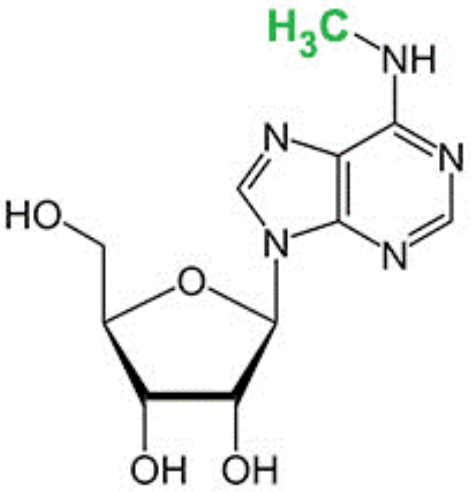
Tutorial 0

# Background

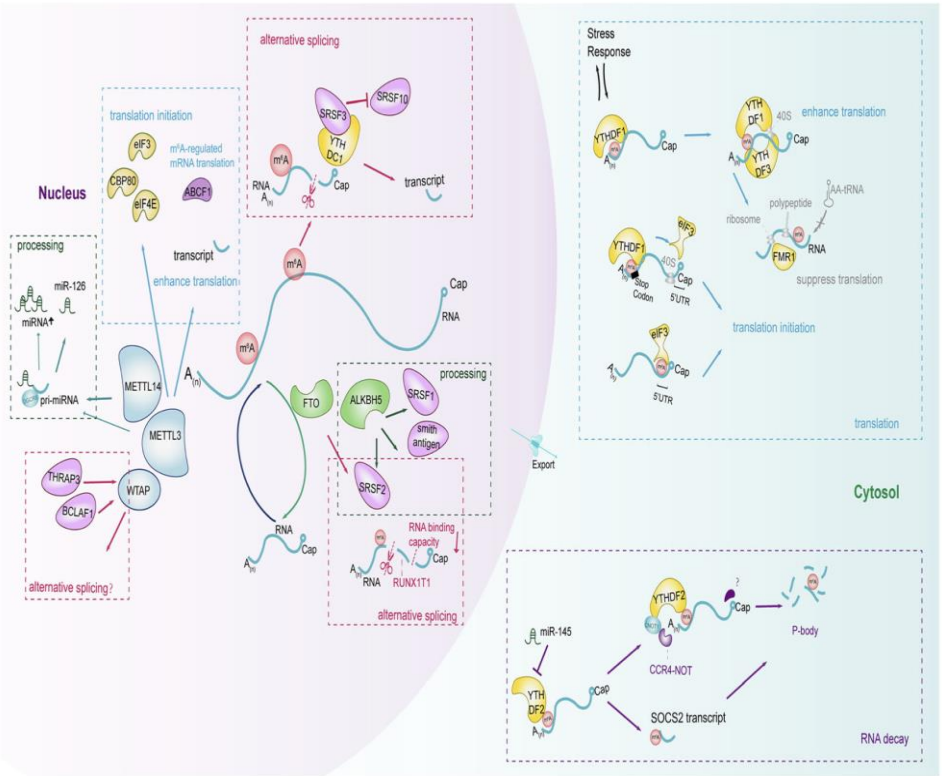
# Background – Chemical Basis of m6A RNA Methylation

- **RNA methylation**
  - A reversible post-translational modification to RNA that epigenetically impacts numerous biological processes.
  - Occurs in different RNAs including tRNA, rRNA, mRNA, tmRNA, snRNA, snoRNA, miRNA, and viral RNA.
  - Different catalytic strategies are employed for RNA methylation by a variety of RNA-methyltransferases

- **N6-methyladenosine (m6A)**
  - The most common and abundant methylation modification in RNA molecules present in eukaryotes and accounts for more than 80% of all RNA methylation.
  - Occurs in the N6-position of adenosine, which is the most prevalent internal modification on eukaryotic mRNA
  - m6A is installed by m6A methyltransferases, removed by m6A demethylases and recognized by reader proteins, which regulate of RNA metabolism including translation, splicing, export, degradation and microRNA processing.



# Background – Regulatory Functions of m6A Modification




## mRNA stability and degradation

- YTHDF2 binds to m6A-modified mRNA, promoting mRNA decay and regulating stability.
- FTO and ALKBH5 can influence mRNA stability by removing m6A marks.

## Translation regulation

- YTHDF1 and YTHDF3 facilitate translation initiation by recruiting translation initiation factors.
- m6A modifications near the stop codon and 3' UTR may enhance translation efficiency.

