Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

The N^6-Methyladenosine RNA modification in pluripotency and reprogramming

Francesca Aguilo[1,2,3] and Martin J Walsh[3,4,5]

Chemical modifications of RNA provide a direct and rapid way to manipulate the existing transcriptome, allowing rapid responses to the changing environment further enriching the regulatory capacity of RNA. N^6-Methyladenosine (m^6A) has been identified as the most abundant internal modification of messenger RNA in eukaryotes, linking external stimuli to an intricate network of transcriptional, post-transcriptional and translational processes. M^6A modification affects a broad spectrum of cellular functions, including maintenance of the pluripotency of embryonic stem cells (ESCs) and the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). In this review, we summarize the most recent findings on m^6A modification with special focus on the different studies describing how m^6A is implicated in ESC self-renewal, cell fate specification and iPSC generation.

Addresses
[1] Wallenberg Centre for Molecular Medicine (WCMM), Umeå University, SE-901 85 Umeå, Sweden
[2] Department of Medical Biosciences, Umeå University, SE-901 85 Umeå, Sweden
[3] Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[4] Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[5] Mount Sinai Center for RNA Biology and Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Corresponding authors: Aguilo, Francesca (francesca.aguilo@umu.se) and Walsh, Martin J (martin.walsh@mssm.edu)

Current Opinion in Genetics & Development 2017, **46**:77–82

This review comes from a themed issue on **Cell reprogramming**

Edited by **Jianlong Wang** and **Miguel Esteban**

http://dx.doi.org/10.1016/j.gde.2017.06.006

Introduction

Massive transcriptome sequencing through high-throughput platforms has revealed that the vast majority of the mammalian genome undergoes transcription into RNA, but many RNA transcripts are never translated into protein [1]. RNA is not only an essential intermediate in the flux of genetic information from DNA to proteins, but rather is a molecule that plays crucial roles in the regulation of fundamental cellular processes, being the dysregulation of certain RNAs implicated in a number of important pathological processes, including cancer [2,3]. The coding and non-coding transcriptome is extensively and dynamically regulated by chemical modification adding a new layer of complexity and functionality to the emerging roles of RNAs in health and disease [4]. Such covalent modifications provide a direct and rapid way to manipulate the existing transcriptome, allowing fast responses to the changing environment [5]. To date, at least 100 chemically distinct RNA modifications have been identified [6–8], among which N^6-methyladenosine (m^6A) is the most abundant in messenger RNA (mRNA) and long non-coding RNA (lncRNA) [9]. Although m^6A was detected in poly(A) + RNA in the 1970s [10–13], it is only recently that transcriptome-wide profiling studies have revealed that m^6A is pervasive throughout the transcriptome and it is distributed within the R(m^6A)CH consensus motif (where R = G or A; H = A, C, or U) [14–17]. Despite the strong consensus, only a small fraction of RACH sites is detectably methylated in vivo, arguing that the sequence motif is not sufficient to determine the distribution of m^6A.

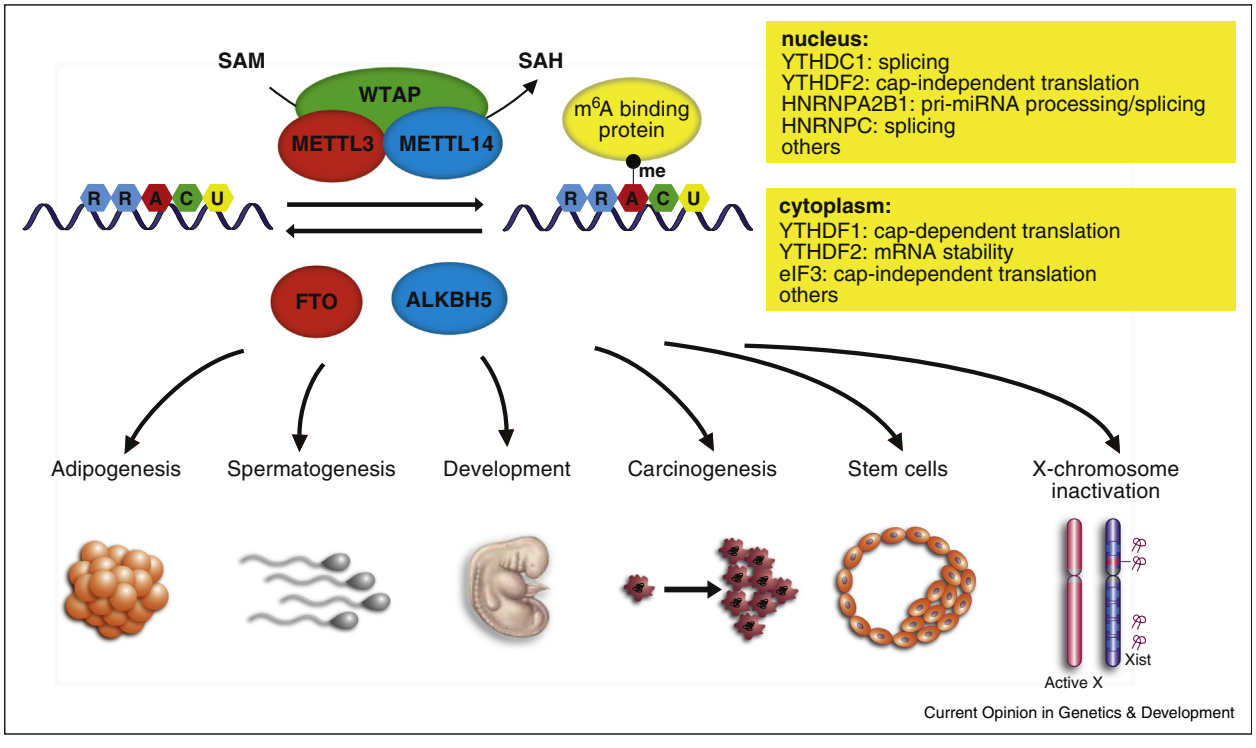
The m^6A landscape is dynamically regulated by a complex interplay between various families of m^6A-specific proteins, termed 'writers', 'erasers' and 'readers' which add, remove and decipher the m^6A mark, respectively [18]. The core mammalian m^6A methyltransferase complex ('writers') includes the methyltransferase-like 3 (METTL3, also known as MT-A70) [19] and the methyltransferase-like 14 (METTL14) [20,21•]. The complex associates with Wilm's tumor 1 associating protein (WTAP), which is necessary for catalytic activity of the m^6A methyltransferase complex in vivo [20,22]. The presence of m^6A erasers suggests that the effects of m^6A can potentially be reversed, and that cellular demethylation pathways might be a mechanism of dynamic m^6A regulation within mRNAs. Fat mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alkB homologue 5 (ALKBH5) are the two m^6A demethylases ('erasers') identified to date [23–25]. The discovery of the 'readers' has been a milestone in elucidating the impact of such modification in RNA. Different 'readers' with different cellular localization, influence almost all aspects of RNA metabolism. Proteins with YTH-domain located in the cytoplasm (YTHDF1, YTHDF2 and YTHDF3) and in the nuclei (YTHDC1 and YTHDF2) directly recognize the m^6A mark [15,26]. YTHDF1 promotes cap-dependent translation [27••], and YTHDF2 regulates mRNA stability by localizing

