Correlating the conformational dynamics of the DEAD-box helicase eIF4A with translation efficiencies

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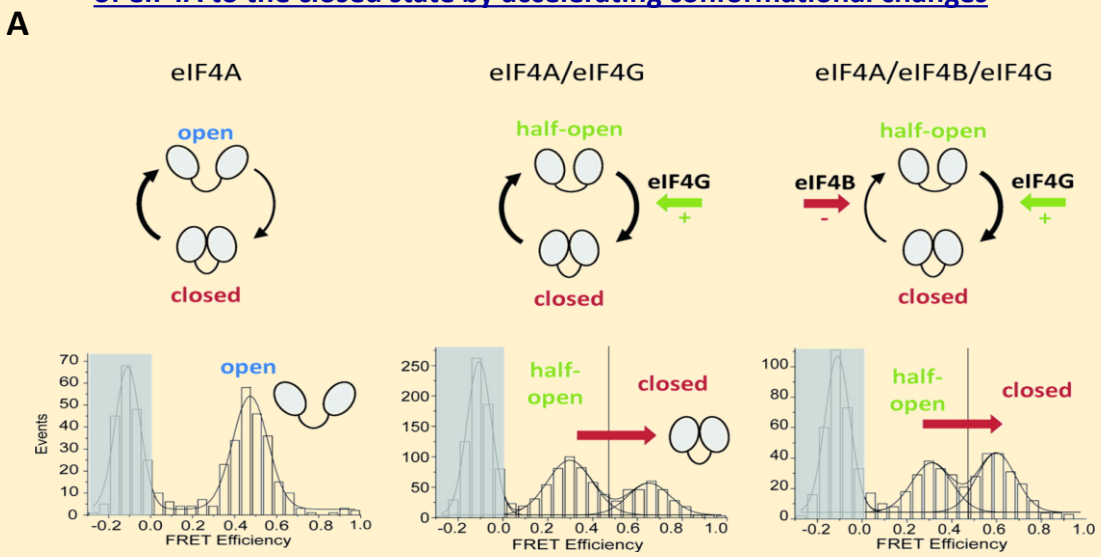
Introduction

eIF4B and eIF4G shift the conformational equilibrium of eIF4A to the closed state by accelerating conformational changes



The RNA substrate affects the conformational dynamics of eIF4A

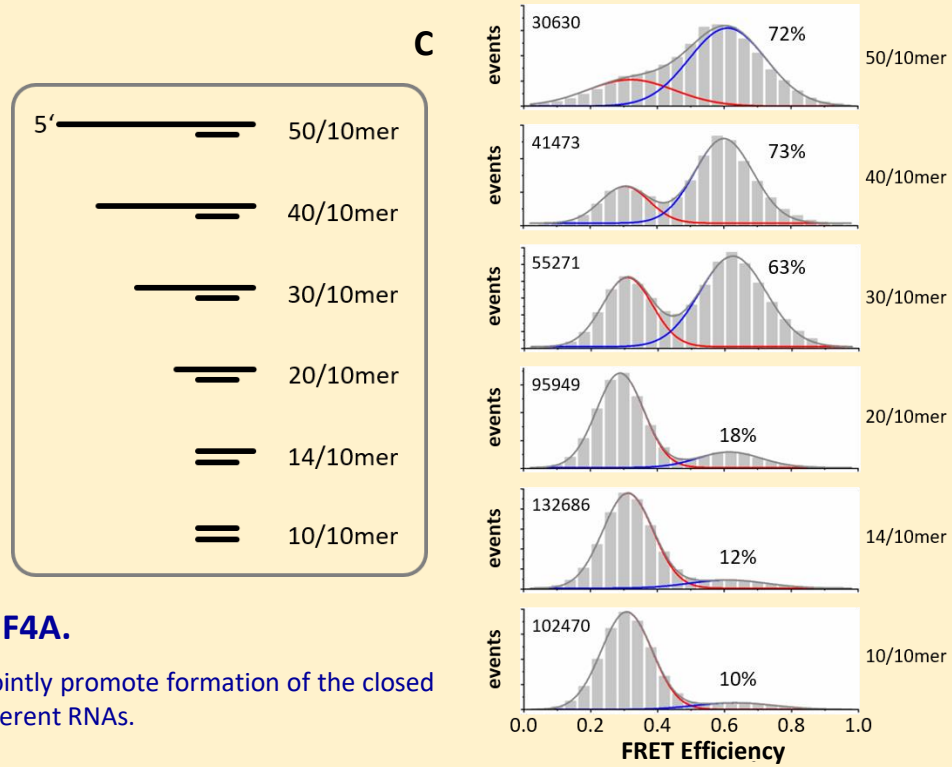


Figure 1: **eIF4B, eIF4G and double-stranded RNA substrates modulate the conformational cycle of eIF4A.**

