

Enabling Exploration of the Eukaryotic Epitranscriptome (E^4)

**John Satterlee, NIDA
on behalf of the Common Fund
Epitranscriptomics Work Group**

E4 Work Group Members

Co-chairs: **Nora Volkow (NIDA), Dinah Singer (NCI)**

Co-coordinators: **John Satterlee (NIDA), Randy Knowlton (NCI)**

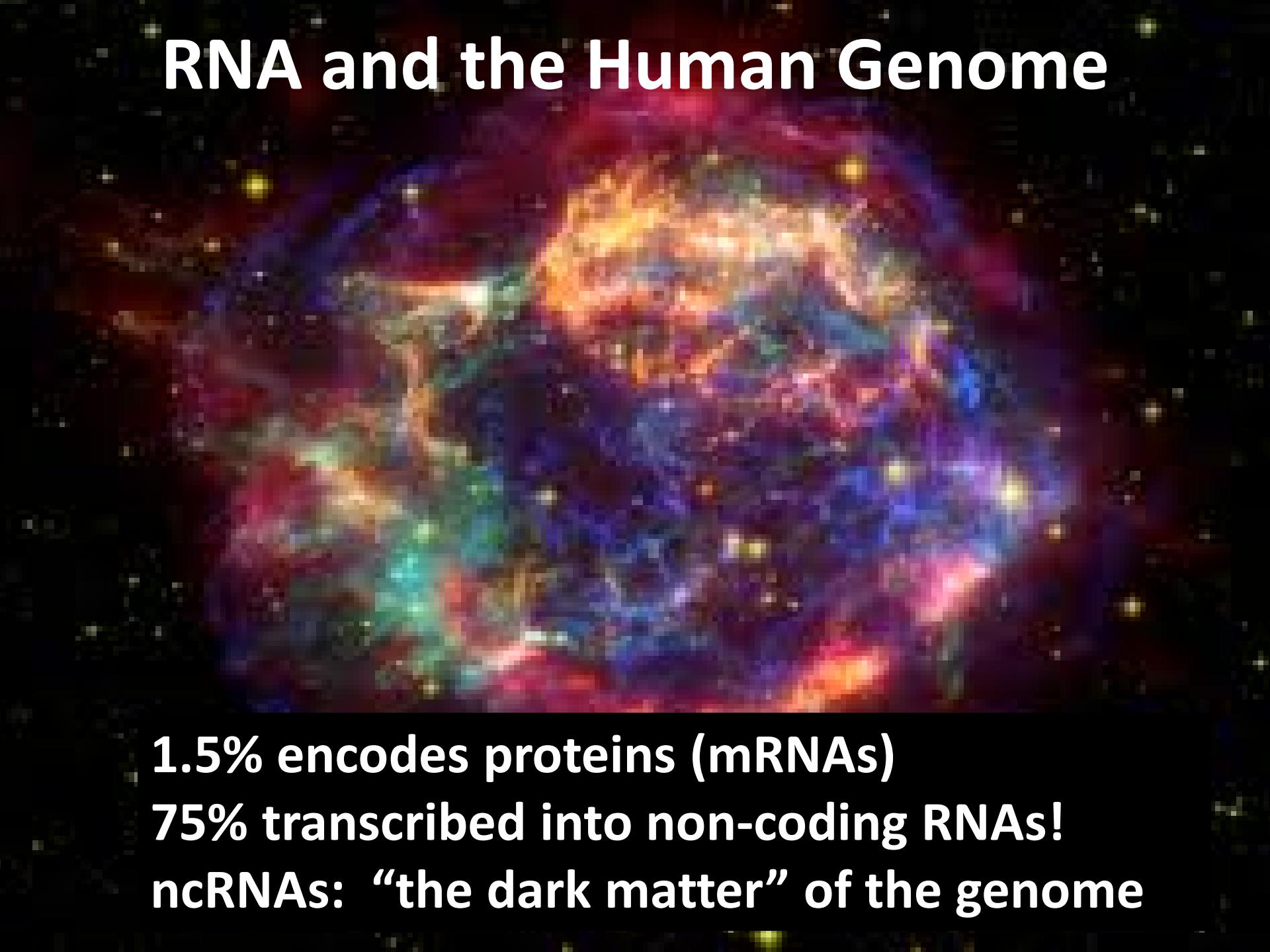
Richard Panniers	CSR	Dena Procaccini	NIDA
Lillian Kuo	NCATS	Silvio Gutkind	NIDCR
Dorit Zuk	NCATS	Isaac Rodriguez-Chavez	NIDCR
Carol Pontzer	NCCAM	Terry Bishop	NIDDK
Sean Hanlon	NCI	Lisa Chadwick	NIEHS
Lisa Neuhold	NEI	Peter Preusch	NIGMS
Michael Smith	NHGRI	Darren Sledjeski	NIGMS
PJ Brooks	NCATS	Anjene Addington	NIMH
Conrad Mallia	NIAID	Geetha Senthil	NIMH
Elizabeth Stansell	NIAID	David Owens	NINDS
James Coulombe	NICHD	Leslie Derr	OD
Stuart Moss	NICHD	Taylor Gilliland	OD
Roger Little	NIDA	Rebecca Lenzi	OD

MISSION: Identify key scientific issues in the area of Epitranscriptomics
for development into a potential new Common Fund program.

Outline

- **Background**
- **RNA Modification Functions**
- **E4 Gaps and Opportunities**
- **E4 Program Components & Impact**

RNA and the Human Genome



1.5% encodes proteins (mRNAs)

75% transcribed into non-coding RNAs!

ncRNAs: “the dark matter” of the genome

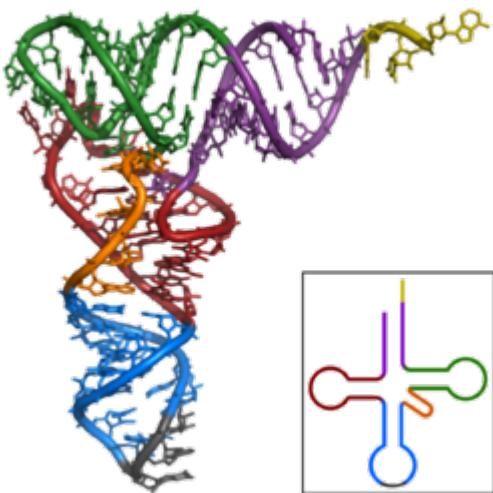
The RNA World

First self-replicating molecule ?

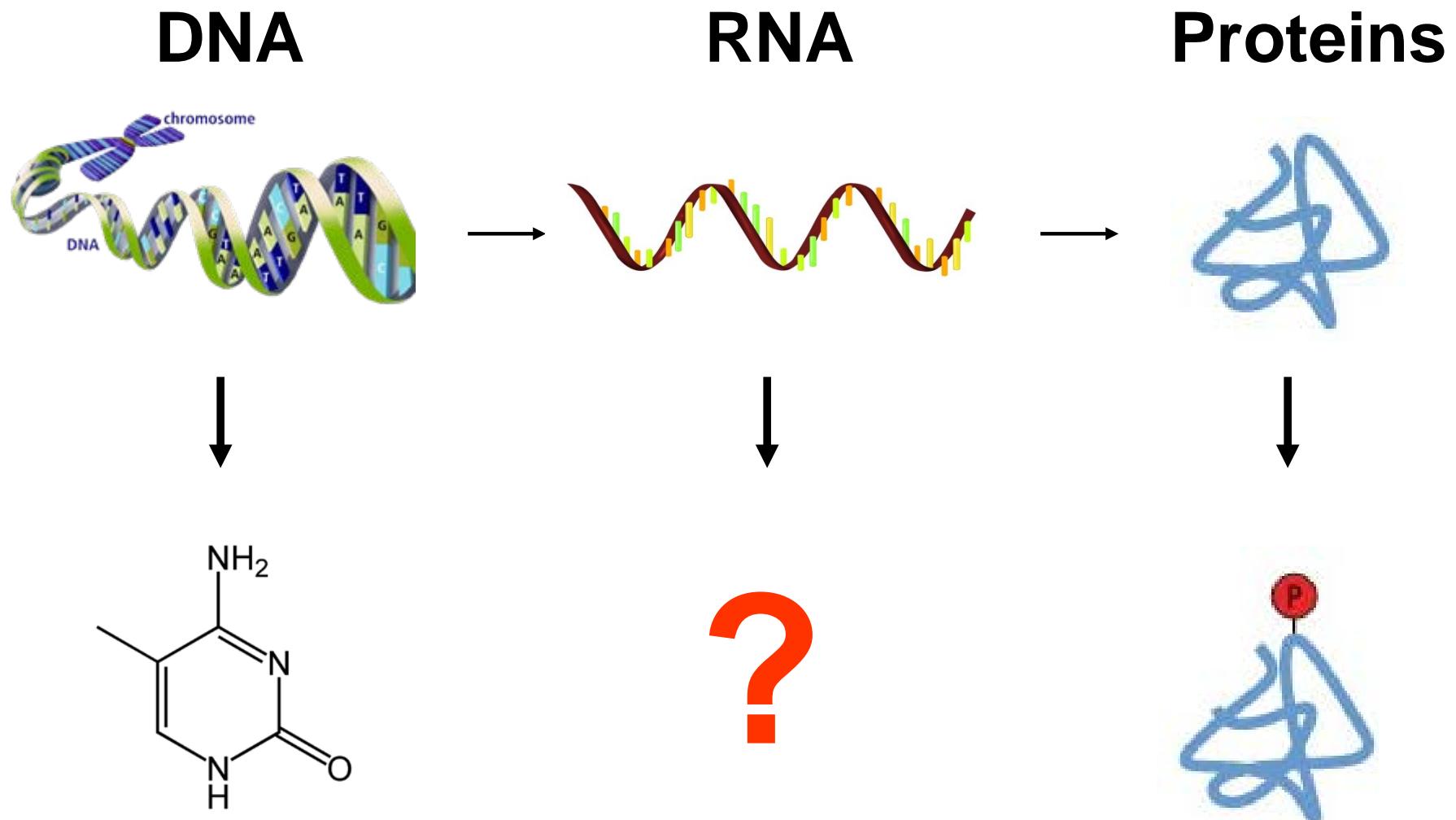
Many classes of RNA: mRNA, tRNA, rRNA, microRNA, siRNA, piRNA, long non-coding RNA, snoRNA, etc.

Many RNA functions:

- Protein translation and localized translation
- Splicing
- Structural scaffold
- Chromatin recruitment
- Ribozymes
- Environmental sensing
- Post-transcriptional regulation
- Gene silencing
- Defense against germline transposons
- Intercellular signaling
- RNA modification

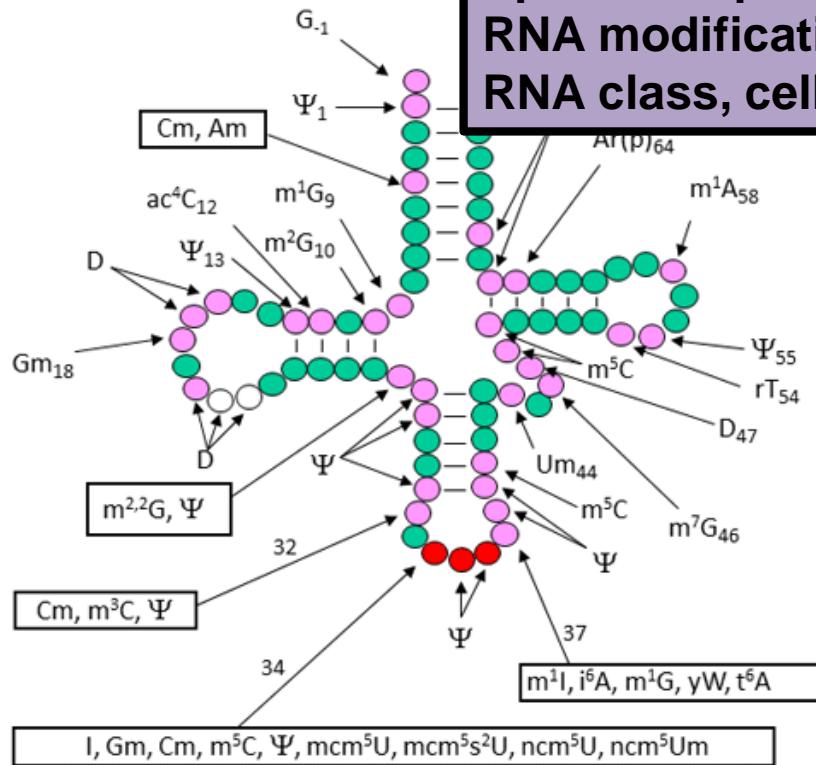


DNA and proteins undergo chemical modifications



Adapted from Samie Jaffrey

>110 RNA Modifications Are Known



Epitranscriptome: all of the RNA modifications for a given RNA class, cell-type, or organism.

