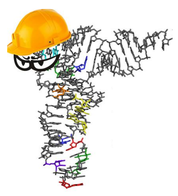
Determination of enrichment factors for modified RNA in MeRIP experiments



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# Equal contribution

I. Introduction and motivation :

RNA contains a large number of modifications which occur post-transcriptionally. They are involved in gene expression regulation and N^6-methyladenosine (m^6A) is among the most abundant modifications in messenger RNAs (mRNA) (Meyer *et al.*, 2012). They are known to affect multiple physiological processes in many different species, ranging from plants to yeast and mammals. Currently, approaches like methylated RNA Immuno-Precipitation (MeRIP) followed by high-throughput sequencing allow researchers to detect and map regions of m^6A in RNA transcriptome-wide (Dominissini *et al.*, 2013). Consequently, the most pertinent feature is the enrichment of methylated or otherwise modified RNA with specific antibodies, of which many are now commercially available. However, antibodies efficiency and specificity towards modification was not very well studied. Furthermore, the mere fact that IgG molecules would develop not only specificity for chemical entities within nucleic acids, but also a selectivity based on the presence of a single methylgroup, is quite remarkable. Figure 1 illustrates, why a deeper understanding of IgG binding to modified versus unmodified RNA is so important. To address these questions, we provide here in detail a procedure to determine enrichment factor in MeRIP experiment exemplified by using a Synaptic System antibody to bind m^6A modified synthetic mRNA. The method consists of the preparation of ^{32}P differential labelling of *in vitro* transcripts, with and without m^6A residues and enrichment factor analysis by thin layer chromatography (TLC).

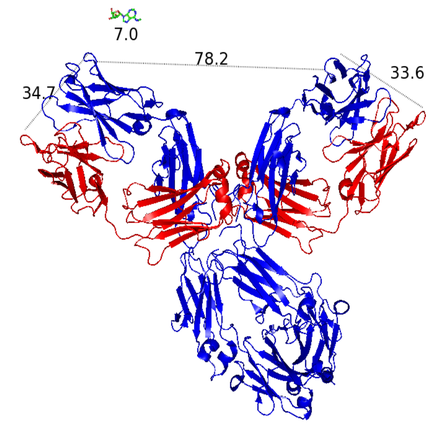

Fig 1. comparaison between IgG molecule interaction surface and m^6A residue.

II. Method:

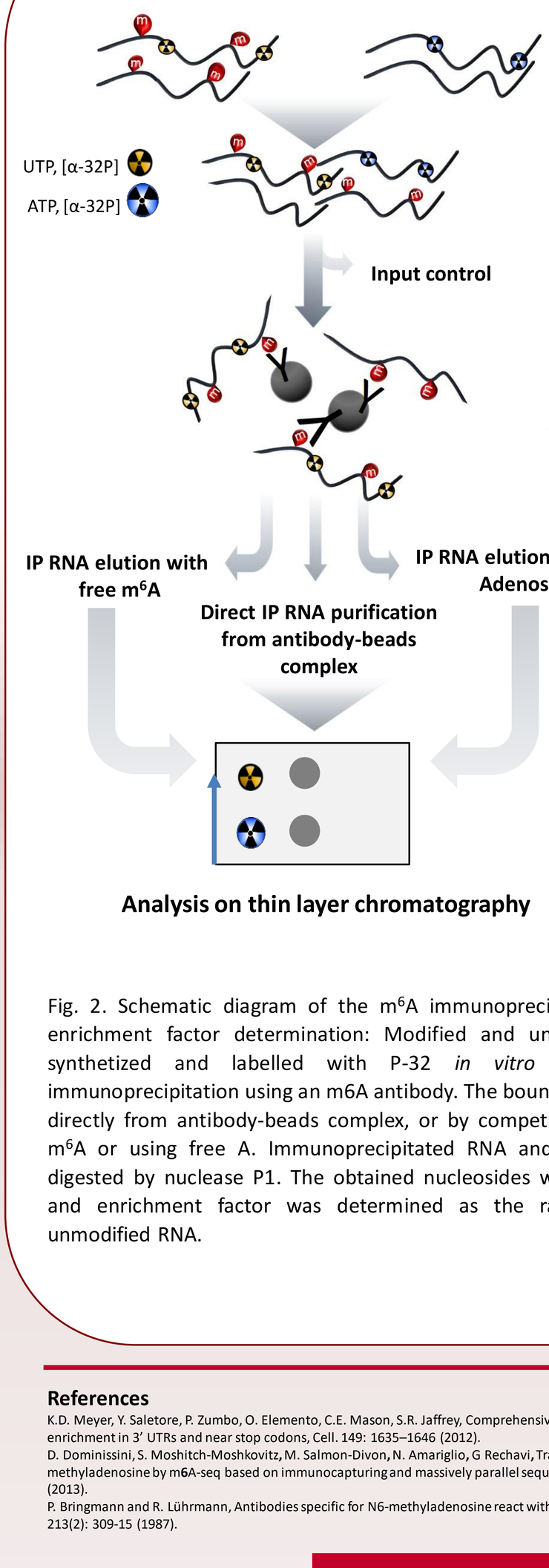

Fig. 2. Schematic diagram of the m^6A immunoprecipitation protocol for enrichment factor determination: Modified and unmodified RNA were synthetized and labelled with P-32 *in vitro* and subjected to immunoprecipitation using an m6A antibody. The bound RNA (IP) was eluted directly from antibody-beads complex, or by competitive assay using free m^6A or using free A. Immunoprecipitated RNA and input control were digested by nuclease P1. The obtained nucleosides were analyzed on TLC and enrichment factor was determined as the ratio of modified to unmodified RNA.

