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## **m6A RNA modification as a new player in R-loop regulation**

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**RNA:DNA hybrids that form across genomes control a wide range of biological processes. A new study shows that *N*6-methyladenosine (m6A) modification on the RNA moiety of these hybrids regulates their formation and genome integrity. This opens up a new avenue of research where the RNA modifications (the “epitranscriptome”) can help control genome maintenance.**

R-loops are tripartite structures that form co-transcriptionally, with the nascent RNA hybridizing back to the DNA template (RNA:DNA hybrid), leaving a displaced non-hybridized single-stranded DNA (ssDNA) (Fig. 1). Besides their prevalence in highly transcribed genes, R-loops also accumulate in repeats such as centromeres, telomeres and retrotransposons. R-loops are beneficial for various biological processes such as transcription initiation and termination, replication, telomere maintenance and immunoglobulin class switch recombination<sup>1</sup>. Yet, they are also potent inducers of DNA damage<sup>2</sup> and roadblocks for repair<sup>3</sup>, thereby making R-loops a source of genome instability. Tight regulation of R-loop levels is therefore critical. This is achieved by both preventing their co-transcriptional formation and favoring their removal via specific nucleases (e.g. RNase H) and helicases (e.g. Senataxin, FANCM)<sup>1,2</sup>. In this issue, Abakir et al. propose an unexpected new regulatory pathway where *N*6-methyladenosine deposition on the RNA moiety of the RNA:DNA hybrid contributes to R-loop removal<sup>4</sup>.

*N*6-methyladenosine (or m6A) is thought to be the most prevalent internal mRNA modification in eukaryotes. This reversible mark plays key roles in the post-transcriptional control of gene expression by affecting nearly every steps of RNA metabolism such as splicing, export, translation, and degradation (Fig. 1). m6A is added co-transcriptionally by “writers” (e.g. METTL3), recognized by specific proteins called “readers” (e.g.: YTHDF1-3, YTHDC1-2) and removed by “erasers” (e.g. FTO)<sup>5</sup>. Importantly, the diverse regulatory functions of m6A are

mainly determined by the identity of the m6A-bound reader. The list of physiological processes affected by m6A is extending and includes response to DNA damage<sup>6</sup>, virus infection, cell differentiation, brain development as well as cell growth and tumor progression<sup>5,7,8</sup>.

### **m6A regulates R-loop stability**

In their study, Abakir *et al.* adapted the antibody-based DRIP technique (that immunoprecipitates RNA:DNA hybrids) to purify m6A-modified RNA:DNA hybrids with the available m6A antibody (m6A-DNA immunoprecipitation, or m6A-DIP) in human pluripotent stem cells<sup>4</sup>. m6A-DIP-seq and DRIP-seq datasets largely overlapped and displayed preferential accumulation on the same genomic features (transcribed regions, repeated sequences), suggesting that most RNA:DNA hybrids are m6A-modified. Importantly, DRIP followed by m6A-DIP-qPCR, also retrieved m6A-modified RNA:DNA hybrids, albeit less efficiently, further attesting that hybrids can be m6A-modified. Furthermore, a recent study by Yang *et al.* also corroborated the physical presence of m6A-modified RNA within R-loops even though their results rather indicate that only a subset of R-loops are m6A-modified<sup>9</sup>. The definitive answer regarding the prevalence of m6A RNA modification in RNA:DNA hybrids will therefore require the development of alternative reagents and strategies, since efficiency of the m6A-DIP-seq procedure relies greatly on the specificity of the antibody and includes a denaturation step which could in theory selectively affect the integrity of a subset of R-loops.

