The dynamic N1-methyladenosine methylome in eukaryotic messenger RNA

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研究背景

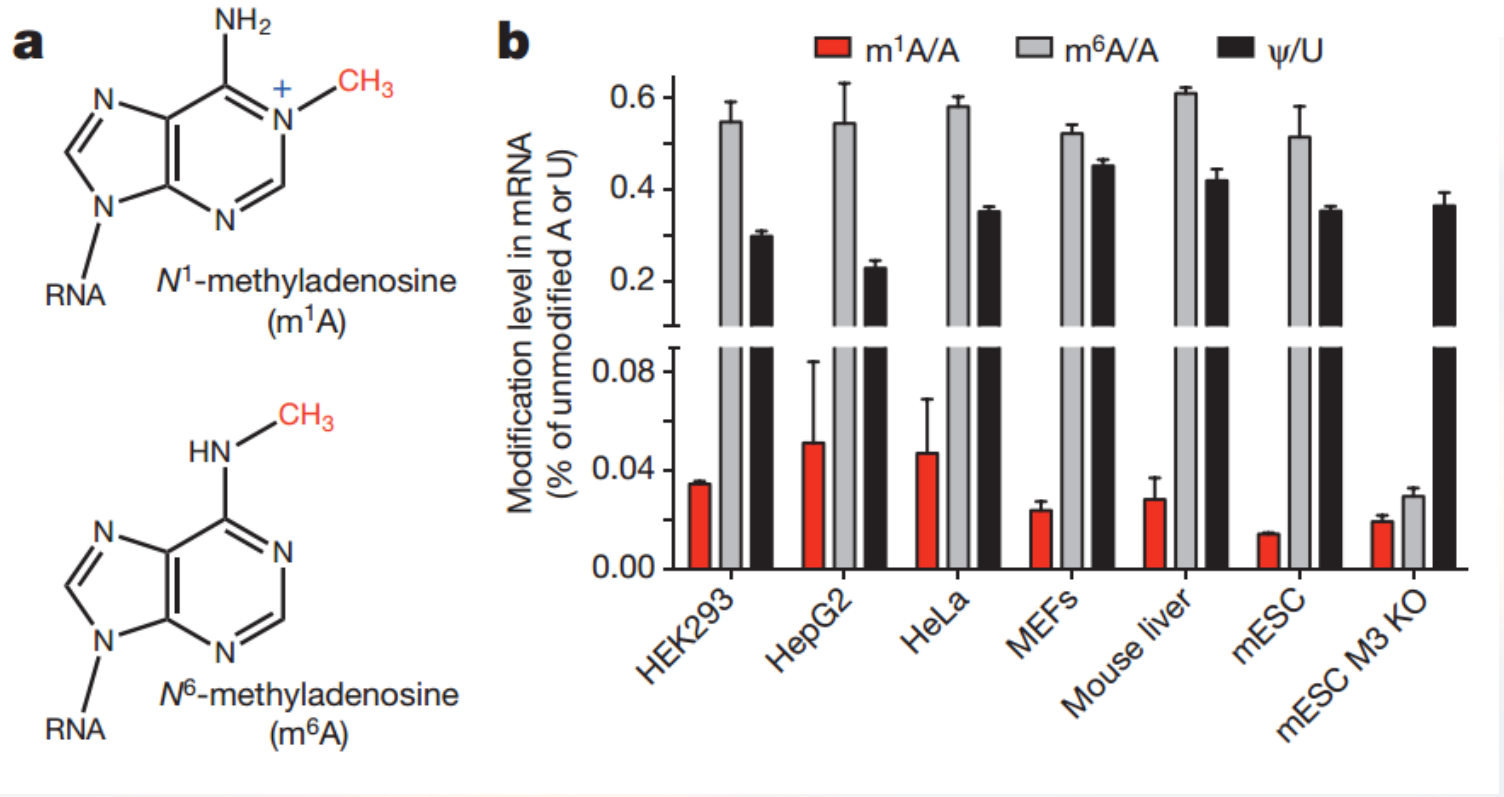
- RNA甲基化作为表观遗传学研究的重要内容之一，是指发生在RNA分子上不同位置的甲基化修饰现象。RNA甲基化在调控基因表达、剪接、RNA编辑、RNA稳定性、控制mRNA寿命和降解等方面可能扮演重要角色。相对于DNA甲基化，RNA甲基化更加复杂、种类繁多、普遍存在于各种高级生物中。由于缺乏有效检测手段，相关研究多局限于非编码tRNA和rRNA，多数的mRNA甲基化功能未知。
- 近些年研究表明N6-methyladenosine (m6A) 在mRNA代谢中扮演重要角色，其广泛地影响了mRNA的定位、稳定、翻译及剪接。此外，pseudouridine (Ψ)和 5-methylcytosine 也被发现在基因表达的转录后修饰中起着重要作用。N1-methyladenosine（ m1A ）在mRNA中的修饰则未见报道。

