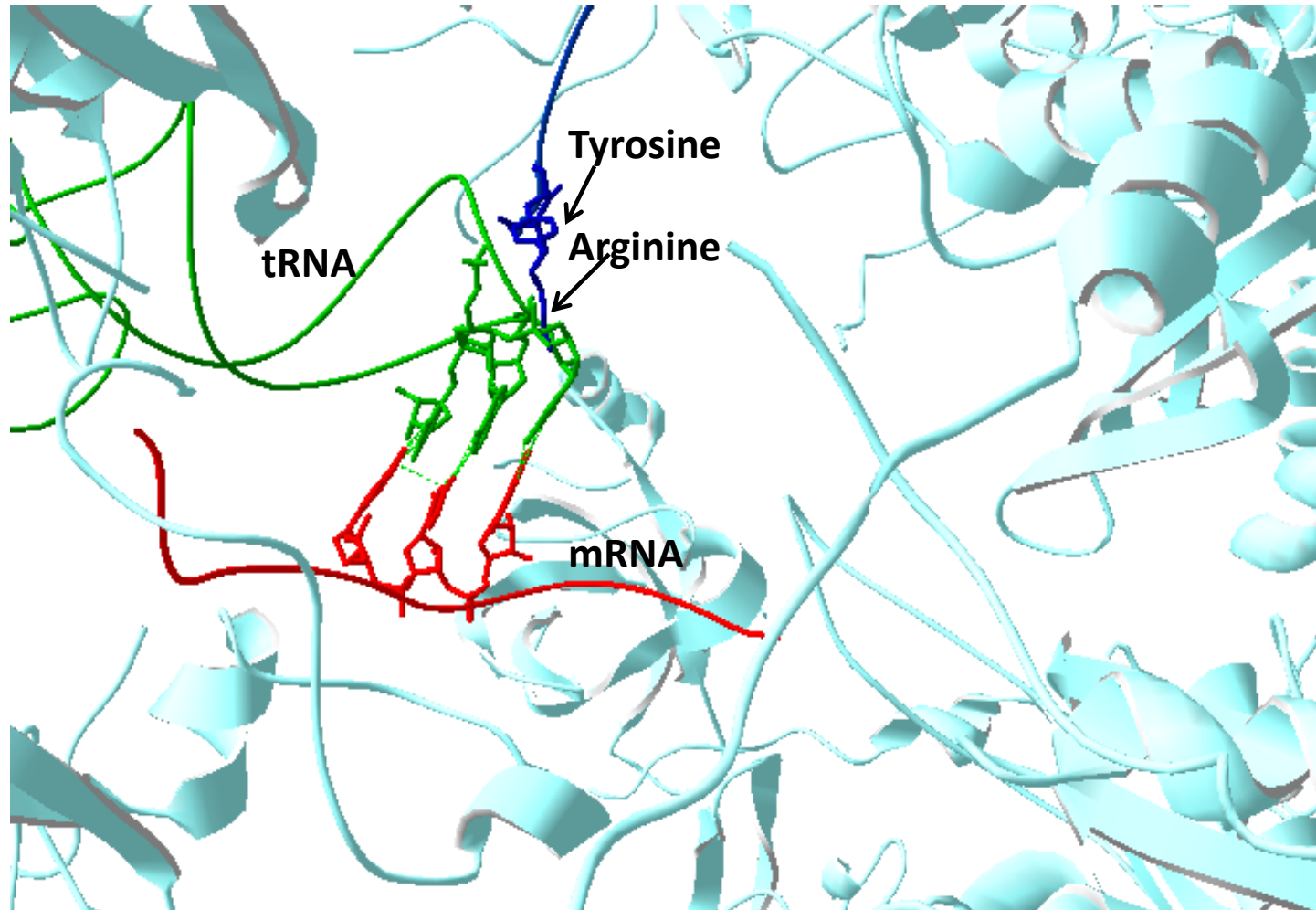
Location of Ribosomal protein uS9 (S16)



Role of uS9 tail in prokaryotic translation



uS9 C-Terminus Location in Eukaryotic Ribosome



Highly Conserved uS9 C-Terminus

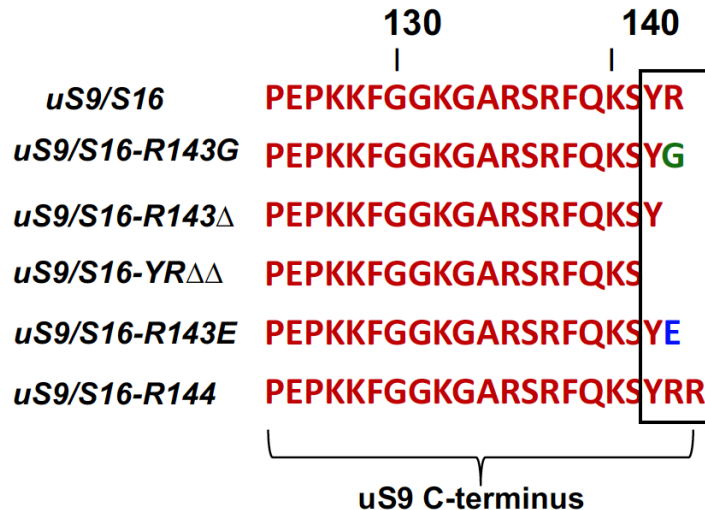
		130	140		Y142	R143
					↙	↘
RS16_Agossyppi	TLLIAD	RRPEPKK	FGGRGARARE	QKSYR		
RS16_Klactis	TLLIAD	RRPEPKK	FGGRGARSRE	QKSYR		
RS16_Scerevisiae	TLLIAD	RRPEPKK	FGGKGARSRE	QKSYR		
RS16_Cglabrata	TLLIAD	ARRPEPKK	FGGKGARARE	QKSYR		
RS16_Calbicans	TLLVAD	SRMEPKK	FGGRGARARE	QKSYR		
RS16_Spombe	TLLVAD	PRMEPKK	FGGHGARARQ	QKSYR		
RS16_Rnorvegicus	TLLVAD	PRCESKK	GGPGARARY	QKSYR		
RS16_Mmusculus	TLLVAD	PRCESKK	GGPGARARY	QKSYR		
RS16_Hsapiens	TLLVAD	PRCESKK	GGPGARARY	QKSYR		
RS16_Dmelanogaster	TLLVGD	PRRCEPKK	GGPGARARY	QKSYR		
RS16_Celegans	SLLVAD	PRRRESKK	GGPGARARY	QKSYR		
RS9_Tacidophilum	TLIVND	VIKLPKK	AGGRGARAKK	QKSYR		
RS9_Tpallidum	-LLTRD	SRMVERKKY	GQRGARRRE	QFSKR		
RS9_Sthermophilus	-LLTRD	ARMVERKKP	GLKKARKAS	QFSKR		
RS9_Ecoli	-FVTRD	ARQVERKKV	GLRKARRRE	QFSKR		
	::	* *	** *	**	* * *	

Objective: 1) To study the significance of uS9 CTT in eukaryotic translation in yeast

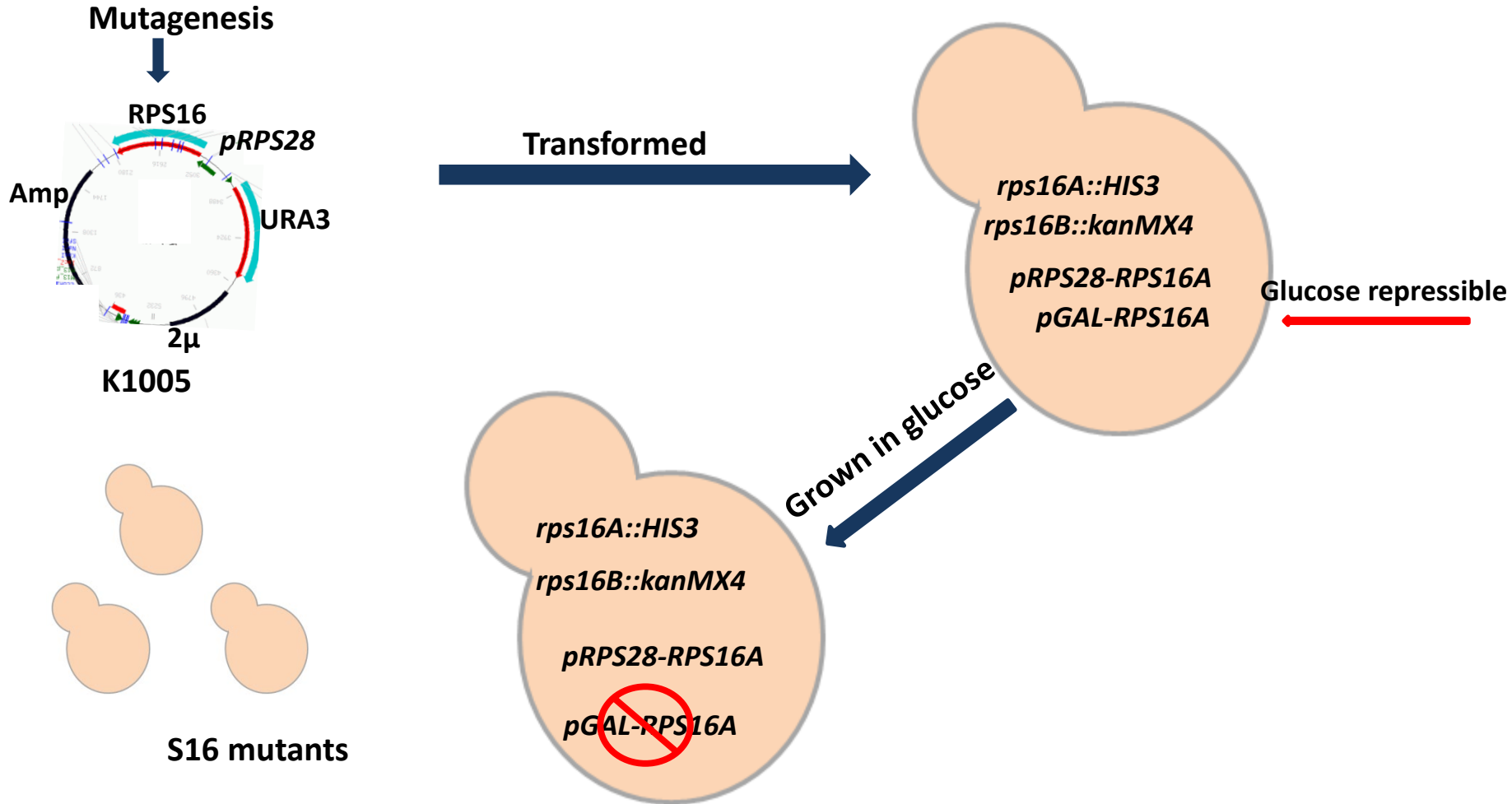
- ✓ the length of C terminal tail of uS9
- ✓ positively charged Arginine

uS9 C-terminal mutants

- To study the importance of the C-terminus length:
 - ✓ R143 Δ - deletion of arginine
 - ✓ YR $\Delta\Delta$ - double deletion of R143 and Y142
 - ✓ R144- Insertion of an extra R at the C-terminal end
- To study the role of arginine in interaction with negatively charged tRNA :
 - ✓ R143G- substitution of arginine by glycine
 - ✓ R143E- substitution of arginine by glutamate



Creating uS9 C-terminal mutants



Experimental scheme

- To assess the impact of different mutations on translation:

Cell growth

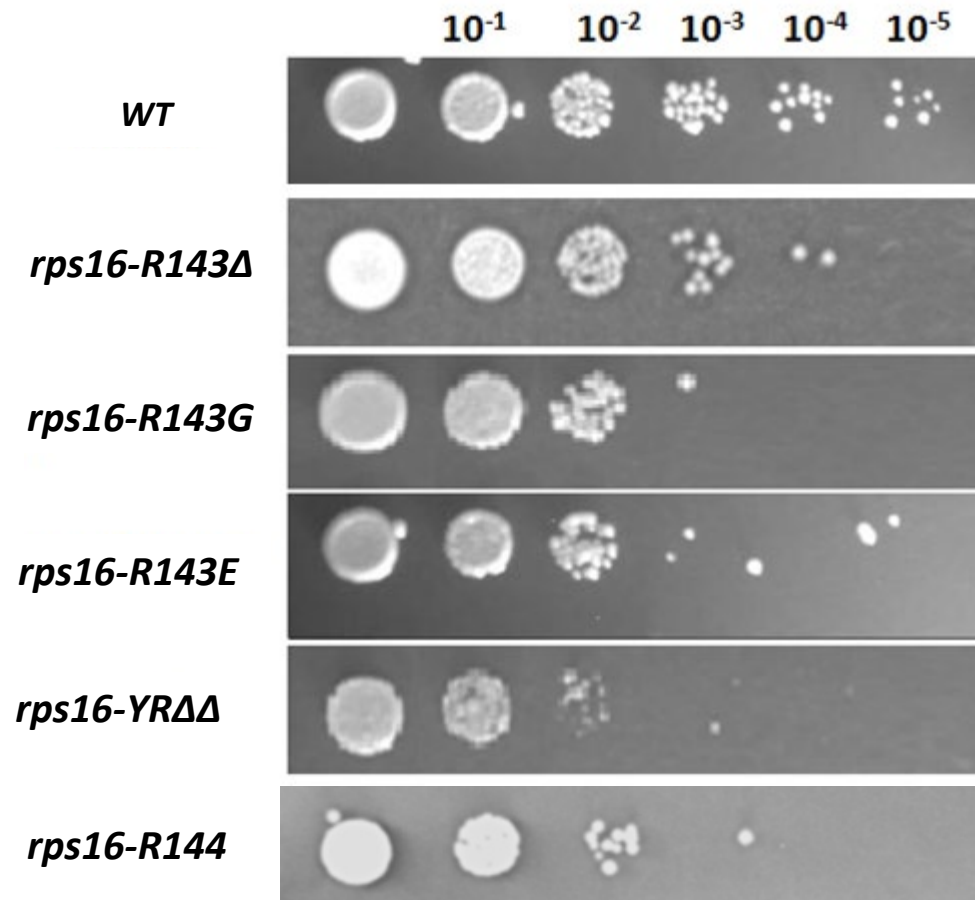
Translation initiation by polysome profiling

