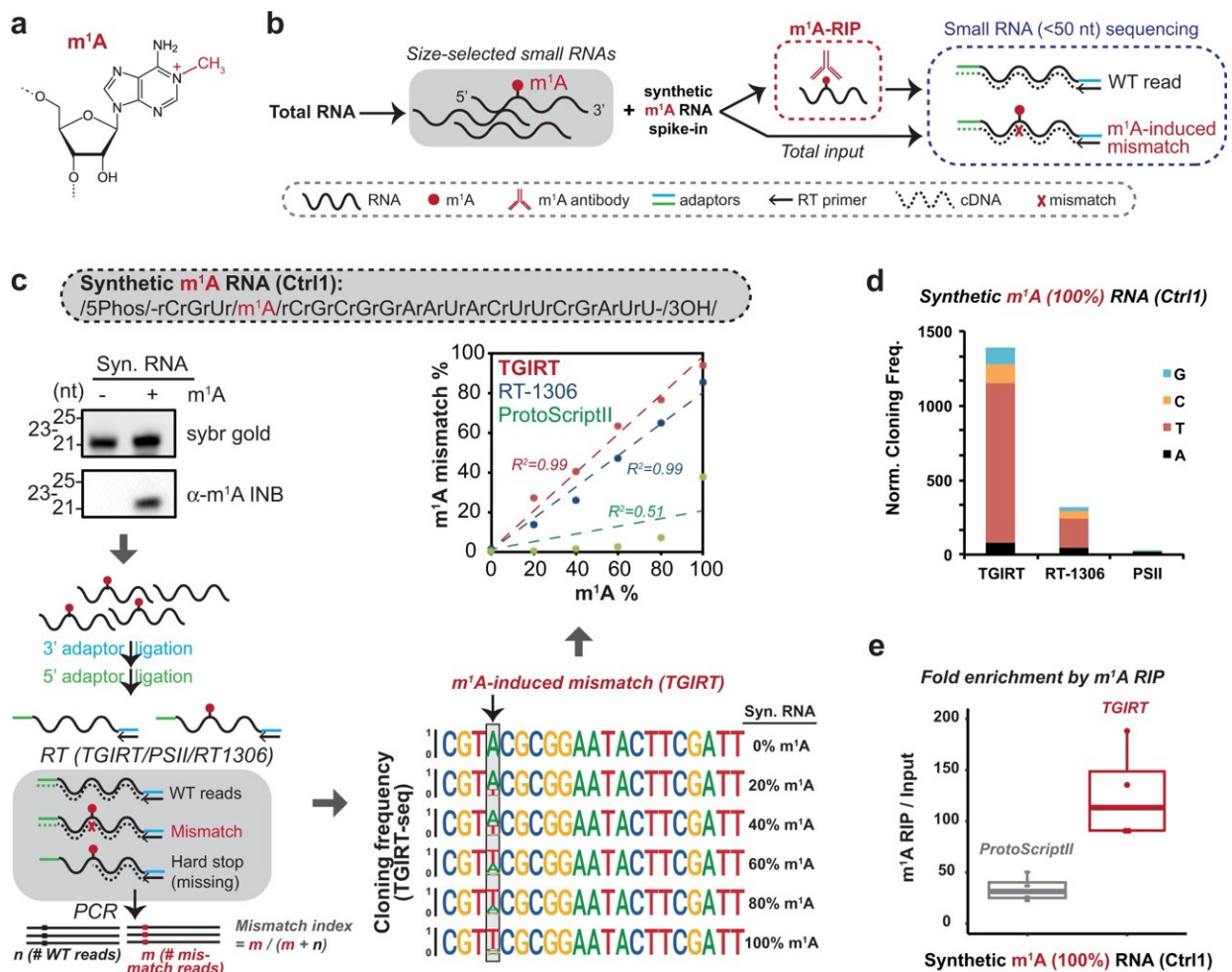


Methylation of tRNA-derived fragments regulates gene-silencing activity in bladder cancer

May 9 2022, by By Jeff Hansen



Systematic mapping of m¹A sites in small RNA space. a) Chemical structure of m¹A (N1-methyladenosine). b) Overall m¹A mapping strategy for small RNAs by combining m¹A RIP and m¹A-induced mismatch analysis. Synthetic m¹A

RNA is included as a positive control. c) TGIRT identified as the optimal reverse transcriptase for m¹A-induced mismatch analysis. Briefly, synthetic m¹A-containing RNA at different m¹A stoichiometry was sequenced by three different reverse transcriptases (RTs): TGIRT (thermostable group II intron reverse transcriptase), PSII (ProtoScriptII, retrovirus RT) and RT-1306 (engineered HIV RT). For each RT, mismatch rate was calculated across all the reads that map to the synthetic RNA sequence (allowing 1 mismatch) as represented by the sequence logo. The mismatch rate at the known m¹A site is then plotted against known m¹A stoichiometry (R² derived from linear fitting forced to cross intercept at zero). d) ProtoScriptII leads to under-representation of m¹A-containing RNAs. Cloning frequency is normalized to spike-ins. e) m¹A RIP successfully enriches synthetic m¹A-containing RNA compared to input, when spiked in with total short RNAs. TGIRT captures enrichment better than ProtoScriptII. Data are based on four independent RIP experiments (two HEK293T and two U251). Boxplot center represents median, bounds represent 25 and 75%, and whiskers show the minimum or maximum no further than 1.5 * interquartile range from the bound. Credit: *Nature Communications* (2022). DOI: 10.1038/s41467-022-29790-8

Anindya Dutta, MBBS, Ph.D., and colleagues have described a novel form of gene regulation that is altered in bladder cancer, leading to the boosting of a gene pathway that helps the cancer cells survive during rapid growth.