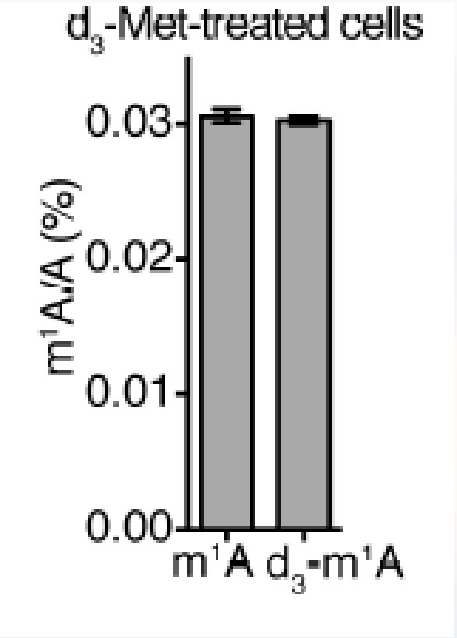
研究方法

- 利用基于MeRIP-seq（ m1A-seq ）的方法研究真核生物mRNA中是否存在着m1A甲基化修饰及其生物学意义。

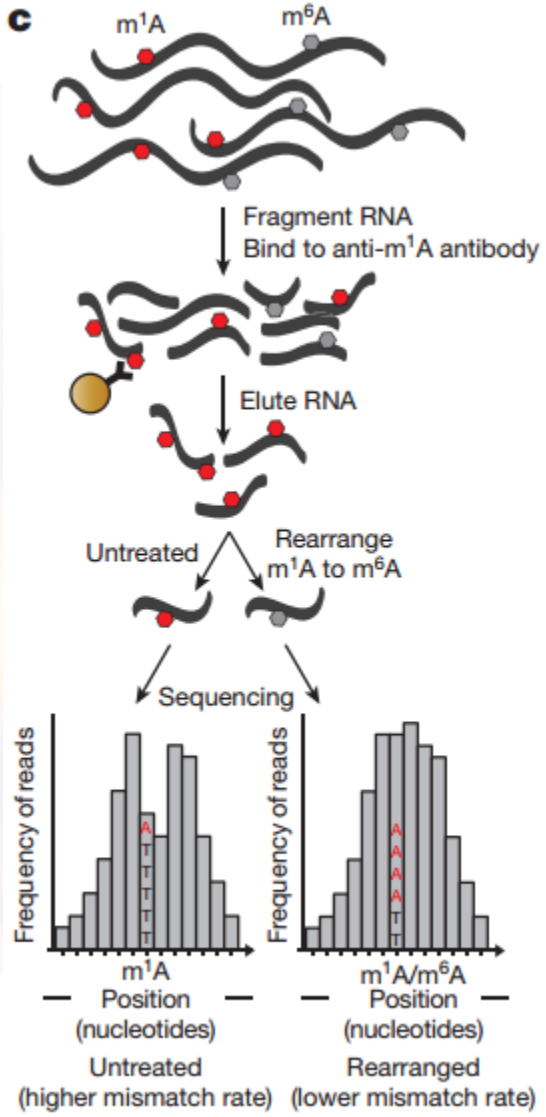
研究结果

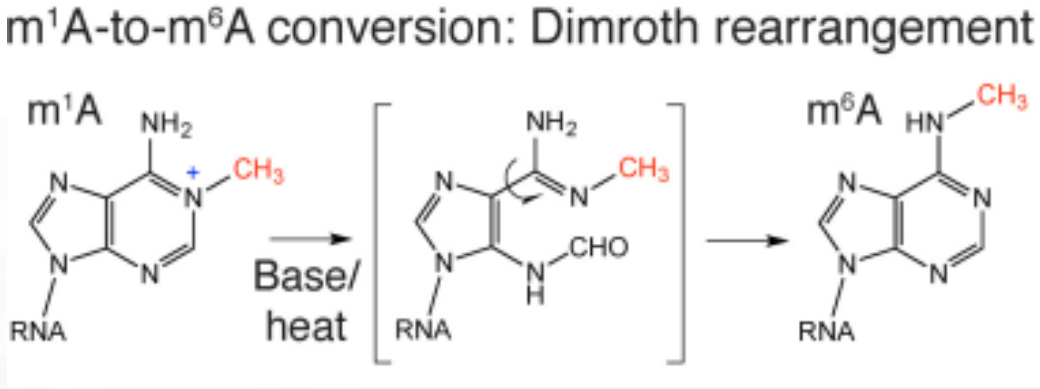
- Transcriptome-wide mapping of m1A

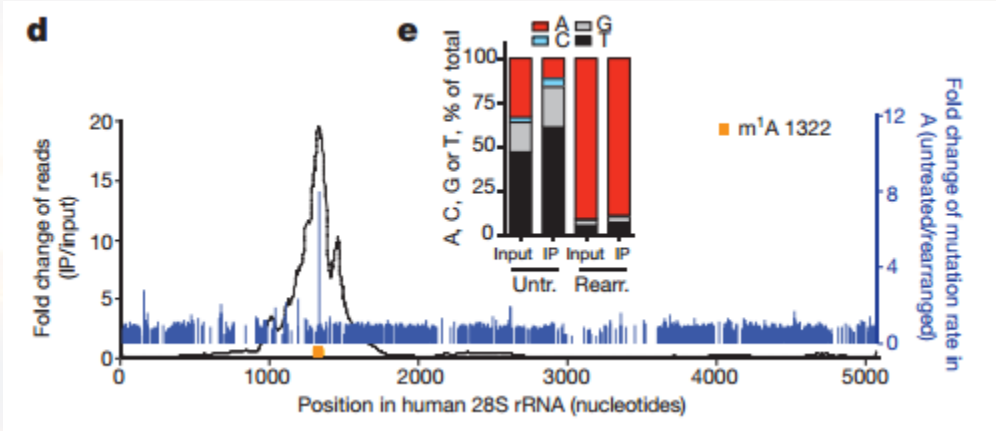




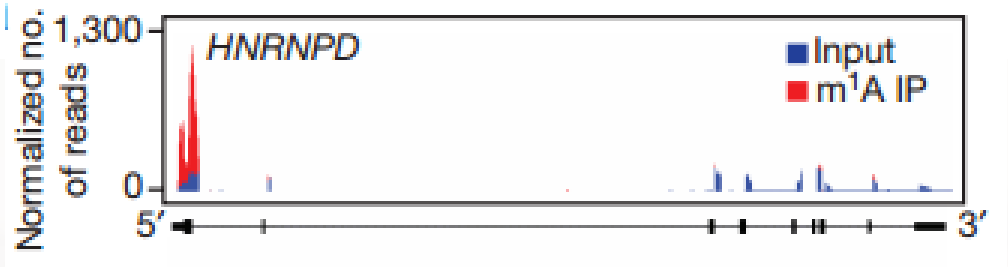
Transcriptome-wide mapping of m1A

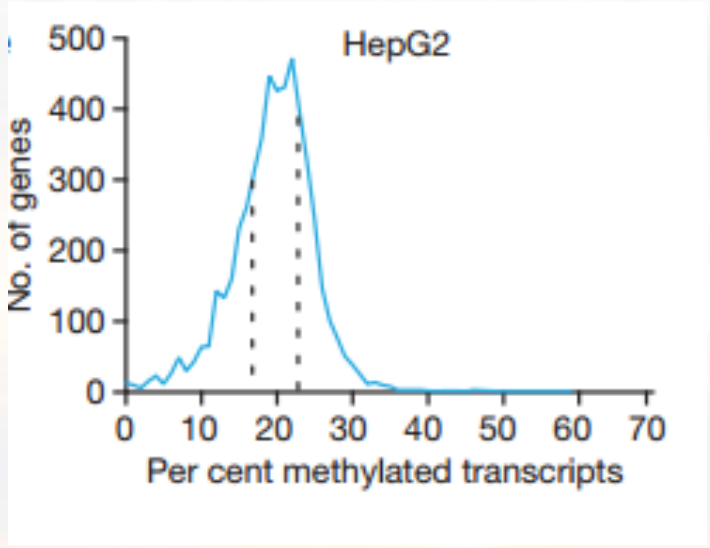


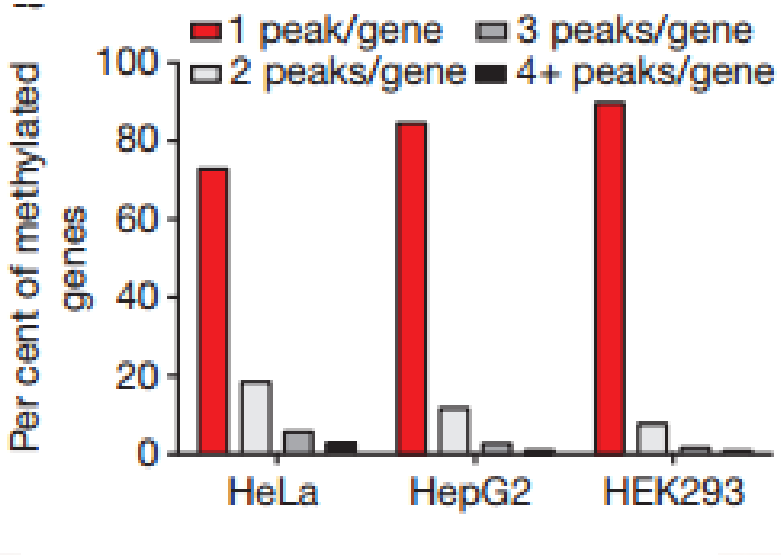


