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mRNA methylation in cell senescence

Gabriel Casella, Dimitrios Tsitsipatis, Kotb Abdelmohsen, Myriam Gorospe

Laboratory of Genetics and Genomics, National Institute on Aging Intramural Research Program, National Institutes of Health, Baltimore, Maryland

Abstract

Cellular senescence, a developmental program central to normal aging and aging pathologies, is robustly regulated at the post-transcriptional level. This regulation involves the interaction of RNA-binding proteins and noncoding RNAs with senescence-associated messenger RNAs (mRNAs). There is increasing evidence that these associations are modulated by chemical modifications of specific mRNA nucleotides which can enhance or reduce the binding of regulatory factors. Recent technological advances in mass spectrometry, next-generation sequencing, and genome mapping have improved markedly the detection of mRNA modifications. Given the rising interest in the epitranscriptomic control of gene expression in aging, we discuss our incipient understanding of the chemical mRNA modifications, specifically m⁶A and m⁵C, that influence cellular senescence.

This article is categorized under:

RNA Export and Localization > RNA Localization

RNA Processing > RNA Editing and Modification

Keywords

5-methylcytosine; aging; mRNA modifications; N⁶-methyladenosine; senescence

1 | INTRODUCTION

1.1 | Cellular senescence

Cellular senescence is a state of permanent growth arrest first identified by Hayflick (1965). Senescence is established in normal tissues in the body following sublethal genomic injury, telomere ablation, oxidative stress, and other types of cell damage, and has been increasingly

Correspondence: Myriam Gorospe, Laboratory of Genetics and Genomics, NIA IRP, NIH, Baltimore, MD 21224. myriam-gorospe@nih.gov.

Gabriel Casella and Dimitrios Tsitsipatis contributed equally to this study.

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implicated in aging physiology and pathology. While cellular senescence is beneficial in tissue remodeling, embryonic development, wound healing, and tumor suppression in young persons (Campisi, 2001; Demaria et al., 2014; Prieur & Peeper, 2008; Storer et al., 2013), it has also been linked to the promotion of age-associated functional declines and diseases. Accordingly, in older persons, senescent cells have been implicated in promoting atherosclerosis, liver fibrosis, insulin resistance, Alzheimer's and Parkinson's pathologies, chronic obstructive pulmonary disease, age-related chronic inflammation, macular degeneration, and cancer (Aravinthan et al., 2013, 2015; Boccardi, Pelini, Ercolani, Ruggiero, & Mecocci, 2015; Campisi & Robert, 2014; Chinta et al., 2013; Dimri et al., 1995; Ito et al., 2014; Kumar, Seeger, & Voswinckel, 2014).

Cellular senescence is studied in the laboratory using model systems that recapitulate the processes that occur in tissues: *damage-induced senescence* is triggered by acute stress agents (ionizing radiation, oxidants, chemotherapeutic drugs, hypoxia, etc.), *replicative senescence* is triggered by proliferative exhaustion in culture, and *oncogene-induced senescence* is triggered by activated oncogenes. The defining hallmarks of senescence are the presence of β -galactosidase activity at pH 6, the induction of the canonical tumor suppressor pathways TP53(p53)/CDKN1A(p21) and/or CDKN2A(p16)/RB1(RB), and morphological changes such as cell flattening, ambiguous cell borders, and the appearance of vacuoles. Additionally, they exhibit a senescence-associated secretory phenotype (SASP) characterized by the production and secretion of cytokines, chemokines, matrix metalloproteinases, growth factors, and angiogenic factors (Dimri et al., 1995; Kuilman, Michaloglou, Mooi, & Peeper, 2010; Panda, Abdelmohsen, & Gorospe, 2017).

1.2 | Messenger RNA modifications

The senescence program is tightly controlled to produce proteins that elicit a senescence-associated growth arrest, a stress-response phenotype, morphological changes, and the SASP. These changes in gene expression programs are governed by both transcriptional and post-transcriptional regulators. The transcriptional regulation of senescence is driven by epigenetic modifications in relevant chromatin regions, and by the action of transcriptional regulatory factors including p53, NF- κ B, ID1, ID2, E2F, B-MYB, and CEBP β (Kuilman et al., 2010). Together, they carry out strong and lasting changes in the transcriptional program of senescent cells (Hernandez-Segura et al., 2017).

