Gene expression regulation is central to sustain life. Post-transcriptional modifications of RNA have recently emerged as a major regulatory layer of gene expression and, thus, a large extent of physiological and disease processes. To understand how an RNA modification contributes to cellular function and gene expression, it is critical to be able to detect the sites of modification in cells. In this issue of *ACS Central Science*, Mikutis and colleagues present a new approach called methyltransferase CLICK-degradation sequencing (meCLICK-seq), which relies on small-molecule click degraders to identify sites of methylated RNA via transcript depletion.\(^1\) Their work provides a new way to identify RNA species that may be methylated in previously inaccessible regions of the transcriptome.

Over 170 post-transcriptional modifications of RNA have been discovered. While their existence has been known for decades, more recently some of these modifications have been found to play broad regulatory roles in gene expression. For instance, N6-methyladenosine (m6A) regulates many aspects of mRNA metabolism, including transcript stability, nuclear processing, translation, and splicing.\(^2\) (Figure 1A). While several other modifications such as pseudouridine, m2G, and m1A have also been studied, the authors focus on m6A, as it is the most abundant internal mRNA modification and the most studied. A majority of mRNA m6A’s are co-transcriptionally and site-selectively installed by a methyltransferase complex of METTL3-METTL14 that uses the S-adenosyl methionine (SAM) cofactor. METTL3 is the catalytic subunit of this main m6A mRNA writer complex.\(^3\) Another methyltransferase, METTL16, was later discovered as an m6A writer that binds U6 snRNA or structured RNA, but its substrate scope is still unclear.\(^4\)

To study how RNA modifications contribute to cellular function and gene expression, transcriptome-wide detection of these sites is critical, and the advent of high-throughput sequencing methods now permits such deeper study of their functions. To date, most researchers have relied on antibody immunoprecipitation to enrich methylated RNAs (Figure 1B). This approach, however, has several drawbacks, such as large sample quantity requirements, nonspecific antibody binding, low resolution, and lack of quantitative information. As a result, simpler, antibody-free methods are needed. Global m6A detection has relied on antibody-based methods due to the inert reactivity of m6A. In this study, however, the authors alter this chemical property by introducing a surrogate of SAM into cells, so that a propargyl group is installed onto the RNA substrate in place of a methyl group (Figure 2A). A previous work installed an allyl group to m6A sites using a SAM homologue for m6A detection.\(^5\) The authors here developed a more general and sensitive approach. The propargyl-modified RNA (Pr6A) undergoes a Cu(I)-catalyzed cycloaddition reaction with azide to form an artificial click-based RNA modification that catalyzes degradation of the RNA (Figure 2B). This click-degrader approach links RNA methylation to degradation and provides the basis for a unique readout of meCLICK-seq: methylated RNAs should exhibit reduced transcript abundance upon RNA-sequencing. Degradation serves as a useful readout because it is easy to identify reduced transcript levels with standard RNA-sequencing technology.

**This click-degrader approach links RNA methylation to degradation, and provides the basis for a unique readout of meCLICK-seq: methylated RNAs should exhibit reduced transcript abundance upon RNA-sequencing.**
The authors first verify in vitro that extensive degradation occurs specifically on RNA functionalized with the click-degrader in the presence of copper and then show that Pr6A can be introduced with subsequent degradation in MOLM13 leukemia and HEK293T kidney cancer cells. Thereafter, the authors apply meCLICK-seq in MOLM13 cells with induced knockdown of either m6A writer METTL3 or METTL16 to map their mRNA substrates. Since meCLICK-seq would label any methylation site that depends on SAM, knocking down a specific methyltransferase helps to identify its substrates. They not only validate that meCLICK-seq yields degradation of known m6A-containing transcripts but also consistently find that a majority of their transcripts overlap with peaks reported from previous m6A cross-linking and immunoprecipitation (miCLIP) sequencing datasets. From their meCLICK-seq method, the authors also report new findings on m6A methylation. For instance, they find a greater number of lncRNA substrates of METTL3 and METTL16 than previous miCLIP data showed. Furthermore, the addition of a bulky propargyl moiety likely hinders RNA processing and recycling pathways. As a result, the authors find m6A methylation on intronic and intergenic regions that were click-degraded with far more peaks primarily due to METTL16. From their intronic sequencing data, the authors discover that intronic polyadenylation sites are linked to methylation by METTL16.

The use of click chemistry for degradation of sites of RNA methylation is clever and simple. After methionine starvation and treatment with the click-degrader, the only steps required to detect methylated sites are RNA extraction followed by sequencing. A drawback of meCLICK-seq, however, may be limited base resolution, or lack of specificity for a particular RNA modification due to its reliance on a SAM surrogate. This issue can be circumvented by knocking
down or expressing a particular RNA modification writer, as the authors did in their study. meCLICK-seq also provides a quantifiable output of methylation, since it harnesses an artificial modification for measurable degradation output. Thus, it may offer a way to study modification stoichiometry in a relatively easy and unbiased manner. Perhaps the more interesting finding is the ability to use meCLICK-seq to study different methylations in general. The authors showed that they can also map internal m^7G on RNA and map m^6A on introns and noncoding RNAs, which could be installed by different methyltransferases. Recent studies have shown that chromatin-associated RNAs, which contain pre-mRNA and noncoding regulatory RNAs, are m^6A methylated and play important roles in chromatin regulation. Studying these less abundant RNA species with m^6A potentially installed by different methyltransferases may be streamlined by meCLICK-seq.

Within the field of m^6A-sequencing, meCLICK-seq joins a series of other recently developed, antibody-free methods. For example, m^6A-REF-seq and MAZTER-seq use a bacterial single-stranded endoribonuclease in which m^6A methylated sites remain uncleaved. DART-seq tethers a bacterial single-stranded endoribonuclease in which m^6A methylated sites remain uncleaved. Similar to meCLICK-seq, m^6A-label-seq introduces a SAM analog to form N^6-allyladenosine, which results in mutations in place of an m^6A site upon sequencing. Meanwhile, m^6A-SEAL co-opts an m^6A demethylase (FTO) to oxidatively modify m^6A for streptavidin-based pulldown. These techniques have benefits, such as lower required input, and limitations, such as required sequence motifs for mutation, complicated post-treatment procedures, or inability to detect all m^6A sites. meCLICK-seq fits a unique niche in that it can identify peaks and genes containing RNA modifications installed by a particular methyltransferase or demethylase relatively easily. Overall, these methods complement one another. Together with existing approaches, meCLICK-seq will help advance our understanding and distribution of the roles of RNA modifications in the epitranscriptome.

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Notes
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