



HAL
open science

eIF3-mediated ribosome recruitment by histone H4 mRNA during translation initiation

Hassan Hayek, Franck Martin, Gilbert Eriani, Christine Allmang

► To cite this version:

Hassan Hayek, Franck Martin, Gilbert Eriani, Christine Allmang. eIF3-mediated ribosome recruitment by histone H4 mRNA during translation initiation. RNA 2019-Krakow. The 24th annual meeting of the RNA society., Jun 2019, Krakow, Poland. hal-03564423

HAL Id: hal-03564423

<https://hal.science/hal-03564423>

Submitted on 21 Feb 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

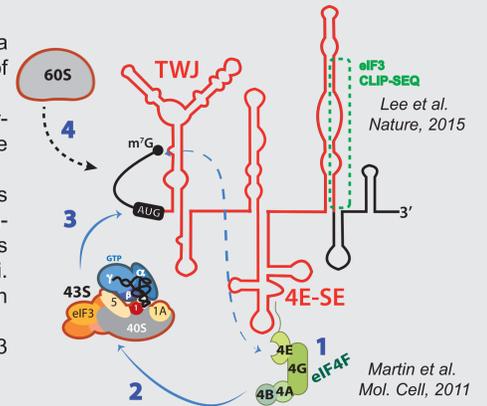
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Hassan Hayek*, Franck Martin, Gilbert Eriani, Christine Allmang

Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR 9002, F-67000 Strasbourg, France
h.hayek@ibmc-cnrs.unistra.fr

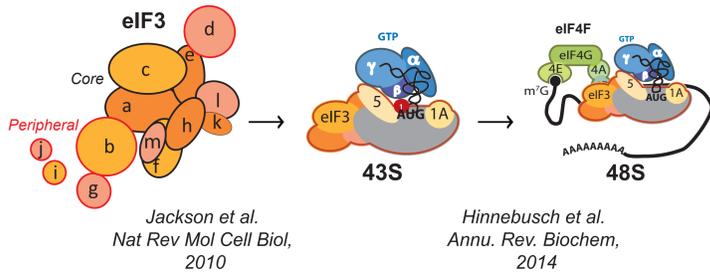
Background

The vast majority of eukaryotic mRNAs initiate translation through a canonical cap-dependent mechanism requiring a free 5' cap and initiation factors (eIF) to form a translationally active ribosome. This tightly regulated process consists of several steps and involves 12 eIFs, 40S and 60S ribosomal subunits until the anticodon of tRNA^{Met} is paired with the AUG codon into the P-site of the 80S ribosome. We have shown that translation initiation of histone H4 mRNA combines canonical features (cap-dependent translation) with viral strategy (lack of scanning and internal recruitment of initiation factors) (Martin et al. Mol. Cell, 2011). H4 mRNA contains a double stem-loop structure called eIF4E-sensitive element that recruits the cap-binding complex eIF4F. In addition, H4 mRNA contains a three-way junction that helps ribosome recruitment and positioning on the AUG start codon. Recent PAR-CLIP experiments showed that eIF3 plays a crucial role in the selection and regulation of some characteristic cellular mRNAs, including histone mRNAs (Lee et al. Nature, 2015). In this work, we decipher the interactions between histone mRNAs and eIF3. To this end we performed ribonucleoprotein immunoprecipitations using HEK293 extracts and showed that all histone mRNAs are able to interact in vivo with eIF3. By UV crosslinking experiments, we identified four eIF3 subunits able to interact with H4 mRNA: c, d, e and g. Next we performed GST-pulldown experiments on HEK293 total RNA using the four isolated subunits produced in E. coli. The mRNA of the linker histone H1 was found to bind tightly the four eIF3 subunits while the octameric core histone mRNAs (H2A, H2B, H3 and H4) interacted with the three subunits c, d, and g, but not eIF3e. Our results also highlighted a marked preference of H4 mRNA for eIF3d binding. Altogether, the data suggest that eIF3 could play a significant role during translation initiation of histone mRNAs. Further investigations will address the effects of eIF3 down-regulation on histone synthesis in vivo.