Nucleosides

adenosine (A)

2'-O-methyladenosine (i6A)

3'-methyladenosine (m6A)

2'-O-methyladenosine (m1A)

inosine (I)

2'-O-methylinosine (m1I)

guanosine (G)

1-methylguanosine (m1G)

7-methylguanosine (m7G)

N2-methylguanosine (m2G)

2'-O-methylguanosine (Gm)

uridine (U)

2'-O-methyluridine (Um)

5-methyluridine (m5 U)

6-methyluridine (m6 U)

5,6-dimethyluridine (m5 m6 U)

2-thiouridine (s2U)

4-thiouridine (s4U)

5-methoxyuridine (mo5 U)

66 known in eukaryotes

5-n

5-

5-

13 in eukaryotic mRNA

1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (m1acp3Y)

dihydouridine (D)

cytidine (C)

5-methylcytidine (m5 C)

2'-O-methylcytidine (Cm)

N4-acetylcytidine (ac4C)

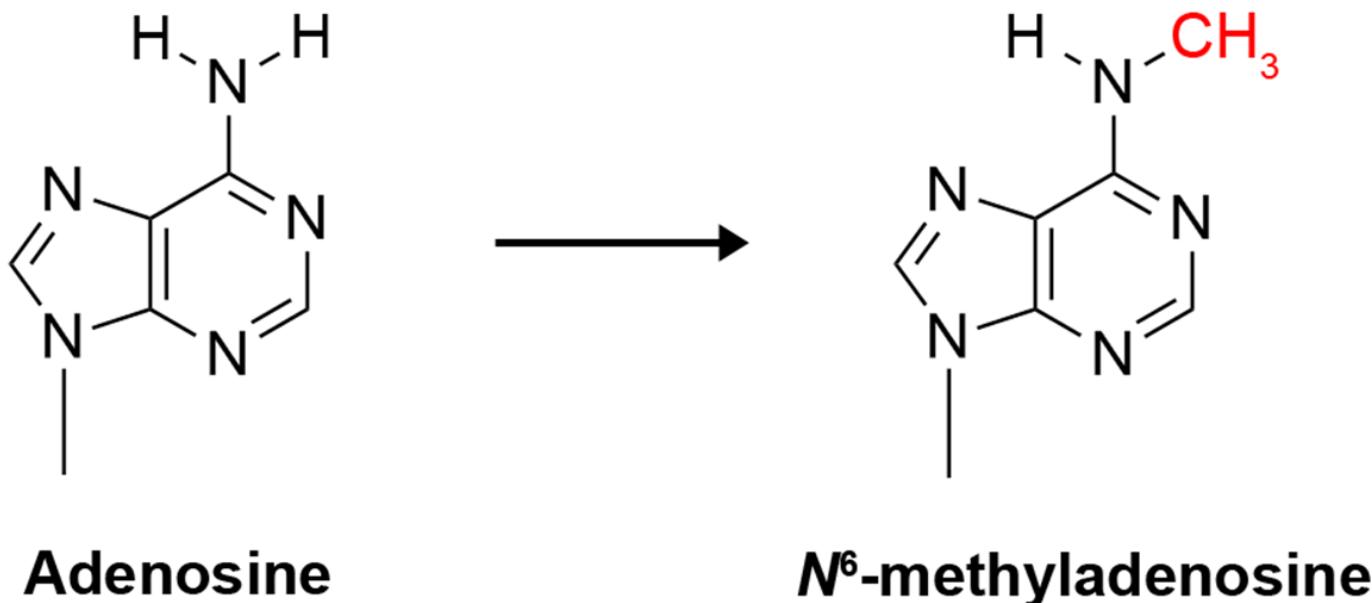
2-thiocytidine (s2C)

5-formylcytidine (f5 C)

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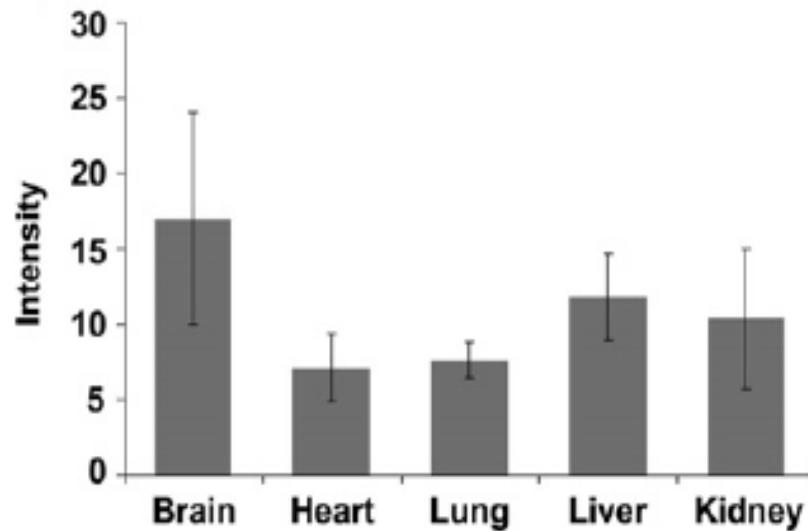
m6A: The 5th base in mRNA



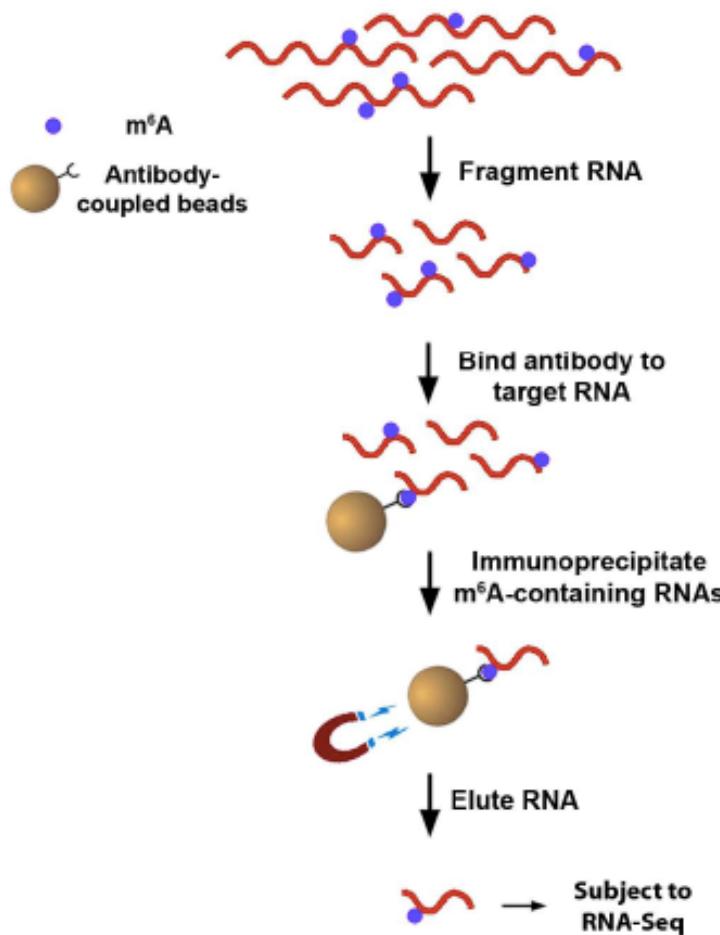
- Discovered in 1975
- Base pairing is not affected, so not easy to detect m6A
- Found in tRNA, snRNAs, ribosomal RNA, one mRNA
- Interest in m6A languished

Adapted from Samie Jaffrey

m6A: Antibodies and Transcriptome-wide Mapping



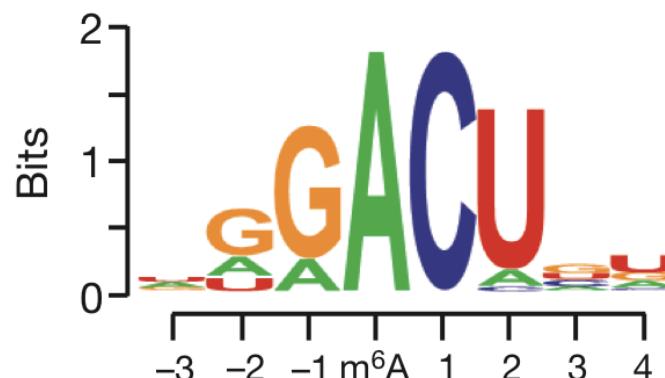
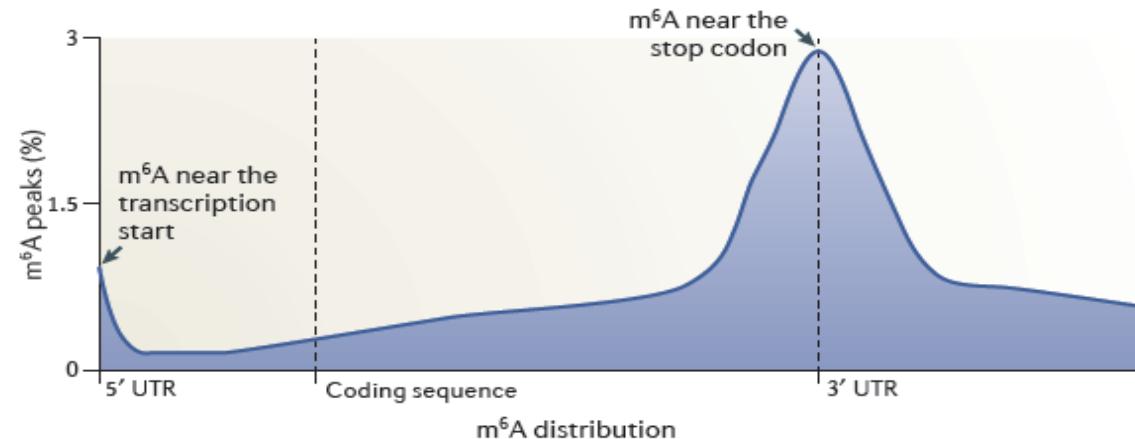
Methylated RNA IP-Seq (MeRIP-seq)



Where is the m⁶A modification found?