## Alternative splicing

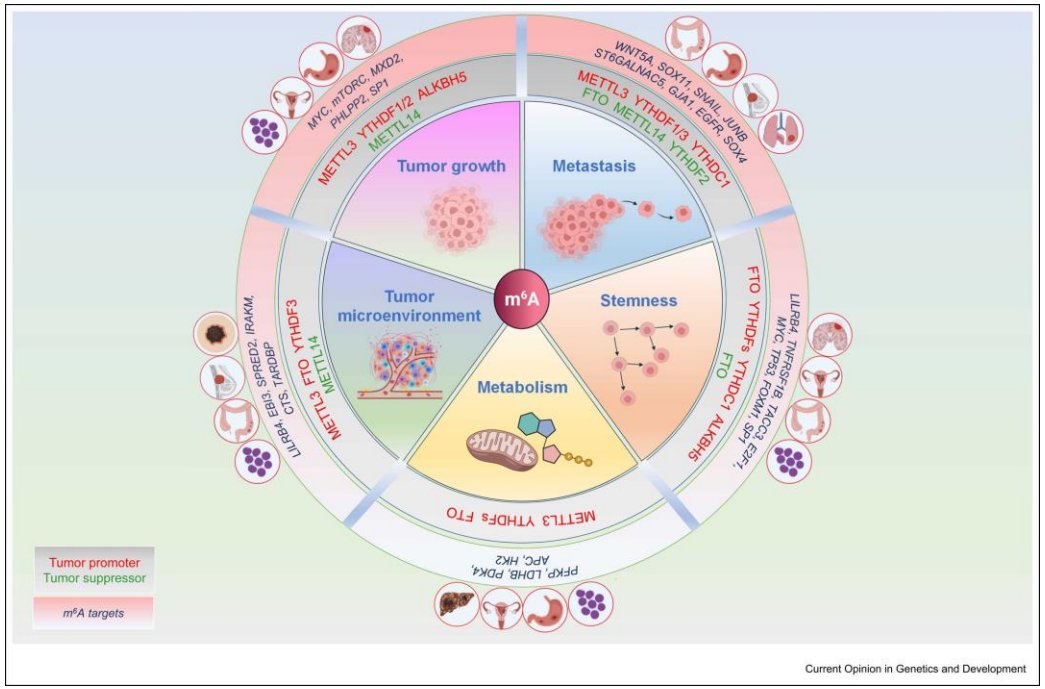
- YTHDC1 modulates alternative splicing by recruiting splicing factors to m6A-modified pre-mRNAs.
- m6A modifications can influence the selection of splice sites and exon inclusion.

## Nuclear export

- YTHDC1 binds to m6A-modified mRNA and facilitates its export from the nucleus to the cytoplasm.

Chen et al. Molecular Cancer (2019) 18:103

# Background – m6A RNA Methylation in Cancers




**m6A modification hinders cancer progression**

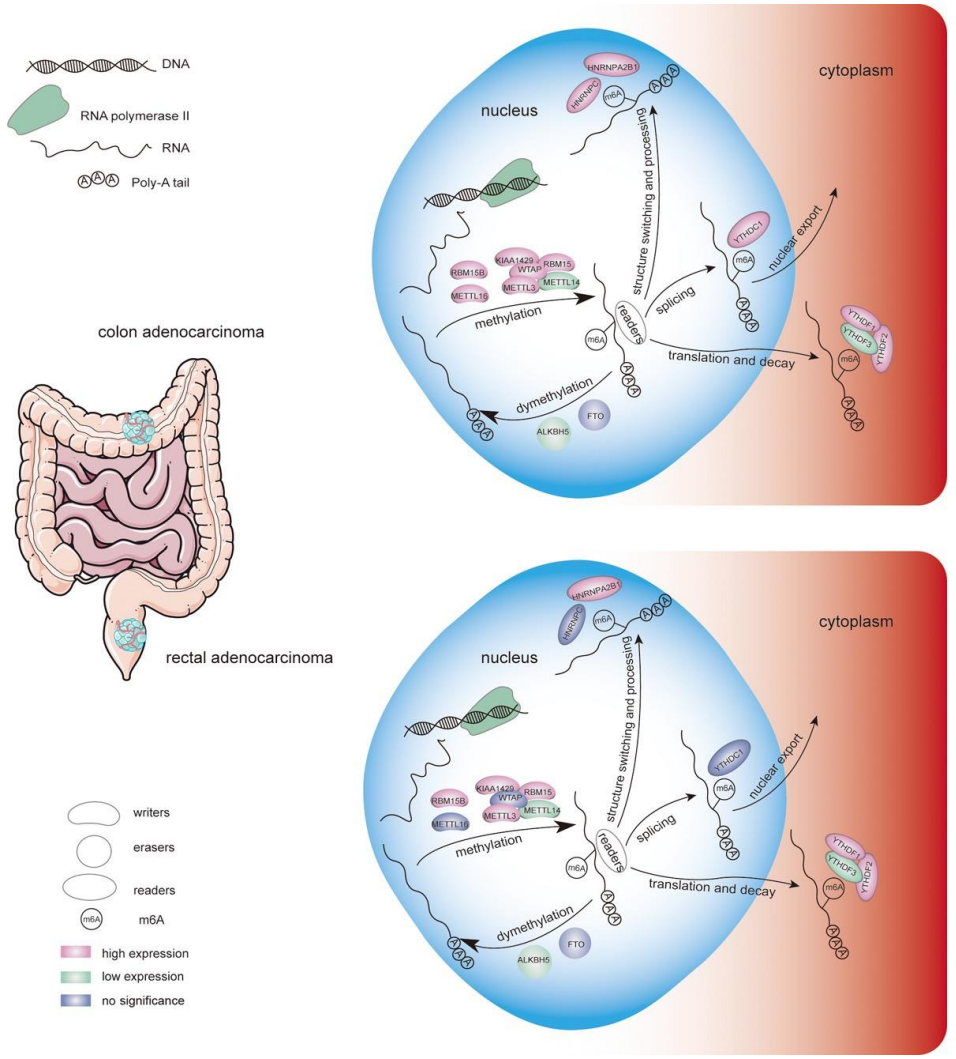
- Glioblastoma: In glioblastoma, m6A modification of the mRNA encoding the transcription factor ADAM19 (a disintegrin and metalloprotease domain-containing protein 19) was shown to enhance its stability and translation, leading to the inhibition of glioblastoma cell proliferation and invasion.
- Hepatocellular carcinoma (HCC): In HCC, the m6A "writer" protein METTL14 has been reported to suppress tumor growth and metastasis. METTL14 can promote m6A modification on the mRNA of the tumor suppressor gene SOCS2 (suppressor of cytokine signaling 2), increasing its stability and expression, which subsequently inhibits the STAT3 signaling pathway and tumor progression.
- Breast cancer: In breast cancer, the m6A "eraser" protein FTO (Fat mass and obesity-associated protein) was found to inhibit cancer progression by demethylating the mRNA of BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3), a pro-apoptotic protein. The increased expression of BNIP3 led to enhanced apoptosis of breast cancer cells.