A: Single-molecule FRET experiments on eIF4A in solution (confocal microscopy). eIF4G stabilizes a half-open state, eIF4B and eIF4G jointly promote formation of the closed state of eIF4A . **B**: Double-stranded RNAs with varying lengths of their 5'-single-stranded tail. **C**: FRET histograms in the presence of different RNAs.

Conformational equilibrium of eIF4A is different for 5’-UTRs of eIF4A-dependent RNA

Motivation

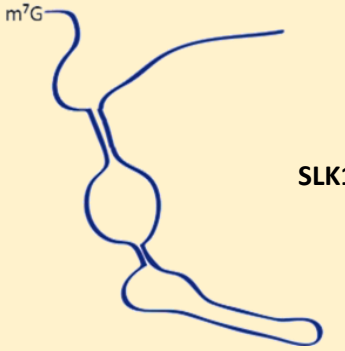
5‘-UTRs used for single-molecule FRET by Confocal microscopy

A

eIF4A-independent

PAP2 5’-UTR

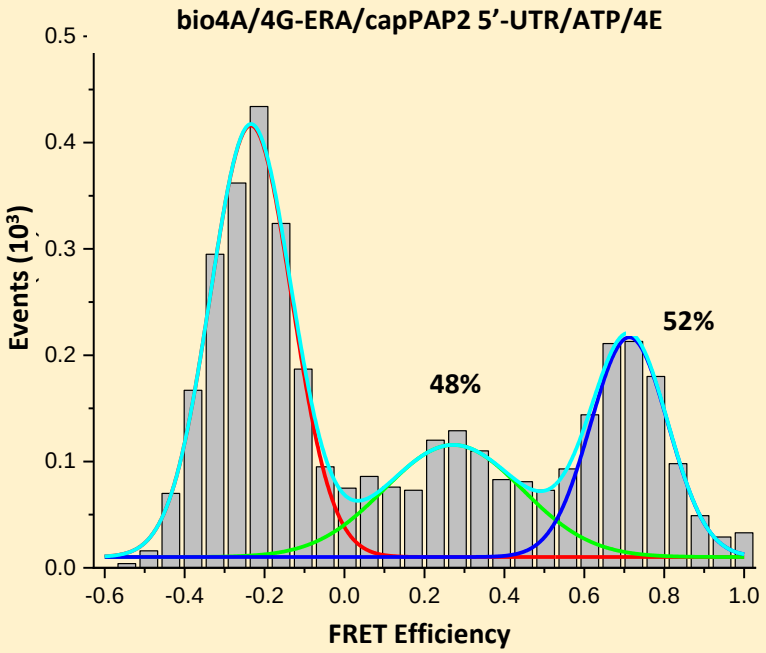
eIF4A-dependent

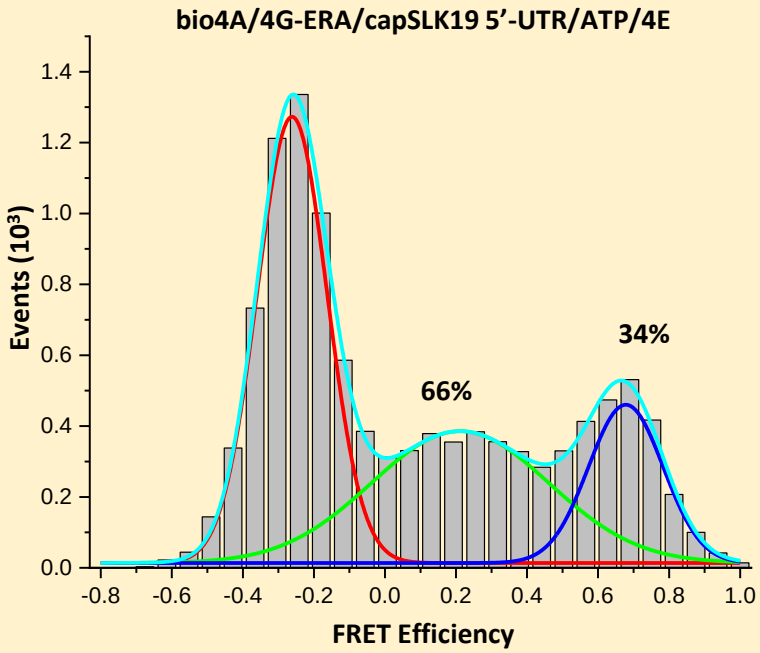


SLK19 5’-UTR

The conformational equilibrium of eIF4A is different for 5‘-UTRs of eIF4A-dependent mRNA

B





bio4A – biotinylated eIF4A