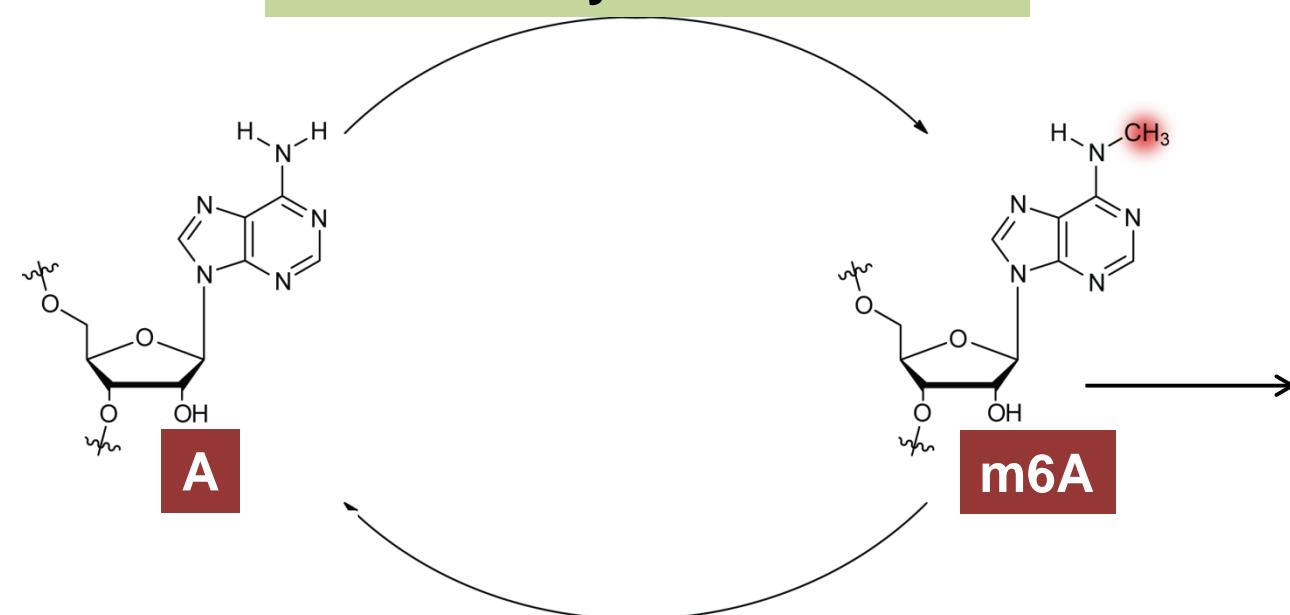
>7,000 genes encode m⁶A methylated mRNAs

>400 m⁶A peaks mapped to non-coding RNAs

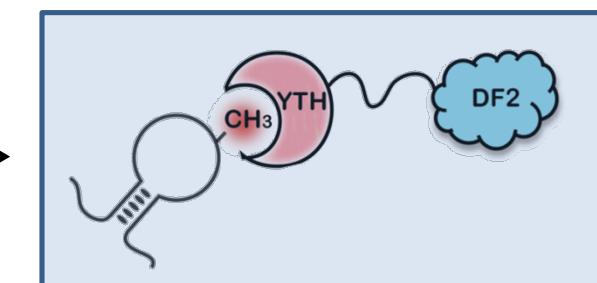


m⁶A: Readers, Writers, and Erasers (RWEs)

**Writers: METTL3/14/WTAP
m6A-methyltransferase**



**Erasers: FTO and ALKBH5
m6A demethylases**



Readers: YTHDF1,2,3

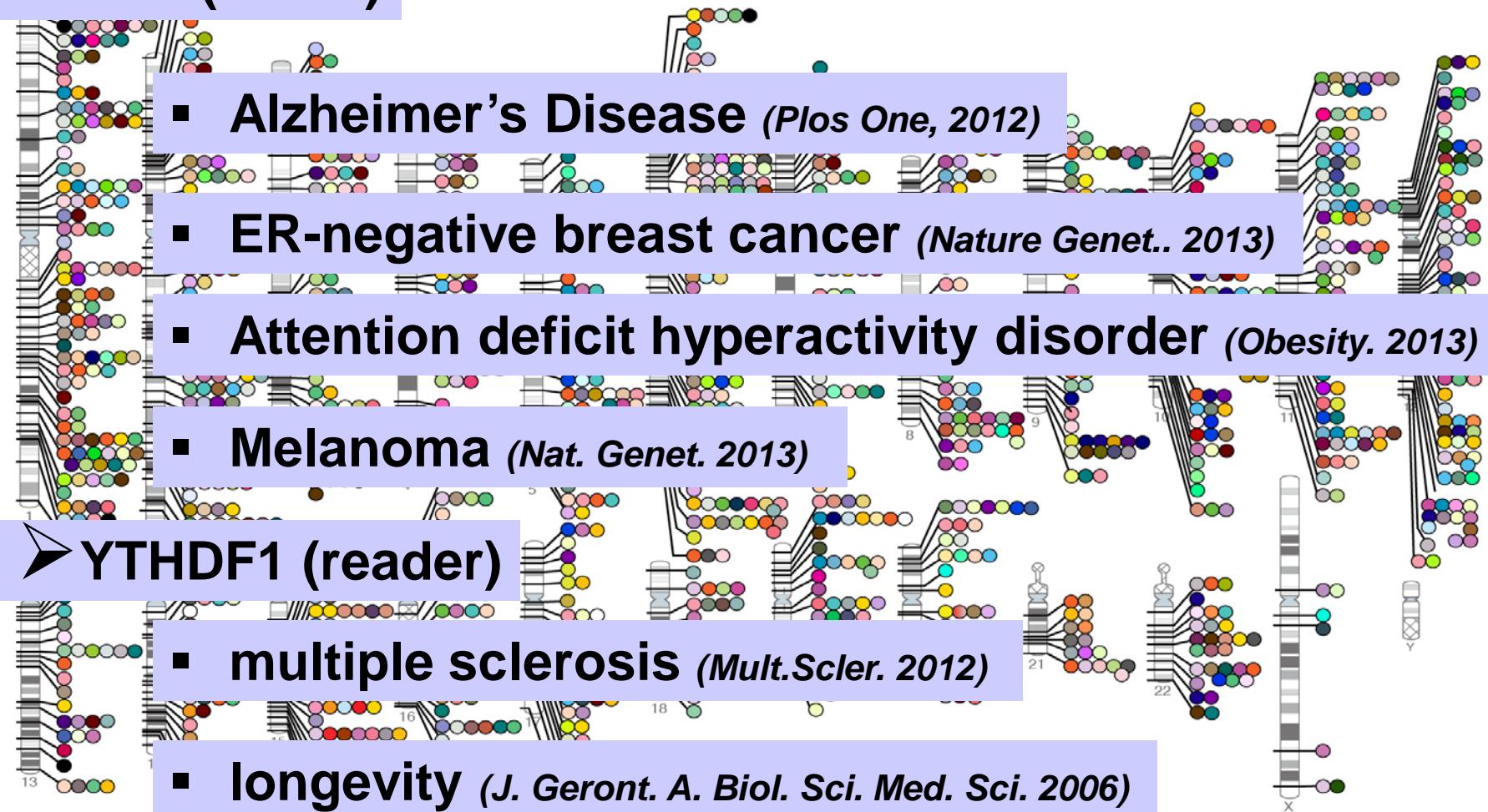
m6A, RWEs and Human Disease

➤ FTO (writer)

- Alzheimer's Disease (*Plos One*, 2012)
- ER-negative breast cancer (*Nature Genet.*.. 2013)
- Attention deficit hyperactivity disorder (*Obesity*. 2013)
- Melanoma (*Nat. Genet.* 2013)

➤ YTHDF1 (reader)

- multiple sclerosis (*Mult.Scler.* 2012)
- longevity (*J. Geront. A. Biol. Sci. Med. Sci.* 2006)



m⁶A: A Diversity of Functions

Scienceexpress

nature

Reports

m⁶A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation

Shay Geula,^{1*} Sharon Moshitch-Moshkovitz,^{2*} Dan Dominissini,^{3*} Abed AlFatah Mansour,^{1*} Nitzan Kol,² Mali Salmon-Divon,² Vera Hershkovitz,² Eyal Peer,² Nofar Mor,¹ Yair S. Manor,¹ Moshe Shay Ben-Haim,² Eran Eyal,² Sharon Yunger,² Yishay Pinto,⁴ Diego Adhemar Jaitin,⁵ Sergey Viukov,¹ Yoach Rais,¹ Vladislav Krupalnik,¹ Elad Chomsky,¹ Mirie Zerbib,¹ Itay Maza,¹ Yoav Rechavi,¹ Rada Massarwa,¹ Suhair Hanna,^{1,6} Ido Amit,⁵ Erez Y. Levanon,⁴ Ninette Amariglio,^{2,4} Noam Stern-Ginossar,¹ Noa Novershtern,^{1†‡} Gideon Rechavi,^{2†‡} Jacob H. Hanna^{1†‡}

¹The Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel. ²Cancer Research Center, Chaim

³Chemica

⁴The Univ

Japan

*Correspondence: ukamuraru@pharm.kyoto-u.ac.jp

<http://dx.doi.org/10.1016/j.cell.2013.10.026>

¹Department of Chemistry, Institute for Biophysical Dynamics, The University of Chicago, 929 East 57th Street, Chicago, IL 60637, USA

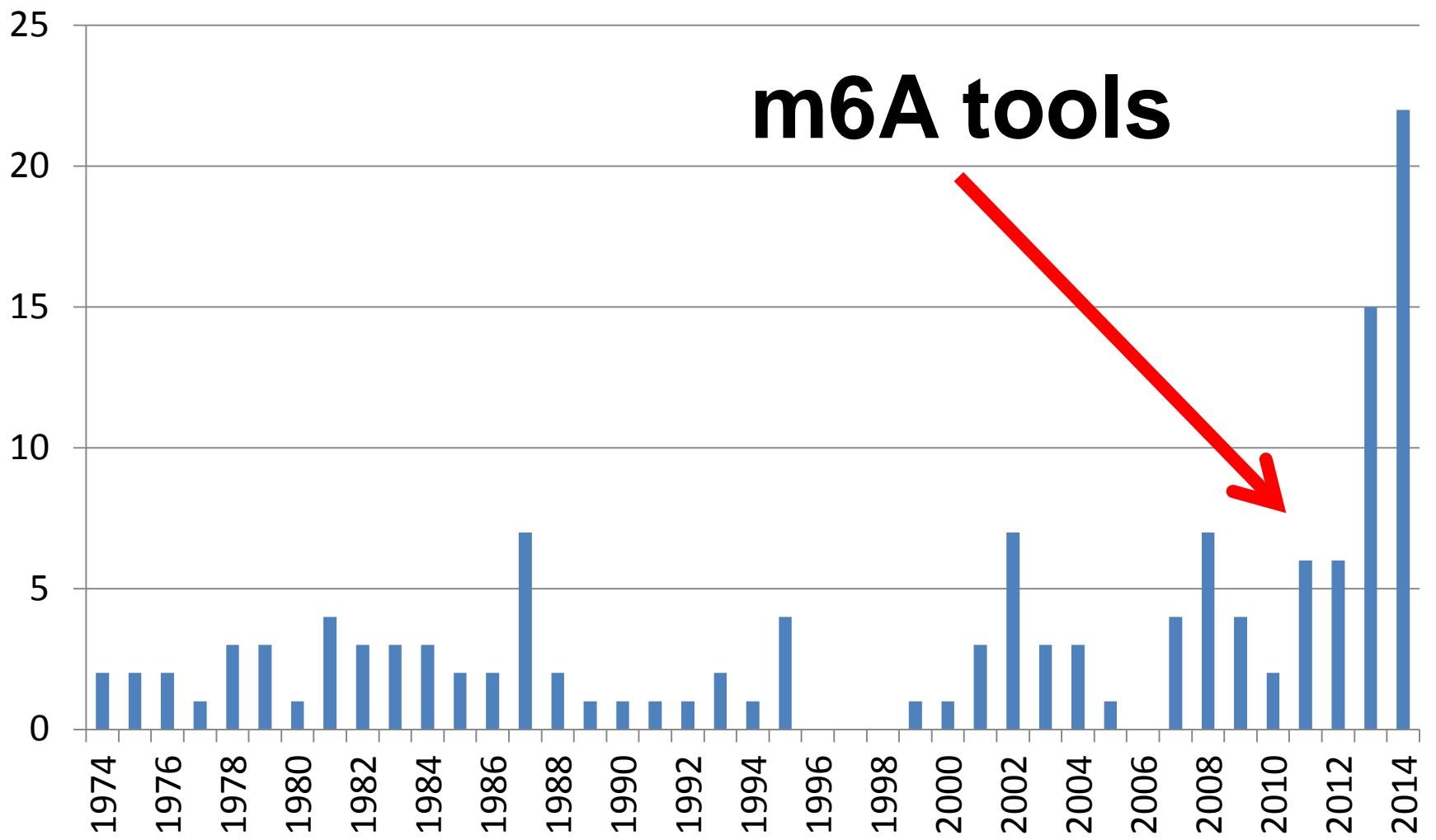
²Genome Structure & Stability Group, BIG CAS-OSLO Genome Research Cooperation, Disease Genomics and Individualized Medicine Laboratory, Beijing Institute of Genomics, Chinese Academy of Sciences, No. 7 Beitucheng West Road, Chaoyang District, Beijing 100029

rely on some of these factors (Fig. 1A and fig. S1). Regulators that specifically inhibited the stability of Oct4-GFP+ primed cells included the epigenetic repressors Dnmt1, Eed, and Suz12 polycomb components, Mbd3, and N⁶-adenosine methyltransferase Mettl3, a component of the N⁶-methyladenosine (m⁶A) mRNA methylating complex (3) (Fig. 1A and fig. S1).

