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



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

Universally conserved ribosomal protein uS9 (S16)

RPS9 T.thermophilus --MEQ-----YYGTGRRKEAVARVFLRPGNGKVTVNGQDFNEYFQGLVRAVAALEPLRA 52 RPS9 E.coli MAENQ-----YYGTGRRKSSAARVFIKPGNGKIVINQRSLEQYFGRETARMVVRQPLEL 54 RPS9 B.subtilis MAQVQ-----YYGTGRRKSSVARVRLVPGEGRIVVNNREISEHIPSAALIEDIKOPLTL 54 RPS9 S.thermophilus MAQAQ-----YAGTGRRKNAVARVRLVPGTGKITVNKKDLEEYIPHADLRLVINOPFAV 54 RPS16 S.cerevisiae MSAVP----SVOTFGKKKSATAVAHVKAGKGLIKVNGSPITLVEP-EILRFKVYEPLLL 54 RPS16 C.glabrata MSTVP----SVOTFGKKKSATAVAHVKAGKGLIKVNGSPITLVEP-EILRFKVYEPLLL 54 RPS16 C.albicans MSTQ-----SVQTFGKKKTATAVAHVKAGKGLIKINGSPITLVQP-EILRFKVYEPLTL 53 RPS16 S.pombe ---MQ-----SVQCFGKKGNATAVAHCKVGKGLIKVNGAPLSLVQP-EILRMKVYEPILV 51 RPS16 C.elegans MTSTV----OSVOTFGRKKTATAVAHCKKGOGLIKVNGRPLEFLEP-OILRIKLOEPLLL 55 RPS16 N.crassa -MATQ----AVQVFGKKKNATAVARCVQGKGLIKVNGVPLKLYAP-EILRAKLYEPILL 53 RPS16 D.melanogaster MOOKRREPVOAVOVFGRKKTATAVAYCKRGNGLLKVNGRPLEOIEP-KVLOYKLOEPLLL 59 RPS16 H.sapiens MPSKG--PLOSVOVFGRKKTATAVAHCKRGNGLIKVNGRPLEMIEP-RTLOYKLLEPVLL 57 RPS16 M.musculus MPSKG--PLQSVQVFGRKKTATAVAHCKRGNGLIKVNGRPLEMIEP-RTLQYKLLEPVLL 57 *:: :.* . * * : :* :*. RPS9 T.thermophilus V--DALGHFDAYITVRGGGKSGOIDAIKLGIARALVOYNPDYRAKLKP-----L 99 RPS9 E.coli V--DMVEKLDLYITVKGGGISGOAGAIRHGITRALMEYDESLRSELRK-----A 101 T--ETAGTYDVLVNVHGGGLSGQAGAIRHGIARALLEADPEYRTTLKR------A 101 RPS9 B.subtilis RPS9 S.thermophilus T--STEGSYDVHVNVVGGGYAGQSGAIRHGIARALLQVDPDFRDSLKR------A 101 RPS16 S.cerevisiae VGLDKFSNIDIRVRVTGGGHVSQVYAIRQAIAKGLVAYHOKYVDEQSKNELKKAFTSYDR 114 RPS16 C.glabrata VGLDKFANIDIRVRVTGGGHVSOVYAIROAIAKGLVAYHOKFVDEOSKNELKKAFTSYDR 114 RPS16 C.albicans VGLDKFOGIDIRVKVTGGGHVSOVYAIROAIAKGLVAYHOKYVDEASKNELKKIFASYDK 113 RPS16 S.pombe AGADKFAGVDIRVRVSGGGHVSOIYAIROAISKAIVAYYOKFVDEHSKAELKKALITYDR 111 RPS16 C.elegans VGKERFODVDIRIRVSGGGHVAQIYAVROALAKALVAYYHKYVDEOSKRELKNIFAAYDK 115 RPS16 N.crassa LGTDKFAEVDIRLKVSGGGHVSQVYAVRQAIAKAIVAYYAKYVDEHSKNTLKTALIQFDR 113 RPS16 D.melanogaster LGKEKFAGVDIRVRVSGGGHVAOIYAIRQAISKALVAFYQKYVDEASKKEIKDILVQYDR 119 RPS16 H.sapiens LGKERFAGVDIRVRVKGGGHVAOIYAIROSISKALVAYYOKYVDEASKKEIKDILIOYDR 117 RPS16 M.musculus LGKERFAGVDIRVRVKGGGHVAOIYAIROSISKALVAYYOKYVDEASKKEIKDILIOYDR 117 * : * *** .* *:: .:::.:: RPS9 T.thermophilus GFLTRDARVVERKKYGKHKARRAPOY KR 128 RPS9 E.coli GFVTRDAROVERKKVGLRKARRRPOFSKR 130 RPS9 B.subtilis GLLTRDARMKERKKYGLKGARRAPOF: KR 130 RPS9 S.thermophilus GLLTRDARMVERKKPGLKKARKASOF; KR 130 RPS16 S.cerevisiae TLLIADSRRPEPKKFGGKGARSRFOK YR 143 RPS16 C.glabrata TLLIADARRPEPKKFGGKGARARFOK YR 143 RPS16 C.albicans TLLVADSRRMEPKKFGGRGARARFQK: YR 142 RPS16 S.pombe TLLVADPRRMEPKKFGGHGARAROOK YR 140 RPS16 C.elegans SLLVADPRRRESKKFGGPGARARYOK YR 144 RPS16 N.crassa TLLVADPRRCEPKKFGGKGARSRFOK YR 142 RPS16 D.melanogaster TLLVGDPRRCEPKKFGGPGARARYOK YR 148 RPS16 H.sapiens TLLVADPRRCESKKFGGPGARARYOK YR 146 RPS16 M.musculus TLLVADPRRCESKKFGGPGARARYOK YR 146