Translation factor association by western blot

Mechanism of translation initiation by expression of reporter constructs

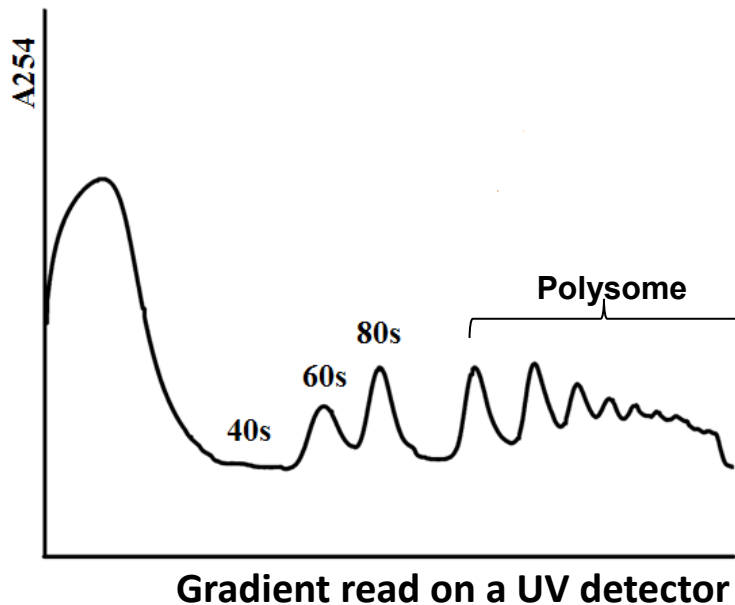
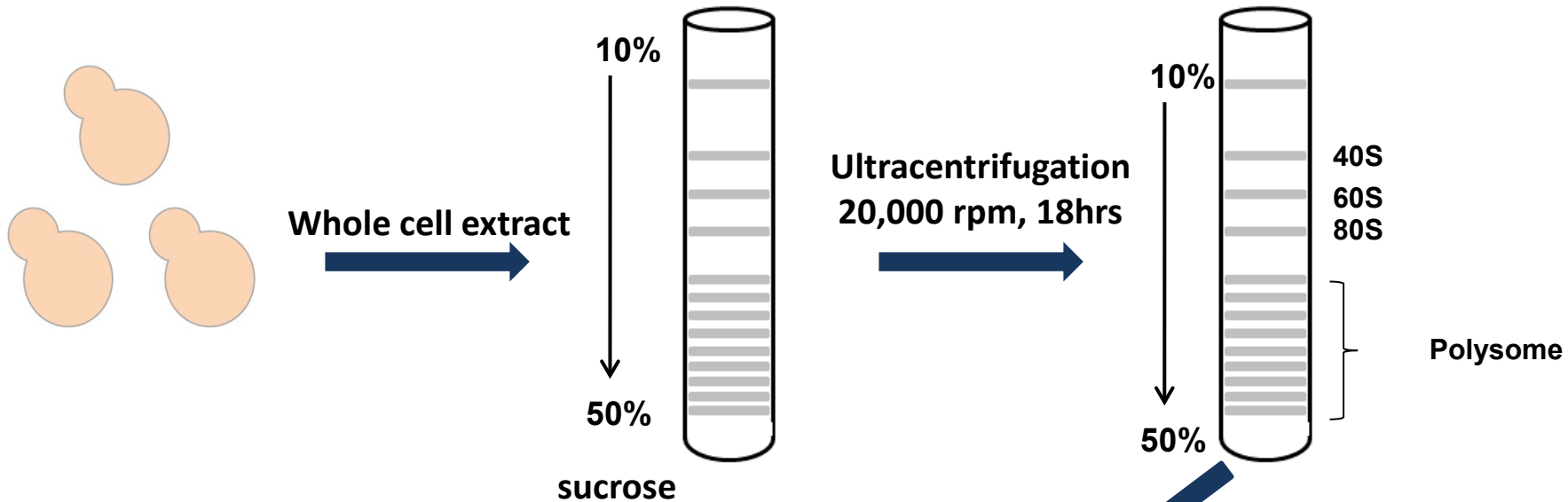
Mechanism of translation initiation by using yeast reconstituted translation initiation system

