

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

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Eukaryotic mRNA translation initiation is a coordinated process, that requires numerous initiation factors (eIFs) that form several complexes, which leads to mRNA attachment to the small 40S ribosomal subunit, mRNA scanning and start codon selection as well as the assembly of the ribosome. Our team showed that translation initiation of histone H4 mRNA is unconventional and combines canonical features (cap-dependent translation) with viral strategy (no scanning) (Martin et al. Mol. Cell, 2011). The ribosomes are recruited by the initiation factors bound to specific structures in the histone mRNA coding region and deposited directly on the initiator AUG codon. This tethering mechanism leads to direct initiation on the AUG without scanning of the 5' untranslated region.

PAR-CLIP experiments showed that H4 mRNA is recognized by the largest initiation factor eIF3 (Lee et al. Nature, 2015). In higher eukaryotes eIF3 is composed of 13 subunits (a to m) and plays a crucial role in the selection and regulation of some characteristic cellular mRNAs. In this work we investigated the eIF3-H4 mRNA interaction in order to decipher and validate its role during translation of histone H4.

To this end formaldehyde cross-linking and ribonucleoprotein immunoprecipitation (RNP IP) experiments were performed *in vivo* and *in vitro*. We showed that H4 mRNA but also other histone mRNAs (H1, H2A, H2B and H3) are able to interact *in vivo* with the entire eIF3 by ribonucleoprotein immunoprecipitation (RNP IP) using antibodies against eIF3b and detection of H4 mRNA by qRT-PCR. UV crosslinking experiments using purified eIF3 and [³²P] α -ATP-labelled thioU H4 mRNA led us to identify the eIF3 subunits that interact with H4 mRNA by western blot and two-dimensional (2D) SDS-PAGE gel. We unambiguously showed that the 4 eIF3 subunits c, d, e and g interact directly with H4 mRNA. Interestingly, among the eIF3 subunits found in direct interaction with H4 mRNA, eIF3d was shown to exhibit a cap binding activity (Lee et al. Nature, 2016). To verify whether eIF3d is able to interact independently of the eIF3 complex, we performed GST-pulldown experiments using a GST-tagged version of eIF3d produced in *E. coli* and total RNAs from HEK293 cells. By qRT-PCR we confirmed that the subunit eIF3d interacts specifically with H4 mRNA.

In short we have demonstrated that eIF3, the largest multisubunit initiation factor, directly interacts with histone H4 mRNA through four eIF3 subunits: c, d, e and g. We also confirmed the interaction of the isolated subunit GST-eIF3d with H4 mRNA. These interactions are likely to play a critical role in the H4 tethering mechanism. Future work will contribute to the elucidation of this unconventional translation mechanisms in eukaryotes.

References:

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