https://doi.org/10.1016/j.gde.2022.101924

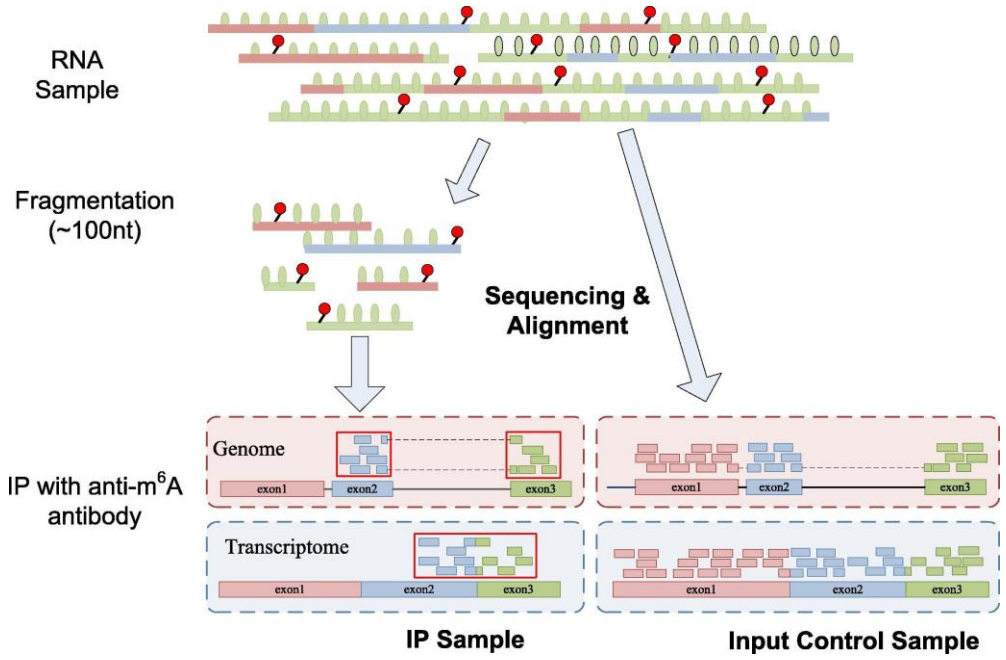
# Background – m6A RNA Methylation in Colorectal Cancers

**m6A RNA methylation (Based on TCGA)**

- Most m6A-related genes were substantially upregulated in CRC tumor tissues
- METTL14, YTHDF3 and ALKBH5 were downregulated in CRC.
- No obvious difference in FTO.
- WTAP, METTL16, HNRNPC and YTHDC1 were abundantly expressed in COAD but not in READ.
- Most of the m6A-related proteins were expressed in the nucleus and cytoplasm.
- The expression levels of METTL3, METTL14, METTL16, FTO and ALKBH5 were associated with the clinical outcomes of CRC patients.