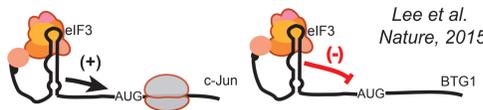
The mammalian eucaryotic initiation factor eIF3

- > eIF3 is a multi protein assembly of 800 kDa
- > Composed of 13 subunits eIF3(a-m)
- > The main role of eIF3 is to orchestrate 43S and 48S particle formation
- > Stabilizes the eIF2-tRNA^{Met} ternary complex association with 43S particle
- > Recruits the 43S complex to the eIF4F cap binding complex
- > Assists in 5'UTR mRNA scanning and AUG recognition
- > Prevents premature joining of the large 60S ribosomal subunit
- > Plays a role in translation reinitiation...



A new role for eIF3 in mRNA selection

eIF3 can activate or repress translation of cap-dependent transcript by direct binding to defined RNA structural elements

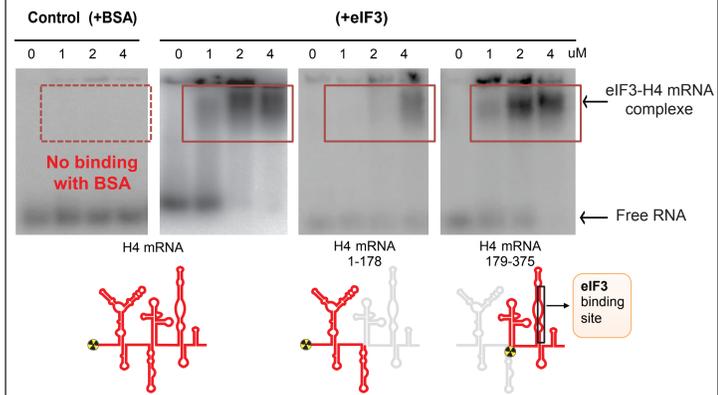


➔ H4 mRNA was found among eIF3 targets

Objective

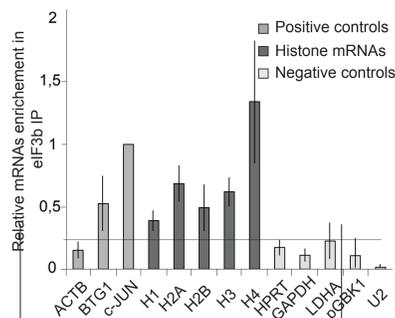
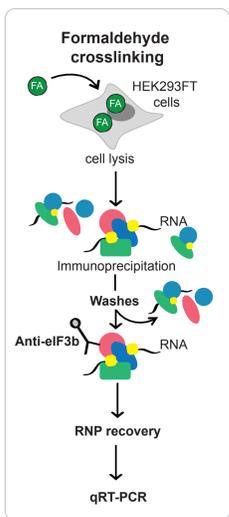
- > Characterization of the eIF3/H4 mRNA interaction
- > Functional role of the eIF3 on histone mRNAs (see poster n°774)

eIF3 interacts with H4 mRNA as shown by EMSA

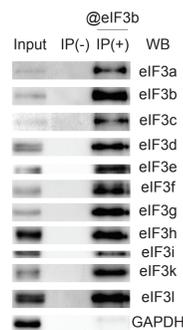


eIF3 directly binds to the H4 mRNA second half containing the predicted eIF3-binding site