The post-transcriptional regulation of senescence is carried out by RNA-binding proteins and a diverse group of noncoding regulatory RNAs, including senescence-associated microRNAs and long noncoding RNAs (Abdelmohsen & Gorospe, 2015; Bilsland, Revie, & Keith, 2013; Panda et al., 2017). These factors bind senescence messenger RNAs (mRNAs) forming ribonucleoprotein (mRNP) complexes capable of directing the nuclear processing of precursor (pre-)mRNAs, and the cytoplasmic stability, storage, and translation of mRNAs. Evidence is emerging that the formation of senescence-relevant mRNPs is influenced by specific chemical modifications of the mRNA, in turn affecting mRNA metabolism and protein production, as reviewed here.

1.2.1 | Post-transcriptional modification of mRNA primary sequences—Pre-mRNA sequences undergo extensive modification before they become mature mRNAs, including 5'-untranslated region (UTR) processing, 3'-UTR processing, alternative splicing, and RNA editing. When nascent pre-mRNA molecules reach up to 30 nucleotides (nt) in length, the 5' end is often capped with 7'-methylguanosine (m⁷G) (Venkatesan & Moss, 1982), and most pre-mRNAs are then cleaved at the 3'-UTR and rapidly polyadenylated afterwards, rendering a mature mRNA that contains a 3' poly(A) tail (E. Wahle & Ruegsegger, 1999). During splicing, pre-mRNA introns are removed and exons are ligated to form a mature mRNA through the action of the spliceosome (Berget, Moore, & Sharp, 1977; Hang, Wan, Yan, & Shi, 2015; M. C. Wahl, Will, & Luhrmann, 2009). RNA can be further edited through the insertion, deletion, or conversion of nucleotides; guanosine-to-adenine (G-to-A) and cytidine-to-uridine (C-to-U) editing have been documented, but the editing most extensively studied on mRNAs is adenosine-to-inosine (A-to-I) nucleotide deamination (Davidson & Shelness, 2000; Nishikura, 2010).

1.2.2 | Chemical mRNA modifications—Although the first modified RNA nucleotides were identified in the 1950s (Davis & Allen, 1957), only recent technological improvements in mass spectrometry, genome mapping, and next-generation sequencing (NGS) have enabled a precise and systematic characterization of RNA modifications (Helm & Motorin, 2017; Ovcharenko & Rentmeister, 2018; Schaefer, Kapoor, & Jantsch, 2017; Schwartz & Motorin, 2017). As a result, there is resurging interest in identifying RNA modifications that affect mRNP complexes implicated in processes like senescence and aging. Currently, a handful of base-specific modifications have been identified on mRNAs, including N⁶-methyladenosine (m⁶A), 5-methylcytidine (m⁵C), N1-methyladenosine (m¹A), and m⁷G (Table 1) (Limbach, Crain, & McCloskey, 1994; Roundtree, Evans, Pan, & He, 2017; Safra et al., 2017; Schaefer et al., 2017; Venkatesan & Moss, 1982; C. Zhang & Jia, 2018; B. S. Zhao, Roundtree, & He, 2017). Specific proteins known as *writers*, *erasers*, and *readers* work in conjunction to establish, remove, and recognize mRNA modifications, respectively. Chemical modifications of pre-mRNA and mRNA have vast and dynamic regulatory potential because they are inducible and reversible, and because their presence can alter the stability and translation efficiency of mRNAs, in turn affecting the production of key proteins (Ke et al., 2017; Limbach et al., 1994; Roundtree et al., 2017; B. S. Zhao et al., 2017). In this review, we focus on the emerging field of senescence epitranscriptomics by focusing on incipient evidence that nucleotide chemical modification impacts gene expression in cellular senescence.