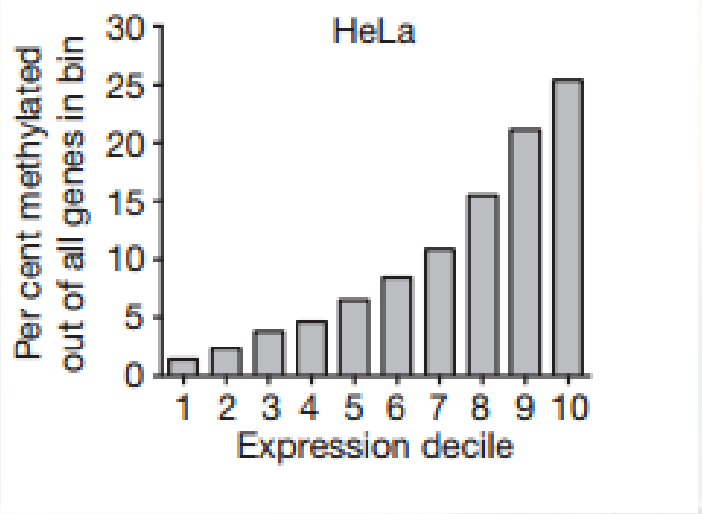


Transcriptome-wide mapping of m1A



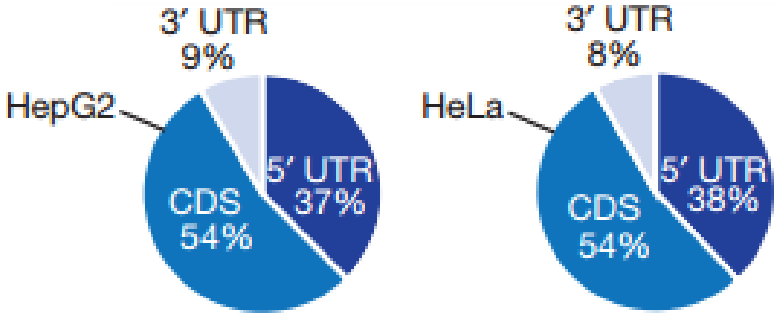


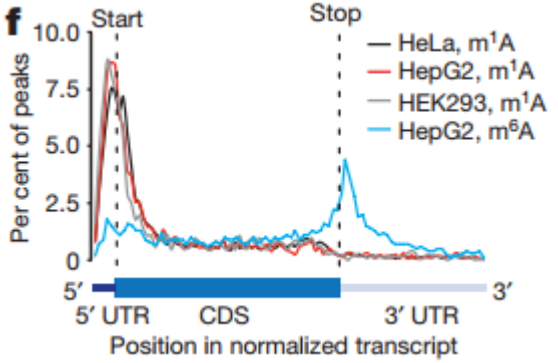


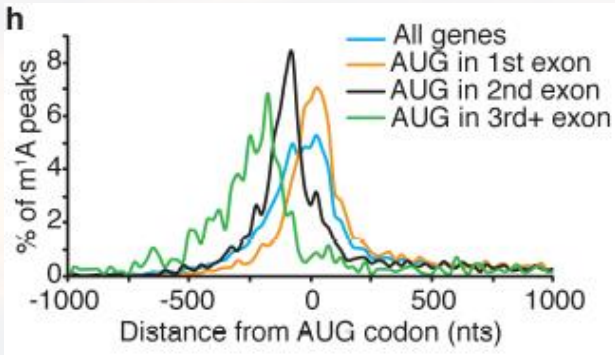


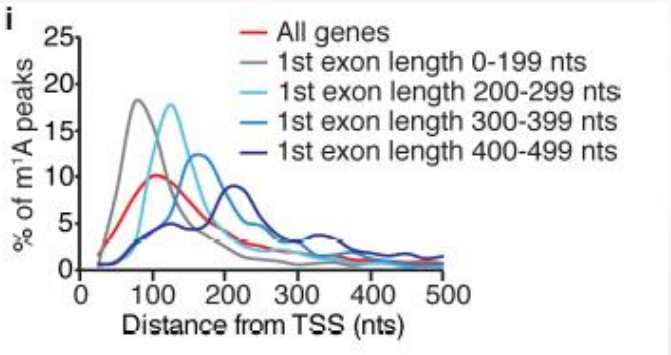
We identified 7,154 peaks (fold change (FC)≥ 2, false discovery rate (FDR)≤5%) in 4,151 coding and 63 non-coding adequately expressed gene transcripts that occurred in both replicates.

