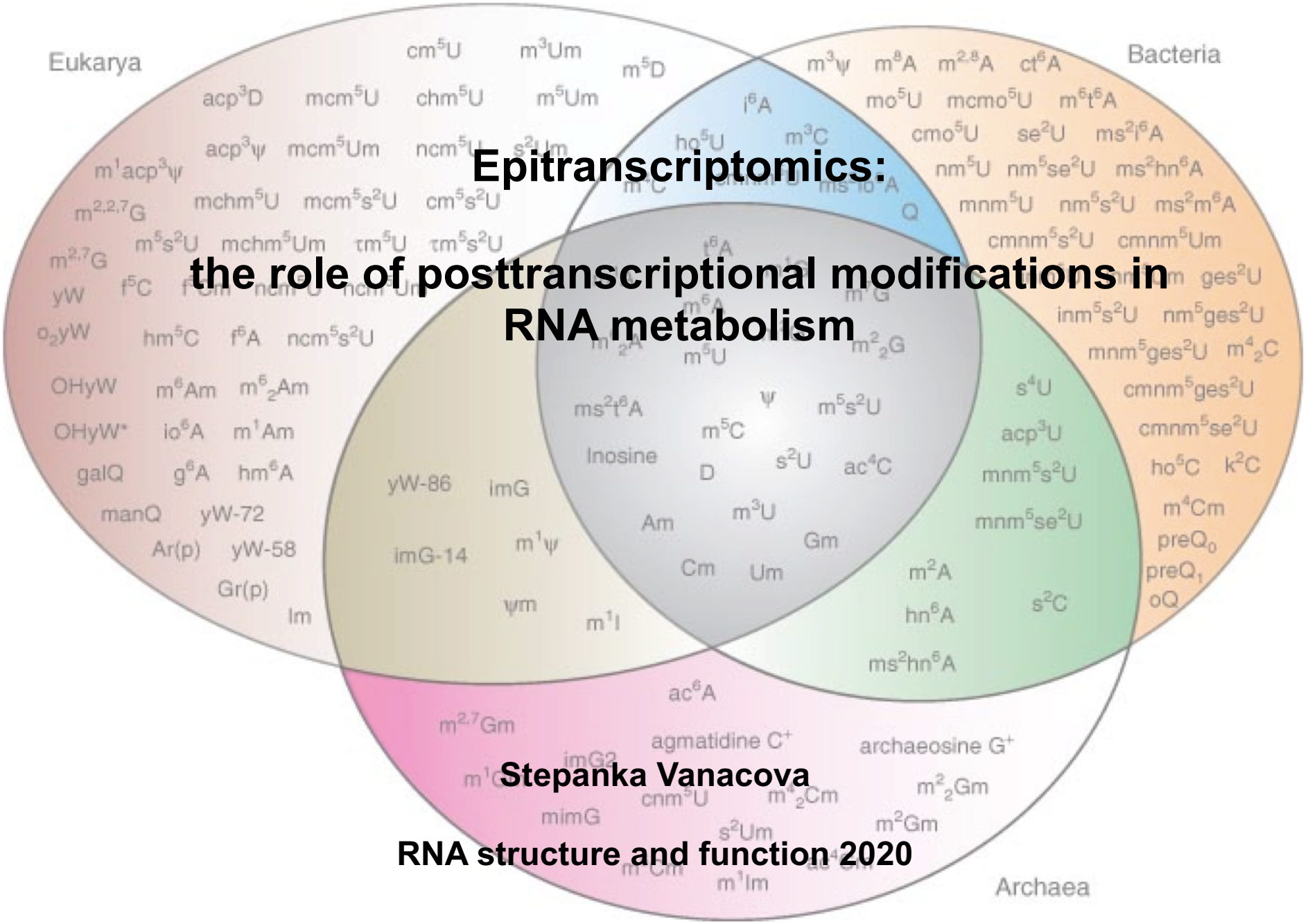


Epitranscriptomics:

