Upstream Open Reading Frames Regulate PERK Translation Initiation

Rafael Fernandes[1,2], Samuel Silvestre[1,2], João Ponte[1,2], Rafaela Lacerda[1] , Luísa Romão[1,2]
[1]Department of Human Genetics, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal
[2]Universidade de Lisboa, Faculty of Sciences, BioISI – Biosystems and integrative Sciences Institute, Lisboa, Portugal

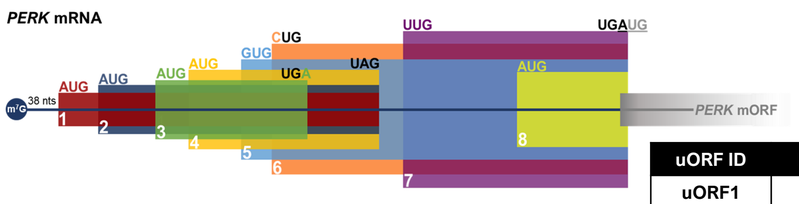
Introduction

Upstream open reading frames (uORFs) are *cis*-acting elements located within the 5' leader sequence (5'UTR) of transcripts, which can regulate translation of the correspondent main open reading frame (mORF). During endoplasmic reticulum (ER) stress, the accumulation of unfolded proteins activates the ER-resident PKR-like ER kinase (PERK), which results in phosphorylation of eIF2α to inhibit global mRNA translation, while allowing the selective uORF-mediated translation of downstream effectors responsible for stress resolution or, ultimately, cell death. The dual role of PERK in regulating cell fate was implicated in human diseases, like diabetes, neurodegenerative disorders and cancer. Moreover, mutations in the *EIF2AK3* gene (encoding PERK) were associated to the rare genetic disease, Wolcott-Rallison Syndrome (WRS).

Aim - Study the functional role of uORFs in PERK expression and evaluate their biological impact in cell physiology and human disease

Results

The PERK 5'UTR contains potentially translated uORFs



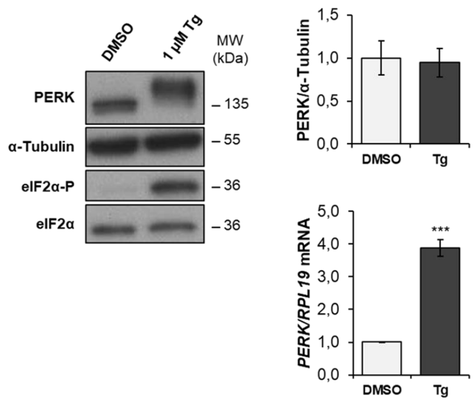
uORF ID	Length (nts)	Start codon strength
uORF1	165	++
uORF2	141	-
uORF3	57	+
uORF4	102	+
uORF5	177	+
uORF6	156	-
uORF7	84	-
uORF8	27	-

The *PERK* mRNA has **at least eight uORFs**:
- **5 AUG-uORFs**
- **3 non-AUG-uORFs**

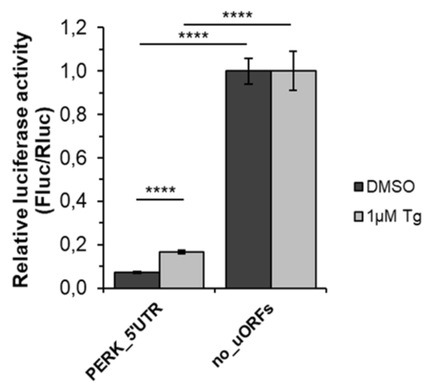
Some of these uORFs are **likely to be translated**, as suggested by the Kozak sequence context strength of their start codons.

PERK uORFs regulate mORF translation during stress

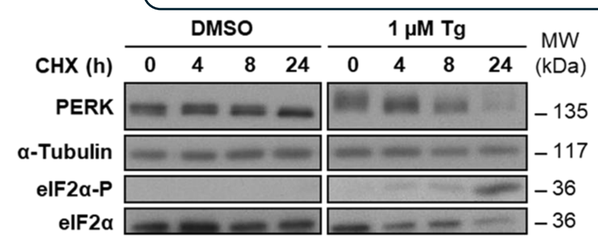
PERK translation is repressed during stress conditions