:: *.* * ** *

Location of Ribosomal protein uS9 (S16)



Ben Sham et al, The Structure of the eukaryotic ribosome at 3.0 A resolution ,2011

Role of uS9 tail in prokaryotic translation



uS9 C-Terminus Location in Eukaryotic Ribosome



Highly Conserved uS9 C-Terminus

RS16_Agossyppi RS16_Klactis RS16_Scerevisiae RS16_Cglabrata RS16_Calbicans RS16_Calbicans RS16_Spombe RS16_Spombe RS16_Rnorvegicus RS16_Mmusculus RS16_Mmusculus RS16_Hsapiens RS16_Dmelanogaster RS16_Celegans RS9_Tacidophilum RS9_Tpallidum RS9_Sthermophilus RS9_Ecoli

130 140 TLLIADSRRPEPKKFGGRGARARFOKSYF TLLIADSRRPEPKKFGGRGARSRFOKSYR TLLIADSRRPEPKKFGGKGARSRFOKSYR TLLIADARRPEPKKFGGKGARARFOKSYR TLLVADSRRMEPKKFGGRGARARFOKSYR TLLVADPRRMEPKKFGGHGARARCOKSYR TLLVADPRRCESKKFGGPGARARYOKSYR TLLVADPRRCESKKFGGPGARARYOKSYR TLLVADPRRCESKKFGGPGARARYOKSYR TLLVGDPRRCEPKKFGGPGARARYOKSYR SLLVADPRRRESKKFGGPGARARYOKSYR TLIVNDVRIKLPKKAGGRGARAKKOKSYR -LLTRDSRMVERKKYGORGARRRFOFSKR -LLTRDARMVERKKPGLKKARKASOFSKR -FVTRDAROVERKKVGLRKARRRFOFSKR

Y142

R143

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∆- deletion of arginine
- \checkmark YRDD- 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

	130	140
uS9/S16	I I PEPKKFGGKGARSRFQK	SYR
uS9/S16-R143G	PEPKKFGGKGARSRFQK	SYG
uS9/S16-R143∆	PEPKKFGGKGARSRFQK	SY
uS9/S16-YR∆∆	PEPKKFGGKGARSRFQK	S
uS9/S16-R143E	PEPKKFGGKGARSRFQK	SYE
uS9/S16-R144	PEPKKFGGKGARSRFQK	SYRR
	ιγ	J
	uS9 C-te ['] rminus	

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





elF2 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



Eukaryotic Translation Initiation



uS9 mutants show failure to resume scanning



Defective 43S complex formation in uS9 mutants



Eukaryotic Translation Initiation



uS9 mutants show leaky scanning of AUG codon



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



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



<TIF5-G31R>

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



• WT • R143E • YRAA • R144

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



Harger et al., Integrated model of programmed ribosomal frameshifting, 2002,

Anisomycin resistance of uS9 mutant ribosomes



uS9/S16 uS9/S16-R143∆ uS9/S16-YR∆∆



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-acyltRNA 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







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