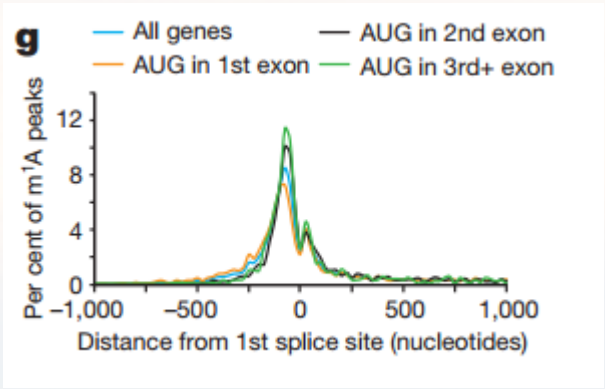
- m1A associates with translation initiation sites and the first splice site



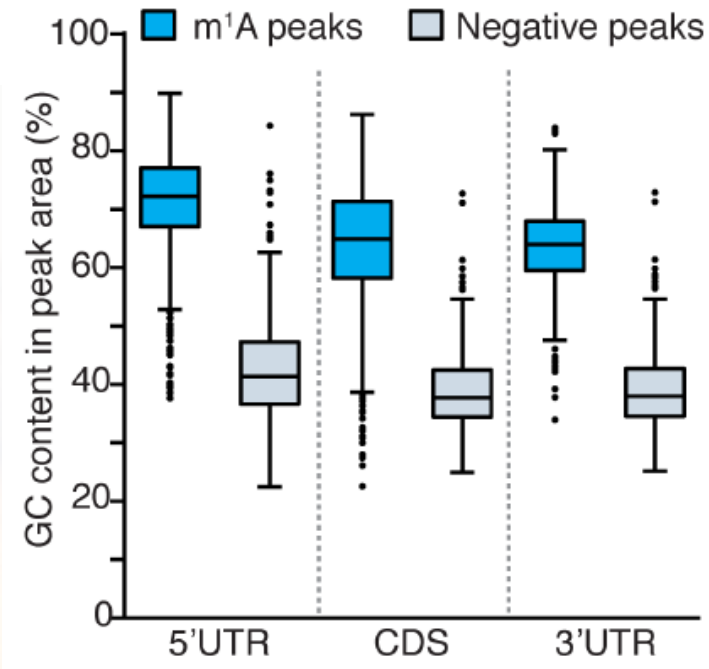








Conserved features of methylated transcripts



m1A peaks have a significantly higher GC content compared to negative peaks in all three transcript segments: 5′ UTR, CDS and 3′ UTR.

Motifs in a 400-nt window centered on the annotated TIS (canonical AUG)

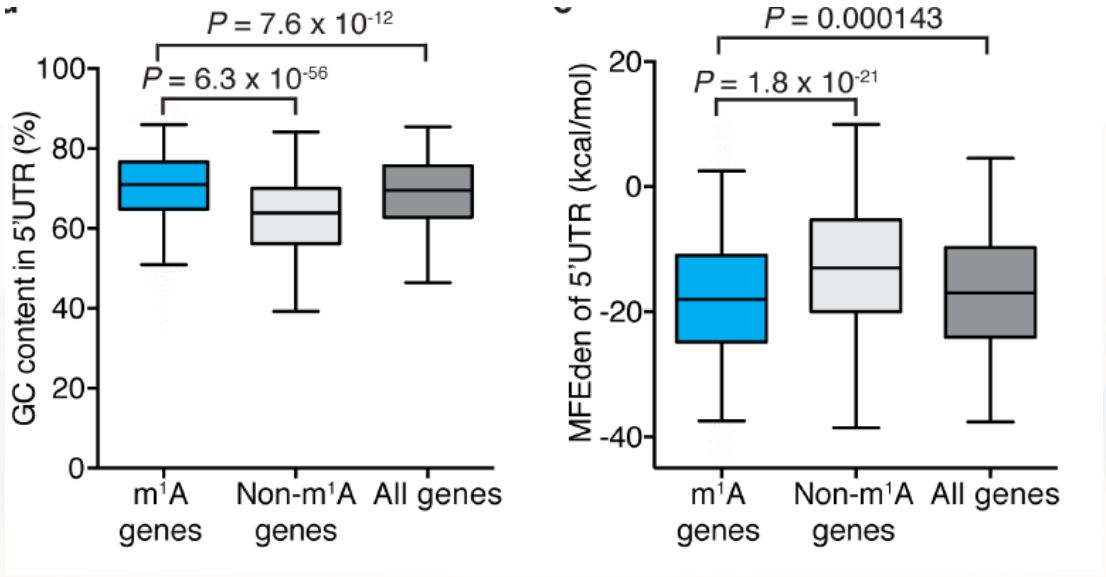
Rank	Motif	P-Value	% Of Peaks	% Of Backgrd.
1		1e-302	89.69%	56.88%
2		1e-268	87.34%	55.57%
3		1e-135	57.32%	32.19%
4		1e-117	65.50%	42.05%
5		1e-105	52.87%	30.82%