# Background – Illustration of MeRIP-Seq Protocol




L. Liu et al. Computational and Structural Biotechnology Journal 18 (2020) 1587–1604

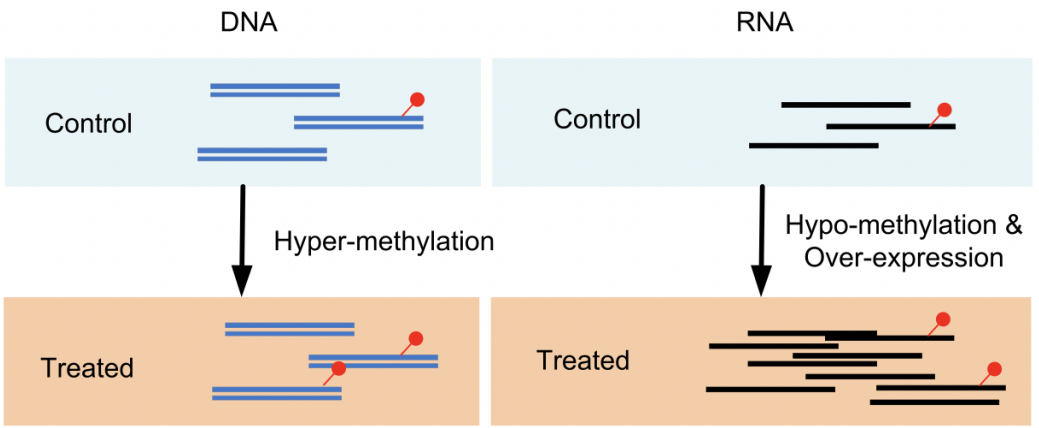
- RNA extraction: Isolate total RNA from cells or tissues.
- Fragmentation: Break extracted RNA into smaller fragments (100-200 nucleotides).
- Immunoprecipitation: Incubate fragmented RNA with anti-m6A antibody to capture m6A-containing fragments.
- Washing and elution: Wash beads to remove non-specific RNA and elute m6A-containing RNA fragments.
- RNA purification: Purify the eluted m6A-containing RNA fragments.
- Library preparation: Convert purified RNA fragments into a cDNA library for sequencing and prepare a separate library from input RNA as control.
- High-throughput sequencing: Sequence libraries using a next-generation sequencing platform.

# Background – MeRIP-Seq Data Analyses Pipeline

| Step | Description |
| --- | --- |
| Quality control | Assess the quality of raw sequencing reads using tools like FastQC and perform adapter trimming and read filtering if necessary. |
| Read alignment | Map the processed sequencing reads to a reference genome or transcriptome using a spliced aligner such as STAR, HISAT2, or TopHat2. |
| Peak calling | Identify m6A-enriched regions (peaks) by comparing immunoprecipitated (IP) and input control samples using tools like MACS2 or exomePeak2. |
| Annotation | Annotate the identified m6A peaks to genomic features (e.g., 5' UTR, 3' UTR, CDS) and associate them with corresponding genes. |
| Differential methylation analysis | Compare m6A methylation patterns between different experimental groups or conditions to identify differentially methylated sites. |
| Gene ontology and pathway enrichment | Perform functional enrichment analysis on genes with differentially methylated sites to identify overrepresented biological processes and pathways. |
| Visualization | Create visual representations of MeRIP-Seq data, such as genome browser tracks, heatmaps, and scatterplots to aid in data interpretation. |
| Integration with other omics data | Integrate MeRIP-Seq data with other omics data (e.g., RNA-seq, ChIP-seq) to uncover potential relationships between m6A methylation and gene expression or chromatin modifications. |
| Validation and experimental follow-up | Validate key findings using orthogonal experimental approaches (e.g., qPCR, RIP-qPCR, luciferase reporter assays) and perform functional studies to investigate the biological consequences of the identified m6A modifications. |

# Background – Differential Methylation Analysis

Compared with the control group, both the absolute and relative amount of methylated DNA in the treatment group increased under the treated condition.




Compared with the control group, the absolute and relative amount of methylated RNA in the treatment group may not be consistent.

The total amount of methylated RNA increased in the treatment group; however, due to the increased expression level (over-expression), the relative amount of methylated RNA decreased (hypo-methylation).

- Differential methylation analysis examines changes in RNA modification status between two biological conditions, primarily focusing on relative abundance or methylation proportion.
- Classic approaches like Fisher's exact test (implemented in the exomePeak2 R package) have been popular, but newer methods such as RADAR and QNB offer higher accuracy through refined statistical models.

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