How m^6A sneaks into DNA

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The biological function and origin of m6A in DNA have been widely debated. A new study demonstrates that the majority of m6A in DNA originates from RNA catabolism via a nucleotide salvage pathway.

Methylation of cytosine (5mC) in DNA plays important roles in genome function by adding to the repertoire of chromatin mechanisms that facilitate transcriptional repression. Discoveries of oxidized 5mC variants in DNA1,2 invigorated researchers to search for additional DNA (and RNA) base modifications in mammalian genomes, leading to the identification of N6-methyladenine (m6A), a DNA base modification previously recognized in bacteria3,4. However, there have been conflicting reports as to whether m6A can be reliably detected in mammalian genomes, challenging its role as a biologically functional epigenetic modification5. In this issue, Musheev et al. make elegant use of mass spectrometry to validate the existence of m6A in mammalian DNA and reveal that the primary source of m6A in mammalian DNA is RNA catabolism6.

The authors used highly sensitive mass spectrometry coupled with a heavy-isotope-labeled precursor (methionine) of the methyl donor S-adenosyl-l-methionine to quantify m6A that is existing in mammalian DNA rather than emerging from contaminating sources such as bacterial DNA. The results demonstrated that m6A does exist in DNA at a relatively low abundance, consistent with previous reports; however, some cell lines have elevated (two orders of magnitude) abundance of this modified base.

Next the authors noticed that accumulation of m6A in DNA was substantially slower than that of the canonical epigenetic mark 5mC, suggesting that unlike DNA-replication-coupled methylation of cytosine, the origin of m6A might be different. The authors speculated that the source of m6A in DNA could be the nucleotide pool, wherein m6dATP would be produced from m6A containing ribonucleotides released from degradation of RNA (Fig. 1). m6A is a well-recognized RNA modification, constituting around 0.1–0.2% of total adenines, making it a possible source of modified adenosines. Employing a battery of orthogonal assays, Musheev et al. demonstrated that ribo-m6A can become a source of deoxyribo-m6A in DNA. Perhaps the most compelling evidence supporting their model comes from double-labeling experiments, which provide quantification of the relative contribution of direct methylation and nucleotide incorporation to the sum of m6A in DNA. Cells were supplied with both heavy-isotope-labeled dAMP, in which heavy atoms were present both in the base and the deoxyribose, and heavy-isotope-labeled methionine. Such labeling enabled the researchers to distinguish m6A originating from direct methylation in DNA (indicated by heavy atoms in deoxyribose, base and methyl group) from m6A originating from catabolism of RNA (which contains labeled base and methyl groups, but not deoxyribose). These experiments demonstrated that the vast majority of m6A in DNA is derived from the nucleotide pool. As an important control, the same method showed that the main origin of 5mC in DNA is direct methylation of cytosines.

Where does this study leave the so-far controversial field of m6A in DNA? First, it corroborates that m6A abundance in DNA is generally very low. Therefore, contamination of m6A originating from

bacterial DNA or reagents used to prepare samples could be one source of conflicting observations, even in the same cell lines3,4,5. The low abundance of a DNA modification per se is not an argument to challenge its biological function. However, the rarity of a modification might make it challenging to prove the biological function even if the enzymes that place or remove the modification are identified. The main complication here is the uncertainty of whether the substrate of interest is the main substrate for the enzyme in vivo.

Second, the demonstration by Musheev et al. that a substantial fraction of m6A originates via nucleotide metabolism challenges its function as an epigenetic mark. For the well-described biological DNA modifications 5mC and 5hmC, a mechanism exists to restrict the salvage of modified nucleotides originating from catabolism of modified DNA7. Thus, mammals have evolved a way to protect DNA from random incorporation of biological DNA modifications, which could be disruptive if placed inappropriately in the genome. The fact that there seem to be no major restrictions on incorporation of m6dA suggests that accurate placement of this modification in the genome may not be important.

Further research will have to consider nucleotide salvage as an important contributor to m6A abundance in DNA when attempting to elucidate whether there is a cell type or cell state in which m6A could serve a biological function. Here, Musheev et al. put forward an intriguing argument against m6A being a true epigenetic DNA modification.

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