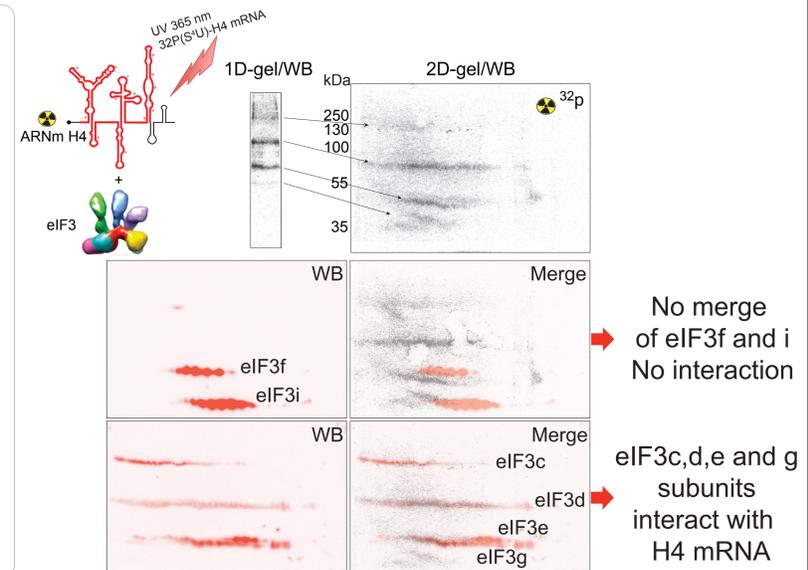
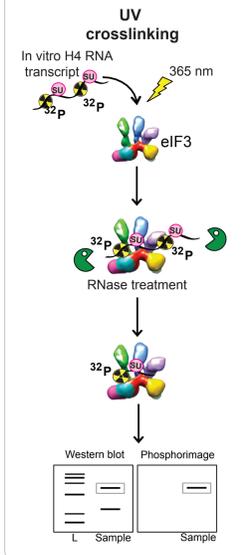
Detection of the interactions between eIF3 and histone mRNAs in vivo by anti-eIF3b immunoprecipitation



The eIF3 complex is able to interact in vivo with H4 mRNA but also the other histone mRNAs



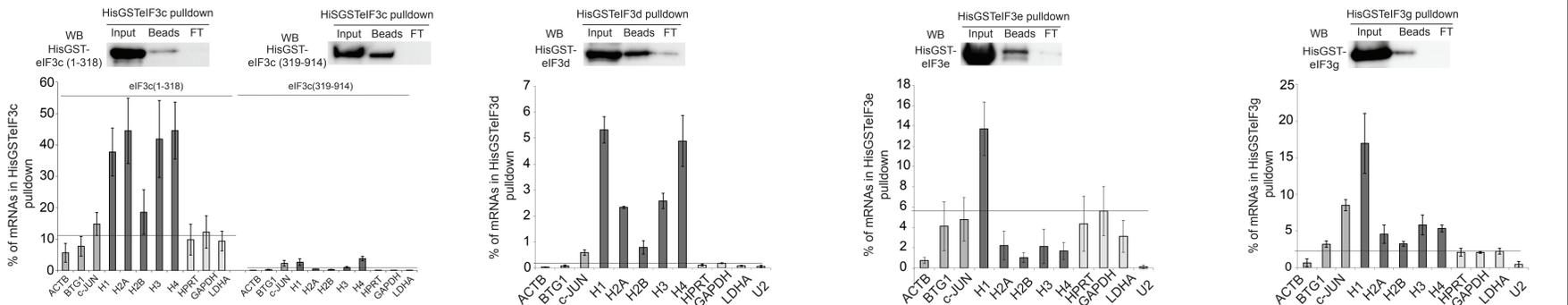
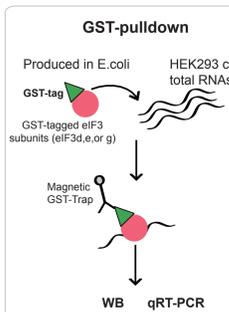
Identification of the eIF3 subunits that interact with H4 mRNA by UV crosslinking



No merge of eIF3f and i
No interaction

eIF3c, d, e and g subunits interact with H4 mRNA

Validation of the interactions between the isolated eIF3c, d, e and g subunits and histone mRNAs by GST-pulldown experiments



> GSTeIF3c, d and g but not GSTeIF3e interact with H4 mRNA and the other histone mRNAs
> Histone H1 mRNA binds tightly the eIF3 subunits tested including eIF3e

Conclusions

- > The eIF3 complex is able to interact in vivo with the histone mRNAs (H1, H2A, H2B, H3 and H4)
- > 4 of the 13 subunits of eIF3 establish direct contacts with H4 mRNA (eIF3c, d, e and g) in crosslinking experiment
- > GSTeIF3c, d and g but not GSTeIF3e interact with H4 mRNA and the other histone mRNAs