4G-ERA – eIF4G containing eIF4E-binding domain, RNA-binding domain and eIF4A-binding domain
4E – eIF4E

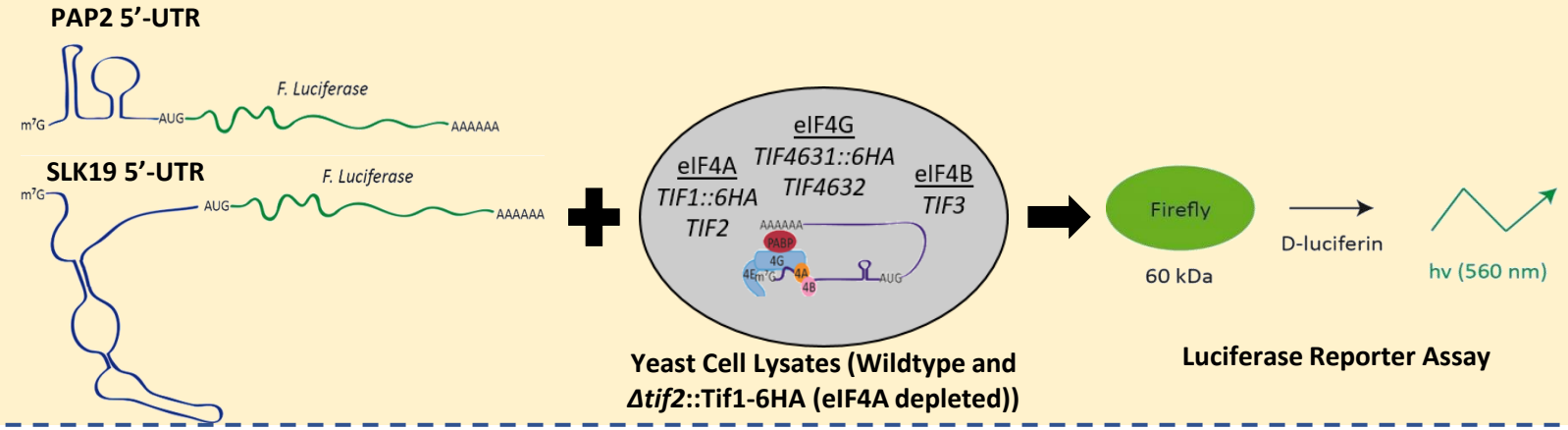
Figure 2: **5’-untranslated regions (UTRs) of eIF4A-dependent mRNAs modulate the conformational equilibrium of eIF4A.**

A: Two 5’-UTRs of eIF4A-dependent mRNAs were investigated based on previous reports (Sen *et al.*, 2015). PAP2 gene expression was found to be independent of eIF4A while SLK19 was found to be around 50 times downregulated . **B**: Single-molecule FRET experiments with eIF4A in solution (confocal microscopy). Both PAP2 5’-UTR and SLK19 5’-UTR affects the conformational equilibrium of eIF4A differently.

Are the changes we observe on eIF4A conformational dynamics mirrored in the efficiency of mRNA translation?