the role of posttranscriptional modifications in RNA metabolism

Stepanka Vanacova

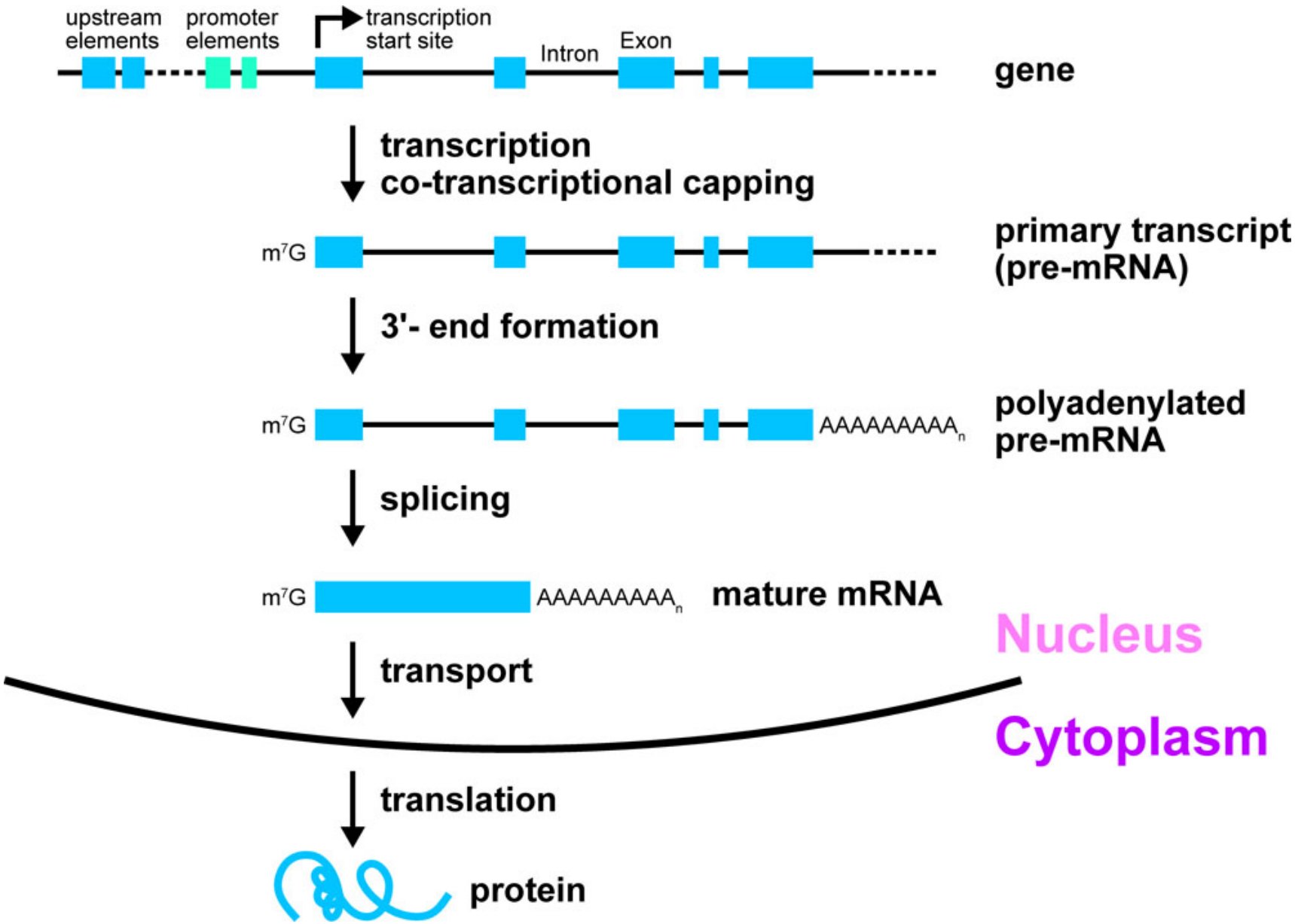
RNA structure and function 2020

Lecture contents:

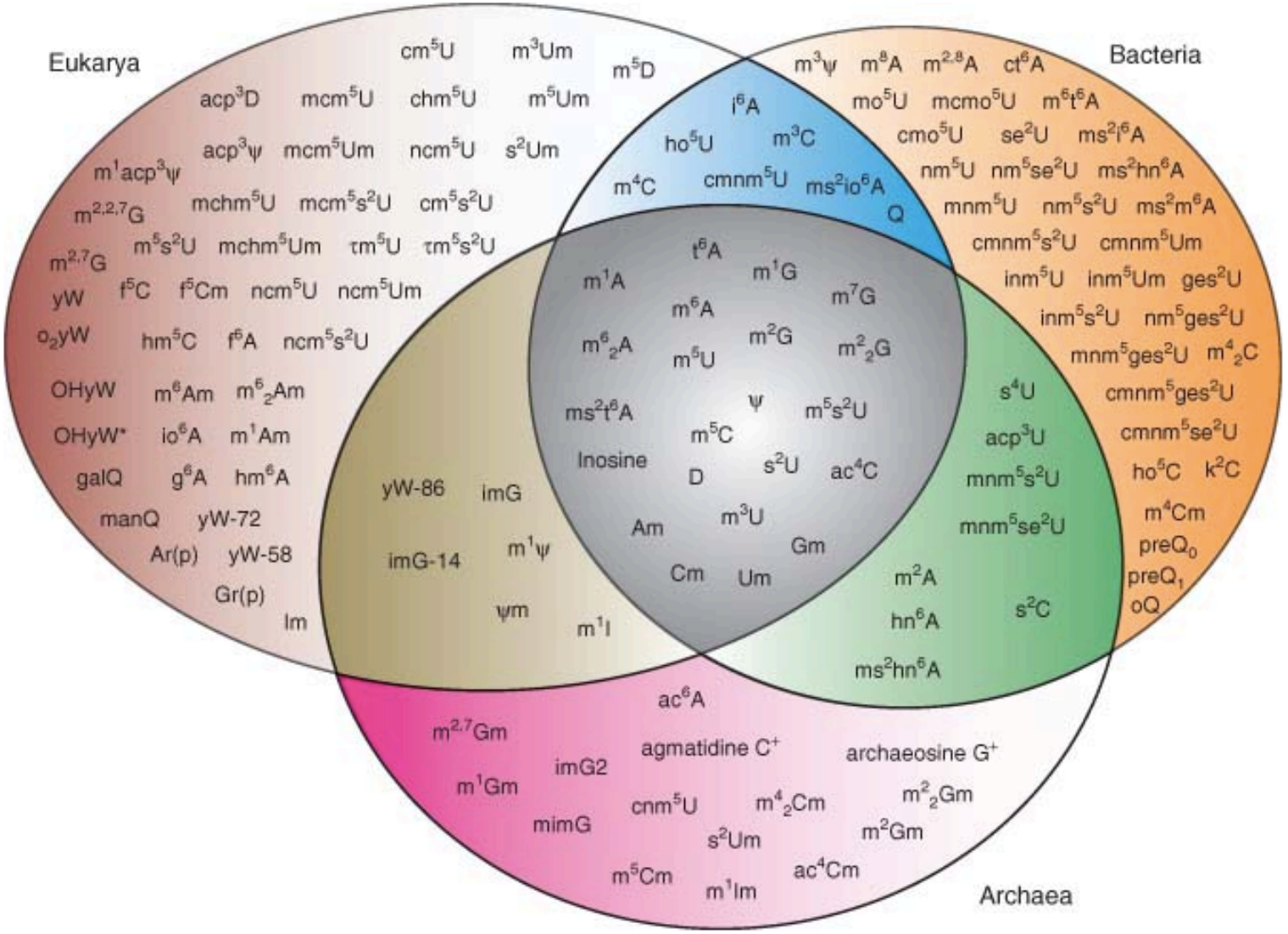
1. Overview of the different (m)RNA modification

