Stabilization of ERK-Phosphorylated METTL3 by USP5 Increases m^{6}A Methylation

Hui-Lung Sun[1,2,9], Allen C. Zhu[1,2,3,9], Yawei Gao[4], Hideki Terajima[1,2], Qili Fei[1,2], Shun Liu[1,2], Linda Zhang[1,2], Zijie Zhang[1,2], Bryan T. Harada[1,2], Yu-Ying He[5], Marc B. Bissonnette[6], Mien-Chie Hung[7], Chuan He[1,2,8]

[1]Department of Chemistry and Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL 60637 USA.
[2]Howard Hughes Medical Institute, The University of Chicago, Chicago, IL 60637, USA.
[3]Medical Scientist Training Program, The University of Chicago, Chicago, IL 60637, USA.
[4]Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, Shanghai Key Laboratory of Signaling and Disease Research, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China.
[5]Department of Medicine, Section of Dermatology, University of Chicago, Chicago, IL, 60637, USA.
[6]Department of Medicine, The University of Chicago, Chicago, IL 60637, USA.
[7]China Medical University, Taichung 404, Taiwan.
[8]Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL, USA.
[9]These authors contributed equally.

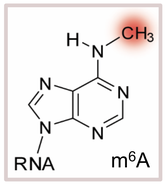
1 INTRODUCTION

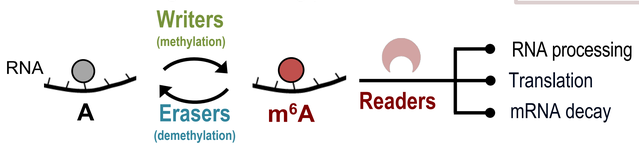
A. Epitranscriptomics—codes along RNA sequences

Like the epigenetic regulation of DNA and histones, RNA has been shown to carry reversible biochemical modifications that mediate gene expression regulation.

B. N^6-methyladenosine (m^{6}A) on mRNAs

- Prevalence: ~3-5 m^{6}A sites per mRNA
- Distribution: RRACH sequence motif; enriched near stop codons and 3'UTRs
- Dynamic: enzymes reversibly install m^{6}A
- Functions: through m^{6}A binding proteins[1]





The formation of m^{6}A is catalyzed by the "writer" enzyme METTL3, which functions as part of the METTL3-METTL14-WTAP methyltransferase complex.

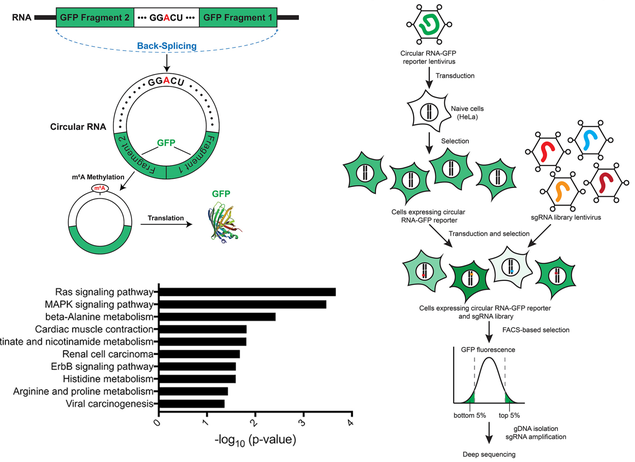
How METTL3 activity is post-translationally regulated is not well understood. Understanding these effects may show how METTL3 controls gene expression through m^{6}A.

C. Previous Studies: m^{6}A & METTL3 in Stem Cells and Cancer

- METTL3 has an oncogenic role in lung cancer, leukemia, bladder cancer[3]
- METTL3 has been shown to promote differentiation and priming in stem cells. [4-5]
- Post-translational modifications such as SUMOylation have been shown to modulate METTL3 activity. [6]

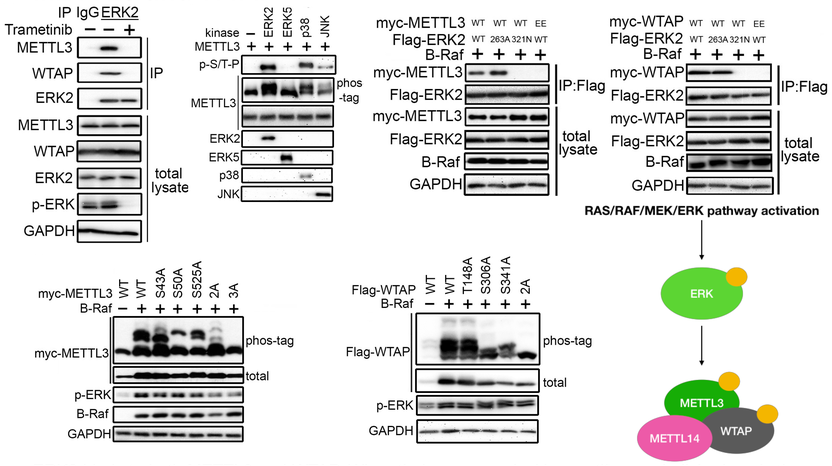
2 HYPOTHESIS & STRATEGY

We hypothesized that phosphorylation of METTL3 by a kinase signaling pathway regulates m^{6}A methyltransferase activity and employed a CRISPR screen with GGACU-dependent GFP expression to find regulators of the m^{6}A pathway.