2 | RNA METHYLATION AT m⁶A IN SENESCENCE

2.1 | N⁶-methyladenosine (m⁶A) *writers*, *erasers*, *readers*

N⁶-methyladenosine (m⁶A) is considered one of the most abundant base modifications in eukaryotic mRNA (Bokar, Rath-Shambaugh, Ludwiczak, Narayan, & Rottman, 1994; Desrosiers, Friderici, & Rottman, 1974). Initial studies suggested that methyltransferase-like 3 (METTL3), and 14 (METTL14), Wilms Tumor 1-Associated Protein (WTAP), and KIAA1429 form a multimeric methyltransferase “*writer*” complex that is responsible for the addition of the methyl group in m⁶A (Bokar et al., 1994; Bokar, Shambaugh, Polayes,

Matera, & Rottman, 1997; Geula et al., 2015; J. Liu et al., 2014; Ping et al., 2014). More recent studies revealed that RBM15, Hakai, and ZC3H13 also interact with WTAP and contribute functionally to establishing m⁶A modifications (Knuckles et al., 2018; Ruzicka et al., 2017; Y. Yang, Hsu, Chen, & Yang, 2018). Although METTL3 has direct methyltransferase capacity (K. I. Zhou & Pan, 2016), knockdown studies indicate that recruitment of METTL14, WTAP, and KIAA1429 is also necessary for establishing the full in vivo m⁶A methylation program in mammals (Schwartz et al., 2014; Sledz & Jinek, 2016; P. Wang, Doxtader, & Nam, 2016; X. Wang, Feng, et al., 2016).

Most m⁶A residues are found within a “GGACU” consensus sequence (J. Liu et al., 2014), which is highly enriched in the 3′-UTRs, near stop codons, and in the last exon (Meyer et al., 2012), suggesting a potential role for m⁶A in modulating translation efficiency, nuclear export, alternative splicing, mRNA turnover, and microRNA biogenesis and metabolism (Alarcon, Goodarzi, et al., 2015; Alarcon, Lee, Goodarzi, Halberg, & Tavazoie, 2015; Geula et al., 2015; N. Liu et al., 2015; Meyer et al., 2015; X. Wang et al., 2015; X. Zhao et al., 2014; Zheng et al., 2013; J. Zhou et al., 2015). m⁶A modifications are dynamic and reversible, indicating that they may have important adaptive roles. The AlkB homologue 5 (ALKBH5) is a nuclear demethylase that exhibits m⁶A demethylation capacity as an “eraser” (Zheng et al., 2013). Several studies have suggested an eraser role of m⁶A for FTO (Gerken et al., 2007; Vujovic et al., 2013; Wei et al., 2018; X. Zhao et al., 2014), but it has also been suggested that FTO may predominantly demethylate m⁶A_m instead (Mauer et al., 2017).

The m⁶A modification is recognized specifically by a class of RNA-binding proteins known as “readers”. It is through *readers* that m⁶A can affect different cellular processes by interacting with protein complexes to induce signaling events and catalyze enzymatic reactions (Cao, Li, Yin, & Flavell, 2016). The proteins YTHDC1, YTHDF1, YTHDF2, and YTHDF3 have been identified as important m⁶A readers (Cao et al., 2016; Y. Wang et al., 2014; K. I. Zhou & Pan, 2018). In one example, YTHDF2-induced destabilization of methylated mRNAs was shown to influence stem cell differentiation by regulating the expression of pluripotency factors (Geula et al., 2015; Y. Wang et al., 2014). Indeed, germline *Ythdf2*^{-/-} knockout mice showed an impaired maternal transcriptome and lower oocyte competence (Ivanova et al., 2017). In another example, J. Zhou et al. (2015) proposed that YTHDF2 may modulate the heat shock response by promoting HSP70 translation through its interaction with the m⁶A modification in the 5′-UTR of *Hsp70* mRNA; however, subsequent studies revealed that m⁶A modification at the *HSP70* 5′-UTR enabled the eukaryotic initiation factor 3 (eIF3)-mediated recruitment of the 43S complex and the start of translation independently of eIF4E (Meyer et al., 2015).

In addition, two members of the HNRNP family, HNRNPC and HNRNPG, displayed higher binding affinity to RNA following m⁶A-induced alterations in RNA structure. In the nucleus, m⁶A methylation was reported to alter the local RNA structure, increasing the binding of HNRNPC (N. Liu et al., 2015) and HNRNPG (N. Liu et al., 2017), and possibly affecting their actions in alternative splicing. HNRNPA2B1 was suggested to be a nuclear reader of m⁶A methylation and influenced both microRNA processing and alternative splicing (Alarcon, Goodarzi, et al., 2015). However, it was recently found that HNRNPA2B1 bound

to motifs located near m⁶A modifications due to the higher access enabled by m⁶A-mediated RNA unfolding (Wu et al., 2018). Thus, HNRNPA2B1 is not itself an m⁶A reader, but rather responds to the presence of m⁶A by recognizing local structural changes induced by the chemical modification. Interestingly, m⁶A has been reported to also prevent the binding of other proteins, as shown for G3BP1, in turn affecting mRNA stability (Edupuganti et al., 2017).