2. Our current data on the role of m6A demethylase in mRNA metabolism

Gene expression in eukaryotes



Phylogenetic distribution of modified nucleosides in RNA originating from the three domains of life



Helm, Motorin, Nat Rev Gen 2017

- Cellular RNAs are post-transcriptionally modified in all life kingdoms
- RNA modification alters physico-chemical properties of nucleotides, including their conformation, polarity, hydrophobicity, chemical reactivity and base-pairing interactions
- RNA modification is performed by highly specific and regulated enzymatic mechanisms involving pure protein enzymes and catalytic RNA–protein complexes (RNPs)
- RNA modification is important for regulation of gene expression
- Transcription-wide RNA modification is dynamic and regulated cellular process
- Deregulation of RNA modification may lead to important human pathologies

Types of RNA modifications

1. RNA editing — insertional & deletional
2. Base modifications — multiple different types
   substitutional RNA editing

Types of RNA modifications

1.	RNA editing	insertional & deletional
2.	Base modifications	multiple different types substitutional RNA editing

Types of RNA editing

Insertion/deletion

U

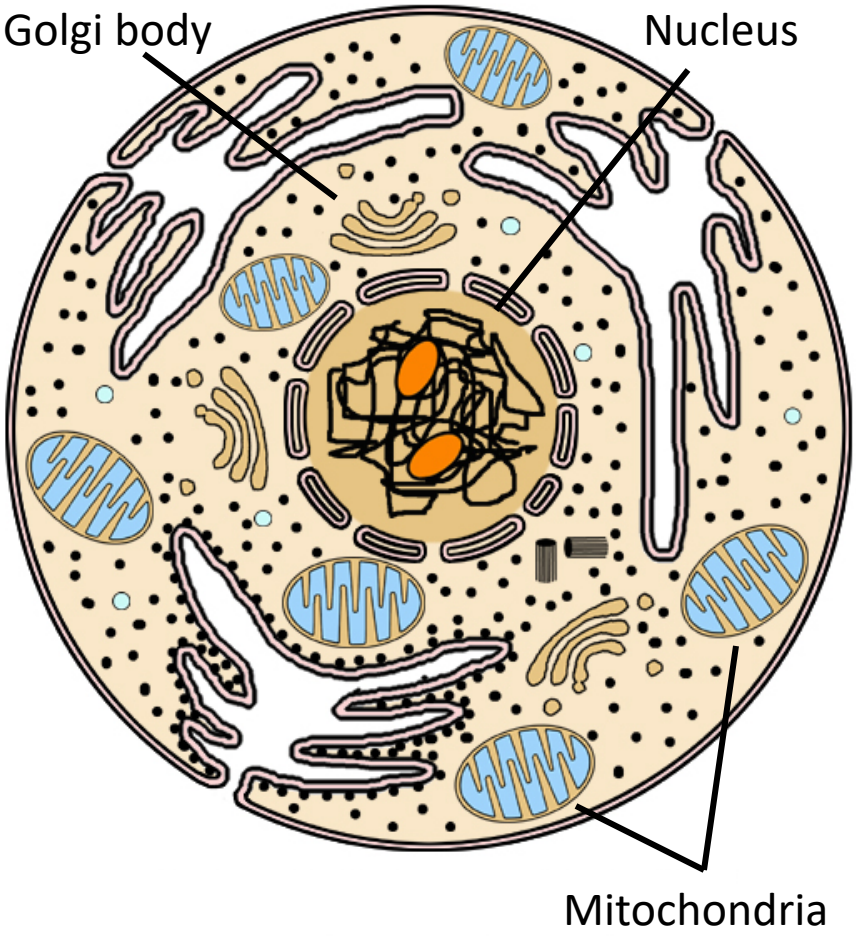
Insertion or deletion

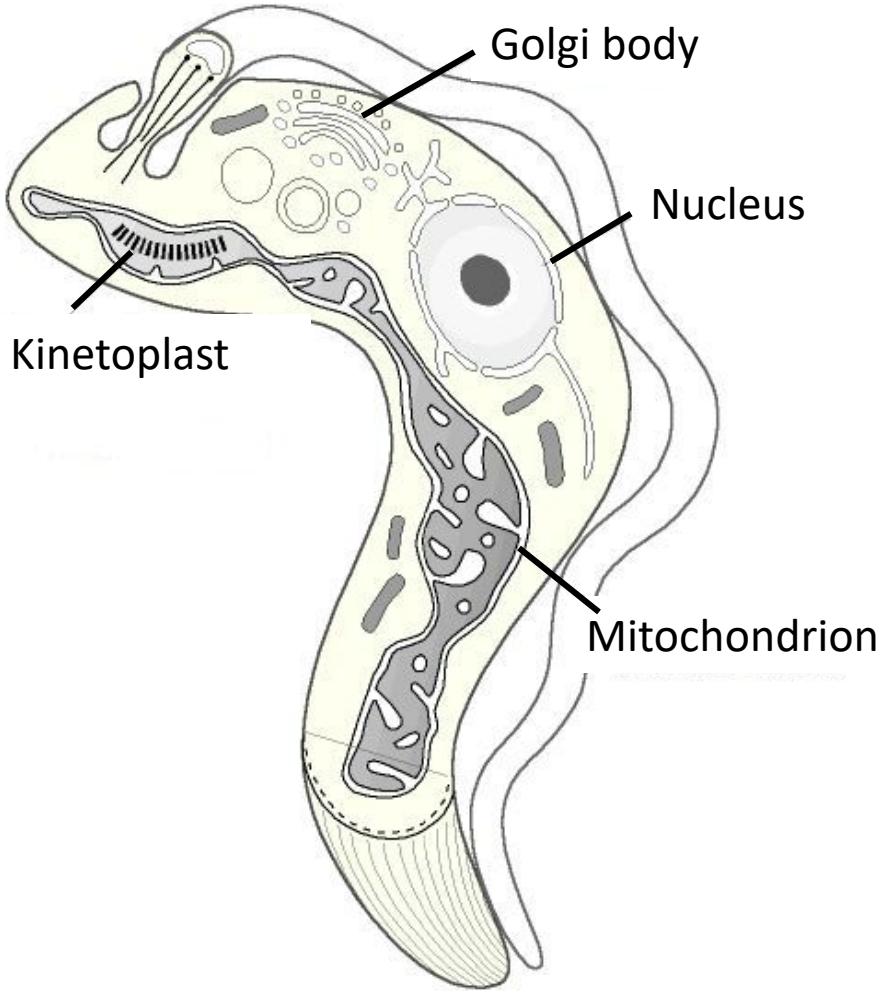
Conversion

A → I

C → U

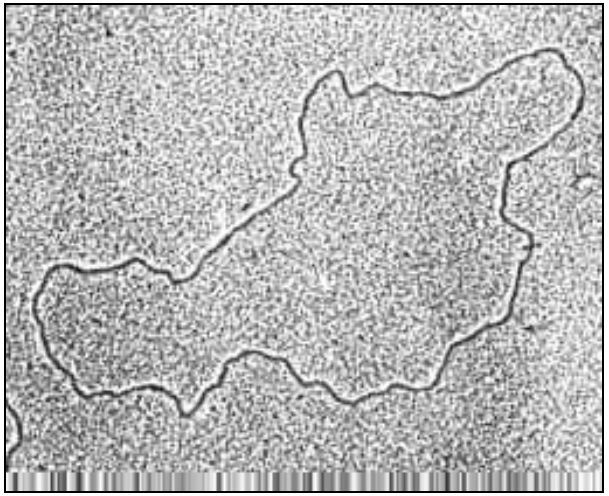
Trypanosomes have only one mitochondrion



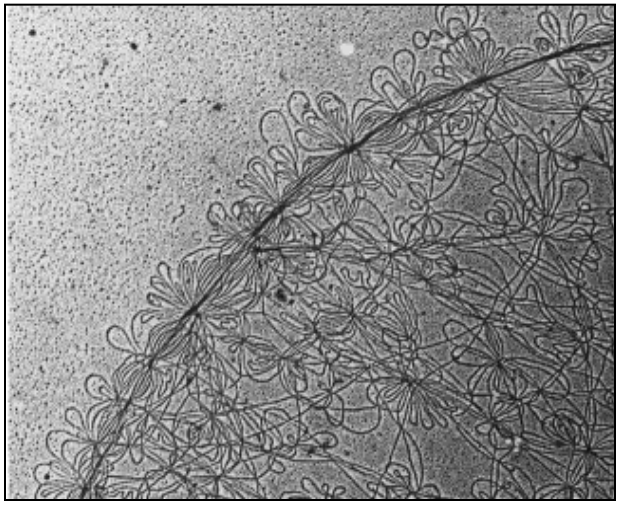


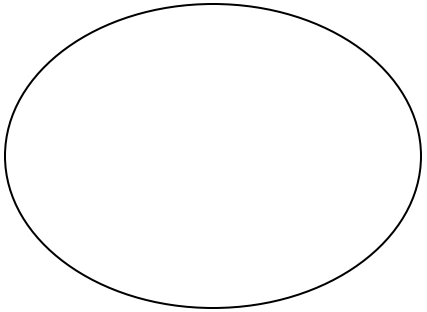
A unique mitochondrial DNA architecture: The kinetoplast