In this study, Abakir *et al.* also identified YTH-domain family member 2 (YTHDF2) to be the main reader of m6A-modified R-loops. One of their key observation is that YTHDF2 functions by preventing the accumulation of m6A-modified R-loops. It remains to be determined whether YTHDF2 destabilizes R-loop via the recruitment of known RNA:DNA hybrids nuclease/helicase or by specifically targeting the endoribonuclease RNaseP/MRP, which has

been shown to promote the cytoplasmic decay of specific m6A-containing mRNAs<sup>10</sup>. This hypothesis is appealing since RNAseP depletion triggers replication stress and genome instability in flies<sup>11</sup>, two processes also involving R-loops. By contrast, Yang *et al.* recently proposed that instead of downregulating R-loop stability, m6A rather promotes R-loop formation at transcription termination sites<sup>9</sup>. The discrepancy between these studies calls for additional work to characterize in which instance m6A modifications destabilize or promote R-loop formation.

### **Consequences on genome stability, and beyond**

One of the most remarkable finding of this study is that depletion of YTHDF2 and METTL3 (the writer that deposits m6A) triggered increased  $\gamma$ H2AX level, a marker of DNA double-strand breaks, suggesting that pathological R-loops accumulation in the absence of the m6A RNA methylation pathway challenges genomic integrity. This is in line with a large number of studies that clearly points towards the potential of R-loops to induce DNA double-strand breaks<sup>2</sup>. Moreover, dysregulation of R-loops is emerging as a critical factor driving genome instability in a large variety of pathological contexts, including following oncogenic stress<sup>12</sup>, in Kaposi's sarcoma-associated herpesvirus infected cells, in neurological disorders associated with trinucleotide repeat expansion (such as Huntington's disease and Fragile X syndrome) and in multiples other inherited ataxias (e.g. ataxia with oculomotor apraxia 2)<sup>2</sup>. The use of existing drugs targeting the m6A pathway (e.g.: FTO inhibitors) could therefore be envisaged as a new therapeutic approach to treat R-loops-related diseases<sup>13</sup>.

Beyond genome stability, the finding that m6A methylation controls R-loop levels across the genome considerably expands the biological functions of the m6A modification pathway to potentially all R-loop-related functions. These include DNA topology, as R-loops were recently

proposed to relieve superhelical stress, immunoglobulin class switch recombination (and therefore the immune response), replication initiation and transcription<sup>1,2,14</sup>. Additionally m6A also accumulates at sites of UV-induced DNA damage<sup>6</sup>, raising the interesting possibility that this modification also regulates R-loops during DNA repair and thus the frequency of chromosomal translocations<sup>15</sup>.

In summary, the results presented by Abakir *et al.* unveil an unexpected interplay between RNA modifications (the epitranscriptome) and the maintenance of genome integrity. Re-analyzing the pathological contexts implicating dysregulation of the m6A RNA pathway through the prism of genome instability will therefore warrant further investigation (Fig. 1).

**Figure 1: Expanding the functions of the m6A RNA pathway.**

m6A is the most abundant internal modification of eukaryotic mRNA. This co-transcriptionally added epitranscriptomic mark plays multiple roles in mRNA metabolism and expectedly, m6A has been associated with a wide variety of pathologies. Notably, nascent RNA can also hybridize back to the DNA template to form the so-called “R-loop”. Abakir *et al.* propose that R-loops are m6A-modified on their RNA moiety. The m6A-RNA reader YTHDF2 is recruited to destabilize RNA:DNA hybrids through a mechanism that remains to be determined (action of nucleases, helicases, RNaseP). The tight regulation of R-loops level is critical to ensure genome stability, and accordingly, stabilization of m6A-modified R-loops (upon YTHDF2 depletion) leads to increased DNA double-strand break formation. This broadens the previously acknowledged functions of m6A pathway in RNA metabolism to the maintenance of genome integrity and opens up new therapeutic strategies for R-loops related diseases.

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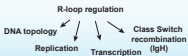
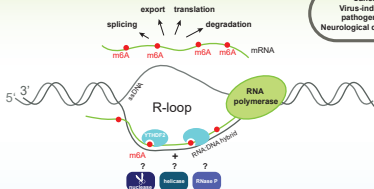
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## EPITRANSCRIPTOME

m6A-associated pathologies

Cancer  
Virus-induced  
pathogenesis  
Neurological disease (?)



R-loop diseases

Cancer  
Neurological disorders  
Autoimmune diseases

GENOME STABILITY