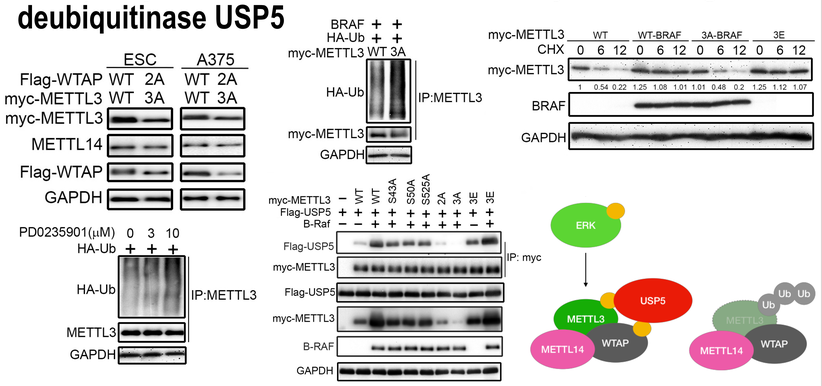
3 RESULTS

The RAS/MAPK pathway kinase ERK2 phosphorylates METTL3 and WTAP



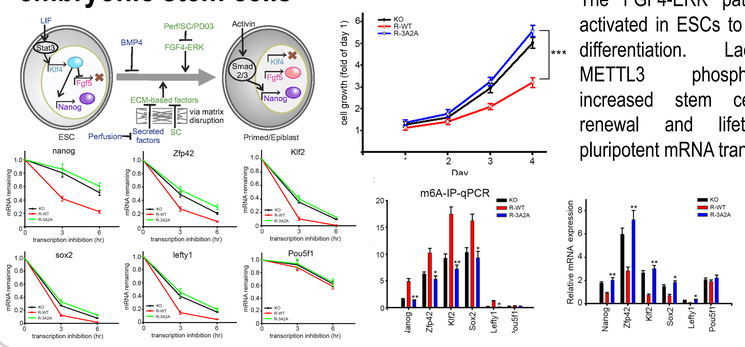
ERK2 binds to both METTL3 and WTAP. When their common docking motifs are abolished, so is the interaction. In vitro, ERK2 is able to phosphorylate METTL3. This occurs at S43, S50, and S525 on METTL3 and at S306 and S341 on WTAP.

Phosphorylation stabilizes METTL3 by recruiting the deubiquitinase USP5



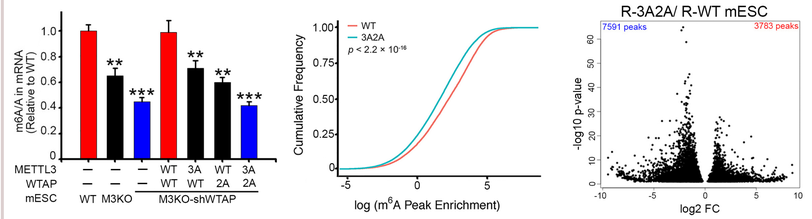
Phosphorylation of METTL3/WTAP stabilizes the writer complex. Lack of phosphorylation by ERK2 increases ubiquitination, due to decreased interaction with deubiquitinase USP5.

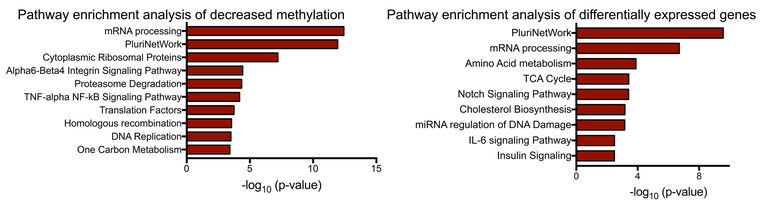
METTL3 phosphorylation promotes differentiation of embryonic stem cells



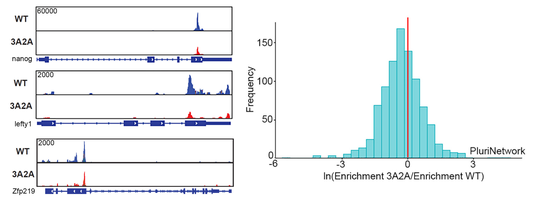
The FGF4-ERK pathway is activated in ESCs to promote differentiation. Lack of METTL3 phosphorylation increased stem cell self-renewal and lifetime of pluripotent mRNA transcripts.

METTL3 phosphorylation increases m^{6}A methylation





Loss of METTL3 phosphorylation leads to significantly reduced m6A methylation. This especially affects methylation of genes involved in pluripotency and affects their gene expression level.



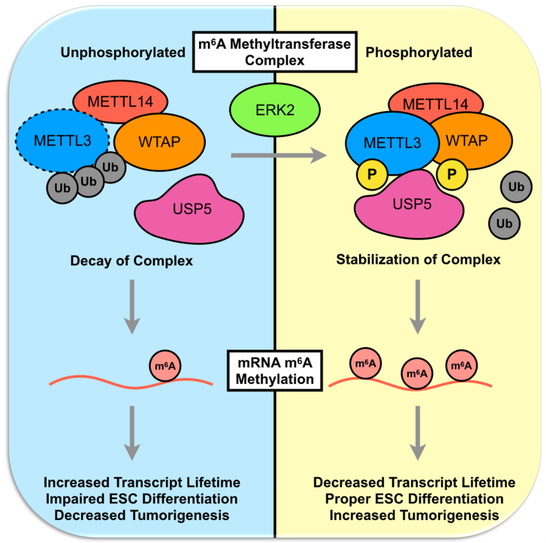
4 CONCLUSION & FUTURE STUDIES

Future Studies:

How do other kinases such as ATM/ATR or p38 affect METTL3 phosphorylation and activity?

Could other GGACU-GFP CRISPR screen top hits reveal more regulators of m^{6}A?

What is the structural basis for phosphorylation-dependent METTL3-WTAP interaction with USP5?



5 References

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6 Acknowledgements

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