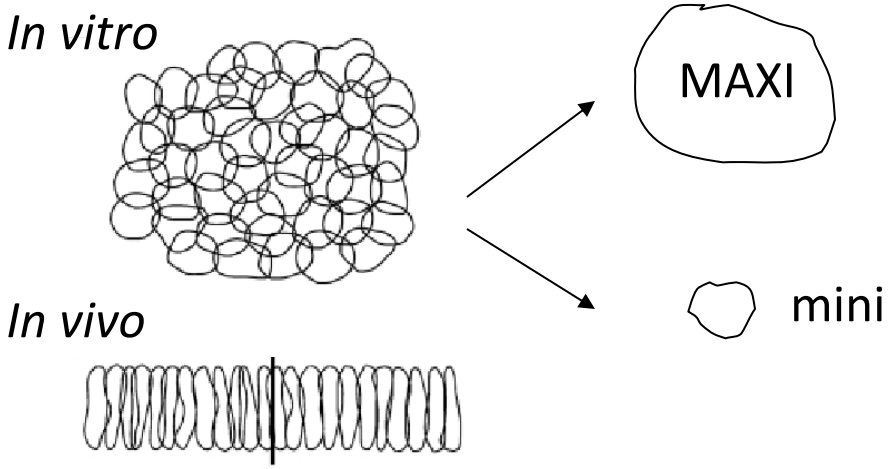
human



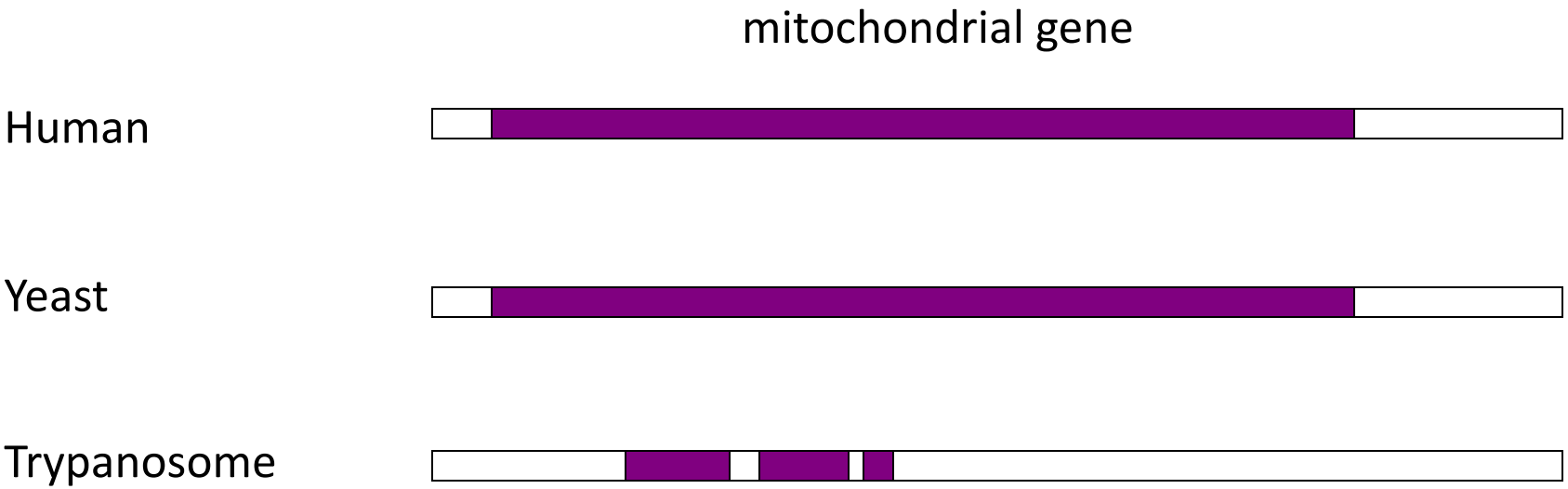
trypanosome







RNA editing by trypanosomes: The mystery of missing genes



DNA

ACCAGAGAGGAGAGUGAGGAAAGGCG

mRNA

AUCCAGUAUUGUUUUUUUAUGGUUUUUAUGUAGUGAGUUUGUUUUAUUUAUGGCG

T. brucei ATPase 6 mRNA

edited

```
                                    M  F  L  F  F  F  C  D
L  F  W  L  R  L  L  L  C  M  Y  Y  C  V  W  S  R  L  C  F
   I  V  Y  F  N  C  L  M  L  I  F  D  F  L  L  F  C  L   F
   D  L  Y  L  F  V  G  L  C     L  F  L  L  L  W  F  M  L
 F  N  L  Y  S  L  I  L  Y  Y   C  I  T  Y  L     N  L   Y
   L  L  F  C  I  V  F  L  L  Y  I  A  F  L  F  L  F  C  F
 L  C  D  F  F  L  F  N  N  L  L  V  G  D     S  F  M  D
 V  F  F  I     R  F  L  L  C  F  L  E  C  F  S  L  L  C  R
   C  L  S  T  F  L  R  L  F  C  N  L  L  S  S  H  F  L  L
 L  M  F  F  D  F  F  Y  F  I  F  V  F  F  F  W  C  F  L
L  L  I  Y  F  I  Y  F  C  V  L  F  L  F  I  I  L  C  V  F
   I  F  V  G  F  I  C     R  H  I  T     V  I  Y  F  L ter
```

