Trends in Genetics

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Spotlight

Resolving m^{6}A epitranscriptome with stoichiometry

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A recent study by Hu *et al*. describes *N*^{6}-methyladenosine (m^{6}A)-selective allyl chemical labeling and sequencing (m^{6}A-SAC-seq), which allows for quantitative, stoichiometric, and positional analyses of m^{6}A at single-nucleotide resolution across the whole transcriptome level. Information on the m^{6}A stoichiometry will provide additional layers of gene regulatory pathways mediated by m^{6}A modification during diverse molecular, cellular, and physiological events.

More than 170 different RNA modifications have been discovered in eukaryotic RNAs. Among them, around ten RNA modifications have been characterized at the transcriptome level, mostly based on approaches using next-generation sequencing (NGS) and antibodies that specifically recognize modified RNAs [1]. These antibody-based NGS approaches provide very useful transcriptome-wide information, such as the identity of modified RNAs and the position of modified bases in the target RNAs. However, they also have several drawbacks, including low sensitivity, a lack of stoichiometric information, and a biased quantitative comparison. These limitations make it difficult to interpret data precisely, especially to evaluate the extent of contribution of RNA modification to a variety of biological events.

Among naturally occurring mRNA modifications, *N*^{6}-methyladenosine (m^{6}A) is known to be the most abundant internal modification found in eukaryotic mRNAs. Approximately one-fourth of endogenous mRNAs contain at least one m^{6}A site. Recently, several antibody-independent methods using selective ribonuclease cleavage were developed [2,3], but these methods cannot cover the entire m^{6}A sites in the transcriptome due to the biased selectivity of ribonucleases. The aforementioned drawbacks were overcome by m^{6}A-selective allyl chemical labeling and sequencing (m^{6}A-SAC-seq) recently developed by Hu *et al*. [4]. In the m^{6}A-SAC-seq, specific detection of m^{6}A over A is achieved by two independent steps. First, this method employs a MjDim1, *Methanocaldococcus jannaschii* homolog of dimethyltransferase that transfers the methyl group from *S*-adenosyl-L-methionine (SAM) to adenosine or m^{6}A. In the presence of a chemically modified *S*-adenosyl-L-methionine (allylic-SAM) cofactor, MjDim1 exhibits ~tenfold more ally transfer to m^{6}A over A, generating allyl-modified m^{6}A and allyl-modified A, respectively. Second, through a subsequent chemical reaction, allyl-modified m^{6}A and allyl-modified A are converted into allyl-labeled and cyclized adducts, cyclized a^{6}m^{6}A and cyclized a^{6}A, respectively. During a reverse transcription (RT) reaction using HIV-1 RT, ~tenfold greater mutation rate is observed at cyclized a^{6}m^{6}A than cyclized a^{6}A. In summary, the state-of-art m^{6}A-SAC-seq allows the determination of m^{6}A sites with ~100-fold preference for m^{6}A over A. More importantly, this method provides precise quantitative information on m^{6}A with stoichiometry.

m^{6}A modification is dynamically and reversibly regulated by the actions of m^{6}A writers (METTL3 and METTL14) and erasers (ALKBH5 and FTO) and it contributes to a fate decision of m^{6}A-modified mRNA via m^{6}A-recognizing reader proteins such as YTH domain-containing proteins. At the molecular level, m^{6}A influences multiple layers of regulation of gene expression, including pre-mRNA splicing, mRNA export, translation, and mRNA stability. Such molecular functions of m^{6}A affect various biological processes, including cell differentiation, embryogenesis, neurogenesis, and carcinogenesis. Thus far, these findings have been obtained mainly from studies based on positional information of m^{6}A sites. For instance, the previous results from a CLIP-seq of YTHDF2 showed that the YTHDF2-bound m^{6}A-tagged mRNA (m^{6}A mRNA) is rapidly degraded independently of the m^{6}A position within mRNA [5′ untranslated region (UTR), coding sequence (CDS), or 3′UTR] [5]. By contrast, another group claimed that m^{6}A promotes the translation of m^{6}A-tagged mRNA at a global level without significantly affecting mRNA stability [6]. However, a careful reanalysis with stoichiometric information from m^{6}A-SAC-seq by Hu *et al*. revealed that, whereas the global level of m^{6}A-tagged mRNAs is reduced by YTHDF2, the mRNAs harboring m^{6}A only in the 5′UTR are instead stabilized by YTHDF2 [4]. Therefore, these observations emphasize that a previous contradictory interpretation of m^{6}A functions should be reconsidered in a stoichiometric aspect.

