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Fluorescein-Based Single-Nucleotide Resolution Monitoring Of RNA N6-Methyladenosine

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Introduction

Since the pre-molecular era, the emergence of new genes with novel functions has been regarded as a significant contributor to adaptive evolutionary innovation. In this paper, I discuss the origin and evolution of new genes and their functions in eukaryotes, a field of study that has advanced rapidly in the last decade thanks to the genomics revolution. Indeed, recent research has provided preliminary whole-genome views of various types of new genes for a wide range of organisms [1]. The range of mechanisms underlying the origin of new genes is enticing, going far beyond the previously well-studied source of gene duplicatiRNA probe pull-down is a common method for finding readers, but its application is still limited to determining kinetically stable RNA-protein interactions. A photo-crosslinking reaction that converts unstable non-covalent interactions into stable covalent bonds has resulted in a significant improvement. However, the attached crosslinking group may cause steric hindrance, altering the recognised motif in mRNA and, as a result, affecting the binding and identification of reader proteins. To address the issue, we developed a DNA templated technique that can guide the desired groups in a site-specific manner via the self-assembly of complementary double-stranded DNA, which has been used in the screening of protein-small molecule interactionson [2].

Description

We present a technique for repeatable conductance measurements on conformation-constrained single nucleotides as well as an advanced algorithmic approach for distinguishing the nucleobases. Our method employs combed and electrostatically bound single DNA and RNA nucleotide nucleotide conductance sequencing (QPICS) on a self-assembled monolayer of cysteamine molecules. We show that by varying the bias and pH conditions, molecular conductance can be turned on and off, resulting in reversible nucleotide perturbation for electronic recognition (NPER). We use NPER to achieve >99.7% accuracy for DNA and RNA base calling at low molecular coverage using unbiased single measurements on DNA/RNA nucleotides, which is a significant improvement over current sequencing methods [3].

According to estimates, m6A is present in over 7000 coding and 300 noncoding RNAs and 0.1-0.4% of total adenine nucleotide content in mammalian transcripts is methylated. Furthermore, m6A is enriched in 3′ UTRs, near stop codons in mRNAs and near the last exon in noncoding RNA. m6A modification, like DNA methylation, is reversible and catalysed by corresponding enzymes, namely "writers," "erasers," and "readers." Abnormalities in m6A have been linked to a number of diseases, including type 2 diabetes mellitus (DM2) and cancer. This review focuses on m6A's possible role in cancer progression [4].

Many computational methods for identifying m6A sites have been developed over the last few decades. Using the motif discovery algorithm, the researchers discover that m6A peaks have a consensus motif in the form of DRACH (where D = A, G, or U; R = A, G, or U; H = A, C, or U). These findings indicate that m6A writers, which are adenosine methyltransferases such as METTL3, METTL14, WTAP and KIAA1429 and m6A erasers, which are demethylases such as FTO and ALKBH5, may constitute a limited repertoire with dominant and a few less abundant elements. At the same time, a large number of consensus motifs are not methylated. To identify methylated m6A sites, high-resolution data for predicting m6A sites is required.

m6A is the most common, abundant and conserved internal modification in mRNAs, non-coding RNAs and ribosomal RNA32 in mammalian cells. In mRNA33, m6A has been found near stop codons, long internal exons, transcription start sites and the 5′ untranslated region (UTR). A series of enzymes and proteins regulate m6A modification at different developmental stages: 1) methyltransferases ("writers"), 2) demethylases ("erasers") and 3) m6A binding proteins ("readers"). The abnormal expression and function of these modifiers has been observed in a variety of cancers and has been linked to the development and progression of these diseases [5].

Conclusion

m6A marks on RNA, unlike other epitranscriptomic codes, do not efficiently block transcription elongation or generate readable mutations during RT. Crosslinking m6A-containing RNA with specific antibodies, on the other hand, would result in a certain number of truncated cDNAs during RT. With this principle in hand, we first tested the feasibility of SuperScrip III-mediated SMART with a pair of synthetic RNA oligos containing GGAC or GGm6AC. The RT signal marked as 'probe + 1' produced by GGm6AC RNA with crosslinking was significantly weaker (28% relative to GGAC) than that produced by GGm6AC RNA without crosslinking (69% relative to GGAC), indicating that antibodies fixed to m6A residue post crosslinking could significantly block fluorescein-dUTP incorporation

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Conflict of Interest

There are no conflicts of interest by author.

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