Types of RNA editing

Insertion/deletion

U

Insertion or deletion

Conversion

A

C

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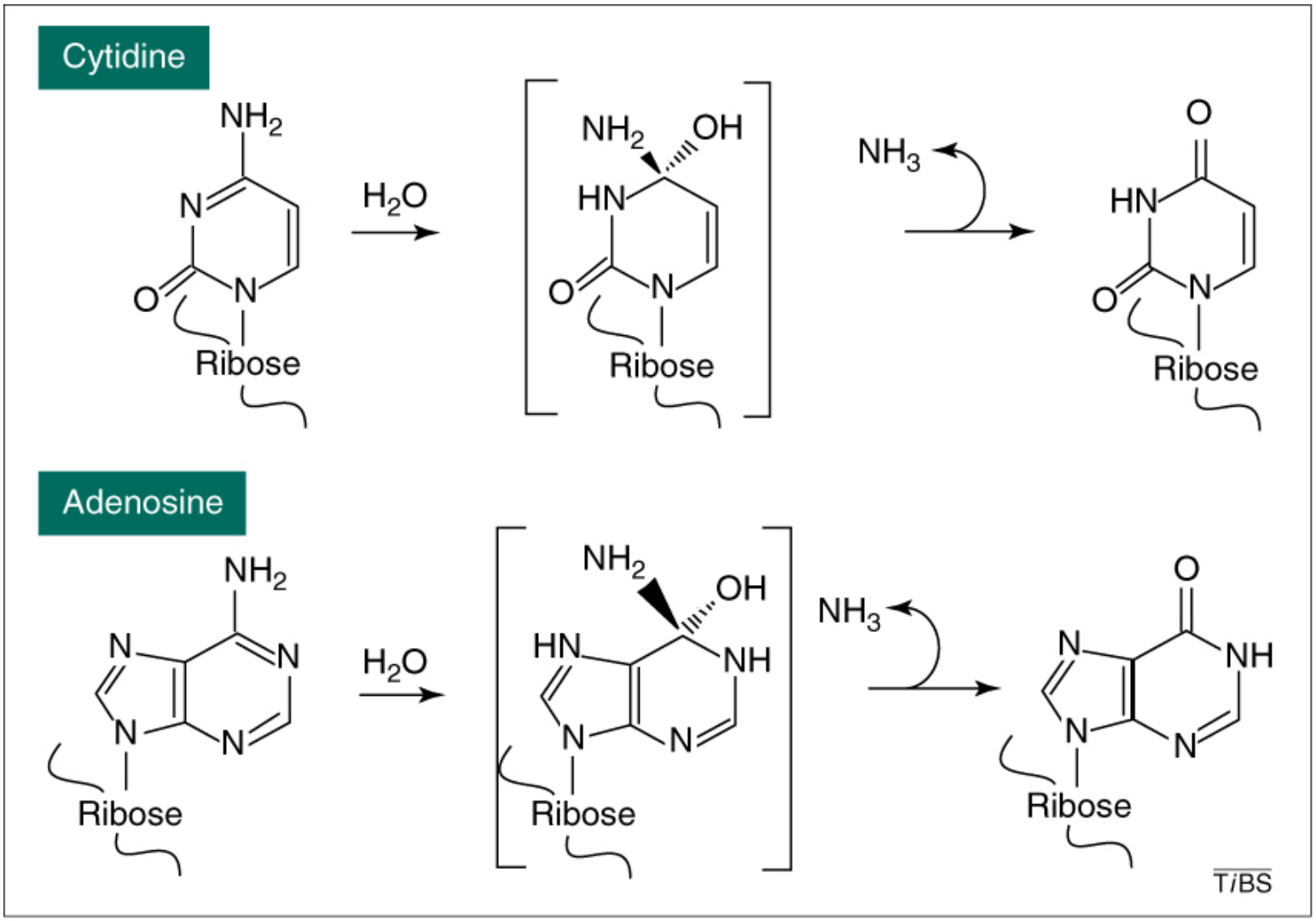
1. RNA editing — insertional & deletional
2. Base modifications — many different once
   substitutional RNA editing