We subsequently focused on the role of m⁶A in pluripotency transitions since the biological role of RNA modifications is only starting to be unveiled (4). m⁶A is an RNA modification catalyzed by Mettl3 (methyl trans-

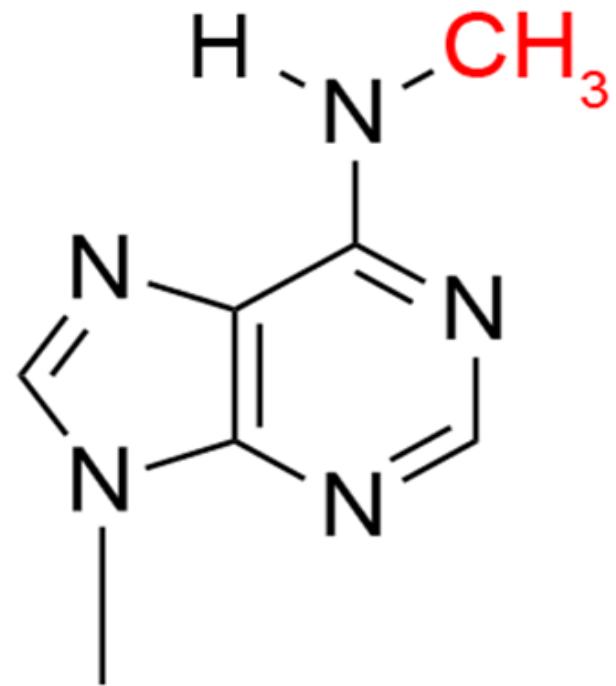
16, 2015

m6A Publication History



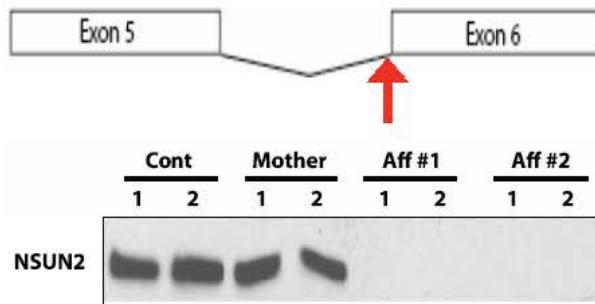
Pubmed search using 6-methyladenosine

Is m⁶A special?



***N*⁶-methyladenosine**

The 5mC Methyltransferase NSUN2 and Intellectual Disability



Neurological

microcephaly, intellectual disability, behavioral deficit, abnormal gait

Development

growth retardation

Skin

Cutaneous abnormalities, sparse hair

Fertility

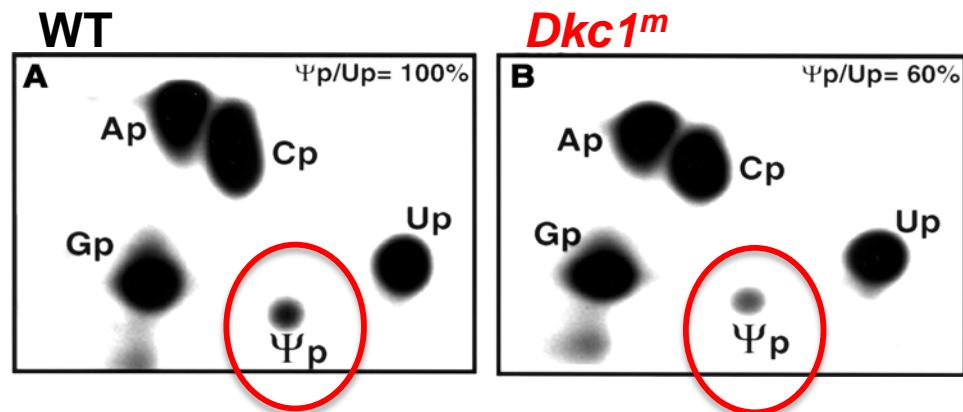
ovaries atrophy

Pseudouridine (Ψ)

DKC1 mutations reduce Ψ levels in rRNA

Dyskeratosis congenita:

- often fatal
- premature aging
- bone marrow failure
- increased suspect. to cancer



DKC1 and p27 genetically interact in pituitary tumorigenesis

Ψ present in mRNA (238 coding transcripts) and non-coding RNA

Regulated by environmental signals

Circular RNAs

ARTICLE

doi:10.1038/nature11928

Circular RNAs are a large class of animal RNAs with regulatory potency

Sebastian Memczak^{1*}, Marvin Jens^{1*}, Antigoni Elefsinioti^{1*}, Francesca Torti^{1*}, Janna Krueger², Agnieszka Rybalk¹, Luisa Maier¹, Sebastian D. Mackowiak¹, Lea H. Gregersen³, Mathias Munschauer³, Alexander Loewer⁴, Ulrike Ziebold¹, Markus Landthaler³, Christine Kocks¹, Ferdinand le Noble² & Nikolaus Rajewsky¹

Circular RNAs (circRNAs) in animals are an enigmatic class of RNA with unknown function. To explore circRNAs systematically, we sequenced and computationally analysed human, mouse and nematode RNA. We detected thousands of well-expressed, stable circRNAs, often showing tissue/developmental-stage-specific expression. Sequence analysis indicated important regulatory functions for circRNAs. We found that a human circRNA, antisense to the cerebellar degeneration-related protein 1 transcript (CDR1as), is densely bound by microRNA (miRNA) effector complexes and harbours 63 conserved binding sites for the ancient miRNA miR-7. Further analyses indicated that CDR1as functions to bind miR-7 in neuronal tissues. Human CDR1as expression in zebrafish impaired midbrain development, similar to knocking down miR-7, suggesting that CDR1as is a miRNA antagonist with a miRNA-binding capacity ten times higher than any other known transcript. Together, our data provide evidence that circRNAs form a large class of post-transcriptional regulators. Numerous circRNAs form by head-to-tail splicing of exons, suggesting previously unrecognized regulatory potential of coding sequences.

>2000 human circular RNAs predicted

Mature messenger RNAs are linear molecules with 5' ends that reflect start and stop of the RNA polymerase or poly-A tails. In cells, different RNA molecules are sometimes

LETTER

doi:10.1038/nature11993

Natural RNA circles function as efficient microRNA sponges

Thomas B. Hansen¹, Trine I. Jensen¹, Bettina H. Clausen², Jesper B. Bramsen^{1,3}, Bente Finsen², Christian K. Damgaard¹ & Jørgen Kjems^{1,3}

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression that act by direct base pairing to target

miRNA-mediated endocleavage^{12,13} (Supplementary Fig. 1b). The miR-671 target site exhibits near-perfect complementarity and very little



m6A 5mC pseudouridine

Table 1 - List of Base Modifications Covered by Claims

Abbreviation	Chemical name
m ⁶ A	1-methyl-3-(3-amino-3-carboxypropyl) pseudouridine
m ⁶ A	1-methyladenosine
m ⁵ G	1-methylguanosine
m ⁵ C	1-methylcytidine
m ⁵ C	1-methylcytidine
m ⁵ C	1-methylcytidine
m ⁵ Gm	1,2'-O-dimethylguanosine
m ⁵ Cm	1,2'-O-dimethylcytidine
m ² A	2-methyladenosine
m ² Am	2-methyladenosine, 2'-O-methyladenosine
m ² Am ⁶ A	2-methylthio- ⁶ A'-hydroxynorvalyl carbamoyl adenosine
m ² Am ⁶ A	2-methylthio- ⁶ A'-methyladenosine
m ² Am ⁶ A	2-methylthio- ⁶ A'-methyladenosine
s ² Gm	2-methylthio- ² '-O-methylguanosine
s ² Gm	2-methylthio- ² '-O-methylguanosine
s ² U	2-thiouridine
Am	2'-O-acetylguanosine
Cm	2'-O-acetylcytidine
Cm	2'-O-acetylcytidine
Im	2'-O-methylinosine
Um	2'-O-methyluridine
Um	2'-O-methyluridine
Gr(p)	2'-O-ribosylguanosine (phosphate)
c ² A ⁴ C	3,4'-cyclic-3'-carboxypropyl cytidine
m ² C	3-methylcytidine
m ² C	3-methylcytidine
m ³ U	3-methyluridine
m ³ Am	3,2'-O-bis(methylamino)-2'-O-methyluridine
img-14	4-demethylwyoisine
m ³ Am	4-demethylwyoisine
chm ⁵ U	5-(carboxyhydroxymethyl)uridine
chm ⁵ U	5-(carboxyhydroxymethyl)-2'-O-methyluridine
imhm ⁵ U	5-(isopentenylaminoethyl)-2'-thiouridine
cmhm ⁵ U	5-(carboxymethylaminoethyl)-2'-O-methyluridine
cmhm ⁵ U	5-(carboxymethylaminoethyl)-2'-O-methyluridine
cmhm ⁵ U	5-(carboxymethylaminoethyl)uridine
c ⁵ Am	5-formyl-2'-O-methylcytidine
hmc ⁵ C	5-hydroxymethylcytidine
hmc ⁵ C	5-hydroxymethylcytidine
mcm ⁵ U	5-methoxycarbonylmethyl-2'-thiouridine
mcm ⁵ U	5-methoxycarbonylmethyl-2'-O-methyluridine
mcm ⁵ U	5-methoxycarbonylmethyluridine
m ⁵ C	5-methylcytidine
m ⁵ U	5-methyluridine
m ⁵ Um	5,2'-O-dimethyluridine
prc ⁶ A	7-cyano-7-deazaguanosine
arch ⁵ A	archaeosine
eQ	epoxyguanosine
hgyW	hydroxybutyrylaine
i	isopyrimidine
img ²	isow-yosine
manQ	mannosyl-taurine
m ² Am	N ² -methyl-2'-O-methyluridine
m ⁵ G	N ¹ -methyl guanosine
m ⁵ G	N ¹ -methyl guanosine
m ⁵ G	N ¹ -methyl guanosine
m ⁵ G	N ¹ ,N ² -dimethyl guanosine
m ⁵ G	N ¹ ,N ² -dimethyl guanosine
ac ² C	N ² -acetyl cytidine
ac ² C	N ² -acetyl cytidine
m ² Cm	N ² ,2'-O-dimethylcytidine
con ⁶ A	N ⁶ -(2-hydroxypropyl)adenosine
s ² A	N ² -acetyl adenosine
g ² A	N ² -glycylcarbamoyladenosine
is ² A	N ² -isopentenyladenosine
ca ² A	N ² -cyclopentyladenosine
m ² A	N ² -methyl adenosine
m ² Am	N ² -O-allyl-2'-O-methyluridine
m ² Am	N ² ,2'-O-dimethyladenosine
m ² Am	N ² ,2'-O-dimethyladenosine
p ² U	pseudouridine
Ch ² W	2-chlorodihydro-2H-pyran-2-one

110
known
RNA
mods

How Do RNA Modifications Impact RNA Classes and Functions?

RNA Classes:

mRNA, tRNA, rRNA, microRNA, siRNA, piRNA, long non-coding RNA, snoRNA, etc.

RNA Functions:

Protein translation and localized translation

Splicing

Structural scaffold

Chromatin recruitment

Ribozymes

Environmental sensing

Post-transcripti

Gene silencing

Defense against

Intercellular signaling

RNA modification

Outline

- **Background**
- **RNA Modification Functions**
- **E4 Gaps and Opportunities**
- **E4 Program Components & Impact**

Scientists Consulted

Paul Agris, RNA Institute

Cheryl Arrowsmith, U. of Toronto

Thomas Begley, U of Albany

Howard Chang

Jim Eberwine

Dan Fabris, SUNY Albany

Chuan He, U of Chicago

Samie Jaffrey, Cornell U

Stuart Legrice, NCI

Patrick Limbach, U of Cincinnati

Chris Mason, Cornell U

Thomas Misteli, NCI

John Ngai, U of Rochester

David Newsham, Austr. Natl. U

Tamar Schlik, NYU

Gabriele Varani, U of Washington

Kevin Weeks, U of North Carolina

Jamie Williamson, Scripps

Crystal Zhao, Sanford Burnham

RNA structure-function

RNA modifying enzymes

Small molecules

Antibody development

Mass spectrometry

Chemistry

Obesity

Neurodevelopmental disorders

Cancer biology

Embryonic stem cells

Single cell imaging

Computational technology

30 RFI responses

What are the Scientific Needs in Epitranscriptomics?