Slow growth phenotype of uS9 mutants

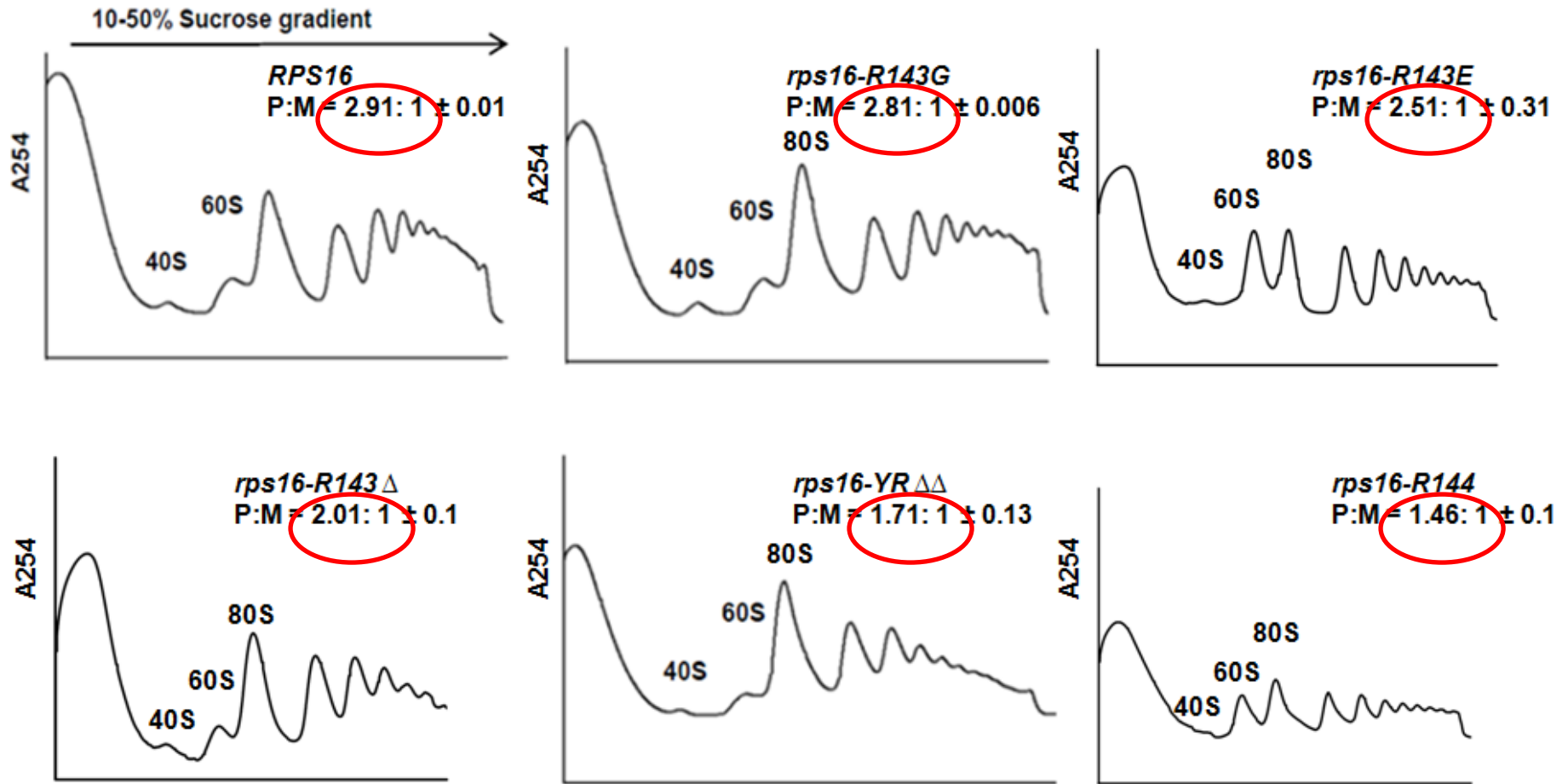


Translation initiation defect in uS9 mutants

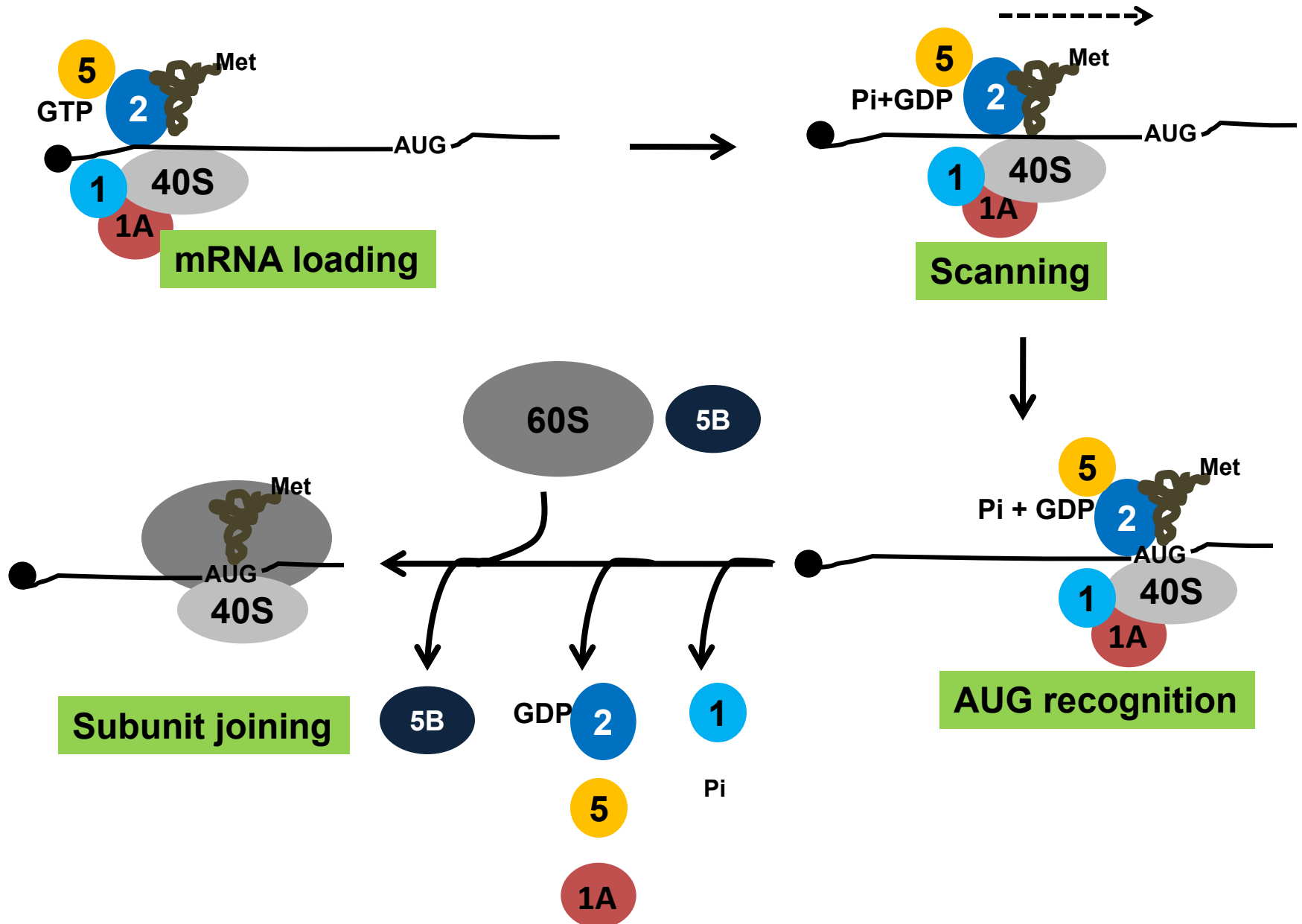
- Studied by polysome profiling



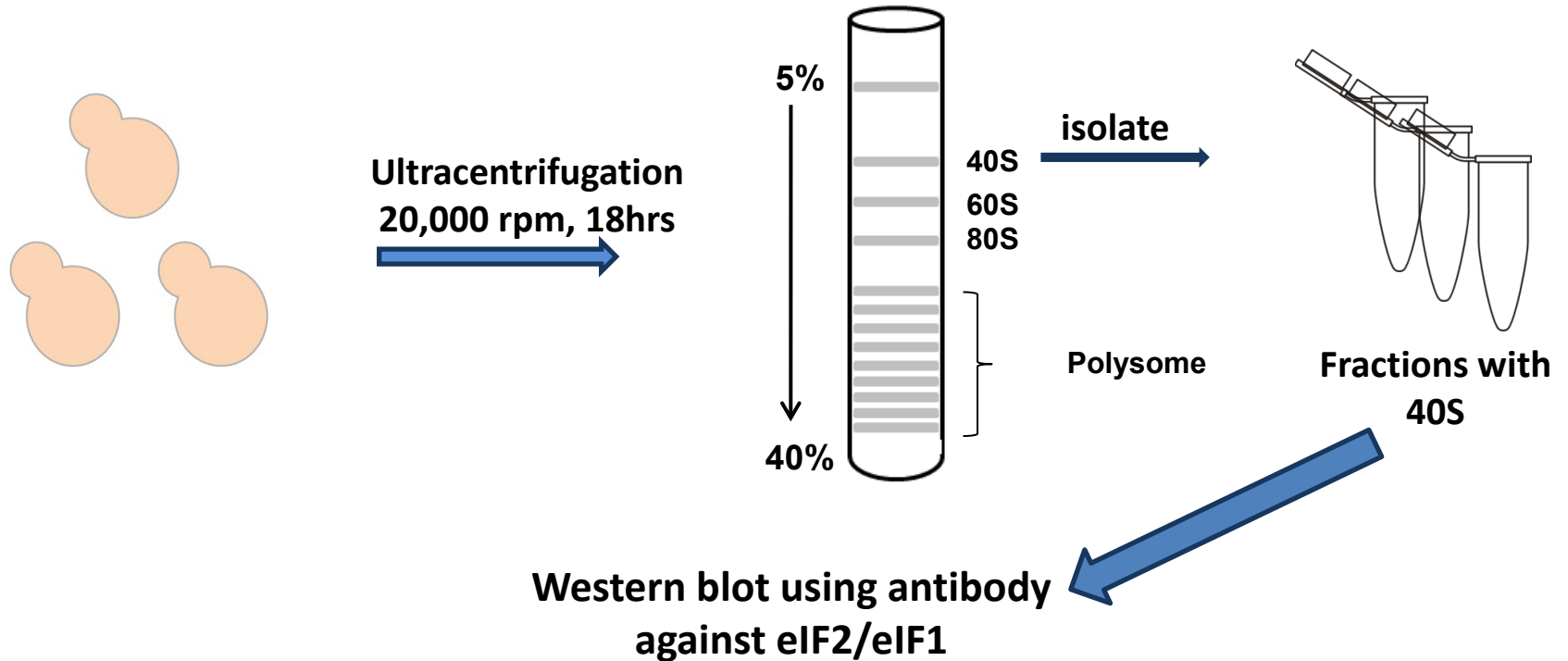
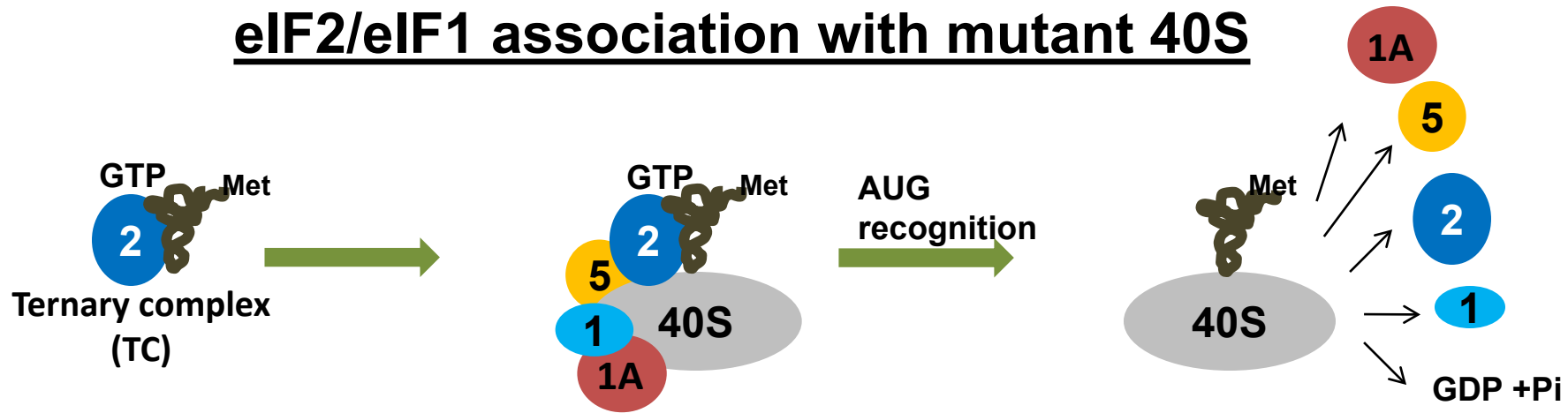
Translation initiation defect in uS9 mutants



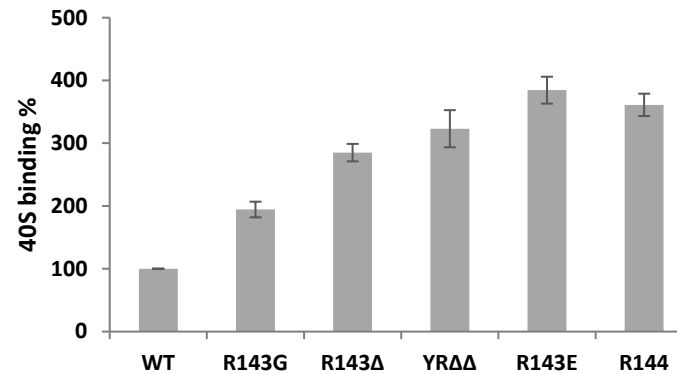
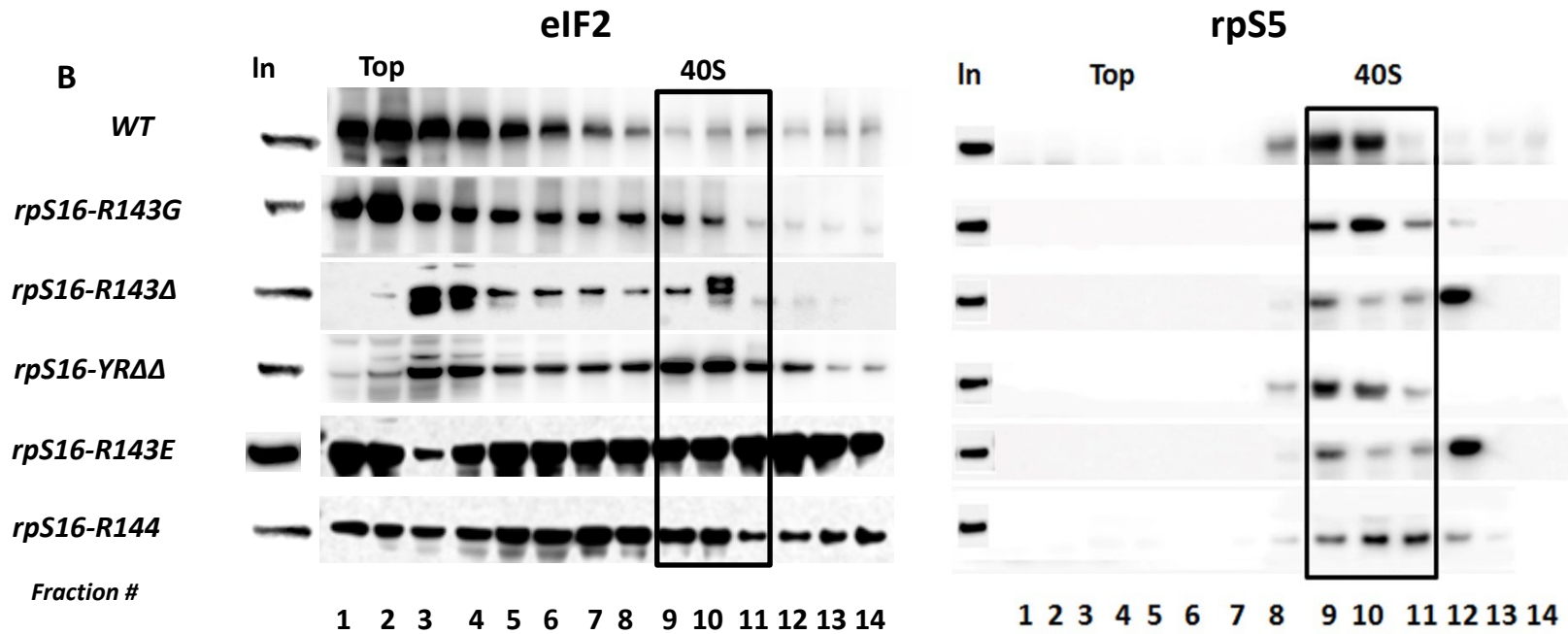
Eukaryotic Translation Initiation



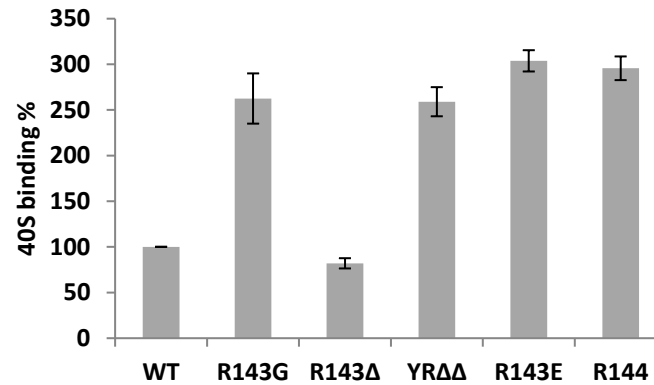
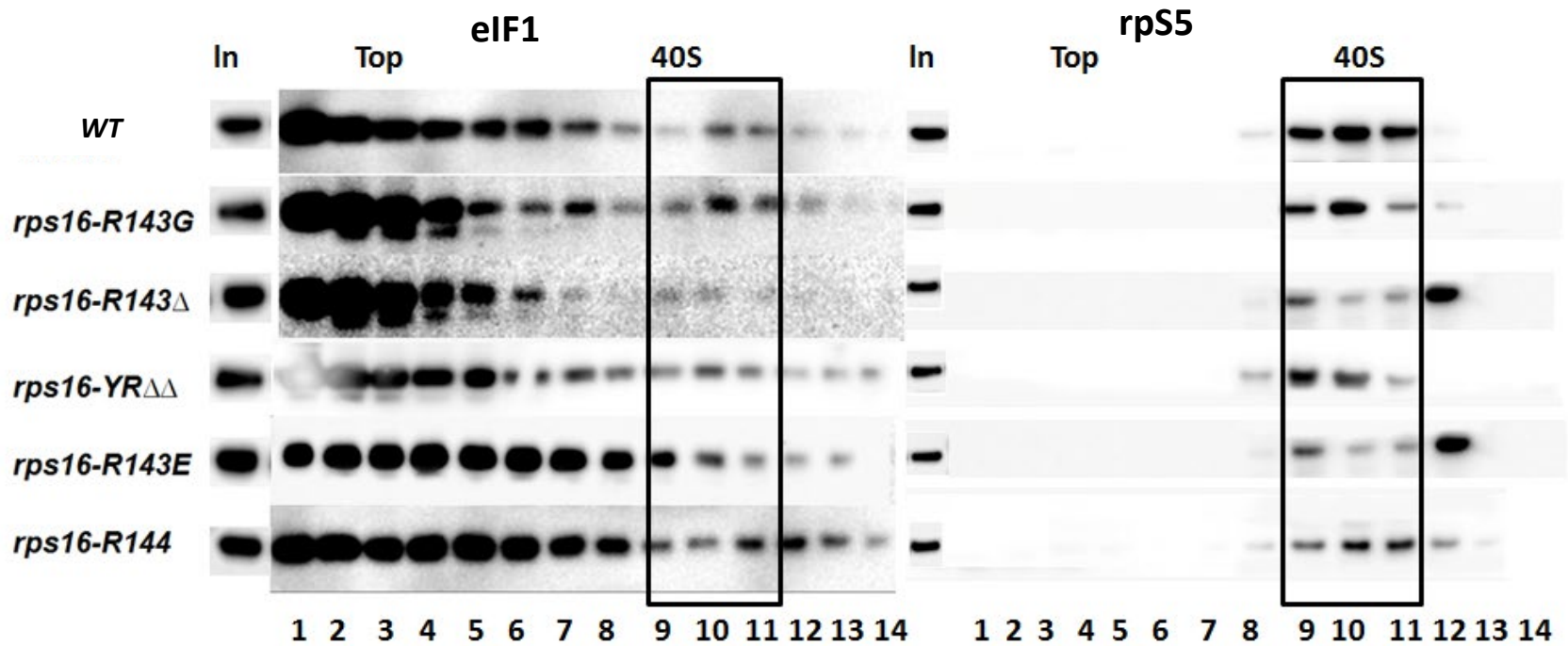
eIF2/eIF1 association with mutant 40S