Motifs in CDS

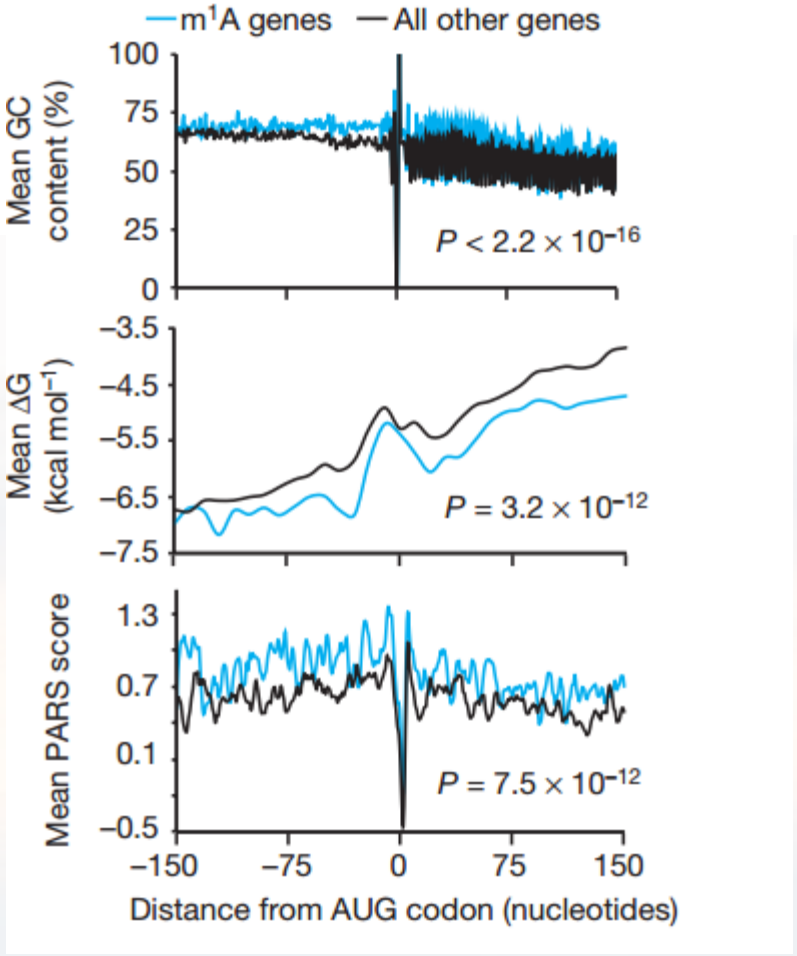
Rank	Motif	P-Value	% Of Peaks	% Of Backgrd.
1		1e-232	88.16%	54.07%
2		1e-230	76.05%	39.30%

Motifs identified in 400-nucleotide windows centred on the canonical AUG start codon in genes with m1A peaks in this window (upper table), or around m1A peaks located in the CDS, outside the AUG start codon window (lower table).

Conserved features of methylated transcripts

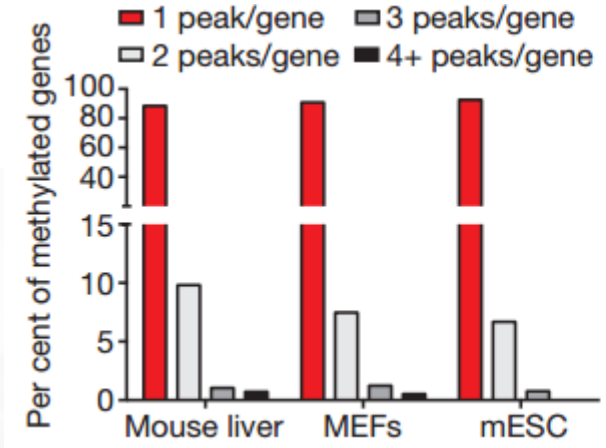


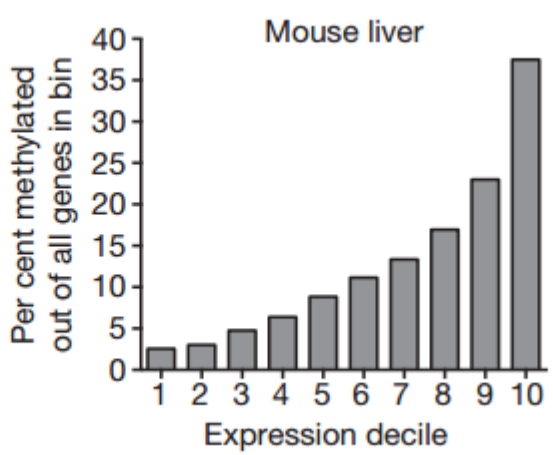
Attributes of the 5′ UTR such as length, GC content, and associated minimum free energy (MFE), are known to affect the efficiency of translation initiation. 5′ UTRs of methylated transcripts differ significantly from those of non-methylated ones in that their GC content is higher and their length-adjusted MFE (aMFE) is lower.

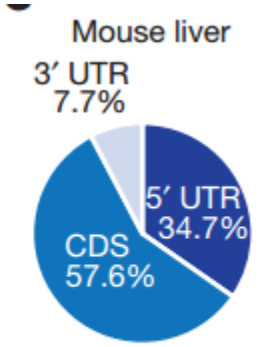


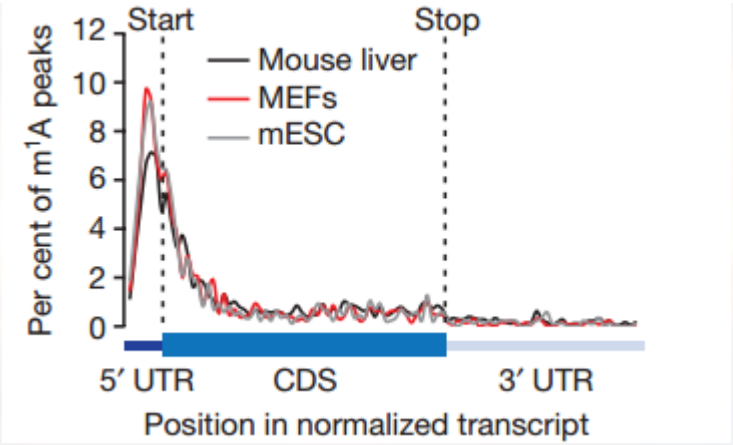
We employed a sliding window approach to further characterize the continuous structural landscape of the AUG window (±150 nucleotides) in terms of GC content, MFE and experimental parallel analysis of RNA structure (PARS) score . Both the experimental and calculated parameters agree that m1A decorates highly structured AUG windows.