2.2 | m⁶A mRNA methylation in senescence

Li et al. (2017) recently reported a link between m⁶A methylation and cell senescence. Using human cervical carcinoma HeLa cells, the authors showed that METTL3/METTL14-dependent m⁶A methylation of *p21* mRNA increased the levels of p21 protein without affecting total *p21* mRNA levels, implicating m⁶A methylation in controlling the efficiency of p21 translation. Interestingly, the authors found that METTL3/METLL14 elicited m⁶A modification of *p21* mRNA in HCT116 p53^{-/-} cells, indicating that this regulation and the ensuing rise in p21 levels were independent of p53 status. Residues A2044 and A2061 in the *p21* 3'-UTR were identified as methylation sites. Exposure of HCT116 p53^{-/-} cells to hydrogen peroxide significantly increased the levels of p21 and METTL3/METLL14 and the activity of senescence-associated β -galactosidase [(SA)- β -gal], further supporting a p53-independent role for METTL3/METLL14 in triggering senescence in response to oxidative damage (Figure 1).

Lewinska, Adamczyk-Grochala, Deregowska, and Wnuk (2017) studied the exposure of three different breast cancer cell lines, MCF-7, MDA-MB-231, and SK-BR-3, to sublethal concentrations of sulforaphane (SFN), which triggered cell cycle arrest. Exposure to SFN increased p53, p21, and p27 protein levels, but not the levels of the respective mRNAs, and elevated (SA)- β -gal activity. Interestingly, exposure to SFN caused DNA hypomethylation, decreased global RNA m⁶A methylation levels, and altered the abundance of many microRNAs. Although the underlying mechanisms are not well understood at present, the authors hypothesized that SFN elicited senescence by lowering global methylation, which increased genetic instability and possibly contributed to cell cycle arrest, in conjunction with reduced m⁶A RNA levels and modified microRNA pools (Lewinska, Adamczyk-Grochala, Deregowska, et al., 2017).

In another study, Min et al. (2018) showed that global m⁶A methylation levels decreased in peripheral blood mononuclear cells (PBMCs) of older individuals when compared to younger Caucasian males. The authors found that DROSHA, a specific endoribonuclease that cleaves the 3' end of pre-microRNA hairpins during the genesis of microRNAs (Slezak-Prochazka, Durmus, Kroesen, & van den Berg, 2010), and AGO2, a major component of the RNA-induced silencing complex (RISC), were both encoded by highly methylated mRNAs in young PBMCs. However, in aged PBMCs *AGO2* mRNA methylation selectively decreased, while *DROSHA* mRNA methylation remained constant. These findings led the authors to suggest that there is selective, age-specific methylation of mRNAs. The decreased *AGO2* mRNA methylation in older PBMCs was tentatively linked to lower levels of WTAP, a writer of m⁶A methylation, as other methyltransferases, demethylases, and m⁶A-binding proteins were only moderately affected (Min et al., 2018). The fact that *AGO2* mRNA

methylation is selectively reduced in aged PBMCs further supports the existence of mRNA-specific m⁶A modifications in aging (Figure 1).

Subsequently, the same authors showed that the hypomethylation of *AGO2* mRNA correlated with decreased AGO2 protein levels in proliferating human diploid fibroblasts (HDFs), a model of cell senescence (Min et al., 2018). m⁶A methylation was lower in both older PBMCs and late-passage HDFs. However, differently from the findings in older PBMCs, late-passage HDFs not only exhibited lower levels of *AGO2* mRNAs, but also displayed reduced abundance of *DROSHA* and *DICER1* mRNAs. Reducing *AGO2* mRNA methylation at m⁶A by METTL3 silencing destabilized *AGO2* mRNA and decreased the levels of a subset of microRNAs, suggesting that lowering AGO2 impaired the production of specific microRNAs in both old PBMCs and late-passage HDFs. Interestingly, members of *let-7* family of microRNAs, previously found to trigger senescence (Peng et al., 2012), were found to be downregulated in both old PBMCs and in HDFs in which MTLL3 or METTL14 were silenced. In summary, m⁶A methylation of *AGO2* mRNA diminished AGO2 protein levels with aging and cell senescence. While the exact m⁶A writers that mediate *AGO2* mRNA methylation in aging are not yet known, the accumulating evidence points to an important role of *AGO2* mRNA methylation in aging- and senescence-associated microRNA function (Table 2). At the moment, specific senescence-associated mRNAs influenced by m⁶A erasers and readers remain to be identified.