eIF2 accumulation on 40S of uS9 mutants



eIF1 accumulation on 40S of uS9 mutants



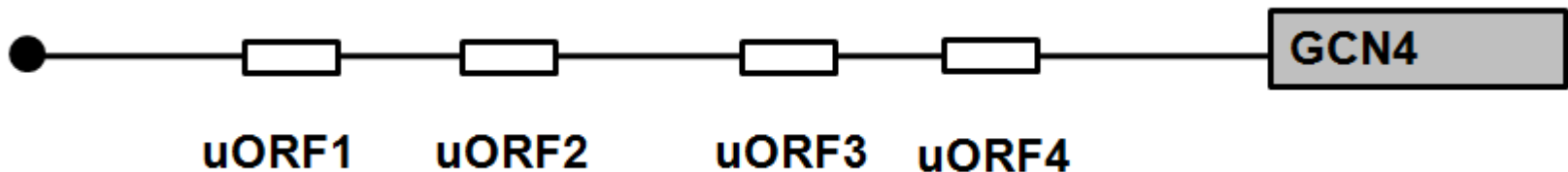
Conclusion

- RPS16 C-terminal tail has a definitive role in translation initiation

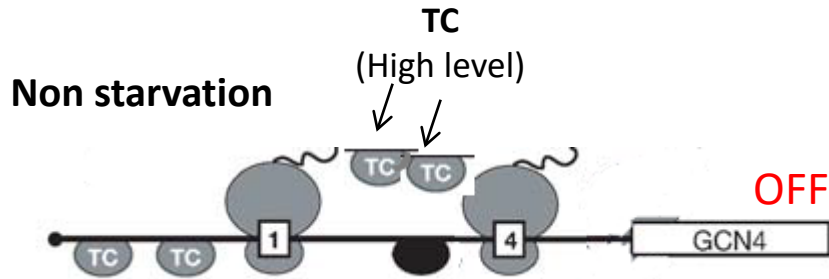
- Which exact step of initiation is compromised?

GCN4 translation control in yeast

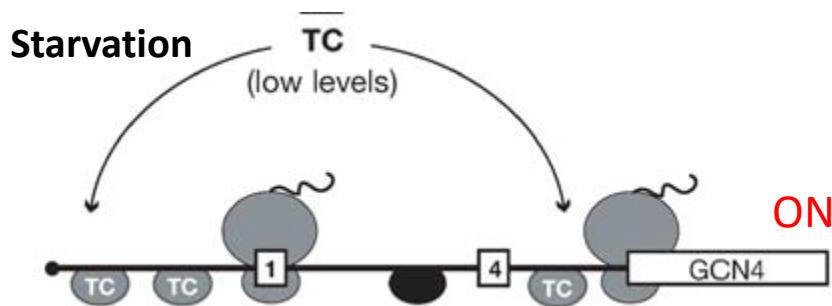
- GCN4 mRNA translation under starvation, repressed under normal conditions
- GCN4 expression control mediated by translation reinitiation
- Extremely sensitive to the activity of TC



GCN4 translation control mechanism

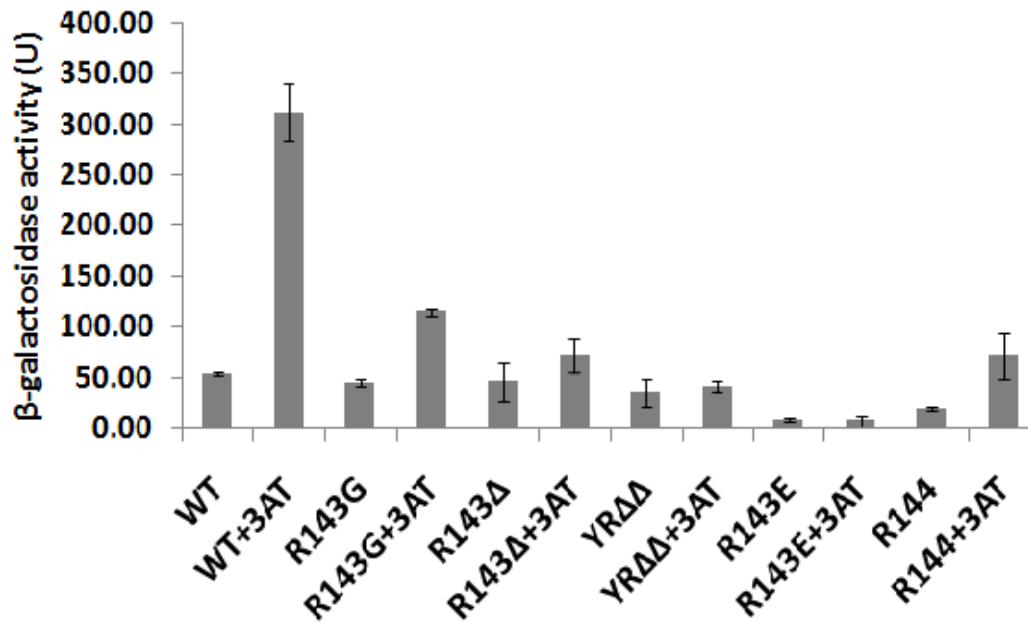
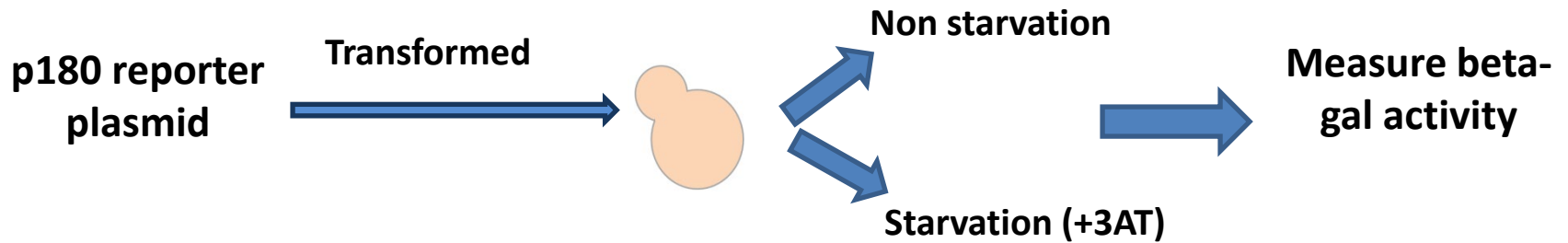


TC conc is high, ~98% of 40S resumes initiation before it crosses AUG4, thus repressing GCN4 expression

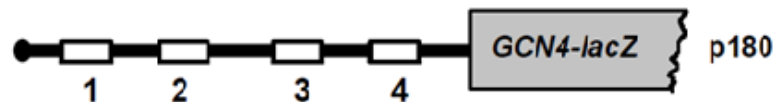


TC conc is low, reinitiating 40S gets loaded with TC after crossing AUG4 and hence available for GCN4 expression

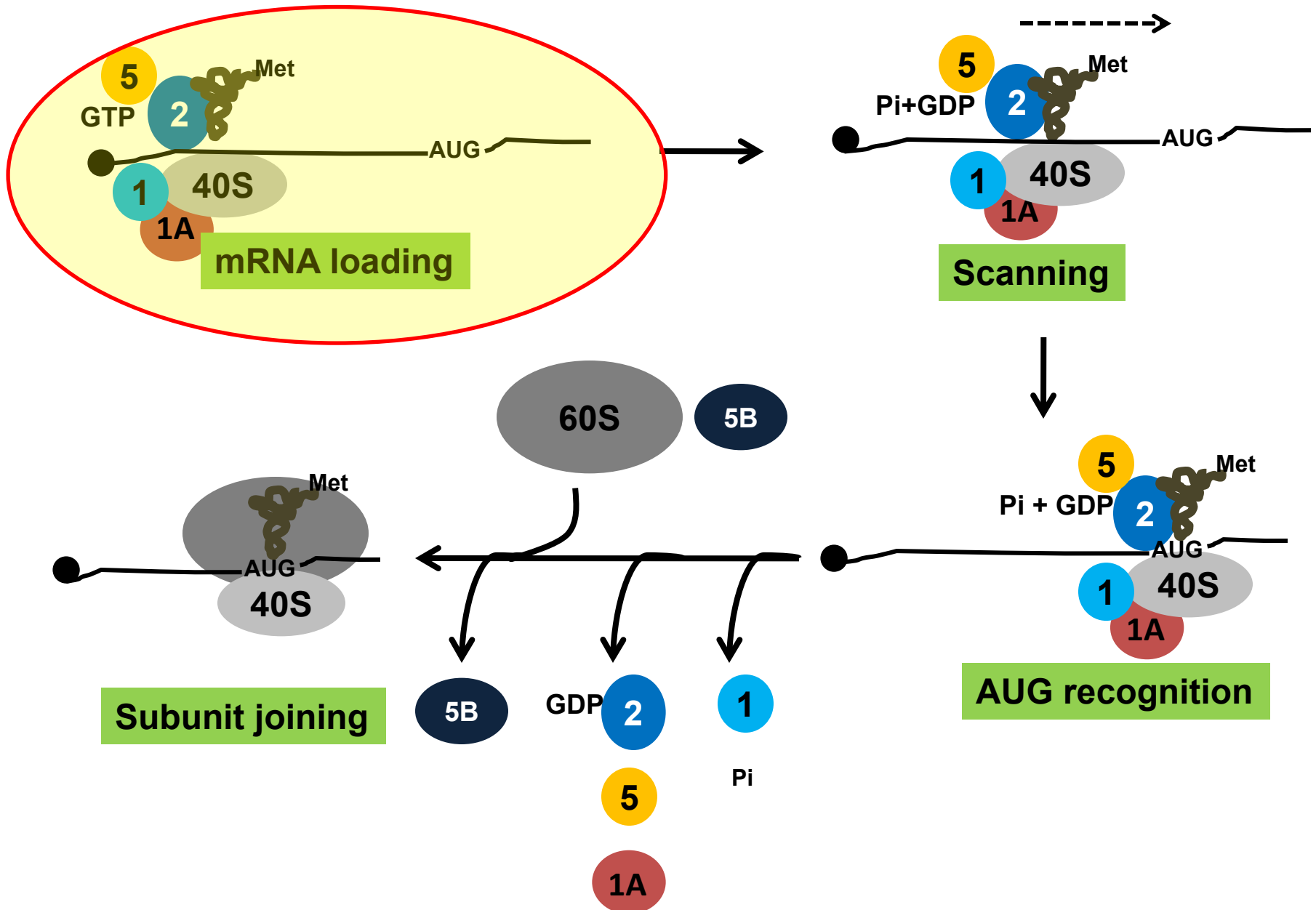
Translation Reinitiation Is Compromised in uS9 mutants