Evolutionary conservation and dynamics



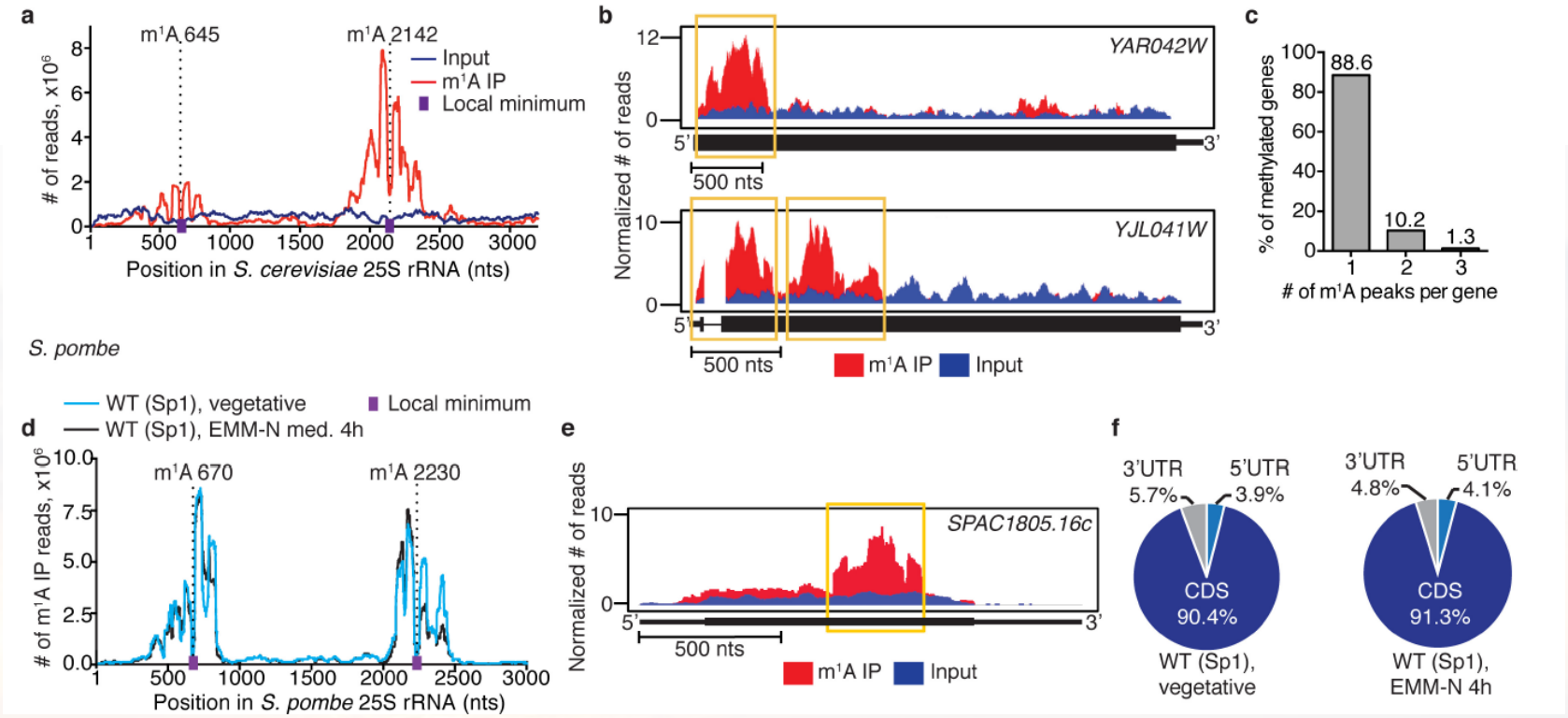






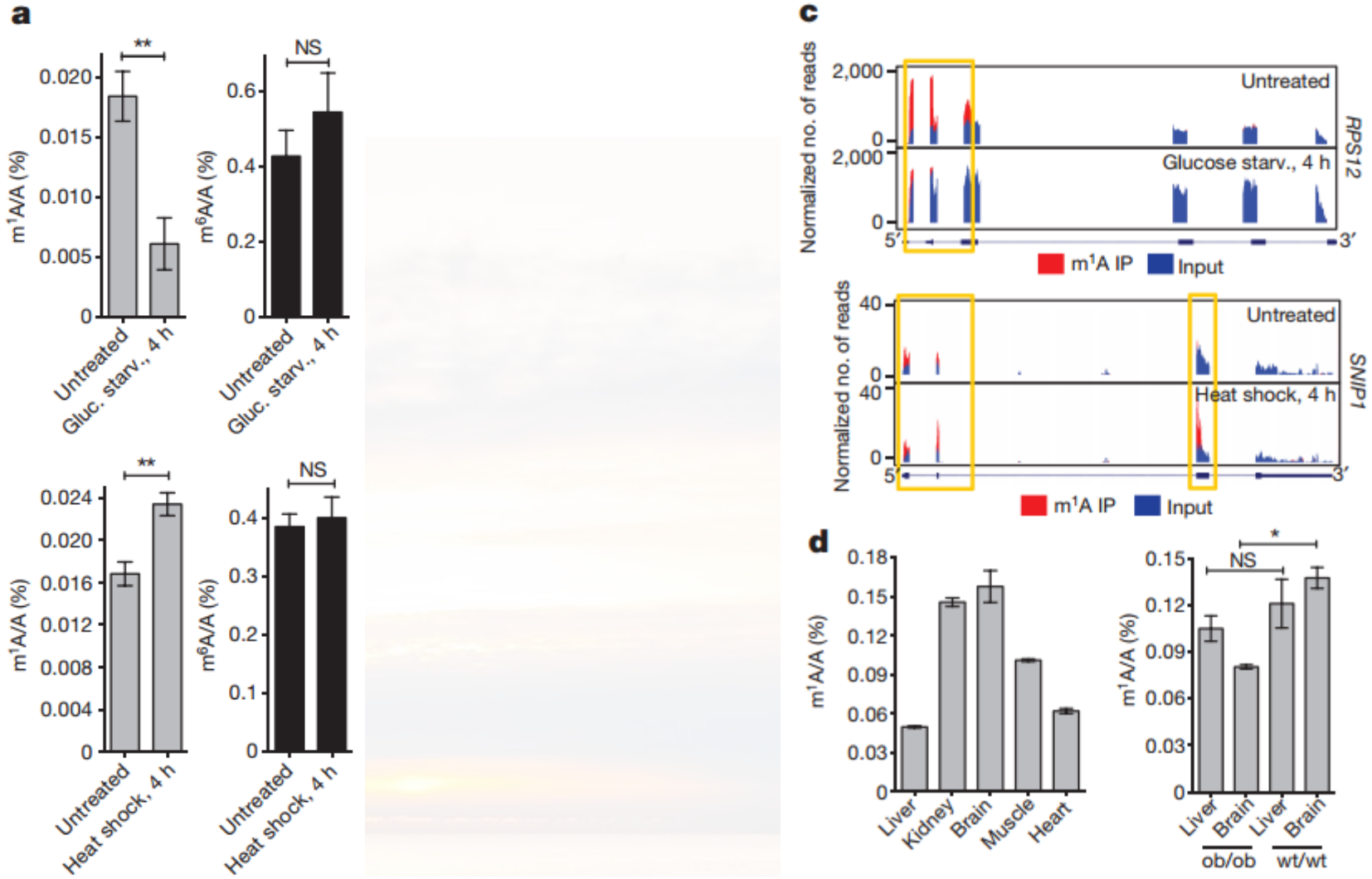
The mouse m1A methylome closely resembles that of human: approximately 15% of adequately expressed genes are methylated, the majority of which carry a single m1A , and the percentage of methylated genes increases with expression level . m1A in mouse mRNA is non-randomly distributed along transcripts with dense clustering around translation initiation sites, mirroring the profile in human, including the association with the nearest splice site.