A. Tools

- **Affinity reagents**
- **Small molecule modulators**
- **Computational tools**



B. Technologies

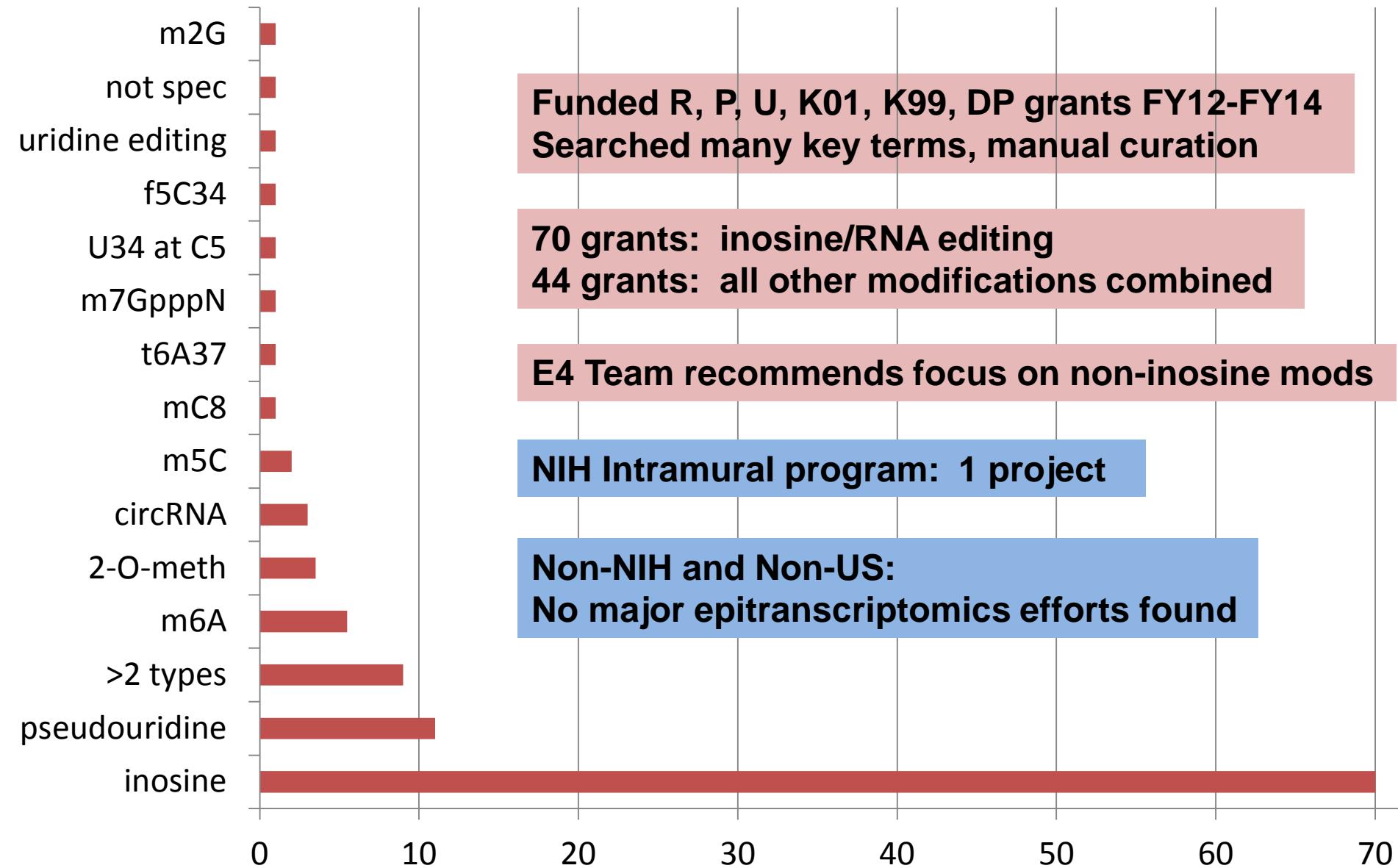
- **RNA mod low abundance detection, genomewide, single base resolution**
- **Detecting effects of RNA mods on RNA structure**
- **Imaging of RNA modifications**
- **Manipulation of RNA modifications**

C. Survey of the RNA modification landscape

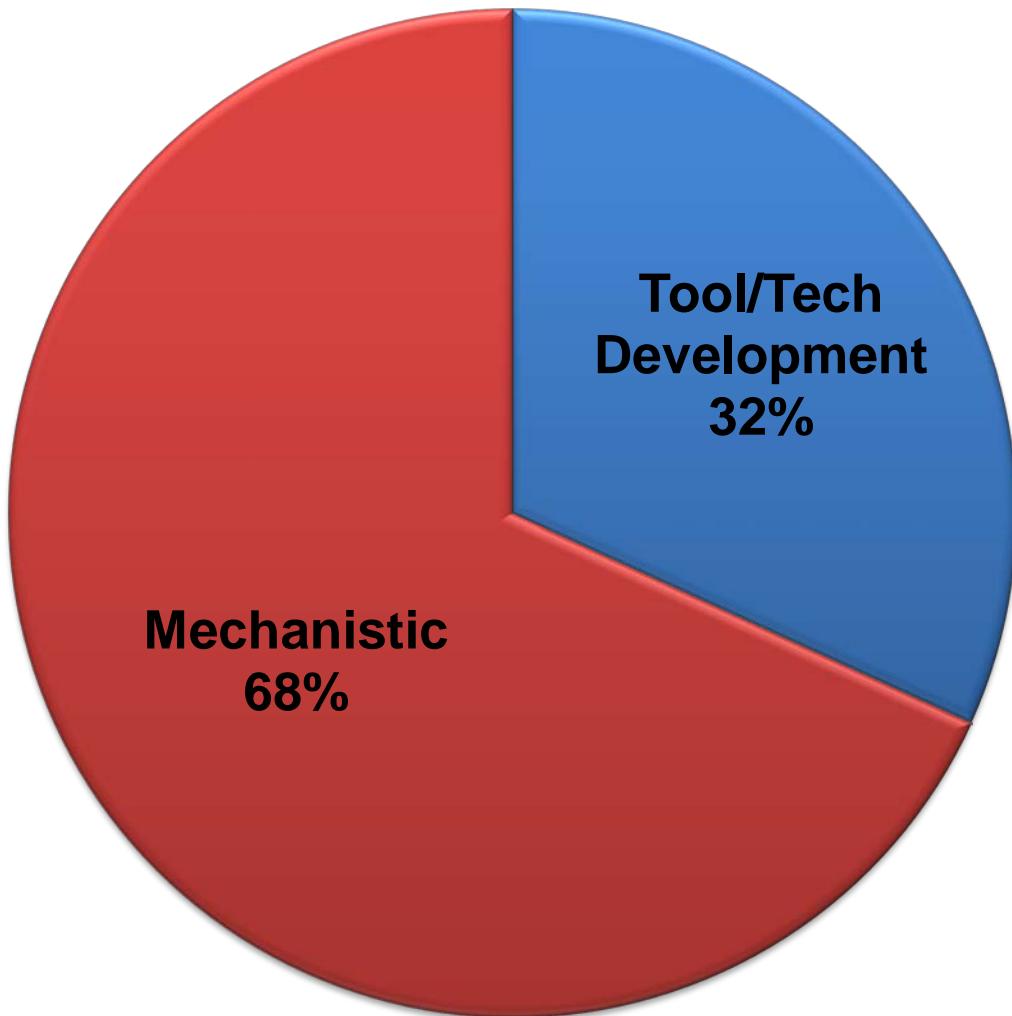
- **Discovery of novel RNA mods, RWEs**
- **Inventory of known RNA mods, RWEs**

D. Functions of RNA mods/RWEs in biological processes, health, and disease

NIH Portfolio in RNA Modifications



Limited NIH Support for RNA Mod Tool & Technology Development

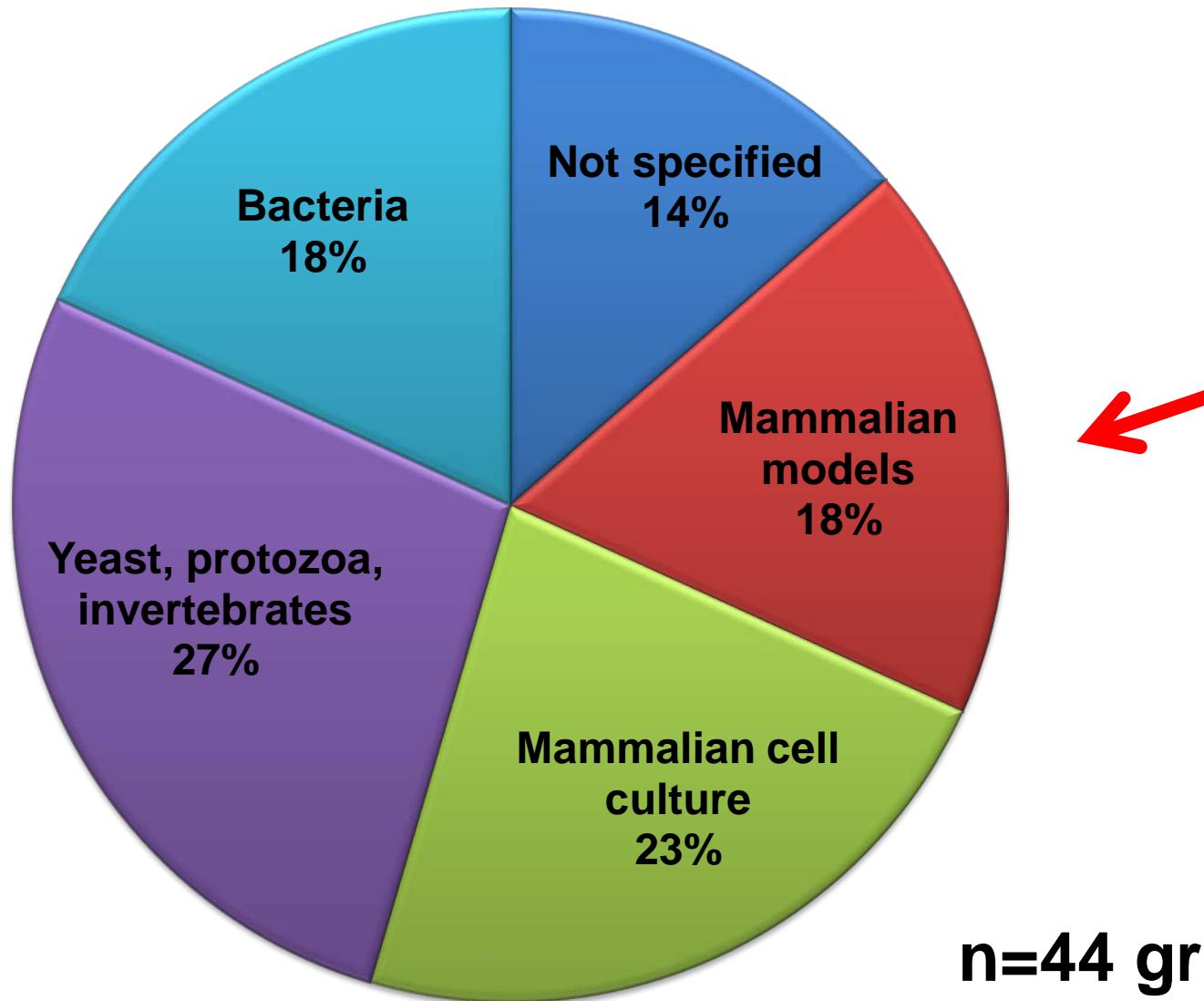


Half are SBIR/STTR grants
from 2014 NIDA RFA



n=44 grants

Comparatively Few Studies Using *in vivo* Mammalian Models



Outline

- **Background**
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- **E4 Program Components & Impact**

Key E4 Program Themes

A. Tools

- Affinity reagents
- Small molecule modulators
- Computational tools



B. Technologies

- RNA mod low abundance detection, genomewide, single base resolution
- Detecting effects of RNA mods on RNA structure
- Imaging of RNA modifications
- Manipulation of RNA modifications