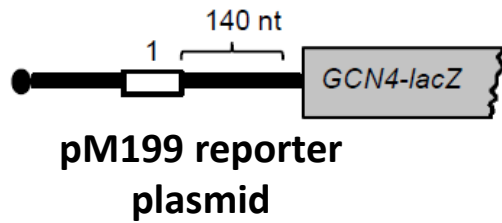
P-value: < 0.001



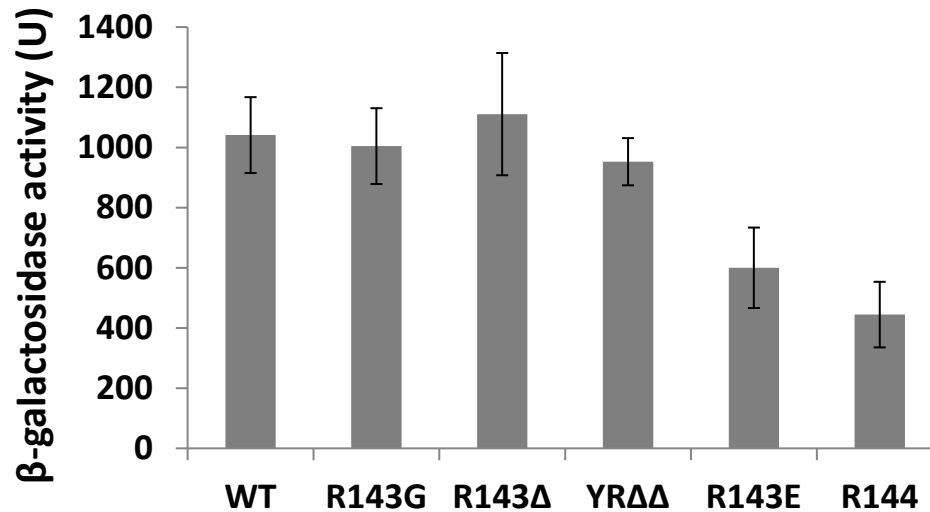
Eukaryotic Translation Initiation



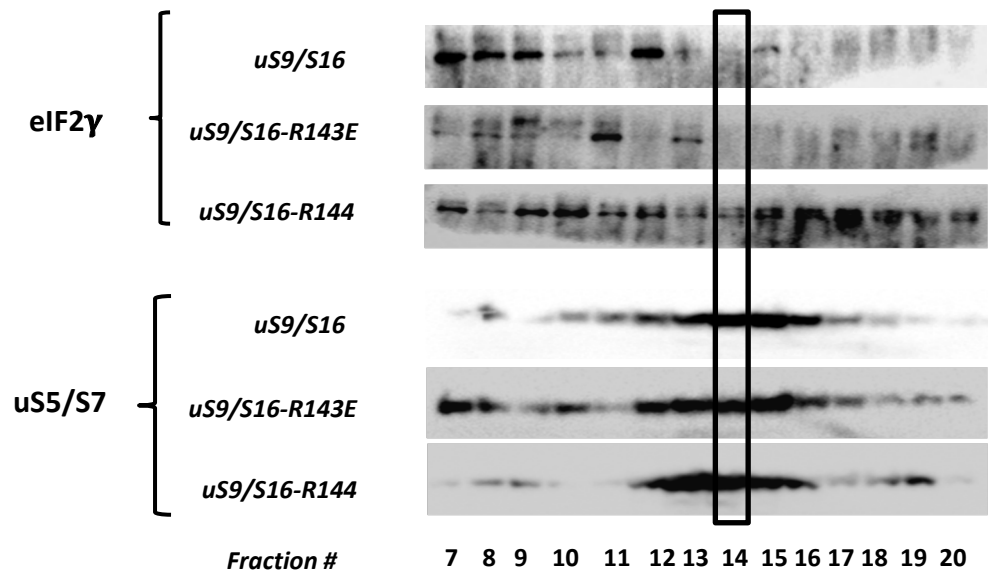
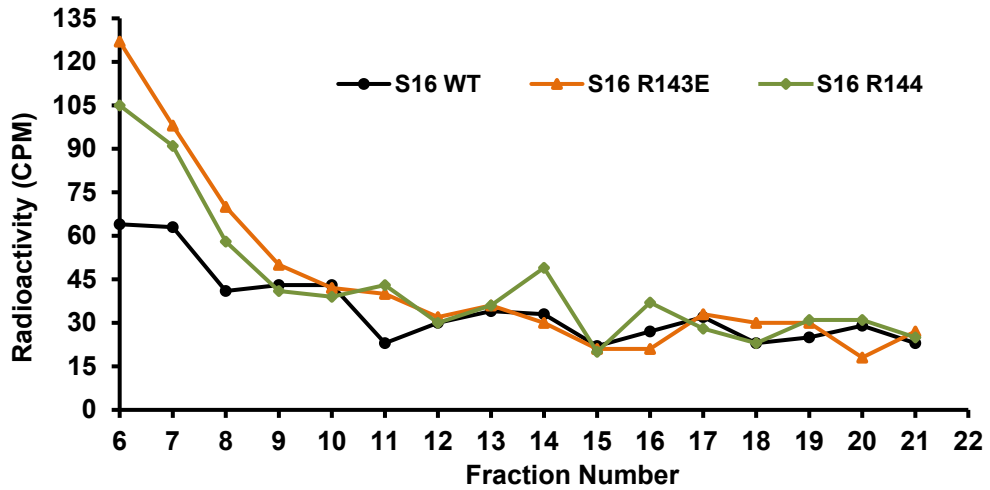
uS9 mutants show failure to resume scanning



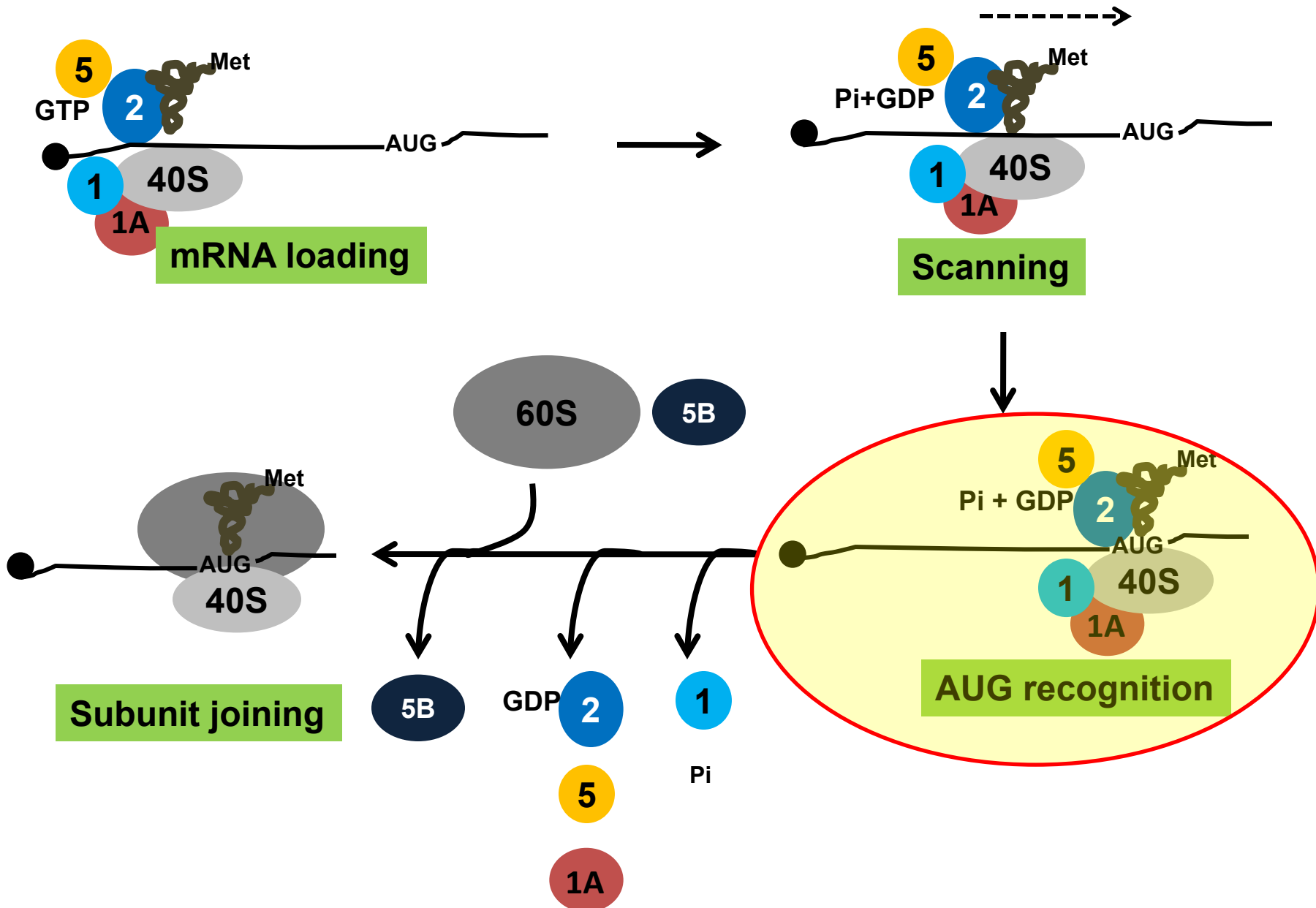
Measure beta-gal activity



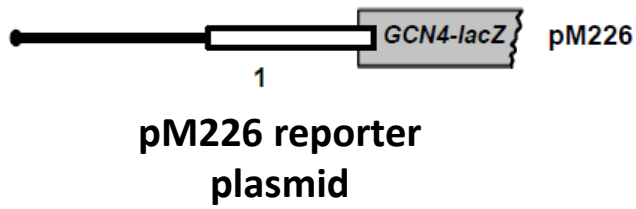
Defective 43S complex formation in uS9 mutants



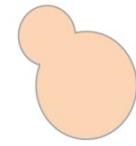
Eukaryotic Translation Initiation



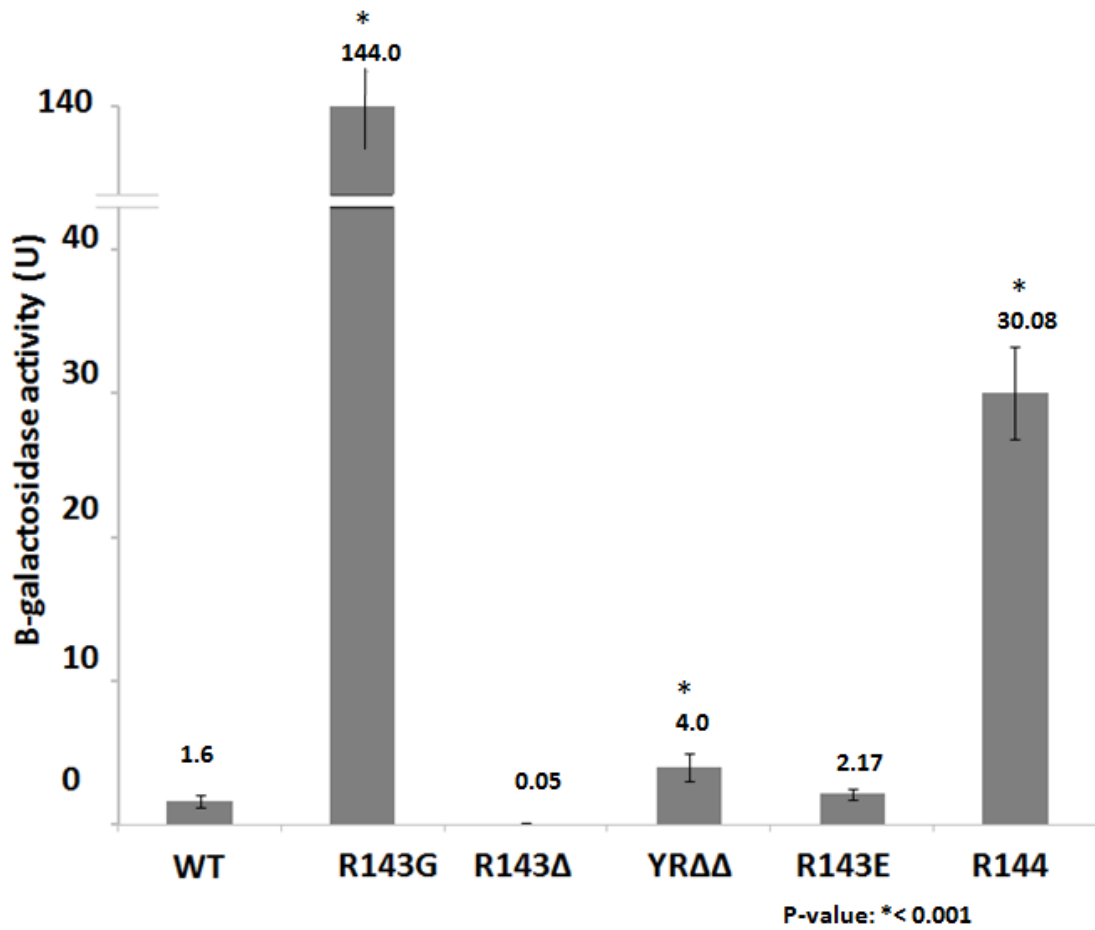
uS9 mutants show leaky scanning of AUG codon