Evolutionary conservation and dynamics



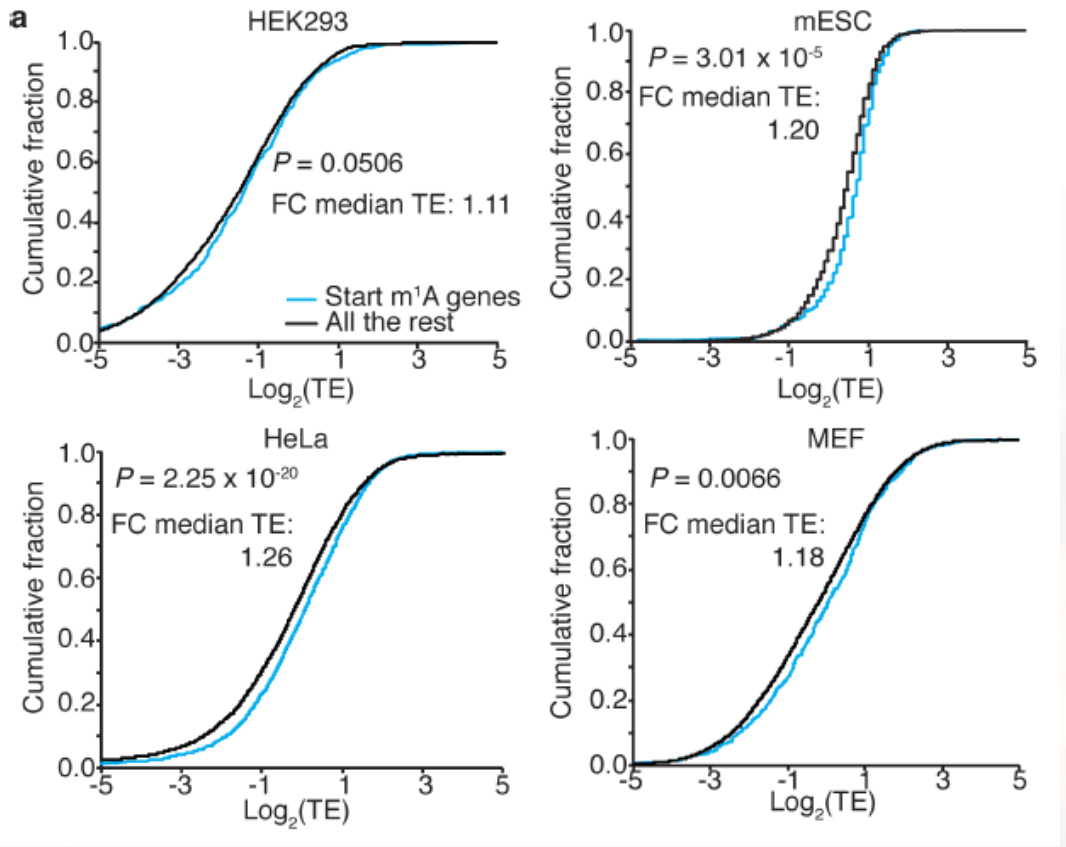
Applied m1A-seq to RNA of Saccharomyces cerevisiae and Schizosaccharomyces pombe to further examine this mark in simple eukaryotes, and found it to be present in their mRNA transcriptomes, albeit without the characteristic mammalian pattern . Importantly, upon transfer to a nitrogen-source deficient medium the S. pombe methylome exhibited noticeable changes in the identity of methylated transcripts (some transcripts gained m1A while others lost it), providing an example of how physiological conditions dynamically shape m1Aas a mark in eukaryotic mRNA, although the distribution pattern and functions in lower eukaryotes could be different from mammals, as also observed for m6A.