m^{6}A-containing mRNA to cellular RNA decay sites [26]. Interestingly, upon heat shock stress, YTHDF2 localizes to the nucleus where preserves 5′ UTR methylation and promotes cap-independent translation initiation [28••], and YTHDC1 directly regulates splicing events through recruitment of pre-mRNA splicing factors to the targeted RNA [29•]. Also in the nuclei, the RNA-binding protein heterogeneous nuclear ribonucleoprotein A2B1 (HNRNPA2B1) recognizes m^{6}A sites and promotes microRNA (miRNA) biogenesis and alternative splicing [30••], and the binding of pre-mRNA splicing factor heterogeneous nuclear ribonucleoprotein C (HNRNPC) to the introns of its targeted mRNAs after 'm^{6}A switch' affects their processing [31••]. In the cytoplasm, a single m^{6}A in the 5′ UTR is sufficient to boost cap-independent translation through interaction with the eukaryotic translation initiation factor eIF3 and subsequent ribosome recruitment [32•]. Therefore, m^{6}A modification operates in a cascade of cellular processes, including circadian rhythm [33], cell meiosis [34], sperm development [24], X chromosome inactivation [35•], embryonic stem cell (ESC) self-renewal and differentiation [36••,37••,38•], and the reprogramming of somatic cells into induced pluripotency stem cell (iPSC) [36••,38•,39••] among others, and thus alteration of m^{6}A homeostasis is implicated in obesity [40], cancer [41–43], and many other human disorders [44–46] (Figure 1).

N^6-Methyladenosine decorates RNAs in mammalian embryonic stem cells

The role of m^{6}A modification in embryonic stem cells (ESCs) has been recently investigated with discrepant results among different studies. The first proposed model describes that m^{6}A methylation is required to keep ESCs in a pluripotent state [21•] whereas a more recent emerging model postulates that m^{6}A is not required for ESC maintenance but for transition of ESCs to differentiated lineages [37••,38•]. Supporting the first model, knockdown of either *Mettl3* or *Mettl14* in mouse ESCs resulted in reduced levels of m^{6}A modification, decreased expression of pluripotency genes, increased expression of developmental regulators, and reduced self-renewal capability [21•]. The same study showed that m^{6}A modification selectively targeted lineage specific markers rather than pluripotency-related genes. The presence of m^{6}A methylation on mRNA encoding developmental regulators promoted their destabilization by preventing Hu-antigen R (HuR; also known as ELAVL1) binding, which blocked microRNA targeting [47,48], thereby maintained the mouse ESC in a pluripotent state [21•] (Figure 2a).

Figure 1



Scheme of m^{6}A reversible methylation and mediated functions. Dynamic m^{6}A modification is regulated by writers (METTL3, METTL14 and WTAP) and erasers (FTO and ALKBH5). Recognition of m^{6}A by different readers in the nucleus or the cytoplasm mediates divergent functions, including splicing, pri-miRNA processing, translation, and RNA stability. Unbalanced m^{6}A regulation will cause defects in adipogenesis, spermatogenesis, development, carcinogenesis, stem cell self-renewal and differentiation, and X-chromosome inactivation among others.