# RNA modifications: methodology and examples in disease

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## RNA modifications: dynamic and reversible



Gokhale and Horner SM, PLOS Pathogens 2017

Shi, Wei and Chuan He, Mol Cell 2019







N<sup>6</sup>-methyladenosine





5-hydroxylmethylcytidine

5-methylcytidine

HO.





## RNA modifications: dynamic and reversible

		s2U	f5Cm	ac4C			
	inm5c211	m5U	ho5C	ac4Cm			
	nm5320	se2U	m4C	m4 4Cr			
<b>(Y)</b>	nmbsezu	tm5U	m3C	m4,401			
m1Y	mcnm5U	nm5U	m5C	m===			
m5Um	nchm5U	ncm511	k2C	mocm			
m1acp3Y	inm5Um	cmo5U	-2C	nmsc		m2 8A	
momEllm	cm5s2U	chro50	520	m4Cm		m6Am	
membom	nm5s2U	cnm5U	t5C		m1l	m6.6A	
mchm5Um	mnm5U	inm50	Cm		m8A	mo,oA	
cmnm5s2U	mcm5U	f5Um	C+	(	m6A	ms2t6A	
mnm5se2U	f5se2U	s2Um		Am	m1A	m6,6Am	
cmnm5se2U	tm5s2U	m3U		g6A	m2A	ms2io6A	
cmnm5ges2U	acp3U	m3Y		i6A	Ar(p)	ms2hn6A	
mnm5gos211	m5s2U	f5U	U Im	f6A	ct6A	ms2m6A	
IIIIIIIJges20	acp3Y	Um		46 A	LOA	ms2i6A	
nm5ges20	cm5U	D		toA	IOOA	m6t6A	
cmnm5Um	m3Um				hn6A	m1Am	
mcm5s2U	ges2U	G+	imG		птбА	m1lm	
mnm5s2U	mo5U	Qbase	e m2G	Gm		0064	
nom511m	f5c211	QtRNA	m1G	mim	IG	acoa	
	15520	oQtRNA	m7G	m20	Sm		
ncm5s2U	no50	galQtRN	imG2	imG	-14	yW	
cmnm5U	mou		yW-58	m2 ·	2 76	Gr(p)	
acp3D	Ym	giuetki	- vW-86		2,70	o2yW	
s4U		preunbas	e JN 00	m∠,	/Gm		
		preQubas	e yw-/2	m2,	2G		
		preQ0tRN	А ОНУЖ	m10	5m		
		preQ1tRN	A OHyW	« m2,	7G		
		manQtRN	A OHyW	/			

Disease related

Detection technique available

Johnkhout et al., RNA 2017 The RNA modification landscape in human disease PMID: 28855326 Me-RIP/m6A-seq

- MeRIP-Seq: Meyer K. D., Saletore Y., Zumbo P., Elemento O., Mason C. E., et al. (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 149: 1635-1646
- m6A-Seq:Dominissini D. et al. (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485: 201-206
- Dominissini D. et al. (2013) Transcriptome-wide mapping of N6-methyladenosine by m6A-seq based on immunocapturing and massively parallel sequencing

#### Me-RIP/m6A-seq



miCLIP -m6A

 miCLIP-m6A: Linder, et al. (2015) Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat Methods 12, 767–772 (2015)



#### Antibody-free methods:

Meyer KD. DART-seq: an antibody-free method for global m6A detection Nature Methods v 16, p 1275–1280 (2019)



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## Meyer KD. DART-seq: an antibody-free method for global m6A detection Nature Methods v 16, p 1275–1280 (2019)

Long-read DART-seq (with PacBio) reveals m6A distribution within individual RNA molecules.



## Direct RNA sequencing with Oxford Nanopore Technology



https://doi.org/10.1016/j.xinn.2021.100153

## Oxford Nanopore Direct RNA sequencing

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Nature Methods 16, p. 1297–1305 (2019) doi: 10.1038/s41592-019-0617-2

## Oxford Nanopore Direct RNA sequencing



Johnkhout et al., RNA 2017 DOI:10.1261/rna.063503.117



With Nanopore DRS study RNA dynamics

doi: https://doi.org/10.1101/2021.09.22.461331

# ✓ Study isoform-specific polyA-tail dynamics



# ✓ Predict RNA modifications



Accurate detection of m6A RNA modifications in native RNA sequences Liu et al. Nature Communications 10: 4079 (2019)

## Long-read sequencing of nascent RNA (combine long read-sequencing with ~chromatin fractionation and ~metabolic labeling)



**Revealing nascent RNA processing dynamics with nano-COP** Drexler et al. Nature Protocols 16, 1343–1375 (2021)



### **Functions of co-transcriptional RNA modifications**

Capturing **nascent** N6-methyladenosine (m6A) RNA modifications:





**Unlabeled RNA** 

**BrU-RNA** 

#### Nascent m6A affects co-transcriptional RNA processing



Louloupi\*, Ntini\* et al., Cell Rep 2018

#### Nascent RNA m6A modification affects co-transcriptional RNA processing



#### Nascent RNA modifications in crosstalk with transcription





## Nascent m6A in crosstalk with transcription

Pausing Index: Read density [0\_+300]/[+300\_+3000]





### Nascent m6A in crosstalk with transcription

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## LETTER TO THE EDITOR m<sup>6</sup>A promotes R-loop formation to facilitate transcription termination

Cell Research (2019) 0:1-4; https://doi.org/10.1038/s41422-019-0235-7

Yang et al. Cell Res 2019



### Nascent RNA modifications on non-coding RNA transcripts

Article

## **Cell Reports**

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# Deposition of 5-Methylcytosine on Enhancer RNAs Enables the Coactivator Function of PGC-1 $\alpha$

Aguilo et al., Cell Rep 2016



- METTL3-dependent RNA m6A dysregulation contributes to neurodegeneration in Alzheimer's disease through aberrant cell cycle events
  Zhao et al., Molecular Neurodegeneration 16: 70 (2021)
- m6A mRNA methylation-directed myeloid cell activation controls progression of NAFLD and obesity (in mice)
  Kin et al., Cell Rep 2021, 37:6 doi.org/10.1016/j.celrep.2021.109968

Review: Epitranscriptomics in liver disease: Basic concepts and therapeutic potential Zhao et al., Journal of Hepatology 2020

Cheng, J. X. et al. RNA cytosine methylation and methyltransferases mediate chromatin organization and 5-azacytidine response and resistance in leukaemia. Nat. Commun. 9, 1163 (2018).



Yankova et al., Nature 2021 Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia, Nature 2021

https://www.stormtherapeutics.com