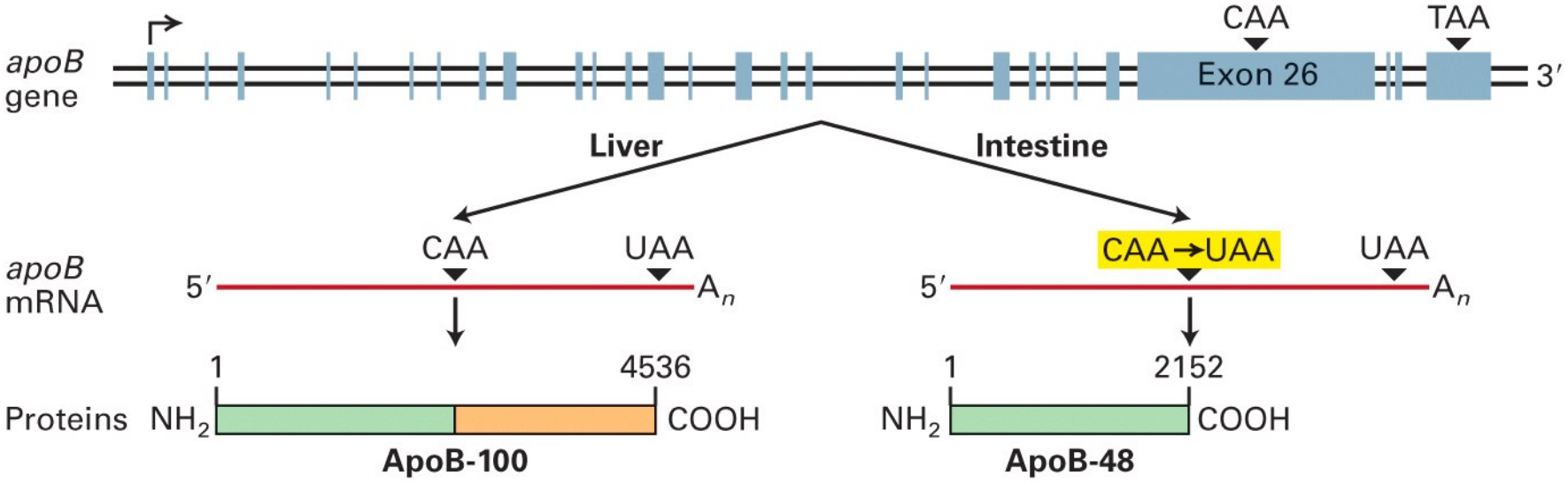
Substitutional RNA editing



Substitutional RNA editing

A to I	ADARs, ADATs (adenosine deaminases acting on RNA/tRNA
C to U	CDARs (Apobec) (cytosine deaminases acting on RNA)

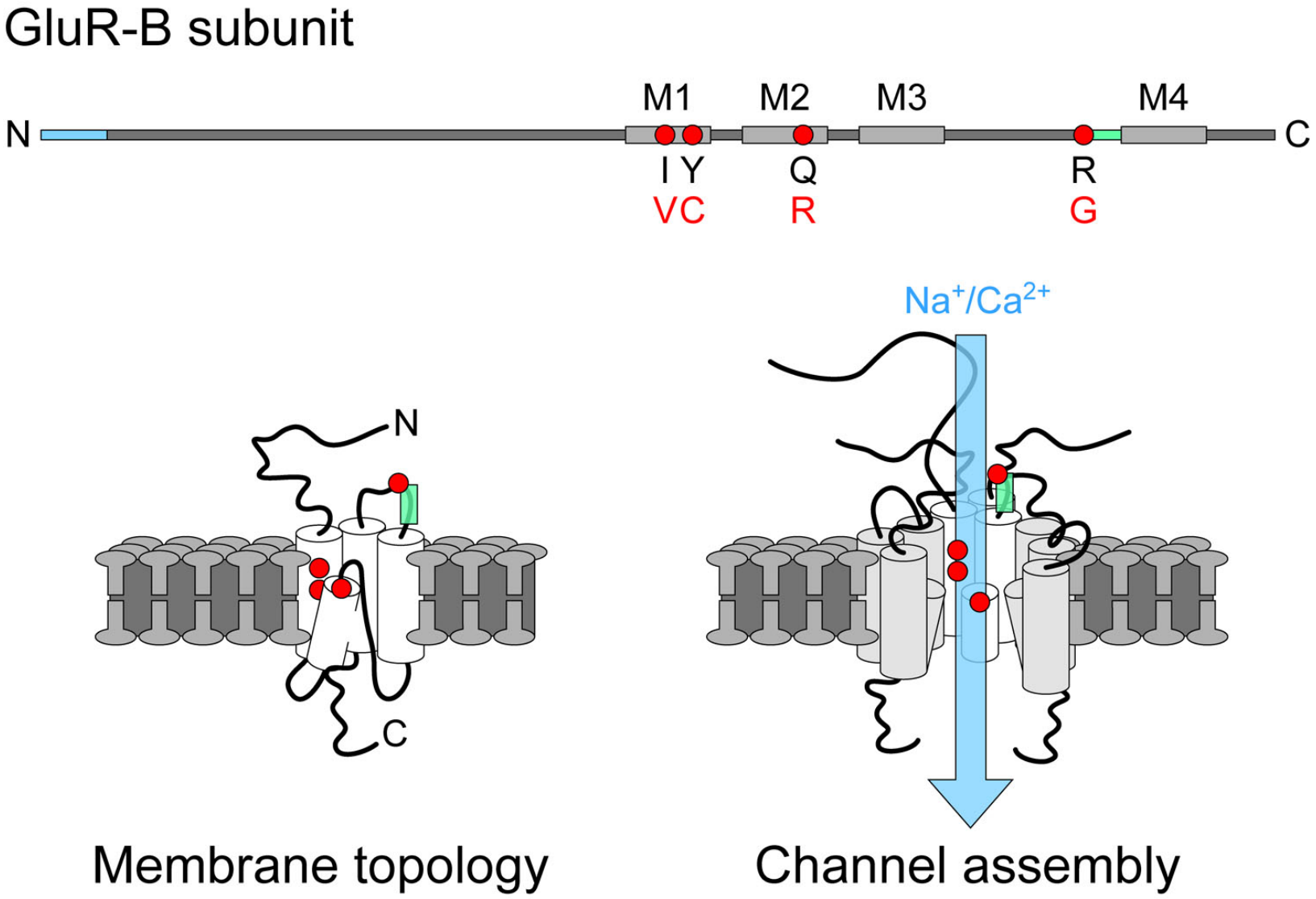
C to U editing often forms additional stop codons