3 | RNA METHYLATION AT m⁵C IN SENESCENCE

3.1 | 5-Methylcytidine (m⁵C) writers, erasers, readers

Despite the fact that the 5-methylcytidine (m⁵C) modification was first identified in DNA in the 1950s (Davis & Allen, 1957) and in mRNA in the 1970s (Sommer et al., 1976), broad interest in the presence of m⁵C modifications in mRNA has only emerged relatively recently (Amort et al., 2017; Squires et al., 2012; X. Yang et al., 2017). In general, m⁵C methylation was found predominantly in noncoding RNAs, in both the 5'-UTR and 3'-UTR of mRNAs, and in the vicinity of AGO-binding regions. Thus far, two prominent m⁵C mRNA methylation writers have been identified, DNMT2 (DNA methyltransferase 2) and NSUN2 (NOP2/Sun RNA Methyltransferase Family Member 2), although a general RNA motif has not yet been identified (Squires et al., 2012). Although DNMT2 and NSUN2 have been mostly studied in m⁵C tRNA methylation (Blanco et al., 2011; Brzezicha et al., 2006; Goll et al., 2006; Schaefer et al., 2010; Squires et al., 2012), NSUN2 has also been implicated in directly methylating coding transcripts, as shown for *CINP* and *NAPRT1* mRNAs (Squires et al., 2012). Despite a considerable amount of work done on DNMT2, its exact role in m⁵C mRNA methylation is not fully understood at present, particularly in light of recent evidence that *Dnmt2* silencing in mouse fibroblasts promotes global DNA and RNA hypermethylation (Lewinska, Adamczyk-Grochala, Kwasniewicz, & Wnuk, 2017). While ALYREF was recently identified as an m⁵C reader (X. Yang et al., 2017), the lack of a more comprehensive knowledge of m⁵C writers, erases, and readers adds to the challenges of elucidating the mechanisms that govern m⁵C modification in mRNA.

3.2 | m⁵C mRNA methylation in senescence

Although relatively less is known about the enzymology of m⁵C mRNA methylation as compared to m⁶A mRNA methylation, m⁵C mRNA modification has also been shown to modulate cellular senescence (Figure 1). Similar to the induction of senescence by m⁶A-regulated rise in p21 levels independently of p53, NSUN2-mediated m⁵C methylation of *p21* mRNA at C2079 (a residue in the *p21* 3'-UTR) also contributed to enhancing p21 protein abundance (Li et al., 2017); in fact, m⁶A and m⁵C modifications of *p21* mRNA synergistically elevated the levels of p21 protein, which in turn promoted senescence (Li et al., 2017). In addition, NSUN2 also increased p16 expression levels by methylating the *p16* 3'-UTR, thereby stabilizing *p16* mRNA (X. Zhang et al., 2012), even though the specific m⁵C site was not identified. These results further implicate NSUN2 in the induction of senescence via both the p53/p21 and the p16/RB pathways.

The role of NSUN2 in senescence is nonetheless complex, as there is evidence that NSUN2 can both promote and suppress senescence depending on the substrate mRNA. Xing et al. (2015) reported that NSUN2 affected the production levels of CDK1, p27, and CDC25C in different ways. NSUN2 was first reported to promote cell growth and delay replicative senescence by elevating CDK1 protein levels. In HeLa cells, Xing et al. found that NSUN2 raised m⁵C methylation of the *CDK1* 3'-UTR, increasing its presence in polysomes and its translation efficiency, eventually elevating CDK1 protein levels. Moreover, NSUN2-dependent methylation of *CDK1* mRNA increased its translation in a cell cycle-dependent manner, peaking during the S and G2/M cell cycle phases (Xing et al., 2015). Interestingly, the same authors subsequently discovered that NSUN2 further delayed replicative senescence (Tang et al., 2015) by methylating the C64 residue at the 5'-UTR of *p27* mRNA (without affecting total *p27* mRNA levels) and reducing p27 translation. In senescent IDH4 fibroblasts (Shay, West, & Wright, 1992), the authors observed decreased levels of NSUN2, CKD1, and CDC25C proteins, while the levels of p27 and p21 proteins increased. Similarly, in human diploid 2BS fibroblasts expressing reduced levels of NSUN2 through shRNA-mediated silencing, p27 protein levels increased, while CKD1 and CDC25C protein levels decreased. As expected, NSUN2 knockout promoted growth arrest and increased in (SA)-β-gal production (Tang et al., 2015). In line with previous observations in HeLa cells, methylation of *CKD1* and *p27* mRNAs was diminished in replicative senescence, further linking NSUN2-induced m⁵C methylation to senescence and aging.