CF NCI Proposal

C. Catalog of the RNA modification landscape

- Discovery of novel RNA mods, RWEs
- Inventory of known RNA mods, RWEs

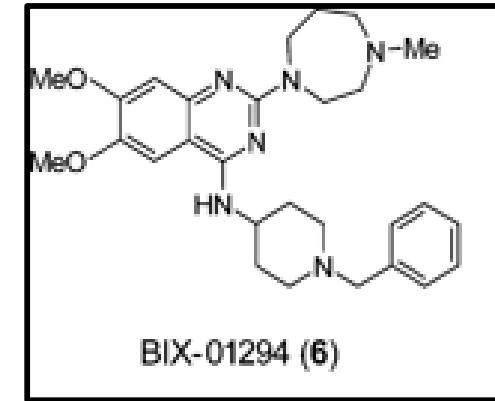
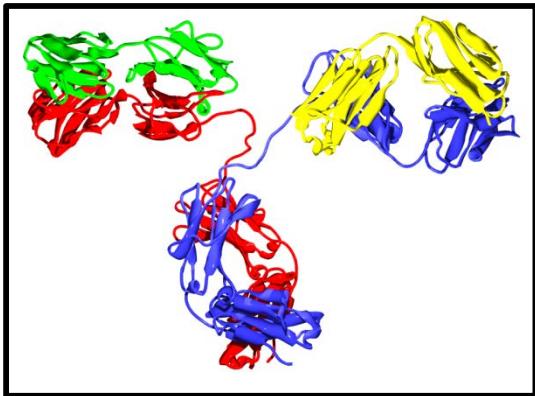
D. Functions of RNA mods/RWEs in biological processes, health, and disease

Epitranscriptomic Tools

GAP: Lack of essential tools to monitor and manipulate most RNA modifications

INITIATIVE: Develop enabling and transformative:

- affinity reagents
- genetic tools and models
- small molecule modulators

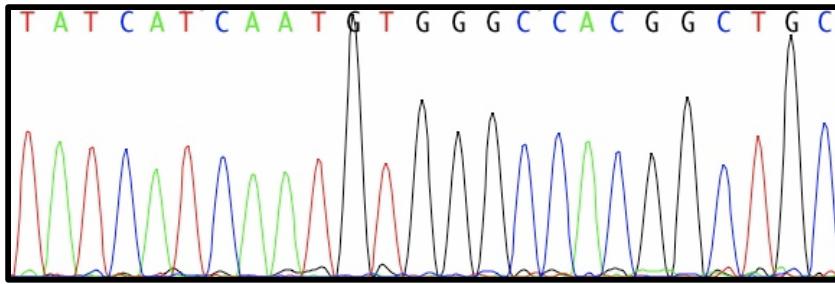


MECHANISM: CF-supported UH2 and IC-supported SBIR/STTR

DELIVERABLES: Defined # of tools for a diversity of RNA mods

Epitranscriptomic Technologies

GAP: Lack critical technologies to monitor & manipulate most RNA mods



INITIATIVE: Develop enabling and transformative technologies:

- Detect low levels of RNA mods
- Transcriptome-wide RNA mod assays at single nucleotide resolution
- Tools to image and manipulate RNA mods or RWEs *in vivo*
- Computational strategies---predict effects on RNA structure/function

MECHANISM: CF-supported UH2/UH3 and IC-supported SBIR/STTR

DELIVERABLES: Defined # of technologies for a diversity of RNA mods

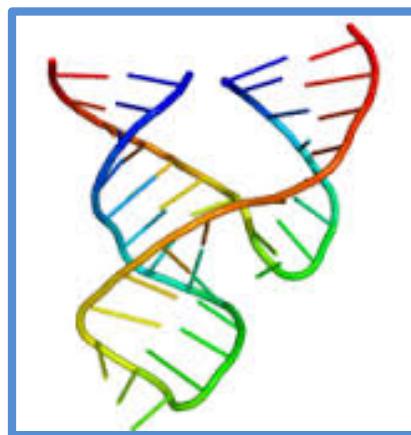
Novel Epitranscriptomic Components

GAP: Only partial inventory of RNA mod reader, writer, and eraser (RWE) (proteins or ribozymes)

INITIATIVE: Support discovery or confirmation of currently unknown readers, writers, erasers, and modifications in any model system (UH2)

Follow up work on function and role in vertebrate systems (UH3)

MECHANISM: UH2/UH3



DELIVERABLES: Defined # new RWEs or RNA mods.

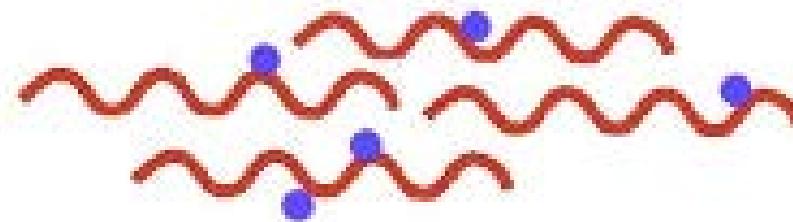
Epitranscriptome Catalog: Phase I and II

GAP: Very limited knowledge of the landscape (abundance and dynamics) of known RNA Mods in different tissues and RNA biotypes

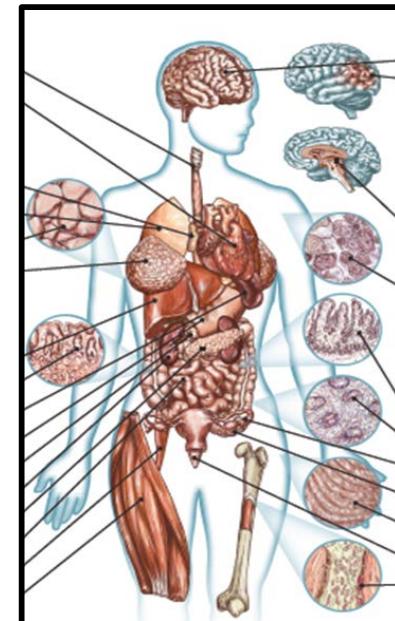
INITIATIVE: Phase I. Exploit current and emerging technologies to inventory known RNA Mods and RWEs

Phase II. Expand to catalog key modified RNAs quantitatively at single nucleotide resolution (the Epitranscriptome). Focus on:

- RNA classes (e.g. mRNA, tRNA, microRNA, long non-coding RNA)
- Disease-relevant mammalian cell types and tissues
- Environmental exposures and RNA mod dynamics



MECHANISM: U01



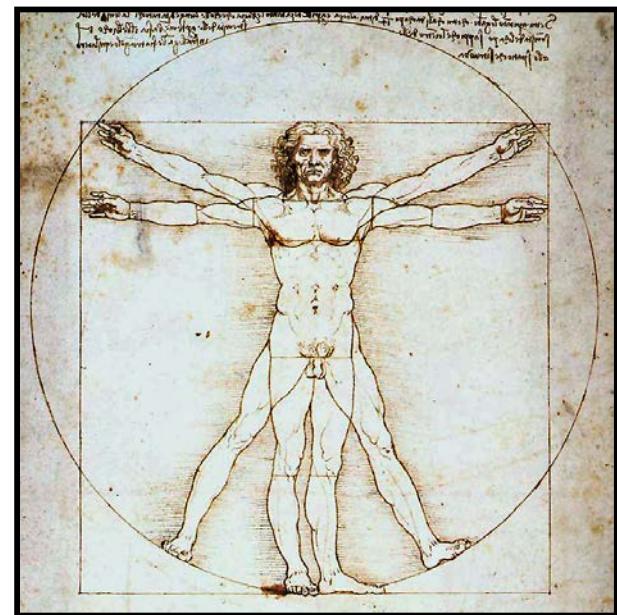
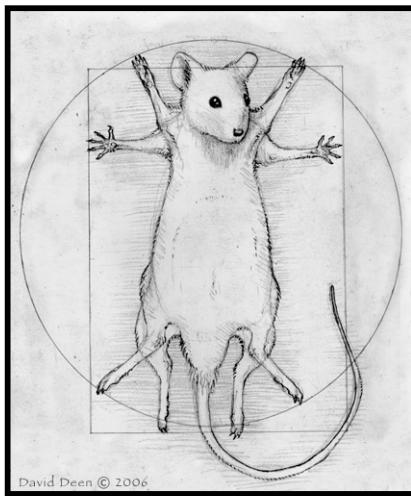
DELIVERABLES: The Epitranscriptome Catalog for a specified # of RNA mods at single nucleotide resolution for a defined #of tissues/conditions.

E4 Demonstration Projects

GAP: We do not fully understand the potential roles of RNA mods in biological processes and disease.

INITIATIVE: Demonstration projects exploiting E4 Program deliverables to investigate the function of RNA modifications.

Emphasize RNA mod quantitation and dynamics



MECHANISM: U01 or R01 Competitive Revision

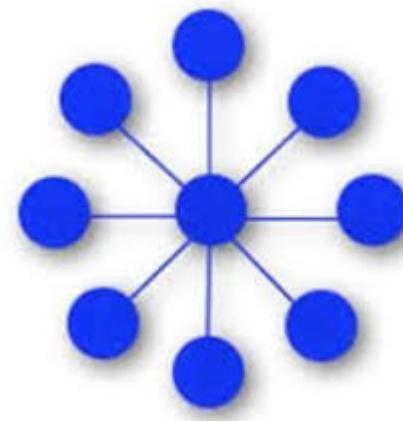
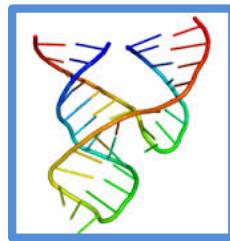
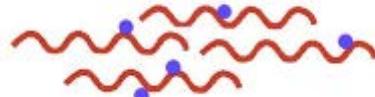
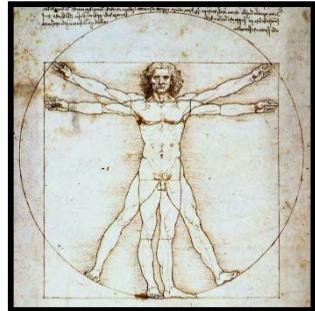
DELIVERABLES: Characterize the roles of a specific # of RNA mods in a diverse selection of biological processes and diseases

Epitranscriptomics Data Hub

GAP: No centralized place to integrate RNA mod knowledge with disease and other datatypes

INITIATIVE: An Epitranscriptomics Data Hub for access to E4 deliverables:

- protocols, tools, technologies, publications
 - links to existing RNA mod resources
 - data coordination and access to Epitranscriptome catalog
 - RNA mod analysis tools and guidance
 - scientific outreach (meetings, workshops, etc)
 - Link RNA mod phenotypes to human disease



MECHANISM: U54

DELIVERABLES: Website, defined # of protocols, data sets, outreach activities
Enable the scientific community to exploit E4 deliverables