III. Enrichment factor determination

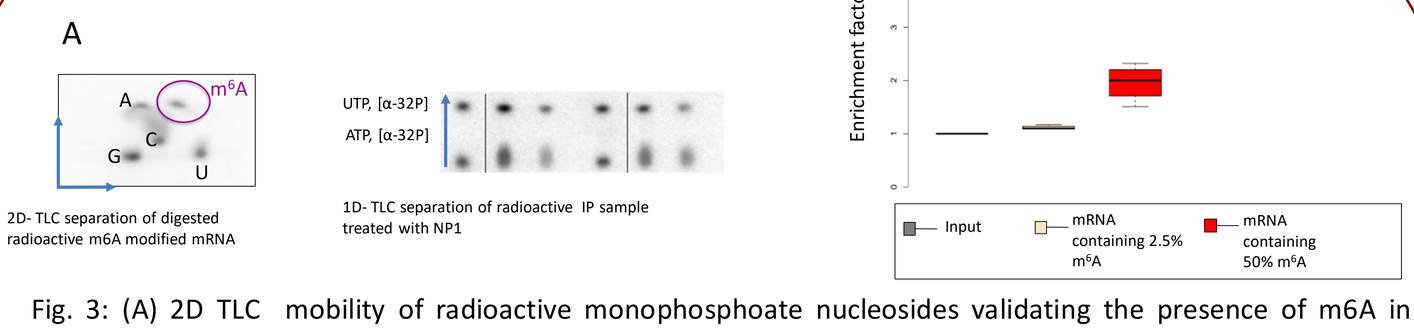

Fig. 3: (A) 2D TLC mobility of radioactive monophosphoate nucleosides validating the presence of m6A in modified mRNA and 1D TLC showing the separation of digested RNA prior (input) and after IP.
After exposure of the TLC to phosphoImager, spots intensity was used for the enrichment factor determination illustrated in figure 3.B and Fig. 4.

IV. Effect of IP optimization enrichment factor

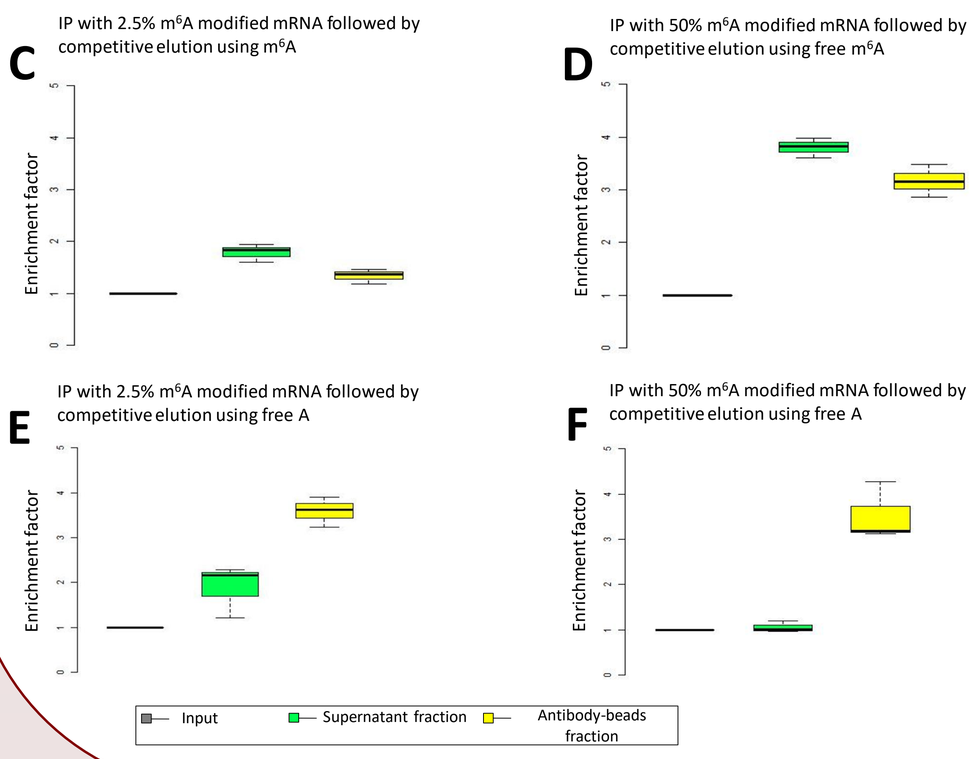

Fig.4:Competitive immunprecipitation was performed using a hot mixture of modified m^6A respectively 2.5% and 50% incorporation) and unmodified mRNA. Subsequently, bound mRNA was eluted using an excess of free m^6A (**C,D**) or free Adenosine (**E,F**). For the competitive assay, eluted RNA in the supernatant was ethanol precipitated and extracted with TRI reagent from the antibody-beads fraction.

IV. Conclusion:

We show here that the isolation of methylated mRNA with m6A was validated by IP using antibody anti-m6A. Enrichment factor revealed that the binding of 2.5% m^6A and/or 50% m^6A modified mRNA was increased by a competition assay elution using an excess of free m^6A (enrichment factor of 1.5 and 4 (in the supernatant) of respectively 2.5% m^6A and 50% m^6A containing mRNA as compared to input). Results demonstrated also that an important fraction of modified mRNA was still bound to the antibody-beads complex. Interestingly, most of the modified RNA was found retained by the antibody-beads complex and was not eluted in the case of competition elution with free non-modified adenosine. In the opposite of elution with free m^6A, elution with adenosine would be harnessed to prevent that free m^6A interferes with downstream sequencing analysis.

References

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