A

***In vitro* translation scheme of 5'-UTRs fused to reporter gene (Firefly Luciferase)**

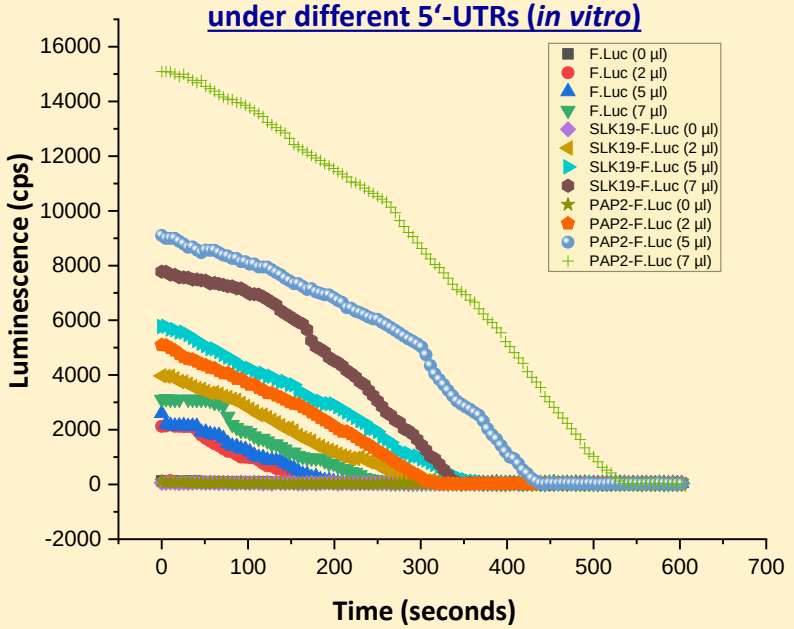


Yeast Cell Lysates (Wildtype and *Δtif2*::Tif1-6HA (eIF4A depleted))

Luciferase Reporter Assay

B

Kinetic Luminescence of Firefly luciferase gene under different 5'-UTRs (*in vitro*)



C

Mean Luminescence (of first 50 cycles) of Firefly luciferase gene under different 5'-UTRs

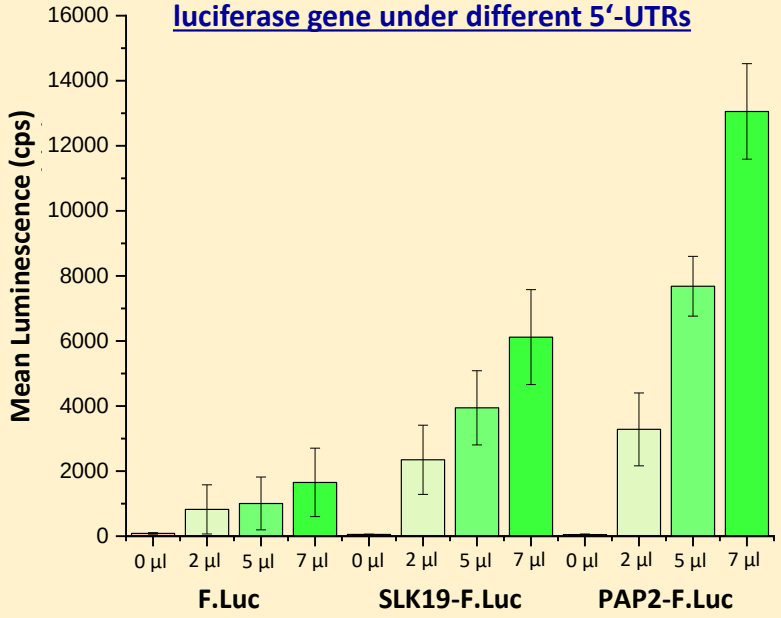


Figure 3: ***In vitro* transcription and coupled *in vitro* translation-Luciferase Reporter Assay of eIF4A-dependent mRNA with Wildtype yeast lysate shows different mRNA translational efficiencies**

A: Two 5'-UTRs of eIF4A-dependent mRNA were fused to a reporter gene (Firefly Luciferase) and were *in vitro* transcribed and translated. Luciferase reporter assay was performed to monitor the translational efficiency of these 5'-UTRs . **B:** Kinetics of Luciferase Reporter Assay showing luminescence of Firefly Luciferase under different 5'-UTRs . **C:** Histogram showing Mean Luminescence (of first 50 cycles) of Firefly Luciferase with change in volume of RNA used. Increase in RNA concentration results in increase in Luminescence and hence, increase in gene expression. PAP2 5'-UTR confers the highest gene expression.