Paradoxically, later reports showed that in human umbilical vein endothelial cells (HUVECs), NSUN2 instead promoted senescence by increasing the translation and production of SHC (Cai et al., 2016), a family of proteins that function as adaptors in the Ras intracellular signaling cascade. Among the three isoforms of SHC, p66SHC in particular enhanced senescence by promoting the formation of reactive oxygen species (ROS), while at the same time its expression correlated negatively with mammalian longevity (Luzi, Confalonieri, Di Fiore, & Pelicci, 2000; Xu et al., 2014; W. Zhang et al., 2010). NSUN2 methylated *SHC* mRNA in multiple sites—the 5'-UTR, the coding region (CR), and the 3'-UTR—both in vivo and in vitro. Methylation of *SHC* mRNA by NSUN2 enhanced SHC translation leading to the activation of p38MAPK, G1 cell cycle arrest, and increased ROS levels, implicating NSUN2 in the induction of oxidative stress-induced

senescence. Furthermore, by increasing *SHC* mRNA methylation, NSUN2 increased SHC production in a p53-independent manner, playing a role in HUVEC oxidative stress and premature senescence. Similar observations were reported in high glucose-induced premature senescence in HUVEC cells. However, since replicatively senescent cells had low levels of NSUN2 and high levels of SHC, the authors proposed that NSUN2 may not regulate SHC levels in replicative senescence, but likely played a different modulatory role in response to oxidant damage (Cai et al., 2016).

Replicative senescence is characterized by telomere shortening (Nelson, McBryan, Jeyapalan, Sedivy, & Adams, 2014). Recently, m⁵C methylation of *TERC*, a noncoding RNA that forms part of the telomerase holoenzyme, was shown to promote the complexing of *TERC* with the protein telomerase (TERT). Given that the RNA-binding protein HuR increases the interaction between TERT-*TERC* and thereby helps to maintain telomere length (Tang et al., 2018), the reduction of HuR levels in senescent cells may help explain the loss of telomerase activity with senescence. Understanding the contribution of mRNA methylation on the integrity of telomeres will shed further light into the role of chemical mRNA modifications in replicative senescence.

The role of DNMT2 in senescence is even less well understood. Lewinska, Adamczyk-Grochala, Kwasniewicz, et al. (2017) reported that *Dnmt2* gene ablation may help mediate cellular senescence in mouse fibroblasts. Compared with WT mice, *Dnmt2*-null mice displayed several senescence-associated traits, such as inhibition of cell proliferation via elevated p53 and p21 protein levels, moderately shorter telomeres, ROS accumulation, genomic instability, and increased (SA)- β -gal production. Interestingly, reducing DNMT2 levels promoted global DNA and RNA hypermethylation and increased the levels of other methyltransferases including DNMT1, DNMT3a, and DNMT3b. Thus, the previously suggested role of DNMT2 as writer of m⁵C for tRNA and mRNA (Dev et al., 2017; Khoddami & Cairns, 2013) seems to be more complex in aging, as suggested by the finding of RNA and DNA hypermethylation in *Dnmt2*-silenced cells. In a related study, silencing DNMT2 in human WI-38 and BJ fibroblasts rendered them more sensitive to oxidative stress, inhibited cell proliferation, and led to the differential upregulation of many microRNAs related to proliferation and tumorigenesis (Lewinska et al., 2018). However, at this time the specific mRNA substrates methylated at m⁵C by DNMT2 that influence the senescent phenotype are not known.