Their work focuses on a 22-base fragment of transfer RNAs known as a tRF-3b, which is modified by an enzyme complex called TRMT6/61A. In [bladder cancer](#), the levels of TRMT6/61A—a methyltransferase enzyme that adds a methyl group onto the fourth base of the tRF-3bs—are elevated. This modification prevents tRF-3bs from silencing the expression of various [genes](#) in the unfolded protein response pathway in the [cancer](#) cells, resulting in increased expression of those genes.

"To the best of our knowledge, this is the first example of microRNA-like gene silencing being regulated by the TRMT6/61 based on an N¹-methyladenosine modification, and our report provides a mechanism by which the elevation of TRMT6/61A seen in cancers can impact gene expression," Dutta said. "Fast proliferating cancer cells synthesize and fold many more proteins than normal cells and thus need to upregulate the unfolded protein response pathway to maintain protein homeostasis. We find that one way bladder [cancer cells](#) activate the pro-survival unfolded protein response to alleviate endoplasmic reticulum stress is by preventing tRFs from silencing the expression of genes involved in this unfolded protein response."

"The unfolded protein response is tightly linked to many aspects of cancer progression and has emerged as a promising therapeutic target," Dutta said. "It has been previously noted that unfolded protein response-related genes are globally upregulated in several cancer types, including bladder cancer, and so our results suggest that inhibiting the TRMT6/61A enzyme may be a new approach to treat bladder cancer."

The study by Dutta and co-corresponding author Rune Ougland, M.D., Ph.D., included analysis of bladder cancer tissue obtained from patients undergoing transurethral resection of bladder tumors. It is published in the journal *Nature Communications*. Dutta is chair of the University of Alabama at Birmingham Department of Genetics, and Ougland is a urologic surgeon and senior research investigator at Oslo University Hospital Rikshospitalet, Oslo, Norway.

An important advance in the study was the workflow used to create a library from human cells of small RNAs with an N¹-methyladenosine modification, or m¹A. The workflow combined two independent approaches—enrichment by m¹A-antibody, followed by small RNA-sequencing and m¹A-induced mismatch signature by sequencing.

The UAB researchers found that a reverse transcriptase enzyme, ProtoScriptII, commonly used for short RNA sequencing, did a poor job of detecting small RNAs that contain m¹A; but the use of two other reverse transcriptases in the workflow revealed that tRNA-derived fragments, including tRF-3b, were enriched among short RNAs. This suggested that small RNAs with an m¹A modification are under-represented in most small RNA-sequence libraries that commonly have used ProtoScriptII.

With the improved workflow, the researchers found that the m¹A modification existed mostly on tRFs among the human small RNAs. They also found that the m¹A modification was highly specific and prevalent on both nuclear-encoded tRFs and mitochondria-encoded tRFs, and the m¹A found on tRF-3b from nuclear-encoded tRNAs was mediated by the TRMT6/61A complex.

How does the m¹A-tRF-3b impede gene silencing? The answer involves an even deeper dive into [molecular genetics](#), but the key appears to be that the N¹-methyladenosine modification disrupts regular Watson-Crick base pairing.

MicroRNAs are known to silence genes by binding to the RNA-induced silencing complex, or RISC. There they act as a template to bind complementary messenger RNAs, and the messenger RNA is then silenced and degraded by RISC. Similar to microRNAs, tRF-3s have been found in diverse biological pathways, in particular gene-silencing pathways that rely on base pairing between the small RNAs, in this case tRF-3s, and the target RNAs.

The researchers created a luciferase reporter assay and found that an unmodified tRF-3 triggered gene silencing, whereas the m¹A-modified tRF-3b abolished the gene silencing. "Since m¹A interrupts canonical base pairing, we hypothesize the weakened base pairing by m¹A in the

tRF-3 with target messenger RNA explains the lowered gene-silencing activity observed for m¹A-containing tRF-3s," Dutta said.

Co-authors with Dutta and Ougland in the study, "TRMT6/61A-dependent base methylation of tRNA-derived fragments regulates gene-silencing activity and the unfolded protein response in bladder cancer," are Zhangli Su, UAB Department of Genetics; Ida Monshaugen and Arne Klungland, Oslo University Hospital Rikshospitalet; and Briana Wilson and Fengbin Wang, University of Virginia.

More information: Zhangli Su et al, TRMT6/61A-dependent base methylation of tRNA-derived fragments regulates gene-silencing activity and the unfolded protein response in bladder cancer, *Nature Communications* (2022). [DOI: 10.1038/s41467-022-29790-8](https://doi.org/10.1038/s41467-022-29790-8)

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