In vitro Translation-Luciferase Reporter Assay with Wildtype and eIF4A immuno-depleted cell extracts

Constructs used for Luciferase reporter Assay with Wildtype and eIF4A immunodepleted yeast cell lysate

A

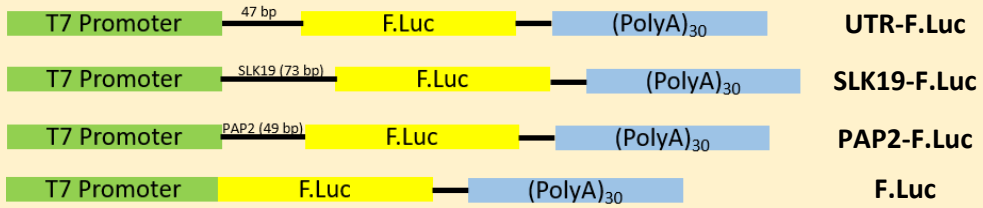
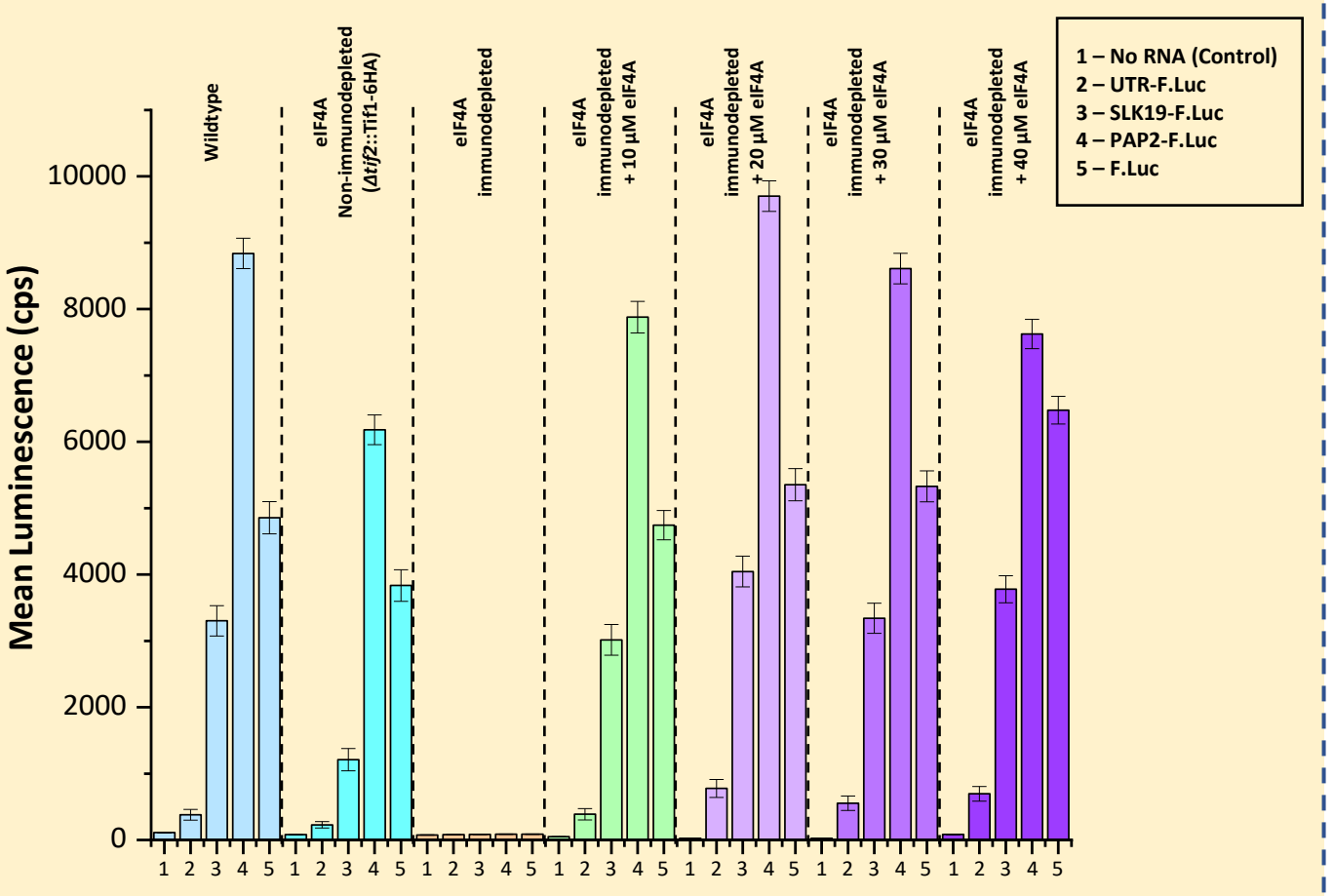