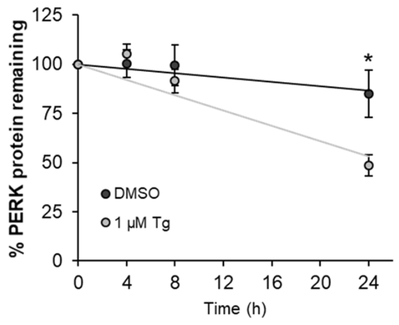


PERK uORF-mediated translational repression is lessened under Tg-induced stress



Thapsigargin-induced stress increases *PERK* protein turnover

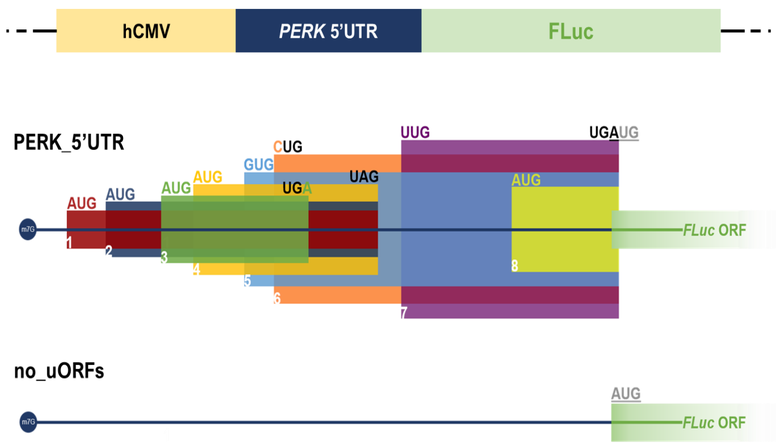




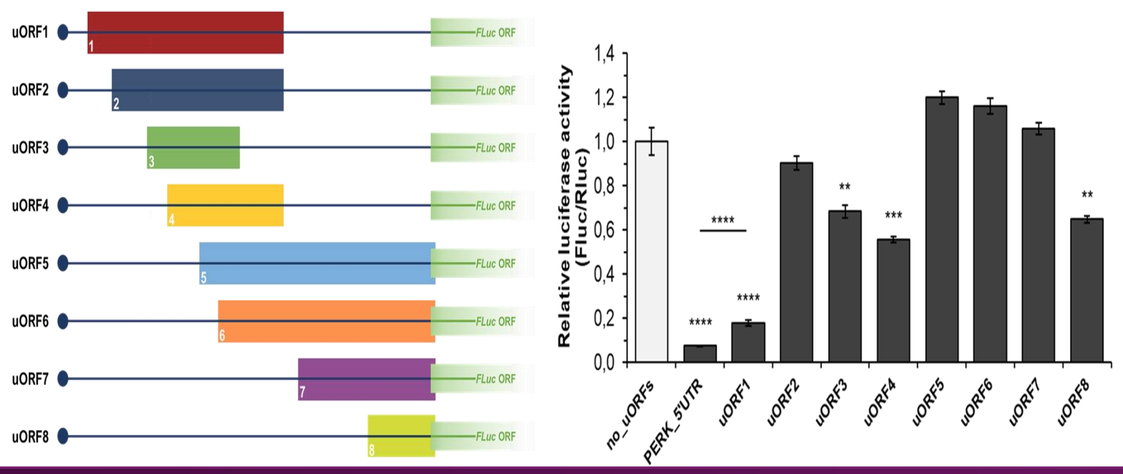
During stress, *PERK* uORFs allow some degree of translational de-repression to occur, nonetheless with a potent repressive effect over mORF translation. But why don't we see that increase in the PERK protein levels?

PERK uORFs regulate mORF translation in basal conditions

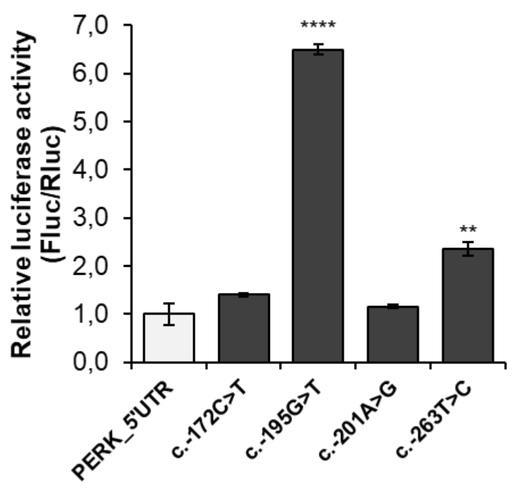
PERK uORFs repress mORF translation

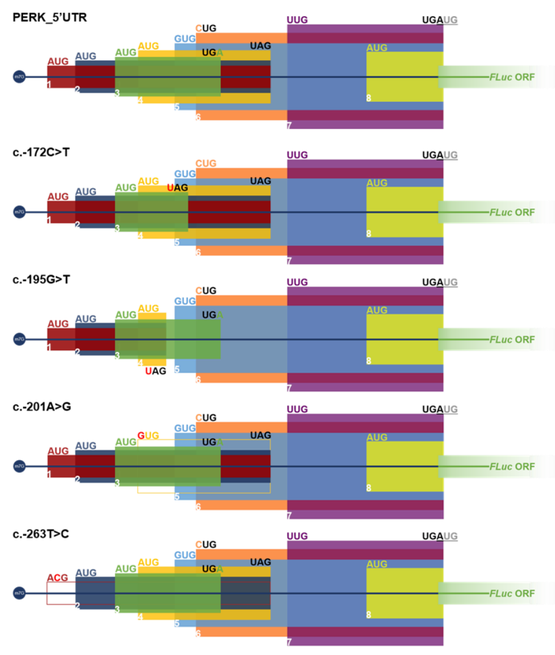


uORFs 1, 3, 4 and 8 have translational repressive activity



PERK uORFs impact in health





Acknowledgements: This work was partially supported by Instituto Nacional de Saúde Doutor Ricardo Jorge and Fundação para a Ciência e a Tecnologia [UIDB/04046/2020 (https://doi.org/10.54499/UIDB/04046/2020) and UIDP/04046/2020 (https://doi.org/10.54499/UIDP/04046/2020) Center grants from FCT (to BioISI)]. R.F. was recipient of a fellowship from the BioSys PhD programme (SFRH/BD/114392/2016) from FCT