OUTLOOK

TRIBE-STAMP reveals new insights into the functions of RNA binding proteins

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RNA binding proteins (RBPs) are important players in RNA metabolism and gene regulation. In this issue of *Genes & Development*, Flamand and colleagues (pp. 1002–1015) developed a new method (TRIBE-STAMP) that detects binding events by two distinct RBPs on single mRNA molecules, which they first applied to the YTHDF family of N^6-methyladenosine (m^6A) reader proteins. The investigators show that these RBPs largely share a common pool of bound transcripts and that an individual mRNA may be bound by multiple YTHDF proteins throughout its lifetime. This single-molecule technique is an exciting new method to study potential synergy and/or antagonism between different RBPs.

To date, >1000 RNA binding proteins (RBPs) have been identified in the human proteome, each with unique mechanisms of RNA binding, repertoires of bound RNAs, and effects on their RNA targets (Hentze et al. 2018). RBPs are key regulators of cellular RNA metabolism at all stages of a RNA's life cycle, and as such have been the subject of frequent study. Methods to detect the repertoire of RBP targets are traditionally based on the CLIP-seq (cross-linking and immunoprecipitation followed by high-throughput sequencing) methodology (for review, see Ramanathan et al. 2019). Although crucial for the expansion of this field, CLIP-based methods can only provide gene-level information about RBP binding events—not identify single mRNA molecules that have been bound. To address this, new technologies have been developed that instead rely on fusion of an RBP of interest to a deaminase enzyme, which permanently marks the bound RNA upon RBP binding: the A-to-I deaminase ADAR for the TRIBE method (McMahon et al. 2016) or the C-to-U deaminase APOBEC1 for the STAMP method (Brannan et al. 2021). RBP binding events can then be detected without the need to enrich RBP–RNA complexes using immunoprecipitation, as the distinct mutation signatures are detectable via standard RNA-seq. This lack of presequencing enrichment, combined with the ability to detect RBP fusion-induced editing events in individual sequencing reads, allows RBP target RNA molecules to be identified among the pool of sequenced RNAs.

Although these editing-based approaches can provide single-molecule resolution, they are only able to detect the RNA targets of one RBP at a time, which limits researchers' ability to see any potential synergistic or antagonistic interactions between RBPs. To address this limitation, Flamand et al. (2022) developed a new method, TRIBE-STAMP, that leverages the unique mutation signatures induced by TRIBE and STAMP to simultaneously identify individual mRNAs that have been bound by two RBPs. The investigators used TRIBE-STAMP to investigate the potential synergy or antagonism between the different members of the YTHDF family of N^6-methyladenosine (m^6A) reader proteins (YTHDF1–3). YTHDF proteins are a major family of cytoplasmic m^6A readers that bind the m^6A modification and mediate functional effects of m^6A to control gene expression. Recently, the functions of the different YTHDF proteins have been a matter of debate in the field, as these proteins have been proposed to have either divergent (Wang et al. 2015) or redundant (Zaccara and Jaffrey 2020) functions. A major factor contributing to this debate has been discrepancies over whether these proteins bind to the same or different sets of cellular mRNAs. Thus, the YTHDF proteins provided an excellent first use case to highlight the features and benefits of the TRIBE-STAMP approach.

Using different combinations of ADAR- and APOBEC1-tagged YTHDF1/2/3, the investigators found a large overlap in the mRNAs targeted by the three YTHDF proteins. The single-molecule resolution of their technique revealed that many mRNAs are bound by more than one YTHDF protein throughout their lifetimes. YTHDF2, however, showed the lowest overlap of targets with the other YTHDF proteins, supporting its role in m^6A-mediated mRNA decay (Du et al. 2016). The elegance of TRIBE-STAMP was made evident when the investigators combined it with

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complementary methods, such as RNA immunoprecipitation (RIP) to assess the order in which different YTHDFs bind a given mRNA molecule, as well as polysome profiling to assess whether YTHDF binding correlates with increased or decreased translation of an mRNA.

It will be exciting to see how this new technique advances the study of RNA binding proteins. One area where this technology could be particularly transformative is in the study of virus–host interactions. All viruses rely on their host cells in order to replicate, which requires hijacking of host translation machinery by viral proteins. In response, host cells take steps to suppress translation of viral mRNAs; much of this battle between the virus and the host cell is carried out by RBPs or other RNA-interacting proteins (Hoang et al. 2021). This scenario of dueling viral and host RBPs is an ideal use case for TRIBE-STAMP, as this technique not only can identify both the overlapping and exclusive interactomes of different host and viral RBPs upon infection, but can also reveal how these interactions facilitate or inhibit viral mRNA translation, as shown by the polysome profiling experiments in this study. Additionally, this technique would be well suited to deciphering the complex orchestration of RBPs required for the functioning of the nervous system. Nerve cell development and function require the proper spatial and temporal localization of mRNAs, which is controlled by numerous RBPs (Fernandopulle et al. 2021). TRIBE-STAMP could be useful for investigating the temporal aspects of RBP binding events in nervous tissue, as the investigators demonstrated that they could extract information about the order of RBP binding events by combining TRIBE with RIP. The high-throughput, single-molecule nature of TRIBE-STAMP holds great promise to reveal synergistic or antagonistic interactions between (essentially) any two RBPs in a wide variety of biological applications.

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