Figure 4: **Luciferase Reporter Assay of eIF4A-dependent mRNA with Wildtype and eIF4A immuno-depleted cell lysate shows different mRNA translational efficiencies due to different 5'-UTRs and difference in UTR length**

A: 5'-UTRs of eIF4A-dependent mRNA were fused to a reporter gene (Firefly Luciferase) and were used for Luciferase reporter assay to monitor the translational efficiency of these 5'-UTRs. Firefly Luciferase gene without any 5'-UTR was used as a negative control . **B**: Graph showing mean Luminescence of *in vitro* translated mRNA containing different 5'-UTRs in Wildtype yeast cell lysate and cell extracts immuno-depleted for eIF4A. Yeast cell lysate immuno-depleted for eIF4A shows no mRNA translation activity while adding 10 µM eIF4A externally restores the translational activity similar to that of Wildtype. Adding 20 µM, 30 µM and 40 µM eIF4A causes no significant change in Luciferase gene expression activity. Moreover, decreasing the 5'-UTR length causes higher gene expression activity in Wildtype and non-immuno-depleted yeast cell extracts supplemented with 10 µM, 20 µM, 30 µM or 40 µM eIF4A.

Mean Luminescence of Firefly luciferase with yeast cell lysate immuno-depleted for eIF4A shows no translational activity

B

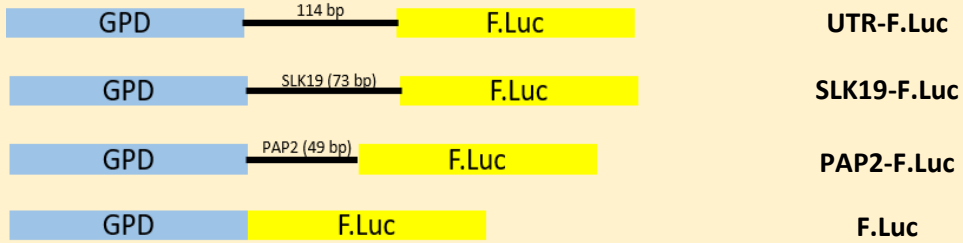


- Yeast cell lysate immuno-depleted for eIF4A shows no gene expression
- 10 µM eIF4A is sufficient enough to restore gene expression similar to the Wildtype
- Decreasing the 5'-UTR length causes increase in gene expression

In vivo Luciferase Reporter Assay in Wildtype yeast

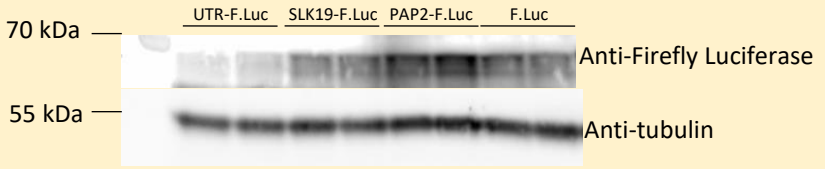
A

Constructs used for Luciferase reporter Assay in Wildtype yeast cell



B

Western Blot for gene expression of Firefly Luciferase under different 5'-UTRs



C

Mean Luminescence of Firefly luciferase under different 5'-UTRs in Wildtype yeast

