

Metabolism of 5-methylcytosine in RNA



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Introduction

More than 150 modified nucleosides exist in RNA, but the precise function and significance of nearly all of them remain unknown.^[1] 5methylcytosine (m⁵C) is one of the most abundant modifications and can influence RNA stability, processing, and protein binding.^[2-4] We probe the dynamics of m⁵C and its metabolic derivatives in RNA to better understand the biological role of modified cytosine residues.



UHPLC-MS/MS nucleoside quantification method

Synthesis of stable isotope labelled standards (SILs)





m/z = 258 → 126.06619 ± 5 ppm m/z = 274 → 142.06110 ± 5 ppm m/z = 262 → 130.09130 ± 5 ppm

Purified RNA is spiked with a known amount of stable isotope-labelled (SIL) standards and enzymatically digested to ribonucleosides. These are separated on an ultra-high performance liquid chromatography (UHPLC) column and quantified using tandem mass spectrometry (MS/MS).

hm⁵C + 3 Da

Synthesis of $[2^{-13}C, 1, 3^{-15}N_2]$ -cytidine (C + 3 Da), 5-methyl-D₃-[6-D]cytidine (m⁵C + 4 Da), and 5-hydroxymethyl-[2-¹³C,1,3-¹⁵N₂]-cytidine (hm⁵C + 3 Da). Asterisks (*) represent a ^{15}N or ^{13}C isotopologue.

In vivo oxidation of m⁵C to hm⁵C and f⁵C



Upon feeding mice with *L*-methionine-(methyl-¹³C,D₃), ¹³C and D isotope incorporation was observed into m⁵C, 5-hydroxymethylcytosine (hm⁵C) and 5-formylcytidine (f^5C), establishing them as metabolic derivatives of m^5C .

hm⁵C is pervasive across all three domains of life



hm⁵C was detected in total RNA from various model organisms. Its levels are species dependent and up to a 1000-fold lower than those of m⁵C.

Transcription-independent formation of hm⁵C

Future perspectives



Freshly transcribed cellular RNA was labelled with ¹⁵N₂-cytidine and methylation and hydroxymethylation thereof was marked with ¹³C and D isotopes. While m⁵C is established co-transcriptionally, its oxidation to hm⁵C boosts at a specific stage during the cell cycle.

- operation of specific RNA types and quantification of their modifications ✓ polyA RNA, tRNA & rRNA, small RNAs
- identification of proteins binding specifically to m⁵C- or hm⁵C-modified RNA \diamond

Bibliography

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