A monoclonal antibody for transcriptome-wide N^6-methyladenosine analysis

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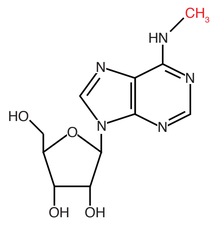
Abstract

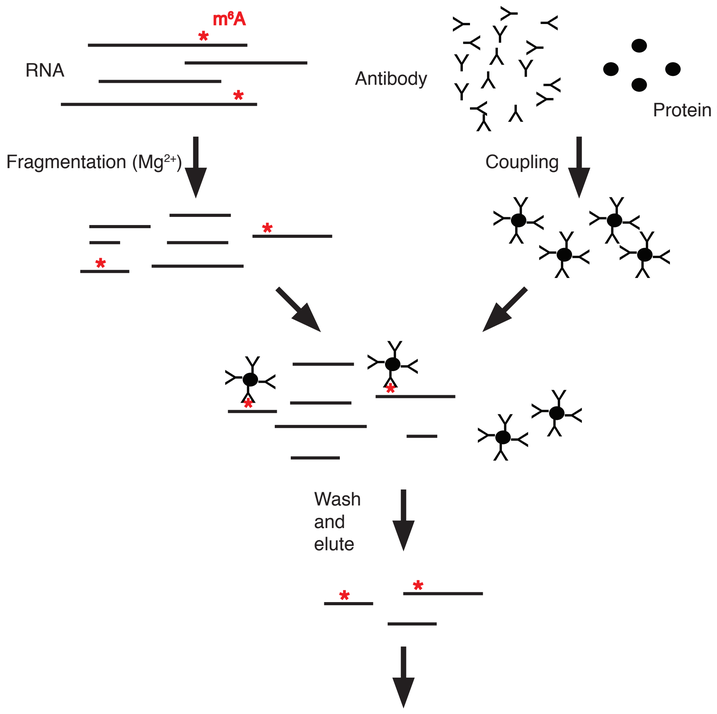
N^6-methyladenosine (m^{6}A) has been shown to be the most common base modification in eukaryotic messenger RNA (mRNA) other than the 7-methylguanosine cap. Recent m^{6}A-RNA immunoprecipitation (m^{6}A-RIP) with polyclonal antibodies combined with RNA high-throughput sequencing (RNA-seq) studies have identified the location of m^{6}A sites in a transcriptome-wide manner in a variety of tissues and have started to analyze the function of m^{6}A in mRNA (1-8). In humans m^{6}A is most commonly associated with a sequence motif in the 3' UTR of mRNAs near stop codons and m^{6}A modification is dependent upon a complex consisting of the methyltranferases METTL3+METTL14 and accessory proteins such as WTAP and KIAA1429. To further advance our understanding of m^{6}A in RNA, it is important to continue improving the tools needed for m^{6}A research. Here we present the generation of a new m^{6}A-specific rabbit monoclonal antibody and its use in m^{6}A-RIP-seq experiments.

Antibody Development



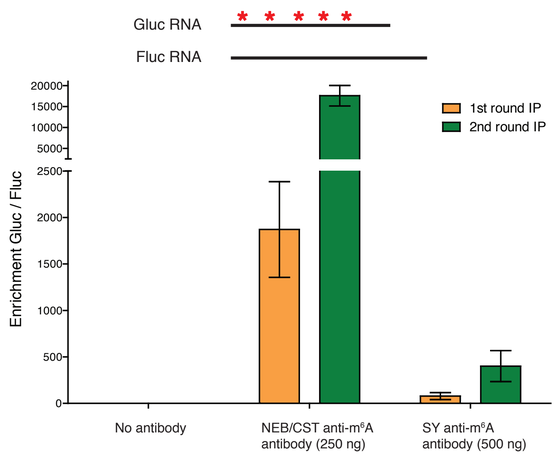
- N^6-methyladenosine (m^{6}A) was coupled to hemocyanin and introduced into rabbits to produce antibodies
- Dot blots and ELISA assays were used to show reactivity towards m^{6}A, but no reactivity with unmodified adenosine or other modified nucleosides (m^{1}A, 2'-OMe A)