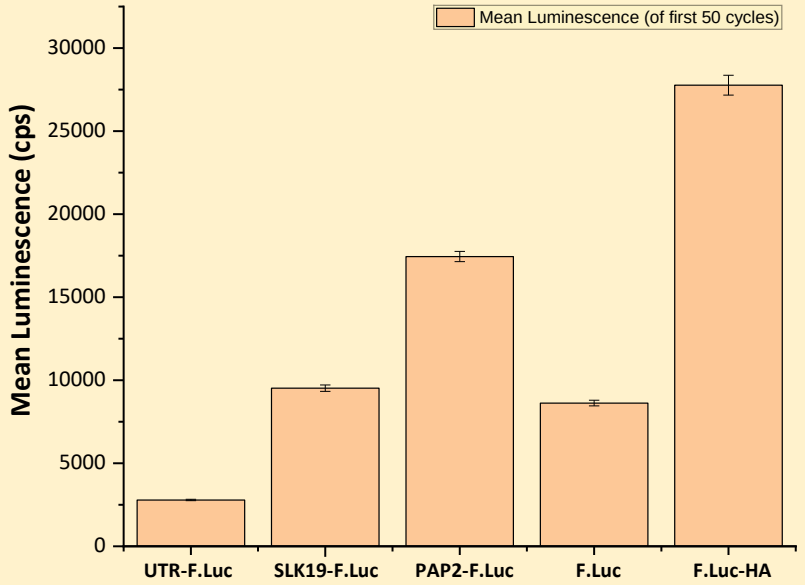


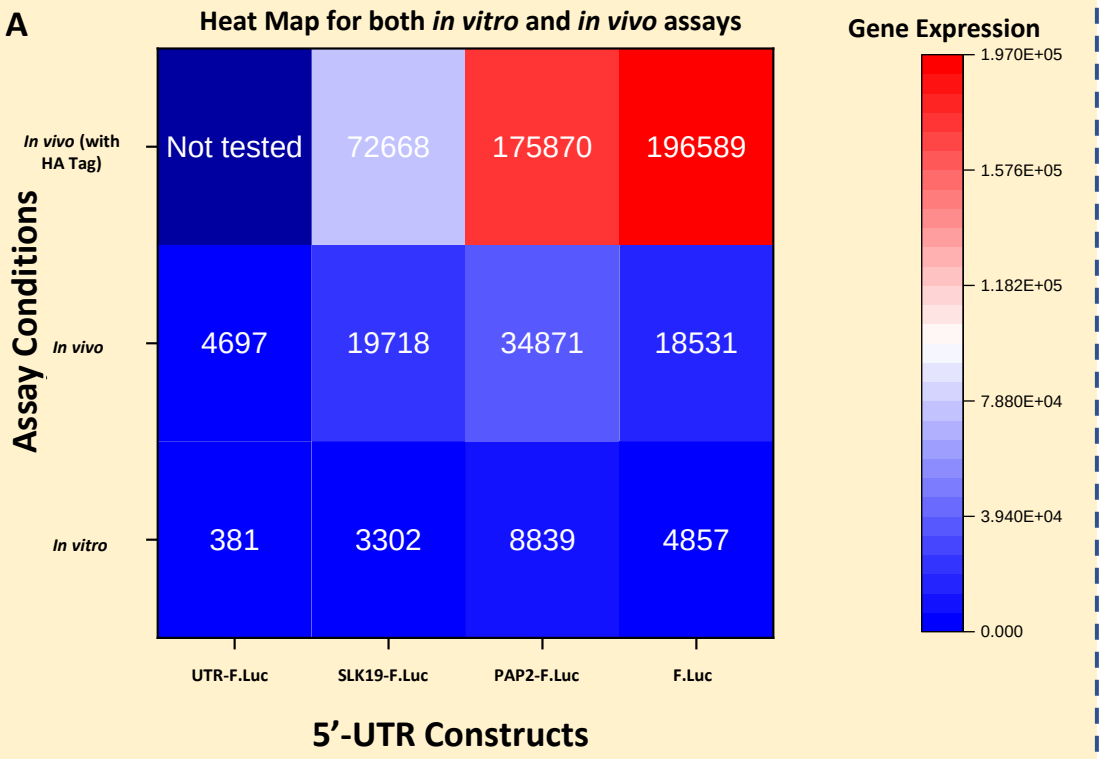
Figure 5: **Luciferase Reporter Assay of eIF4A-dependent mRNA in Wildtype shows effect of 5'-UTR and 5'-UTR length**

A: Plasmids containing 5'-UTRs of eIF4A-dependent mRNA fused to a reporter gene (Firefly Luciferase) were transformed in Wildtype yeast cell and were used for Luciferase reporter assay to monitor the translational efficiency of these 5'-UTRs. Firefly Luciferase gene without any 5'-UTR was used as a negative control . **B**: Western Blot with Anti-Firefly Luciferase showing highest gene expression for PAP2 5'-UTR (PAP2-F.Luc) followed by SLK19 5'-UTR (SLK19-F.Luc). Moreover, when 5'-UTR was deleted from the plasmid (F.Luc), gene expression increased as compared to plasmid containing 114bp 5'-UTR (UTR-F.Luc). Anti-Tubulin was used as a loading control . **C:** Graph showing mean Luminescence of *in vivo* translated mRNA containing different 5'-UTRs in Wildtype yeast cell. The gene expression was highest for PAP2 5'-UTR (PAP2-F.Luc) followed by SLK19 5'-UTR (SLK19-F.Luc) as compared to 114bp UTR-F.Luc. Removing the 5'-UTR (F.Luc) increases the gene expression as compared to UTR-F.Luc containing 114bp 5'-UTR. As a comparison, another construct (F.Luc-HA) was assayed containing Firefly Luciferase gene fused to 3X HA Tag without any 5'-UTR. It showed the highest gene expression.