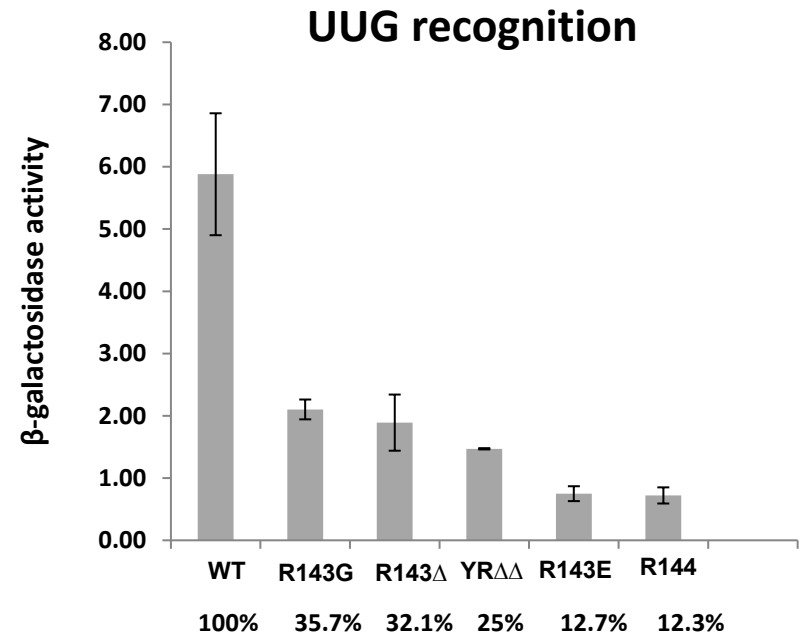
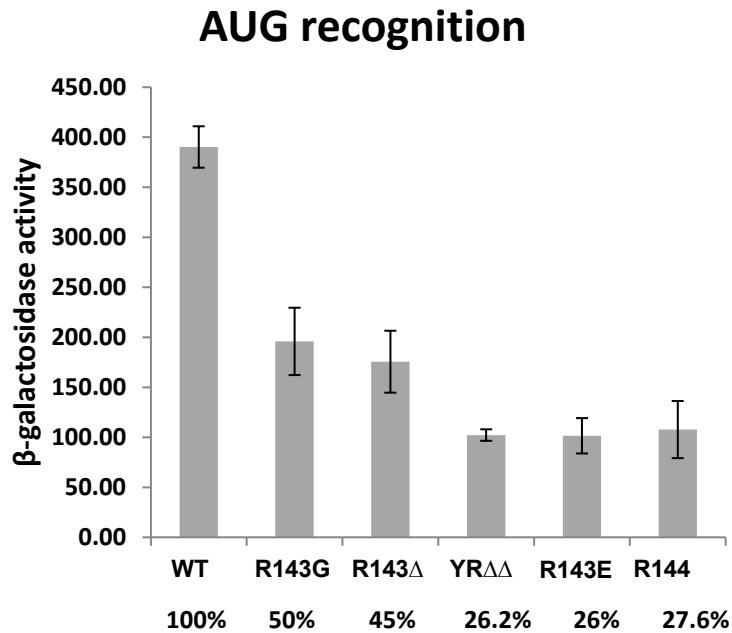
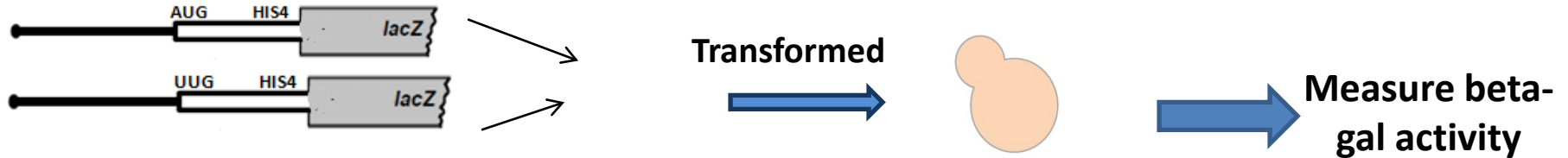
Transformed



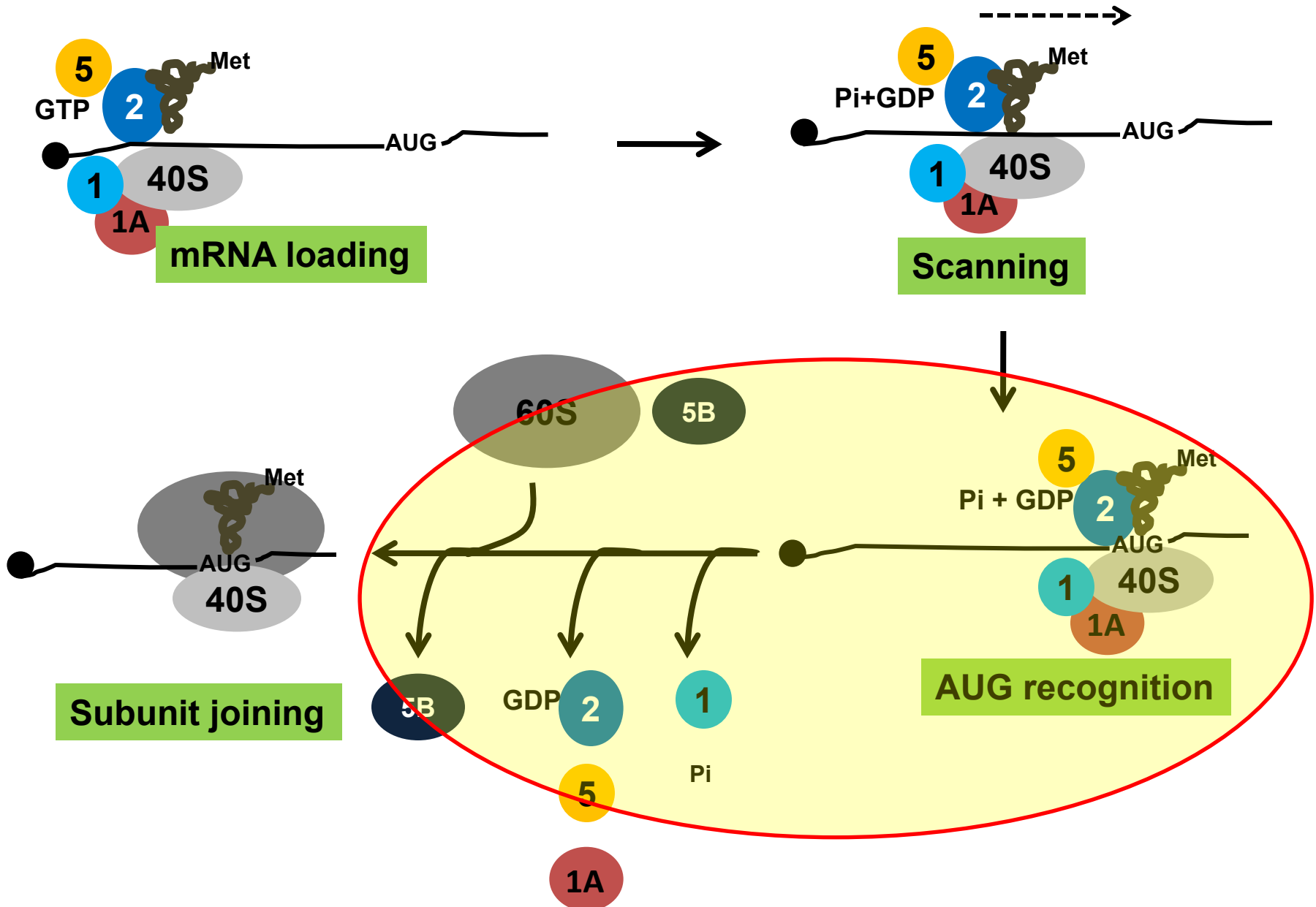
Measure beta-gal activity



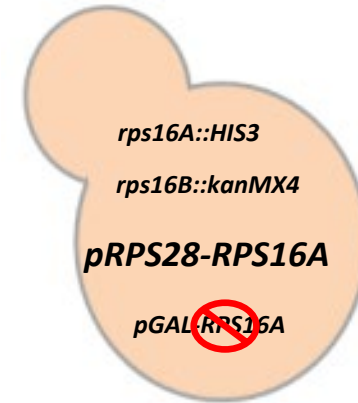
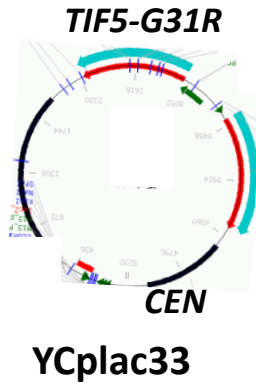
AUG and UUG recognition is Compromised in uS9 mutants



Eukaryotic Translation Initiation



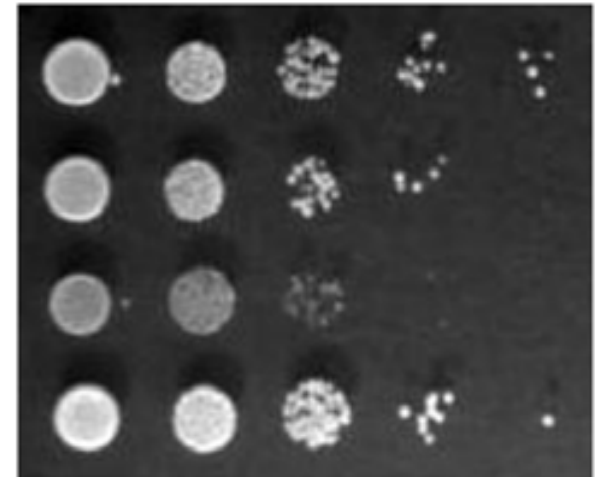
Increased GAP (GTPase activating protein) function of eIF5 rescues slow growth phenotype of uS9 mutant



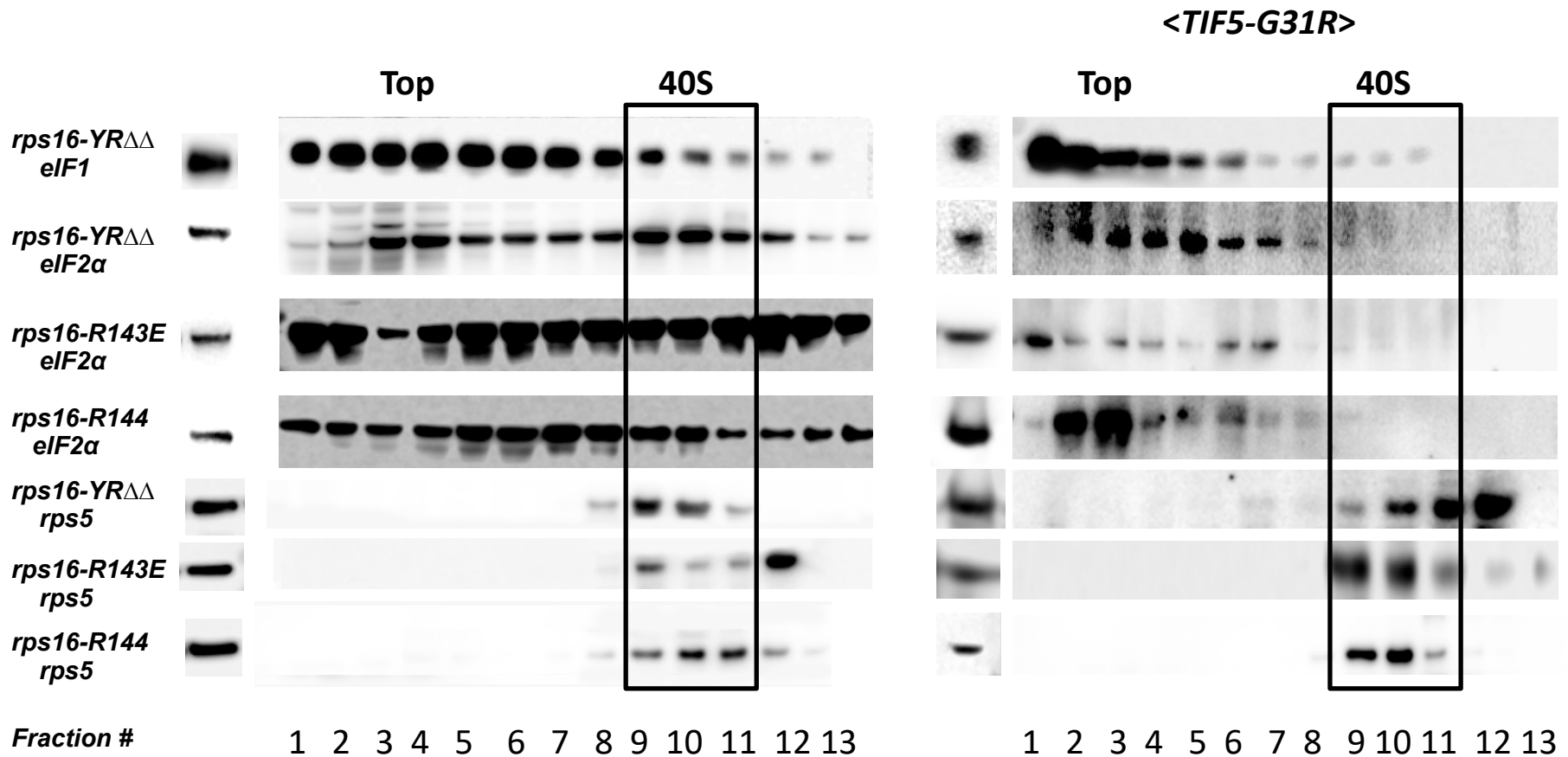
TIF5-G31R:
Stimulate eIF2-GTP → GDP+Pi
hydrolysis