Phase I

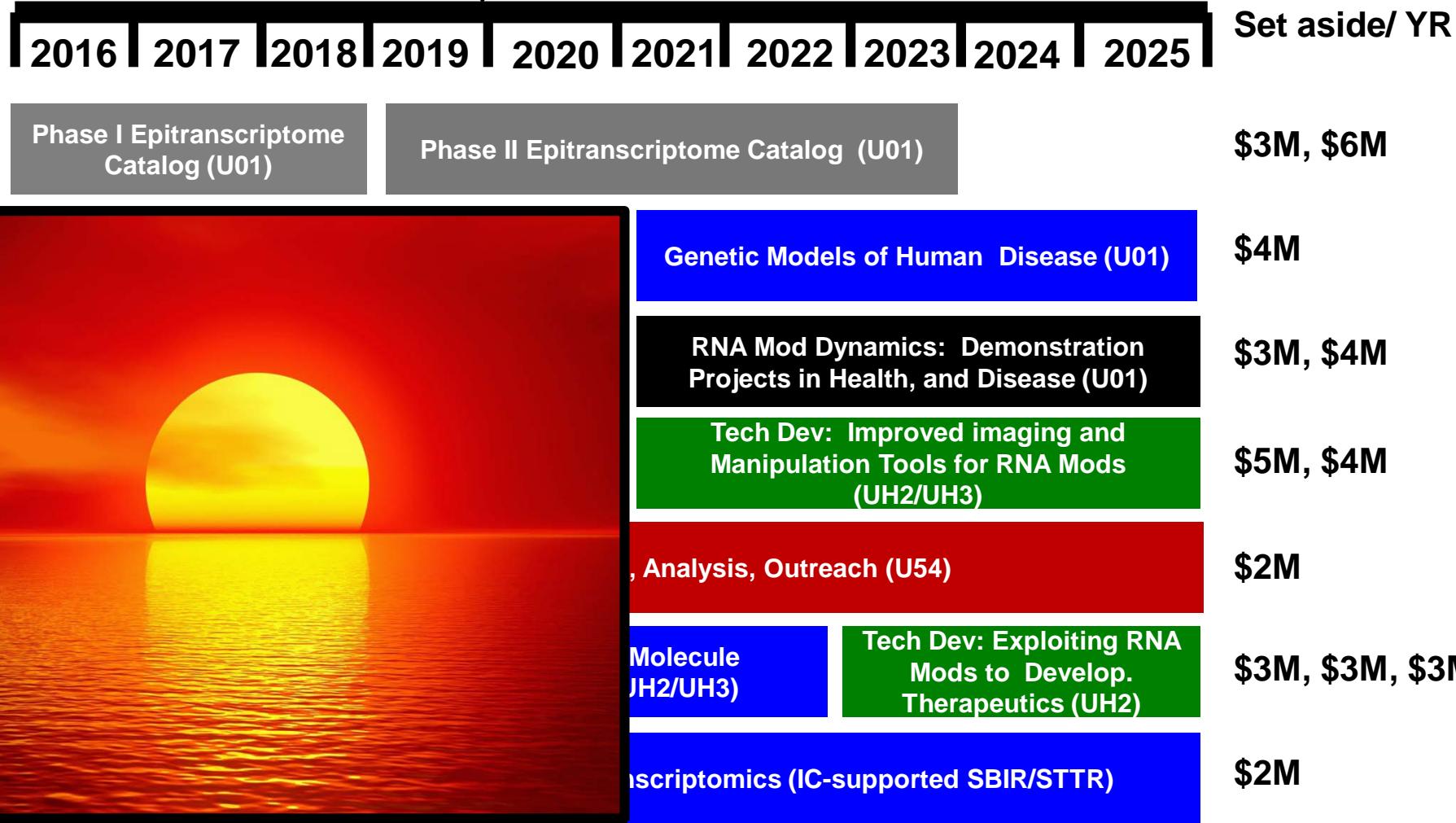
Phase II

	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	Set aside/ YR
Phase I Epitranscriptome Catalog (U01)											\$3M, \$6M
Discovery: Novel RNA Mods, Writers, Erasers, and Readers (UH2/UH3)											\$4M, \$4M
Tech Dev: Improved Computational Tools for RNA Mods (UH2/UH3)											\$3M, \$4M
Tech Dev: Improved Detection/Sequencing of RNA Mods (UH2/UH3)											\$5M, \$4M
Data Coordination, Analysis, Outreach (U54)											\$2M
Tools: Affinity Reagents (UH2)											\$3M, \$3M, \$3M
Tools: Small Molecule Modulators (UH2/UH3)											
Tech Dev: Exploiting RNA Mods to Develop Therapeutics (UH2)											
Develop Tools and Technologies for Epitranscriptomics (IC-supported SBIR/STTR)											\$2M

CF \$	18M	20M	20M	23M	23M	22M	22M	22M	16M	16M	\$185M CF
IC \$	2M	\$20M IC									
Ttl \$	20M	22M	22M	25M	25M	24M	24M	24M	18M	18M	\$205M Total

Phase I

Phase II



CF \$	18M	20M	20M	23M	23M	22M	22M	22M	16M	16M	\$185M CF
IC \$	2M	\$20M IC									
Ttl \$	20M	22M	22M	25M	25M	24M	24M	24M	18M	18M	\$205M Total

Leveraging Other Projects and Resources

- ENCODE/Epigenomics/4DN? Integrate E4 data with their datasets
- BD2K: Align E4 data sets into future BD2K framework
- GWAS? Integration of RNA modification sites with SNPs and gene expression levels for particular tissues and diseases
- GTEx: Leverage GTEx post-mortem tissues samples for inventory if sufficient quantities and collection
- exRNA: Leverage body fluid samples from exRNA program to explore RNA mods in exRNAs
- SGC: Work together to develop small molecule modulators for Epitranscriptomic readers, writers, and erasers
- International: German funding agencies have expressed interest in this topic. Others may also be interested.
- KOMP: use to help develop mouse models of E4 disease

Beautify!

E4 Program Outcomes

New RNA Mod Tools & Technologies:

- Catalyze the scientific community to investigate the role of RNA mods in biological & disease processes

RNA Mod Discovery & Catalog:

- See the RNA Mod “landscape” for the first time
- Enable the scientific community to generate hypotheses about the role of RNA mods in biological processes and diseases

Overall:

- Transform our understanding of the role of RNA Mods in wide range of critical biological & disease processes
- Provide a firm foundation to exploit new knowledge about RNA Mods & their function to prevent, diagnose, & treat disease