4 | CONCLUSIONS AND PERSPECTIVES

It is well established that epitranscriptomic changes, including chemical modifications of RNA, are key regulators of gene expression programs RNA and cell physiology. Early reports identified many chemical base modifications in tRNAs, while m⁶A and m⁵C were found to be among the most prominent chemical modifications in mRNAs (Davis & Allen, 1957; Roundtree et al., 2017; Schaefer et al., 2017; B. S. Zhao et al., 2017). However, systematic studies of mRNA base modifications have only been possible thanks to the recent technological improvements in mass spectrometry, NGS, and genome mapping (Schaefer et al., 2017). Ongoing research on mRNA modifications is predominantly focused on the contribution of mRNA methylation and editing in cancer, obesity, and other age-associated

pathologies (Batista, 2017; Cao et al., 2016; Deng et al., 2018). Indeed, a growing body of evidence indicates that mRNA modifications may contribute to physiological aging and diseases of aging.

NGS-based techniques, currently the best approaches to detect mRNA methylation, require PCR amplification and digestion of the amplified fragments for library preparation and analysis (Schwartz & Motorin, 2017). The recent development of third-generation (or long-read) sequencing technologies, including those by Pacific Biosciences, Oxford Nanopore, Quantapore, and Stratos, is expected to increase the ease of identifying mRNA modifications. Nanopore sequencing, which was first developed to measure RNA modifications on the 16S rRNA of *Escherichia coli* (Smith, Jain, Mulroney, Garalde, & Akeson, 2017), holds great promise as a technology to identify mRNA methylation on individual transcripts (Garalde et al., 2018) as this field progresses.

As reviewed here, two common chemical mRNA modifications, m⁶A and m⁵C, can modulate senescence in different cell culture models through various mechanisms. Writers of methylation at m⁶A (MELLT3/MELLT14 and WTAP), and m⁵C (NSUN2), have been shown to regulate the levels of senescence-associated proteins p21 and p27 by methylating *p21* and *p27* mRNAs (Lewinska, Adamczyk-Grochala, Deregowska, et al., 2017; Li et al., 2017; Tang et al., 2015). Interestingly, p21 protein levels were synergistically increased by methylation of *p21* mRNA at m⁶A and m⁵C (Li et al., 2017). Furthermore, NSUN2 was reported to increase SHC levels, thereby contributing to premature senescence following exposure to oxidative stress or high glucose (Cai et al., 2016). mRNA methylation was also reported to regulate the expression of a subset of cyclins and cyclin-dependent kinases, in turn triggering cell cycle arrest and promoting senescence (Lewinska, Adamczyk-Grochala, Deregowska, et al., 2017; Xing et al., 2015). In addition, the methylation-induced degradation of *AGO2* mRNA was found to affect the differential expression of many microRNAs and further played a role in promoting senescence (Min et al., 2018). Moreover, *Dnmt2*-null mouse fibroblasts were found to have enhanced global m⁵C mRNA methylation, accompanied by the presence of several senescence-associated hallmarks, such as high levels of p53 and p21, shorter telomeres, and elevated (SA)- β -gal activity (Lewinska, Adamczyk-Grochala, Kwasniewicz, et al., 2017). Given the accumulating evidence of m⁶A and m⁵C methylation of mRNAs encoding key senescence-regulatory factors, a comprehensive analysis of mRNA methylation in different senescent models is needed. Genetic animal models will also be essential for investigating the full impact of senescence-associated mRNA methylation in vivo and on aging physiology.

Finally, while other epitranscriptomic changes, such as nucleotide conversions (C-to-U, G-to-A, and A-to-I) have not been studied in the context of senescence, dysregulated A-to-I editing has been linked to pathologies that increase in aging, including amyotrophic lateral sclerosis, glioblastoma multiforme, hepatocellular carcinoma, type 2 diabetes mellitus, and transient forebrain ischemia (Slotkin & Nishikura, 2013). In A-to-I editing, adenosine undergoes a conversion to inosine through the deamination of the C6 carbon of adenine catalyzed by the ADAR (adenosine deaminase acting on RNA) family of proteins (Nishikura, 2010), changing the sequence of the translated protein, since inosine behaves chemically similar to guanosine. A-to-I editing is also involved in many other processes such

as post-transcriptional regulation in organ development, innate immunity, and autoimmunity (Mannion et al., 2014; Pestal et al., 2015; Stellos et al., 2016). Nicholas et al. examined two transcripts that undergo A-to-I editing, *CYFIP2* and *GABRA3* mRNAs, in 25 frontal cortex tissue samples from donors ranging between 22 and 103 years of age. They found that only *CYFIP2* mRNA A-to-I editing decreased with age, indicating that mRNA A-to-I editing decreased selectively for certain transcripts and proposed that this selectivity might arise from the differential expression of ADAR family members with age (Nicholas et al., 2010). Whether nucleotide conversions occur during senescence remains to be investigated.