RPS16
RPS16
<*TIF5-G31R*>
rps16-YRΔΔ
rps16-YRΔΔ
<*TIF5-G31R*>

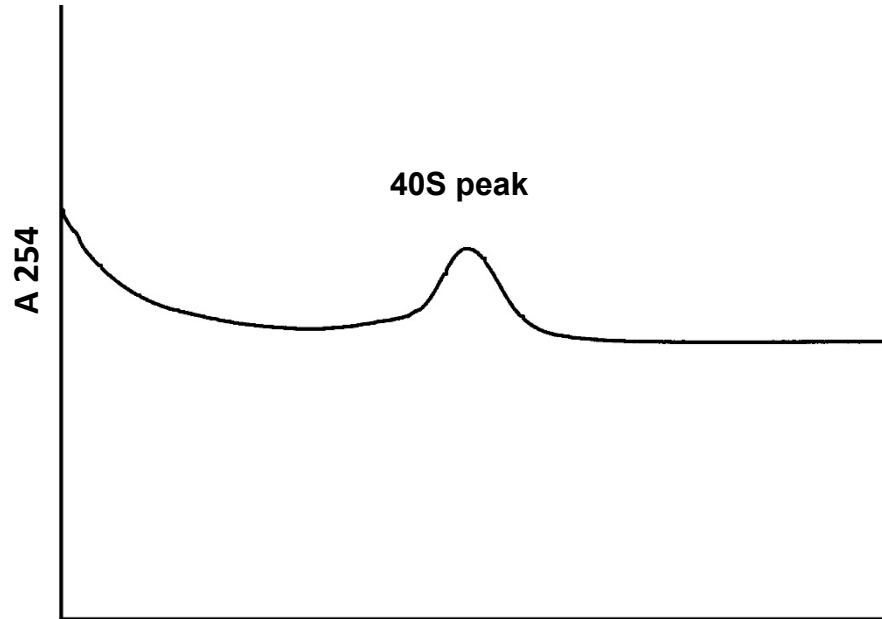
10⁻¹ 10⁻² 10⁻³ 10⁻⁴



Association of eIF2/eIF1 with 40S subunits in uS9 mutants *and* uS9 <TIF5-G31R> yeast strains



Purification of 40S subunits for GTPase assay

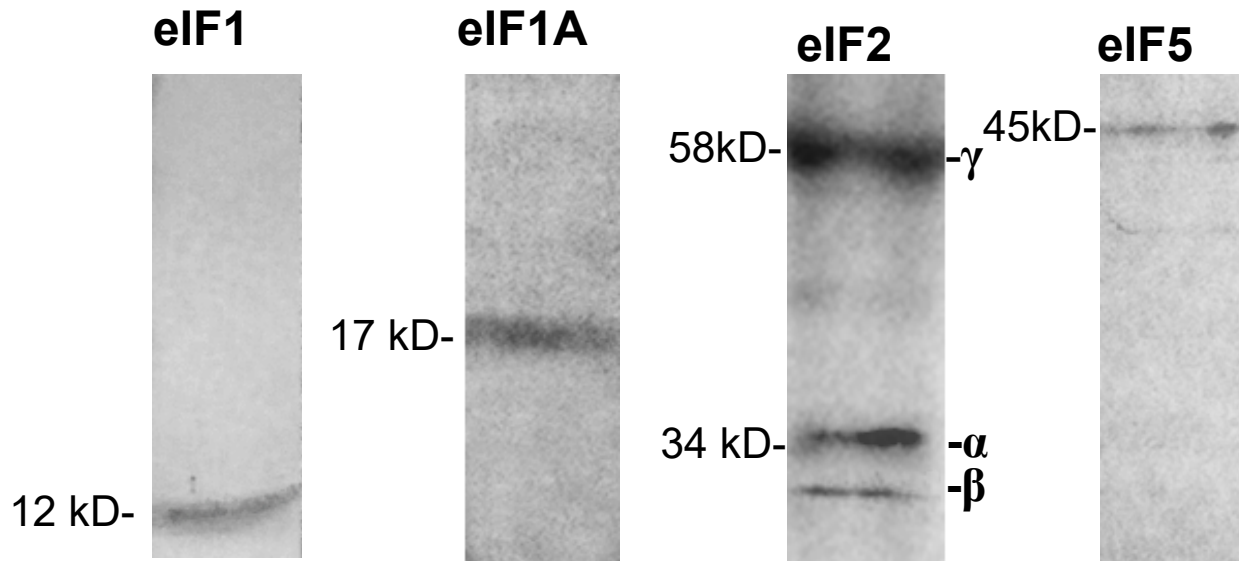


Western for
ribosomal protein S5

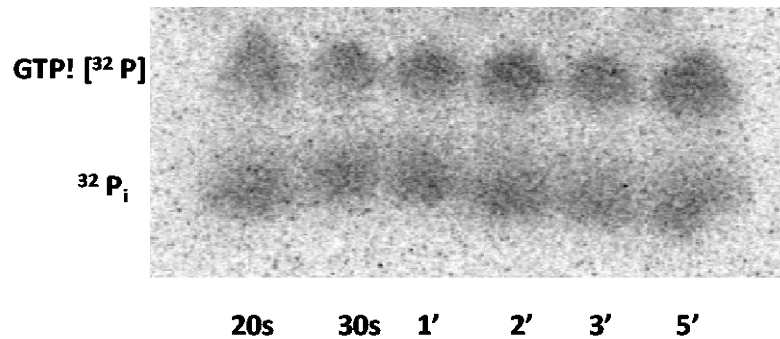
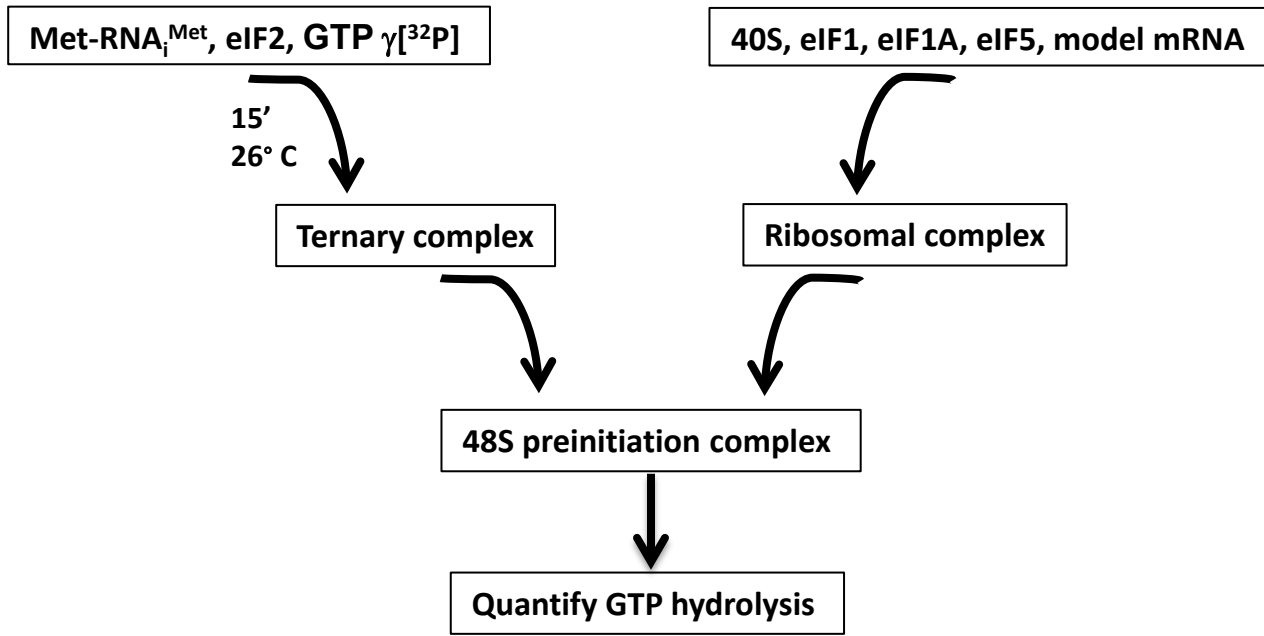


Purification of initiation factors for GTPase assay

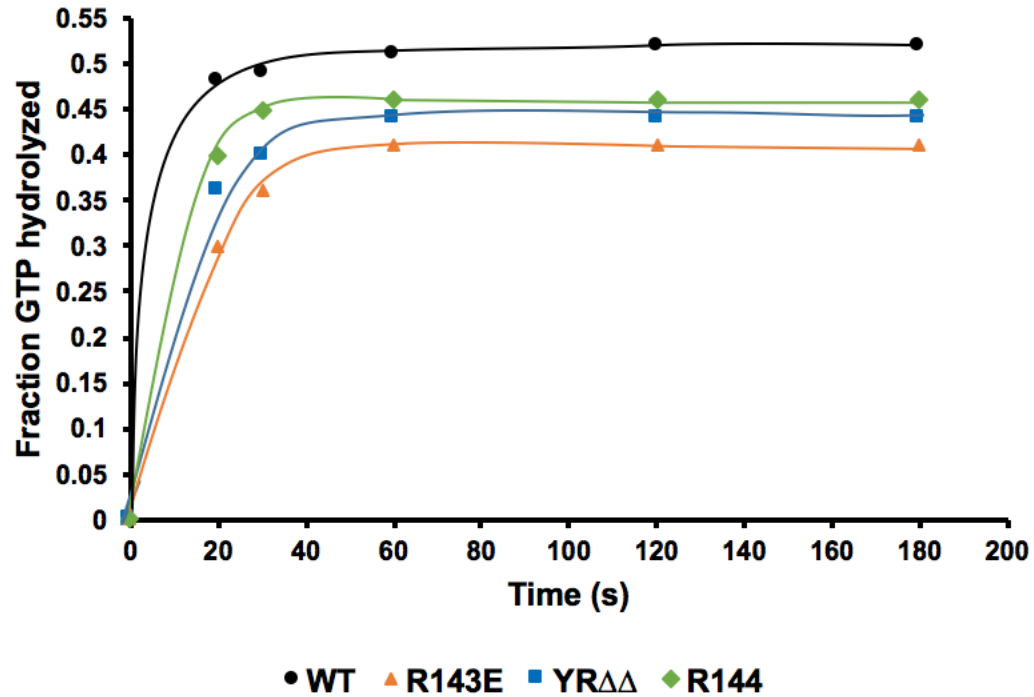
- Expresses fusion protein (desired eIF+ chitin binding domain)
- The chitin binding domain is a high affinity tag.