Table 1 - List of RNA Modifications Covered by Claims	
Abbreviation	Chemical Name
m ¹ A	Smino-1-Aminoimidazole-4-carboxamide ribonucleotide
m ² C	2-Carboxycytidine
m ² G	2-Guanosine
m ² T	2-Thioguanosine
m ³ G	3-Guanosine
m ³ U	3-Uridine
m ⁴ C	4-Cytidine
m ⁵ C	5-Cytidine
m ⁶ A	Smino-6-Aminohypoxanthine-6-ribonucleotide
m ⁶ C	6-Cytidine
m ⁶ G	6-Guanosine
m ⁶ U	6-Uridine
m ⁷ G	7-Guanosine
m ⁷ U	7-Uridine
m ⁸ A	Smino-8-Aminoadenosine
m ⁹ C	9-Cytidine
m ¹⁰ G	10-Guanosine
m ¹¹ U	11-Uridine
m ¹² A	Smino-12-Aminoadenosine
m ¹³ C	13-Cytidine
m ¹⁴ G	14-Guanosine
m ¹⁵ U	15-Uridine
m ¹⁶ A	Smino-16-Aminoadenosine
m ¹⁷ C	17-Cytidine
m ¹⁸ G	18-Guanosine
m ¹⁹ U	19-Uridine
m ²⁰ A	Smino-20-Aminoadenosine
m ²¹ C	21-Cytidine
m ²² G	22-Guanosine
m ²³ U	23-Uridine
m ²⁴ A	Smino-24-Aminoadenosine
m ²⁵ C	25-Cytidine
m ²⁶ G	26-Guanosine
m ²⁷ U	27-Uridine
m ²⁸ A	Smino-28-Aminoadenosine
m ²⁹ C	29-Cytidine
m ³⁰ G	30-Guanosine
m ³¹ U	31-Uridine
m ³² A	Smino-32-Aminoadenosine
m ³³ C	33-Cytidine
m ³⁴ G	34-Guanosine
m ³⁵ U	35-Uridine
m ³⁶ A	Smino-36-Aminoadenosine
m ³⁷ C	37-Cytidine
m ³⁸ G	38-Guanosine
m ³⁹ U	39-Uridine
m ⁴⁰ A	Smino-40-Aminoadenosine
m ⁴¹ C	41-Cytidine
m ⁴² G	42-Guanosine
m ⁴³ U	43-Uridine
m ⁴⁴ A	Smino-44-Aminoadenosine
m ⁴⁵ C	45-Cytidine
m ⁴⁶ G	46-Guanosine
m ⁴⁷ U	47-Uridine
m ⁴⁸ A	Smino-48-Aminoadenosine
m ⁴⁹ C	49-Cytidine
m ⁵⁰ G	50-Guanosine
m ⁵¹ U	51-Uridine
m ⁵² A	Smino-52-Aminoadenosine
m ⁵³ C	53-Cytidine
m ⁵⁴ G	54-Guanosine
m ⁵⁵ U	55-Uridine
m ⁵⁶ A	Smino-56-Aminoadenosine
m ⁵⁷ C	57-Cytidine
m ⁵⁸ G	58-Guanosine
m ⁵⁹ U	59-Uridine
m ⁶⁰ A	Smino-60-Aminoadenosine
m ⁶¹ C	61-Cytidine
m ⁶² G	62-Guanosine
m ⁶³ U	63-Uridine
m ⁶⁴ A	Smino-64-Aminoadenosine
m ⁶⁵ C	65-Cytidine
m ⁶⁶ G	66-Guanosine
m ⁶⁷ U	67-Uridine
m ⁶⁸ A	Smino-68-Aminoadenosine
m ⁶⁹ C	69-Cytidine
m ⁷⁰ G	70-Guanosine
m ⁷¹ U	71-Uridine
m ⁷² A	Smino-72-Aminoadenosine
m ⁷³ C	73-Cytidine
m ⁷⁴ G	74-Guanosine
m ⁷⁵ U	75-Uridine
m ⁷⁶ A	Smino-76-Aminoadenosine
m ⁷⁷ C	77-Cytidine
m ⁷⁸ G	78-Guanosine
m ⁷⁹ U	79-Uridine
m ⁸⁰ A	Smino-80-Aminoadenosine
m ⁸¹ C	81-Cytidine
m ⁸² G	82-Guanosine
m ⁸³ U	83-Uridine
m ⁸⁴ A	Smino-84-Aminoadenosine
m ⁸⁵ C	85-Cytidine
m ⁸⁶ G	86-Guanosine
m ⁸⁷ U	87-Uridine
m ⁸⁸ A	Smino-88-Aminoadenosine
m ⁸⁹ C	89-Cytidine
m ⁹⁰ G	90-Guanosine
m ⁹¹ U	91-Uridine
m ⁹² A	Smino-92-Aminoadenosine
m ⁹³ C	93-Cytidine
m ⁹⁴ G	94-Guanosine
m ⁹⁵ U	95-Uridine
m ⁹⁶ A	Smino-96-Aminoadenosine
m ⁹⁷ C	97-Cytidine
m ⁹⁸ G	98-Guanosine
m ⁹⁹ U	99-Uridine
m ¹⁰⁰ A	Smino-100-Aminoadenosine
m ¹⁰¹ C	101-Cytidine
m ¹⁰² G	102-Guanosine
m ¹⁰³ U	103-Uridine
m ¹⁰⁴ A	Smino-104-Aminoadenosine
m ¹⁰⁵ C	105-Cytidine
m ¹⁰⁶ G	106-Guanosine
m ¹⁰⁷ U	107-Uridine
m ¹⁰⁸ A	Smino-108-Aminoadenosine
m ¹⁰⁹ C	109-Cytidine
m ¹¹⁰ G	110-Guanosine
m ¹¹¹ U	111-Uridine
m ¹¹² A	Smino-112-Aminoadenosine
m ¹¹³ C	113-Cytidine
m ¹¹⁴ G	114-Guanosine
m ¹¹⁵ U	115-Uridine
m ¹¹⁶ A	Smino-116-Aminoadenosine
m ¹¹⁷ C	117-Cytidine
m ¹¹⁸ G	118-Guanosine
m ¹¹⁹ U	119-Uridine
m ¹²⁰ A	Smino-120-Aminoadenosine
m ¹²¹ C	121-Cytidine
m ¹²² G	122-Guanosine
m ¹²³ U	123-Uridine
m ¹²⁴ A	Smino-124-Aminoadenosine
m ¹²⁵ C	125-Cytidine
m ¹²⁶ G	126-Guanosine
m ¹²⁷ U	127-Uridine
m ¹²⁸ A	Smino-128-Aminoadenosine
m ¹²⁹ C	129-Cytidine
m ¹³⁰ G	130-Guanosine
m ¹³¹ U	131-Uridine
m ¹³² A	Smino-132-Aminoadenosine
m ¹³³ C	133-Cytidine
m ¹³⁴ G	134-Guanosine
m ¹³⁵ U	135-Uridine
m ¹³⁶ A	Smino-136-Aminoadenosine
m ¹³⁷ C	137-Cytidine
m ¹³⁸ G	138-Guanosine
m ¹³⁹ U	139-Uridine
m ¹⁴⁰ A	Smino-140-Aminoadenosine
m ¹⁴¹ C	141-Cytidine
m ¹⁴² G	142-Guanosine
m ¹⁴³ U	143-Uridine
m ¹⁴⁴ A	Smino-144-Aminoadenosine
m ¹⁴⁵ C	145-Cytidine
m ¹⁴⁶ G	146-Guanosine
m ¹⁴⁷ U	147-Uridine
m ¹⁴⁸ A	Smino-148-Aminoadenosine
m ¹⁴⁹ C	149-Cytidine
m ¹⁵⁰ G	150-Guanosine
m ¹⁵¹ U	151-Uridine
m ¹⁵² A	Smino-152-Aminoadenosine
m ¹⁵³ C	153-Cytidine
m ¹⁵⁴ G	154-Guanosine
m ¹⁵⁵ U	155-Uridine
m ¹⁵⁶ A	Smino-156-Aminoadenosine
m ¹⁵⁷ C	157-Cytidine
m ¹⁵⁸ G	158-Guanosine
m ¹⁵⁹ U	159-Uridine
m ¹⁶⁰ A	Smino-160-Aminoadenosine
m ¹⁶¹ C	161-Cytidine
m ¹⁶² G	162-Guanosine
m ¹⁶³ U	163-Uridine
m ¹⁶⁴ A	Smino-164-Aminoadenosine
m ¹⁶⁵ C	165-Cytidine
m ¹⁶⁶ G	166-Guanosine
m ¹⁶⁷ U	167-Uridine
m ¹⁶⁸ A	Smino-168-Aminoadenosine
m ¹⁶⁹ C	169-Cytidine
m ¹⁷⁰ G	170-Guanosine
m ¹⁷¹ U	171-Uridine
m ¹⁷² A	Smino-172-Aminoadenosine
m ¹⁷³ C	173-Cytidine
m ¹⁷⁴ G	174-Guanosine
m ¹⁷⁵ U	175-Uridine
m ¹⁷⁶ A	Smino-176-Aminoadenosine
m ¹⁷⁷ C	177-Cytidine
m ¹⁷⁸ G	178-Guanosine
m ¹⁷⁹ U	179-Uridine
m ¹⁸⁰ A	Smino-180-Aminoadenosine
m ¹⁸¹ C	181-Cytidine
m ¹⁸² G	182-Guanosine
m ¹⁸³ U	183-Uridine
m ¹⁸⁴ A	Smino-184-Aminoadenosine
m ¹⁸⁵ C	185-Cytidine
m ¹⁸⁶ G	186-Guanosine
m ¹⁸⁷ U	187-Uridine
m ¹⁸⁸ A	Smino-188-Aminoadenosine
m ¹⁸⁹ C	189-Cytidine
m ¹⁹⁰ G	190-Guanosine
m ¹⁹¹ U	191-Uridine
m ¹⁹² A	Smino-192-Aminoadenosine
m ¹⁹³ C	193-Cytidine
m ¹⁹⁴ G	194-Guanosine
m ¹⁹⁵ U	195-Uridine
m ¹⁹⁶ A	Smino-196-Aminoadenosine
m ¹⁹⁷ C	197-Cytidine
m ¹⁹⁸ G	198-Guanosine
m ¹⁹⁹ U	199-Uridine
m ²⁰⁰ A	Smino-200-Aminoadenosine
m ²⁰¹ C	201-Cytidine
m ²⁰² G	202-Guanosine
m ²⁰³ U	203-Uridine
m ²⁰⁴ A	Smino-204-Aminoadenosine
m ²⁰⁵ C	205-Cytidine
m ²⁰⁶ G	206-Guanosine
m ²⁰⁷ U	207-Uridine
m ²⁰⁸ A	Smino-208-Aminoadenosine
m ²⁰⁹ C	209-Cytidine
m ²¹⁰ G	210-Guanosine
m ²¹¹ U	211-Uridine
m ²¹² A	Smino-212-Aminoadenosine
m ²¹³ C	213-Cytidine
m ²¹⁴ G	214-Guanosine
m ²¹⁵ U	215-Uridine
m ²¹⁶ A	Smino-216-Aminoadenosine
m ²¹⁷ C	217-Cytidine
m ²¹⁸ G	218-Guanosine
m ²¹⁹ U	219-Uridine
m ²²⁰ A	Smino-220-Aminoadenosine
m ²²¹ C	221-Cytidine
m ²²² G	222-Guanosine
m ²²³ U	223-Uridine
m ²²⁴ A	Smino-224-Aminoadenosine
m ²²⁵ C	225-Cytidine
m ²²⁶ G	226-Guanosine
m ²²⁷ U	227-Uridine
m ²²⁸ A	Smino-228-Aminoadenosine
m ²²⁹ C	229-Cytidine
m ²³⁰ G	230-Guanosine
m ²³¹ U	231-Uridine
m ²³² A	Smino-232-Aminoadenosine
m ²³³ C	233-Cytidine
m ²³⁴ G	234-Guanosine
m ²³⁵ U	235-Uridine
m ²³⁶ A	Smino-236-Aminoadenosine
m ²³⁷ C	237-Cytidine
m ²³⁸ G	238-Guanosine
m ²³⁹ U	239-Uridine
m ²⁴⁰ A	Smino-240-Aminoadenosine
m ²⁴¹ C	241-Cytidine
m ²⁴² G	242-Guanosine
m ²⁴³ U	243-Uridine
m ²⁴⁴ A	Smino-244-Aminoadenosine
m ²⁴⁵ C	245-Cytidine
m ²⁴⁶ G	246-Guanosine
m ²⁴⁷ U	247-Uridine
m ²⁴⁸ A	Smino-248-Aminoadenosine
m ²⁴⁹ C	249-Cytidine
m ²⁵⁰ G	250-Guanosine
m ²⁵¹ U	251-Uridine
m ²⁵² A	Smino-252-Aminoadenosine
m ²⁵³ C	253-Cytidine
m ²⁵⁴ G	254-Guanosine
m ²⁵⁵ U	255-Uridine
m ²⁵⁶ A	Smino-256-Aminoadenosine
m ²⁵⁷ C	257-Cytidine
m ²⁵⁸ G	258-Guanosine
m ²⁵⁹ U	259-Uridine
m ²⁶⁰ A	Smino-260-Aminoadenosine
m ²⁶¹ C	261-Cytidine
m ²⁶² G	262-Guanosine
m ²⁶³ U	263-Uridine
m ²⁶⁴ A	Smino-264-Aminoadenosine
m ²⁶⁵ C	265-Cytidine
m ²⁶⁶ G	266-Guanosine
m ²⁶⁷ U	267-Uridine
m ²⁶⁸ A	Smino-268-Aminoadenosine
m ²⁶⁹ C	269-Cytidine
m ²⁷⁰ G	270-Guanosine
m ²⁷¹ U	271-Uridine
m ²⁷² A	Smino-272-Aminoadenosine
m ²⁷³ C	273-Cytidine
m ²⁷⁴ G	274-Guanosine
m ²⁷⁵ U	275-Uridine
m ²⁷⁶ A	Smino-276-Aminoadenosine
m ²⁷⁷ C	277-Cytidine
m ²⁷⁸ G	278-Guanosine
m ²⁷⁹ U	279-Uridine
m ²⁸⁰ A	Smino-280-Aminoadenosine
m ²⁸¹ C	281-Cytidine
m ²⁸² G	282-Guanosine
m ²⁸³ U	283-Uridine
m ²⁸⁴ A	Smino-284-Aminoadenosine
m ²⁸⁵ C	285-Cytidine
m ²⁸⁶ G	286-Guanosine
m ²⁸⁷ U	287-Uridine
m ²⁸⁸ A	Smino-288-Aminoadenosine
m ²⁸⁹ C	289-Cytidine
m ²⁹⁰ G	290-Guanosine
m ²⁹¹ U	291-Uridine
m ²⁹² A	Smino-292-Aminoadenosine
m ²⁹³ C	293-Cytidine
m ²⁹⁴ G	294-Guanosine
m ²⁹⁵ U	295-Uridine
m ²⁹⁶ A	Smino-296-Aminoadenosine
m ²⁹⁷ C	297-Cytidine
m ²⁹⁸ G	298-Guanosine
m ²⁹⁹ U	299-Uridine
m ³⁰⁰ A	Smino-300-Aminoadenosine
m ³⁰¹ C	301-Cytidine
m ³⁰² G	302-Guanosine
m ³⁰³ U	303-Uridine
m ³⁰⁴ A	Smino-304-Aminoadenosine
m ³⁰⁵ C	305-Cytidine
m ³⁰⁶ G	306-Guanosine
m ³⁰⁷ U	307-Uridine
m ³⁰⁸ A	Smino-308-Aminoadenosine
m ³⁰⁹ C	309-Cytidine
m ³¹⁰ G	310-Guanosine
m ³¹¹ U	311-Uridine
m ³¹² A	Smino-312-Aminoadenosine
m ³¹³ C	313-Cytidine
m ³¹⁴ G	314-Guanosine
m ³¹⁵ U	315-Uridine
m ³¹⁶ A	Smino-316-Aminoadenosine
m ³¹⁷ C	317-Cytidine
m ³¹⁸ G	318-Guanosine
m ³¹⁹ U	319-Uridine
m ³²⁰ A	Smino-320-Aminoadenosine
m ³²¹ C	321-Cytidine
m ³²² G	322-Guanosine
m ³²³ U	323-Uridine
m ³²⁴ A	Smino-324-Aminoadenosine
m ³²⁵ C	325-Cytidine
m ³²⁶ G	326-Guanosine
m ³²⁷ U	327-Uridine
m ³²⁸ A	Smino-328-Aminoadenosine
m ³²⁹ C	329-Cytidine
m ³³⁰ G	330-Guanosine
m ³³¹ U	331-Uridine
m ³³² A	Smino-332-Aminoadenosine
m ³³³ C	333-Cytidine
m ³³⁴ G	334-Guanosine
m ³³⁵ U	335-Uridine
m ³³⁶ A	Smino-336-Aminoadenosine
m ³³⁷ C	337-Cytidine
m ³³⁸ G	338-Guanosine
m ³³⁹ U	339-Uridine
m ³⁴⁰ A	Smino-340-Aminoadenosine
m ³⁴¹ C	341-Cytidine
m ³⁴² G	342-Guanosine
m ³⁴³ U	343-Uridine
m ³⁴⁴ A	Smino-344-Aminoadenosine
m ³⁴⁵ C	345-Cytidine
m ³⁴⁶ G	346-Guanosine
m ³⁴⁷ U	347-Uridine
m ³⁴⁸ A	Smino-348-Aminoadenosine
m ³⁴⁹ C	349-Cytidine
m ³⁵⁰ G	350-Guanosine
m ³⁵¹ U	351-Uridine
m ³⁵² A	Smino-352-Aminoadenosine
m ³⁵³ C	353-Cytidine
m ³⁵⁴ G	354-Guanosine
m ³⁵⁵ U	355-Uridine
m ³⁵⁶ A	Smino-356-Aminoadenosine
m ³⁵⁷ C	357-Cytidine
m ³⁵⁸ G	358-Guanosine
m ³⁵⁹ U	359-Uridine
m ³⁶⁰ A	Smino-360-Aminoadenosine
m ³⁶¹ C	361-Cytidine
m ³⁶² G	362-Guanosine
m ³⁶³ U	363-Uridine
m ³⁶⁴ A	Smino-364-Aminoadenosine
m ³⁶⁵ C	365-Cytidine
m ³⁶⁶ G	366-Guanosine
m ³⁶⁷ U	367-Uridine
m ³⁶⁸ A	Smino-368-Aminoadenosine
m ³⁶⁹ C	369-Cytidine
m ³⁷⁰ G	370-Guanosine
m ³⁷¹ U	371-Uridine
m ³⁷² A	Smino-372-Aminoadenosine
m ³⁷³ C	373-Cytidine
m ³⁷⁴ G	374-Guanosine
m ³⁷⁵ U	375-Uridine
m ³⁷⁶ A	Smino-376-Aminoadenosine
m ³⁷⁷ C	377-Cytidine
m ³⁷⁸ G	378-Guanosine
m ³⁷⁹ U	37

Impact of finding a new modification:

266 hmC papers Is an Epigenetic *Cell* mark of Melanoma

Christine Guo Lian,^{1,2,13} Yufei Xu,^{1,13} Craig Ceol,^{3,6} Feizhen Wu,⁹ Allison Larson,⁵ Karen Dresser,⁷ Wenqi Xu,⁹ Li Tan,⁹ Yeguang Hu,¹ Qian Zhan,² Chung-wei Lee,² Di Hu,¹ Bill Q. Lian,^{1,8} Sonja Kleffel,⁵ Yijun Yang,¹⁰ James Neiswender,⁶

CHROMATOGRAPHY IN THE NERVOUS SYSTEM

Dynamics and Inheritance 5-Hydroxyproline

Jamie A. H...
Caroline Le...

Mouse prim...
DNA demet...
of CpG met...
driven by hi...
PGCs at em...
Mechanistic...
with replicati...

fC
cac

5-formylcytosine
5-carboxylcytosine

Joseph R. Ecker*, Patrice M. Milos*, Suneet Agarwal* & Anjana Rao**
redundant systems that drive regulatory elements that escape mechanistic basis for transgenerational epigenetic inheritance.

ford, ORCRB, O...

cLoughlin⁵,
3,

QUESTIONS?