RNA IP procedure

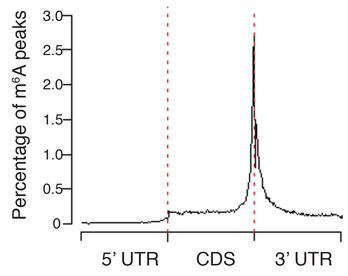


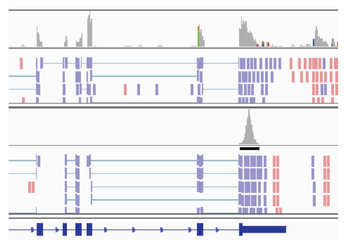
Prepare library for high-throughput sequencing with:
NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina®
(NEB# E7420)

RNA IP-qPCR with control RNAs

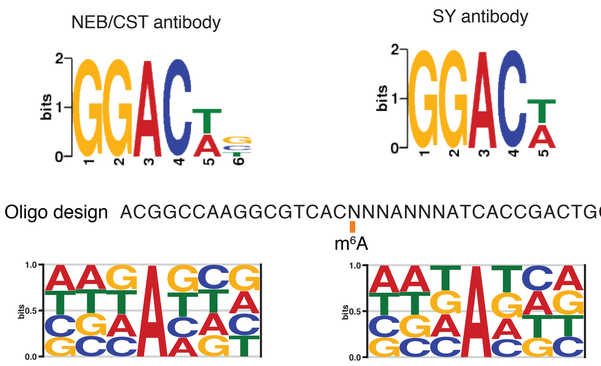


RIP-seq using mouse brain polyA+ RNA



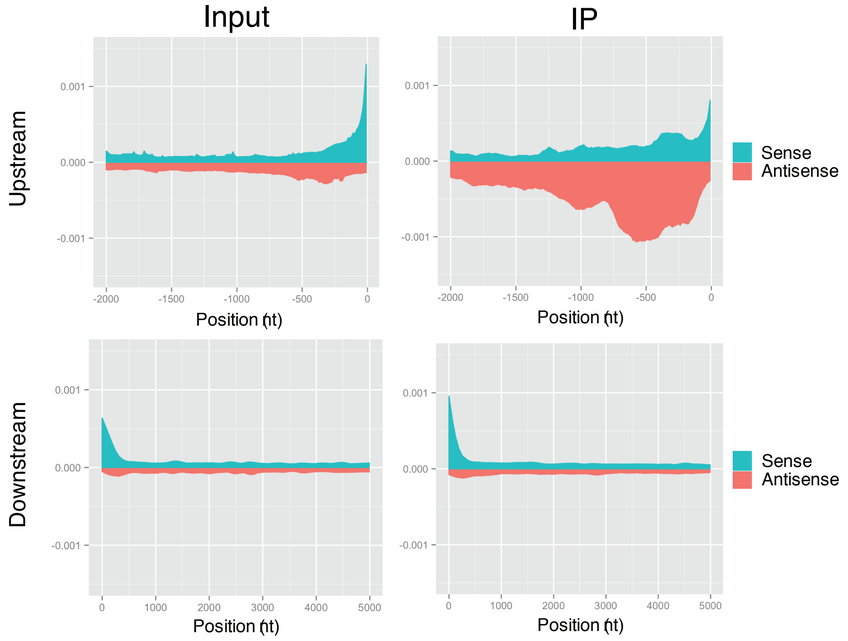


Motif analysis within m^{6}A peaks near stop codons

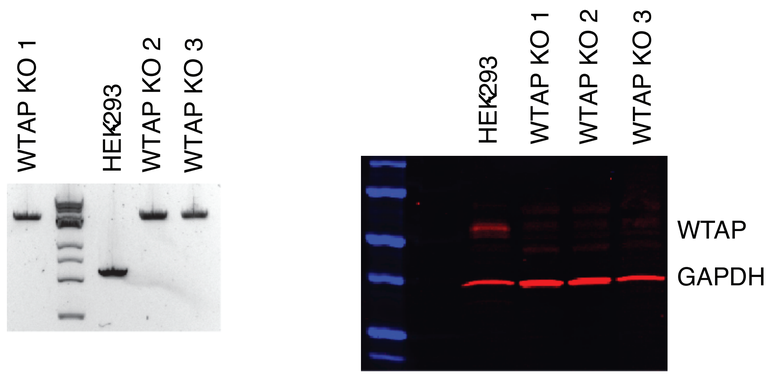


Motif from cellular RNA data is not due to antibody bias

RIP-seq reveals antisense enrichment

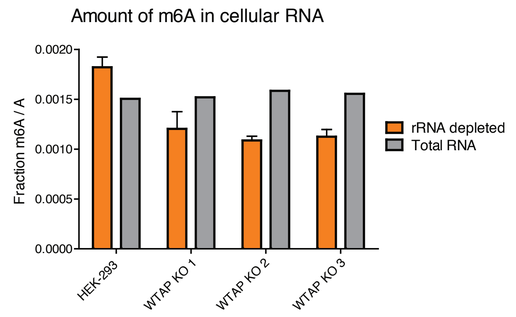


Disruption of WTAP via Cas9 genome editing

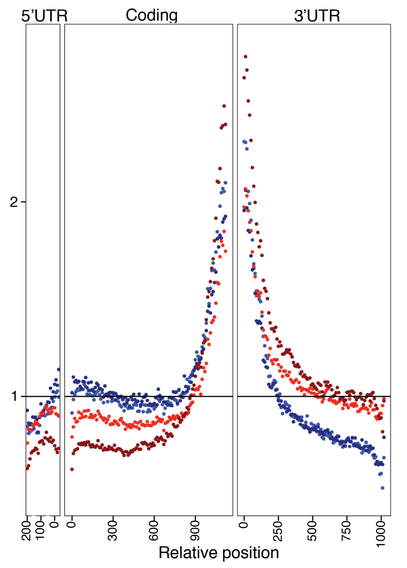


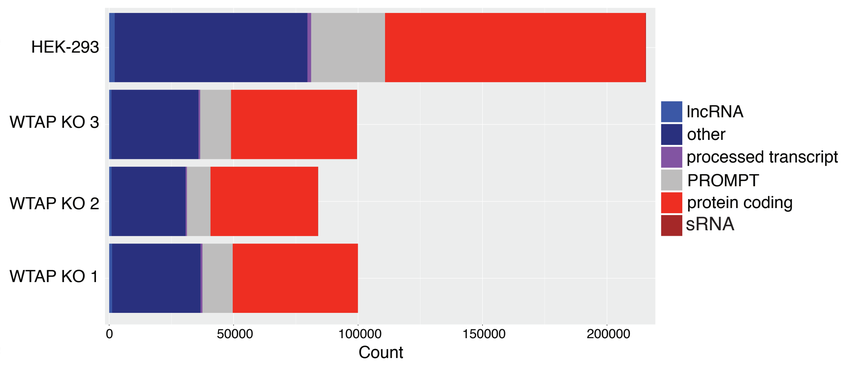
- PCR from genomic DNA shows rearrangement at the WTAP locus
- Western blot analysis shows complete loss of WTAP protein expression

RIP-seq using RNA from WTAP knockout cells



- HPLC analysis of RNA showed ~40-50% decrease in m6a in mRNA in WTAP null cells
- m6A RIP-seq showed a de crase of m6A near stop codons
- The distribution of m6A in classes of RNAs is un changed in WTAP null cells





Conclusions

- We have developed and validated a monoclonal antibody specific for m^{6}A.
- The monoclonal antibody results in better enrichment of m^{6}A-containing control RNA than a currently available polyclonal antibody.
- The monoclonal antibody performs well in m^{6}A-RIP-seq and produces results consistent with published m^{6}A-RIP-seq data. In addition, our analysis revealed enrichment of antisense RNAs.
- WTAP knockout strains show a reduction in RNA m6A levels and reduced enrichment of m6A modified sites after RIP-seq, but the overall pattern of m6A sites does not change.
- This suggests that WTAP is necessary for full methyltransferase complex activity, but not significantly involved in determining which sites are methylated.

References

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