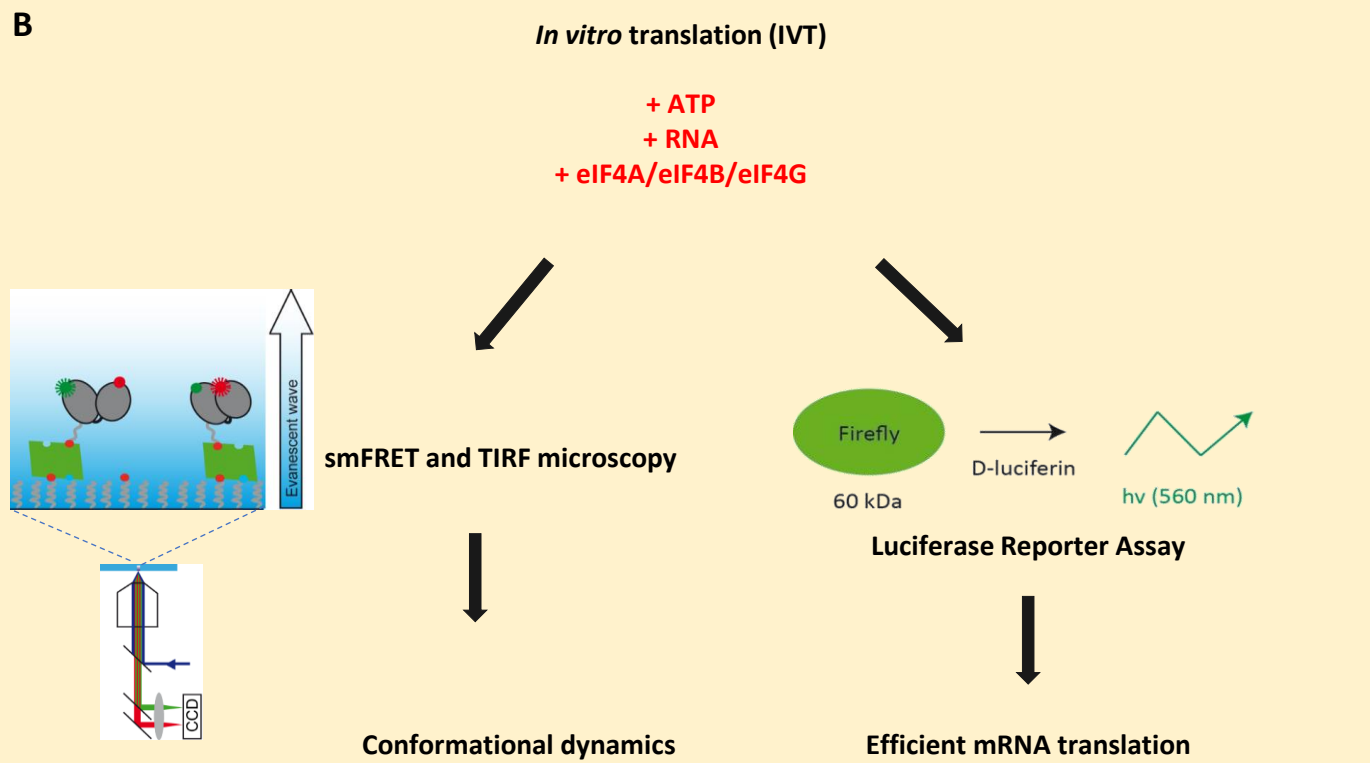
- PAP2 5'-UTR (PAP2-F.Luc) confers the highest gene expression
- Decreasing the 5'-UTR length causes increase in the gene expression (F.Luc)

Summary and Outlook

Summary



Outlook



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Acknowledgements:

This work was supported by the German Research Foundation (DFG). We thank Daniela Schlingmeier for technical assistance.