In closing, thanks to the development of improved animal models and detection technologies, our knowledge of the influence of RNA metabolism on physiology and disease is increasing rapidly. The same tools will be instrumental in current efforts to advance our understanding of the RNA modifications that drive cellular senescence and aging.

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FIGURE 1.

Senescence-associated changes in m⁶A and m⁵C modifications. The main modifications reported in mRNAs encoding senescence-associated proteins, m⁶A and m⁵C, are indicated (gray rectangles). Each modification can promote (green) or repress (purple) protein expression from the respective mRNAs. *mRNA methylation at m⁶A and m⁵C decreases as cells progress towards senescence. mRNA, messenger RNA

TABLE 1

Common modifications of mRNA nucleotides

Symbol	Description	Initial report in mammals
m⁶A	N⁶-methyladenosine	✓
Am	2'-O-methyladenosine	✓
m ⁶ Am	N ⁶ ,2'-O-dimethyladenosine	✓
m ⁶ ₂ Am	N ⁶ ,N ⁶ ,2'-O-trimethyladenosine	
A-I	Adenosine to inosine	✓
m⁵C	5-Methylcytidine	
Cm	2'-O-methylcytidine	✓
m ⁷ G	7-Methylguanosine	✓
Gm	2'-O-methylguanosine	✓
m ^{2,7} G	N ² ,7-dimethylguanosine	
m ^{2,2,7} G	N ² ,N ² ,7-trimethylguanosine	
Um	2'-O-methyluridine	✓
ac4c	N ⁴ -acetylcytidine	
m ¹ A	N ¹ -methyladenosine	
m ³ Um	3,2'-O-dimethyluridine	
Ψ	Pseudouridine	

Among the most prominent RNA modifications, those that have been linked to senescence thus far are highlighted in bold.

Abbreviation: mRNA, messenger RNA.

TABLE 2

mRNA modifications influencing senescence and/or age-related changes

Modification	mRNAs modified (region)	Writers	Processes affected	Impact of modification on senescence and/or aging	Refs.
m ⁶ A	<i>p21</i> (3'-UTR)	METTL3/ METTL14	Translation ↑	Senescence ↑	Li et al. (2017)
	<i>p53</i> (unknown) <i>p21</i> (unknown) <i>p27</i> (unknown)	Unknown	Translation ↑	Senescence ↑	Lewinska, Adamczyk-Grochala, Deregiowska, et al. (2017)
	<i>AGO2</i> (unknown)	METTL3/ WTAP	Translation ↑ (PBMCs)	Aging ↓	Min et al. (2018)
		METTL3	Stability ↑ (HDFs)	Senescence?	
m ⁵ C	<i>p21</i> (3'-UTR)	NSUN2	Translation ↑	Senescence ↑	Li et al. (2017)
	<i>p16</i> (3'-UTR)	NSUN2	Stability ↑	Senescence ↑	Zhang et al. (2012)
	<i>CDK1</i> (3'-UTR)	NSUN2	Translation ↑	Senescence ↓	Xing et al. (2015)
	<i>CDC25C</i>	NSUN2	Translation ↑	Senescence ↓	Xing et al. (2015)
	<i>p27</i> (5'-UTR)	NSUN2	Translation ↓	Senescence ↓	Tang et al. (2015)
	<i>SHC</i> (5'-UTR,CR,3'-UTR)	NSUN2	Translation ↑	Senescence ↑	Cai et al. (2016)
	<i>TERC</i>	Unknown	Telomerase activity↑	Proliferation↑	Tang et al. (2018)

The ribonucleotide modifications (column 1), modified mRNAs and region of the mRNAs that are modified (column 2), enzymes involved (column 3), and consequences on gene regulatory processes (column 4) are indicated. The impact of the modifications on senescence or aging is indicated (column 5). ↑, increased; ↓, decreased.

Abbreviations: mRNA, messenger RNA; UTR, untranslated region.