The detailed stoichiometric information would be more helpful for a comprehensive understanding of m^{6}A-mediated liquid–liquid phase separation (LLPS), such as stress granules and processing bodies. The presence of multiple but not single m^{6}A sites in the mRNA promotes LLPS formation by providing a multivalent scaffold for YTHDF binding [7]. However, previous ensemble analysis of m^{6}A modification lacking stoichiometric information is limited because an individual identical mRNA may have m^{6}A sites at different positions within cells. A more precise analysis of m^{6}A mRNAs based on stoichiometric information might help select mRNAs harboring multiple m^{6}A sites, which are favorable substrates for LLPS formation (Figure 1A).

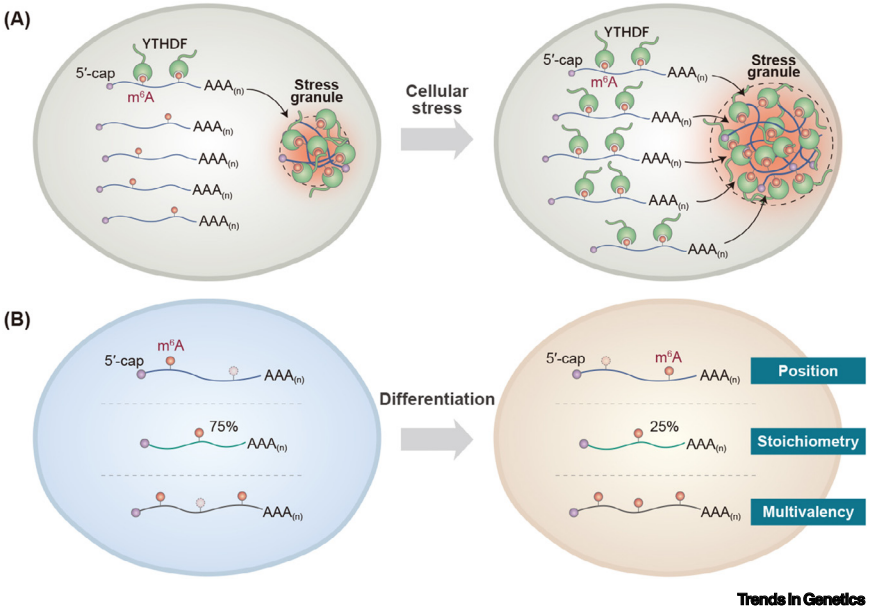

Figure 1. Quantitative determination of N^6-methyladenosine (m^6A) positions and stoichiometry. (A) Dynamic changes of m^6A multivalency may affect the liquid–liquid phase separation upon cellular stress. (B) The m^6A-selective allyl chemical labeling and sequencing (SAC-seq) enables sensitive detection of the changes in the position, stoichiometry, and multivalency of m^6A tags during differentiation.

Another strength of m^6A-SAC-seq is the ability to trace dynamic changes of m^6A stoichiometry among different cellular conditions (Figure 1B). For example, Hu *et al.* examined m^6A dynamics during hematopoietic stem cell differentiation into monocytes [4]. Interestingly, the position of m^6A peaks displayed substantial redistribution among different regions of mRNA during hematopoietic stem cell differentiation, which is contrary to a previous observation [8]. In addition, they found that transcripts with dynamic m^6A stoichiometric changes showed more variations than the transcripts with stable m^6A tags, suggesting changes in m^6A positions may contribute to the alteration of transcriptome abundance during differentiation. A deeper analysis of m^6A stoichiometry dynamics in various cellular systems in the future will provide further evidence to clarify whether m^6A is dynamic or static across conditions.

The m^6A-SAC-seq dataset displayed dynamic changes in positions and stoichiometry of m^6A modification in hematopoietic progenitor differentiation, suggesting cell type-specific m^6A epitranscriptome. Indeed, scDART-seq recently revealed unexpected heterogeneity in the presence and the abundance of m^6A tags across individual cells [9]. Although technical improvement is still required, resolving m^6A stoichiometry at a single cell level will give us a more comprehensive understanding of m^6A dynamics in a complex context, such as developing tissues, pathogenic infections, and cancer. In addition, recent improvements in direct long-read RNA-sequencing have enabled the detection of mRNA modifications in a single transcript [10]. Rich information acquired from these multifaceted approaches will shed light on the veiled dynamic regulation of the epitranscriptome in normal physiology and human diseases in the future.

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Declaration of interests

The authors declare no conflicts of interest.

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