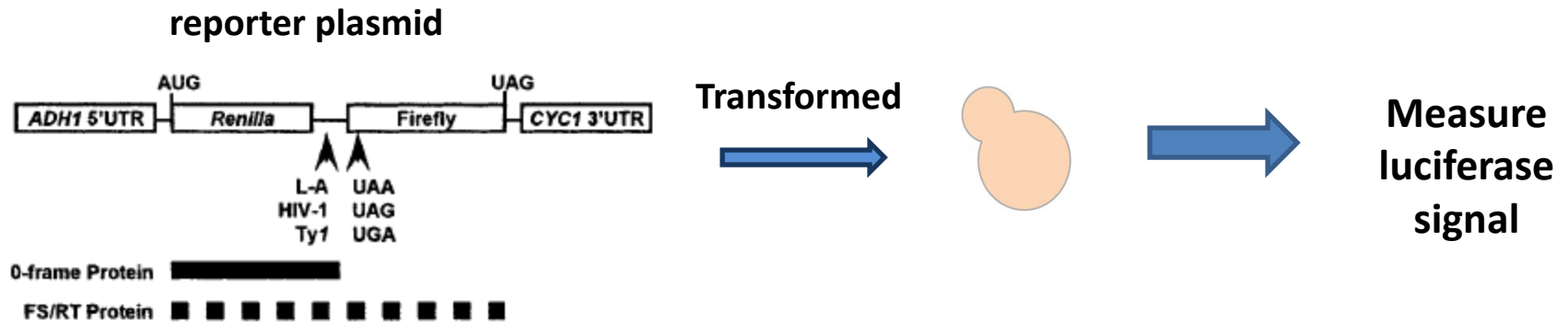
Benchtop GTPase assay



Compromised GTP hydrolysis in uS9 mutants

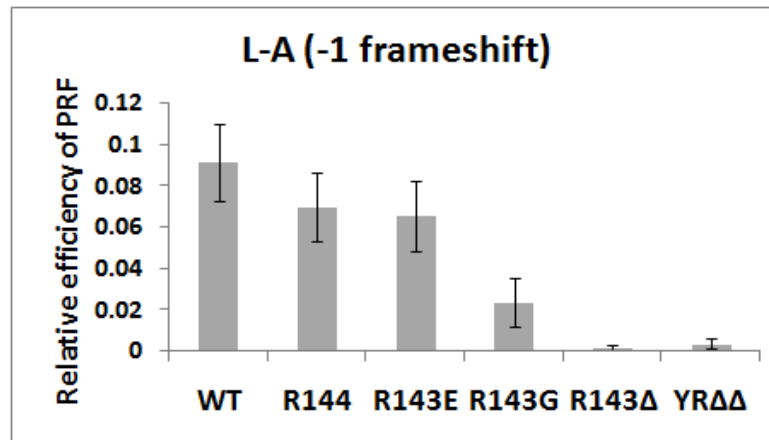
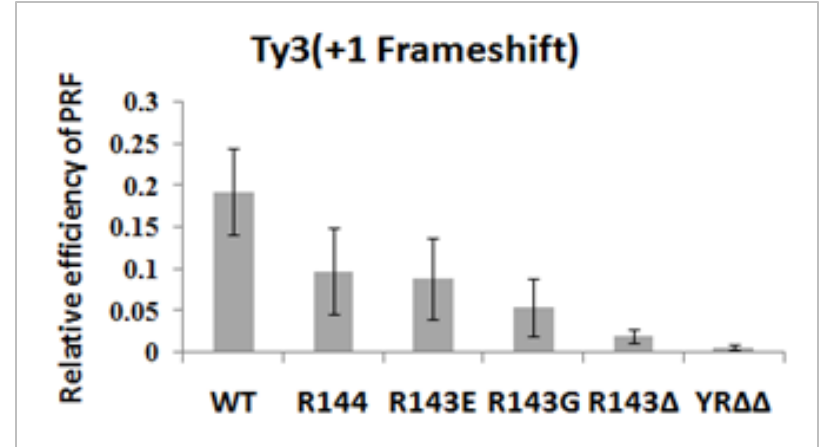
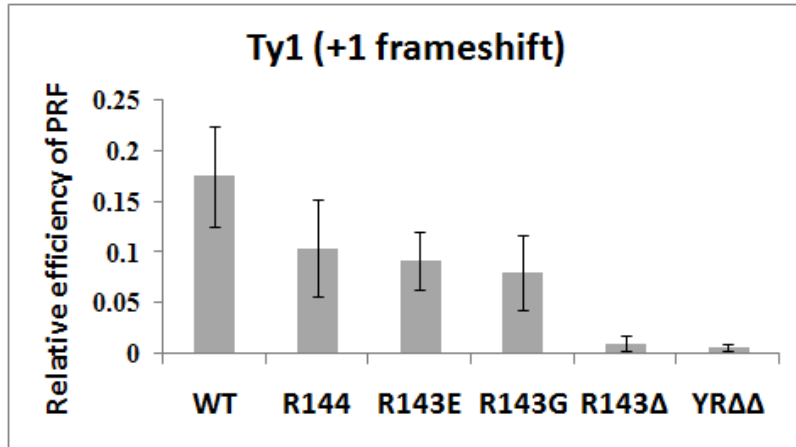


Programmed ribosomal frameshift efficiency

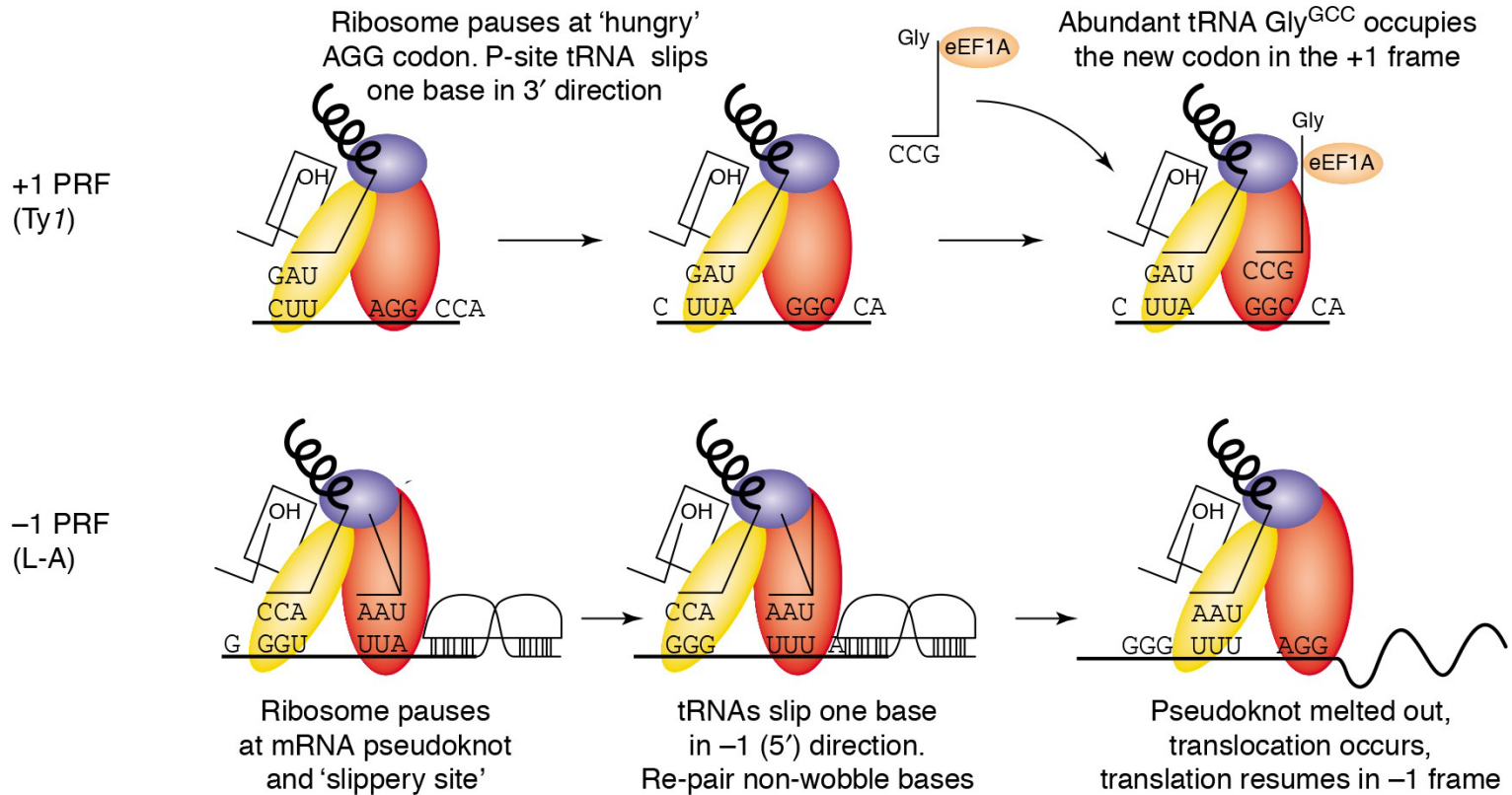


- Luciferase reporter plasmids in which frame shift signals (LA, Ty1, Ty3) inserted between luciferase gene
- Only when ribosomal frame shifting , firefly luciferase will be synthesized
- Hence, high firefly luciferase signal implies more Programmed Ribosomal Frame shift (PRF) efficiency, more translation infidelity

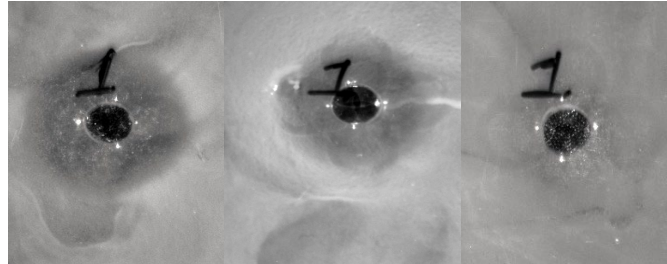
Reduced programmed ribosomal frameshifting



-1/+1 Programmed ribosomal frameshifting



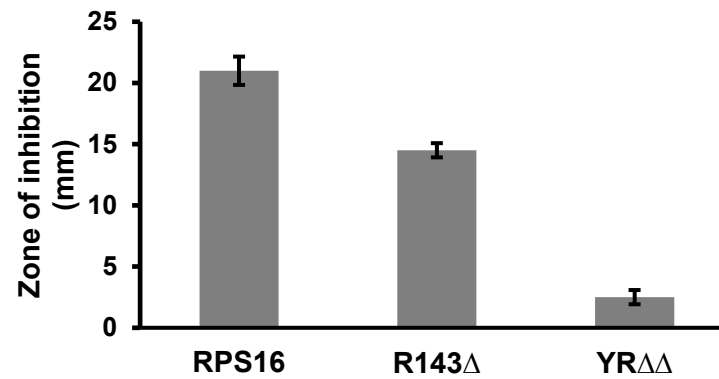
Anisomycin resistance of uS9 mutant ribosomes



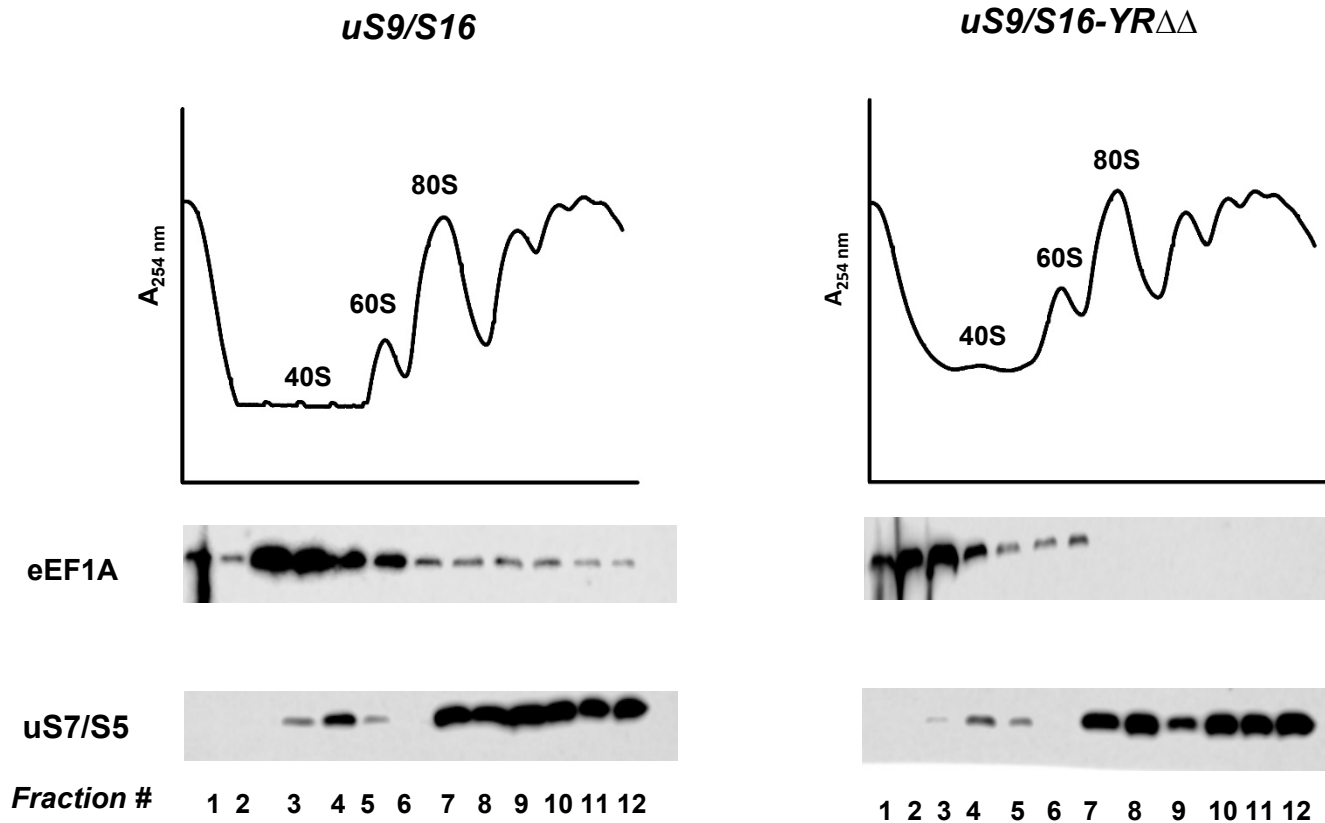
uS9/S16

*uS9/S16-R143*Δ

*uS9/S16-YR*ΔΔ



Reduced association of eEF1A to uS9 mutant ribosomes



Role of C-terminal tail of uS9 during elongation

- eEF1A mutant (N153T) exhibits enhanced resistance to anisomycin and also decreased PRF efficiency, also exhibit stimulated intrinsic GTPase activity (Cavallius and Merrick, 1998, Kinzy et al, 2002).
- Hypothesize uS9 mutants increase GTPase activity and thus accommodation.
- Deletion of Tyrosine (Y) and Arginine (R) residues in the uS9 CTT causes reduced frameshifting, anisomycin resistance and reduced eEF1A association to 40S.
- uS9 CTT might play a role during GTP hydrolysis during elongation, different than initiation.

Conclusions

- uS9 C-terminal tail (CTT) is important during translation initiation in eukaryotes
 - Recruitment of TC and scanning
 - AUG recognition at the P site
 - GTP hydrolysis

- uS9 CTT also important during elongation
 - Accommodation of amino-acyl-tRNA at the A site
 - Possible role in GTP hydrolysis

- uS9 C-terminus potentially forms different set of contacts with elongator tRNA vs initiator tRNA

Acknowledgements



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Arnab



Nishant

Plasmids/ Constructs/ Antibodies etc.:

- Alan Hinnebusch (NICHD, NIH)
- Thomas Dever (NICHD, NIH)
- Leoš Valášek (Academy of Sciences, Czech Republic)
- Phillip Milkereit (Univ. of Regensburg, Germany)