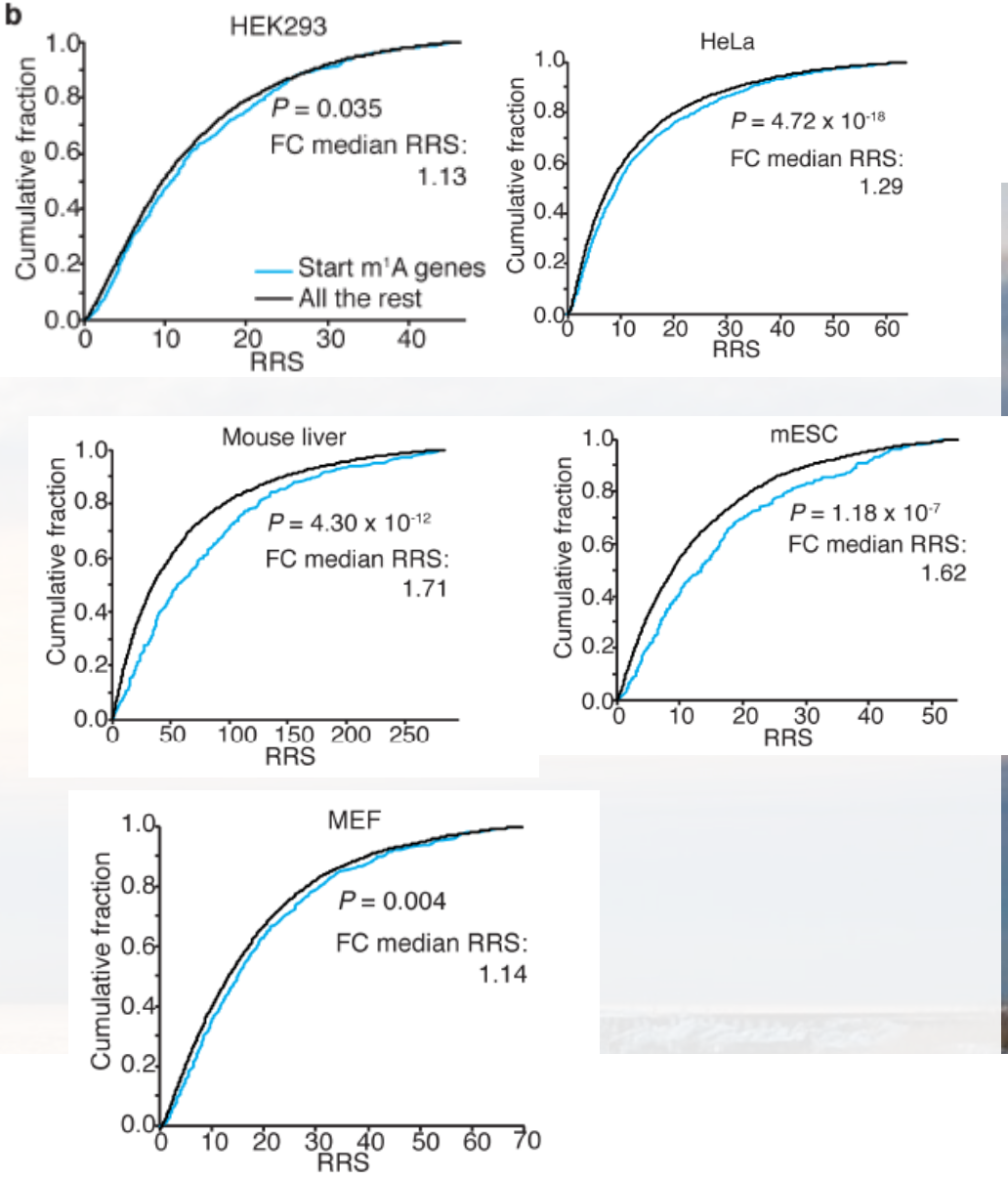
Evolutionary conservation and dynamics



m1A in mRNA is a dynamic modification that responds to changing physiological and stress conditions, and varies between tissues.

m1A correlates with elevated translation





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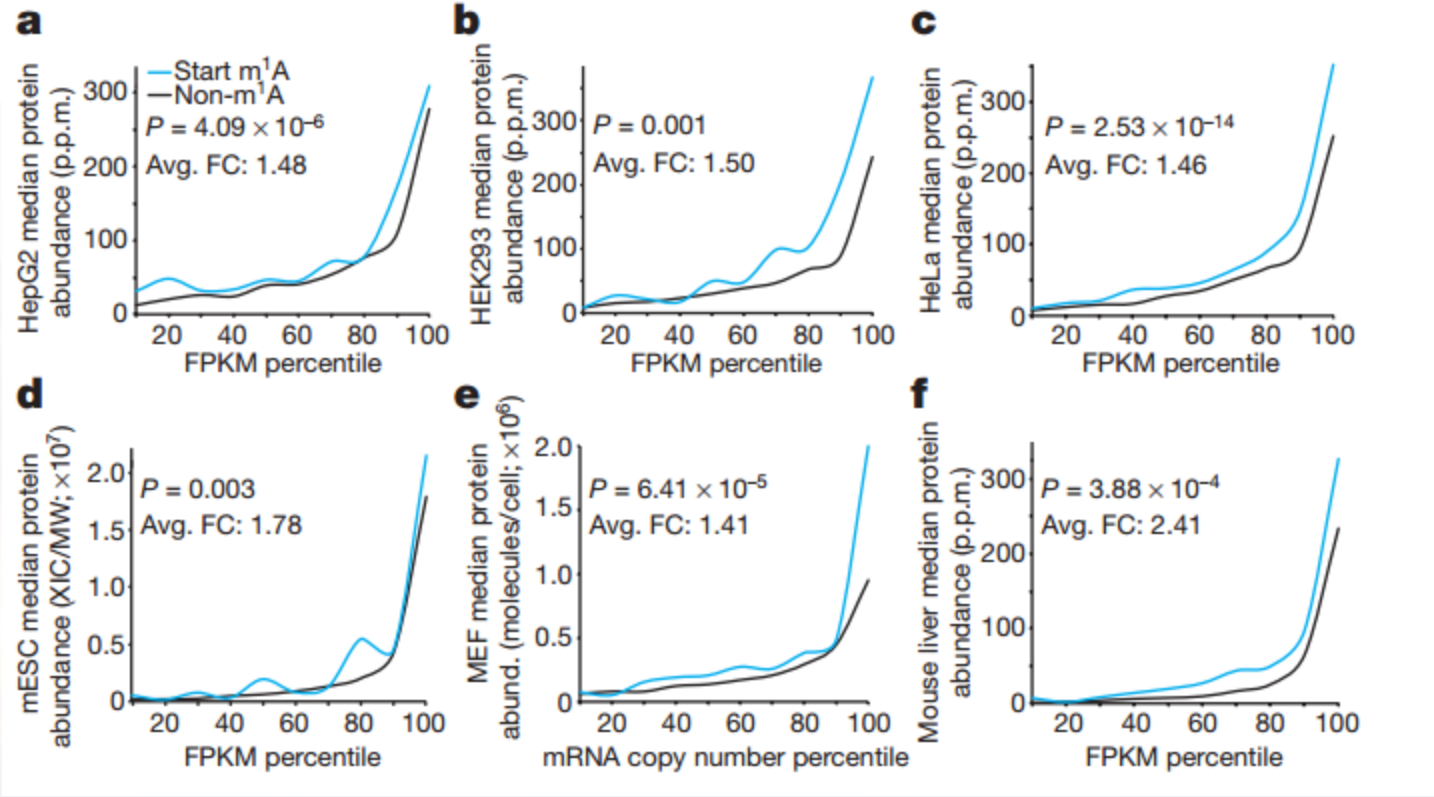

m1A around the start codon correlates with higher protein levels.

Highlights:

- 首次通过MeRIP-seq在真核生物mRNA中对m1甲基化作用进行全面的研究，揭示了其调控RNA代谢的相关机制。
- 本文通过研究发现m1A和m6A会通过不同的通路来实施对mRNA的影响，这两种不同的机制将会提供不同的基因表达转录后调节。
- 研究还发现m1A针对不同的压力反应有一个动态的修饰过程。

Needs to Improve

- m1A甲基化位点的正电荷是如何影响和调控mRNA代谢的可以进行进一步研究。
- 证明m1A可以动态修饰时，只是在酵母中发现了这一现象，并没有在人类和小鼠中阐述具体的动态修饰机制。