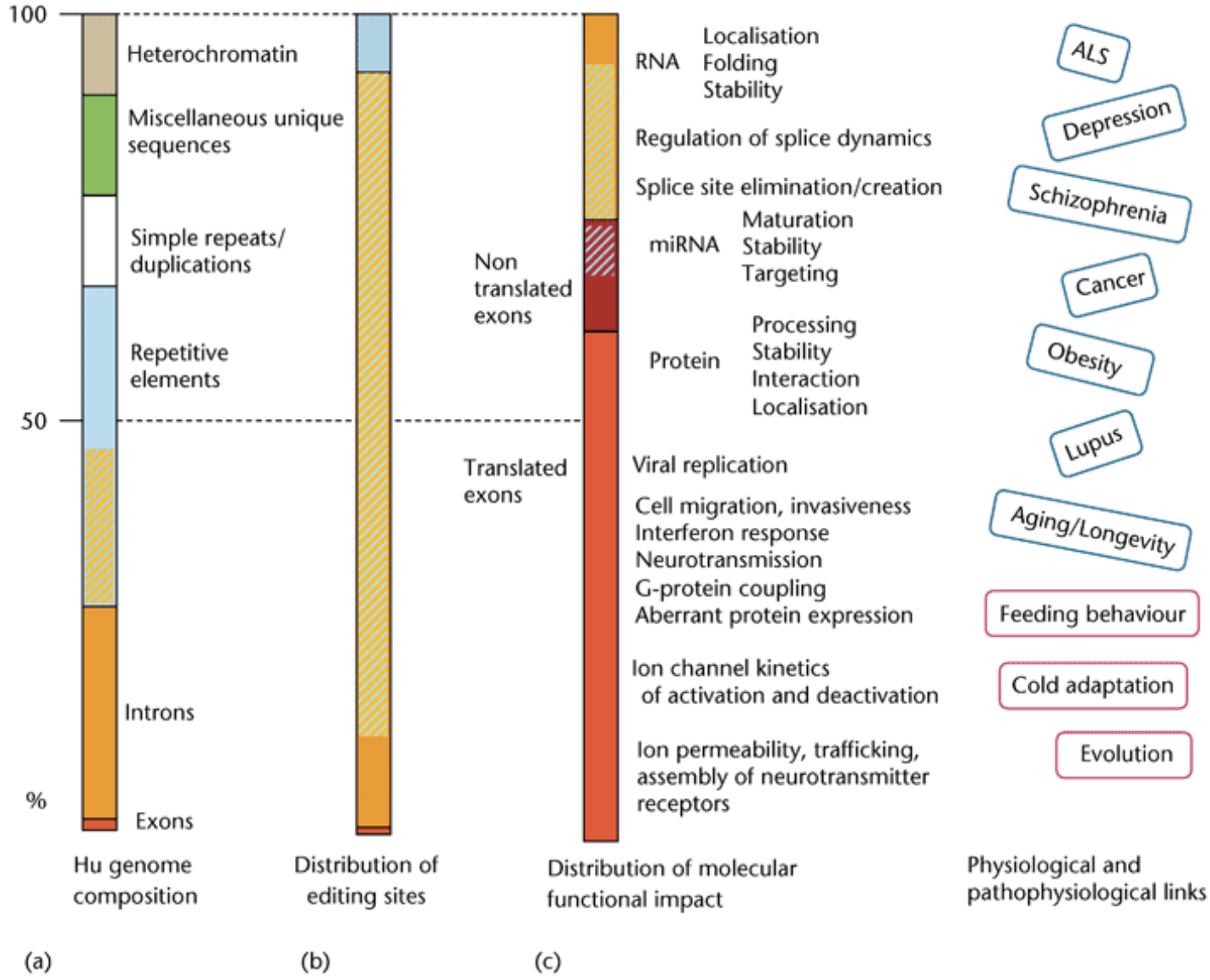
Apolipoprotein B-100
4563 amino acids
Function: transport of cholesterol in the blood

Apolipoprotein B-48
2152 amino acids
Function: absorption of lipids from the intestine

Organization of the glutamate-gated ion channel receptors



Landscape of A-to-I RNA editing occurrence and impact



Maas, Stefan(Sep 2013) A-to-I RNA Editing and Human Genetic Disease. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net [doi: 10.1002/9780470015902.a0024625]

Types of RNA modifications

1. RNA editing — insertional & deletional
2. Base modifications — multiple different types
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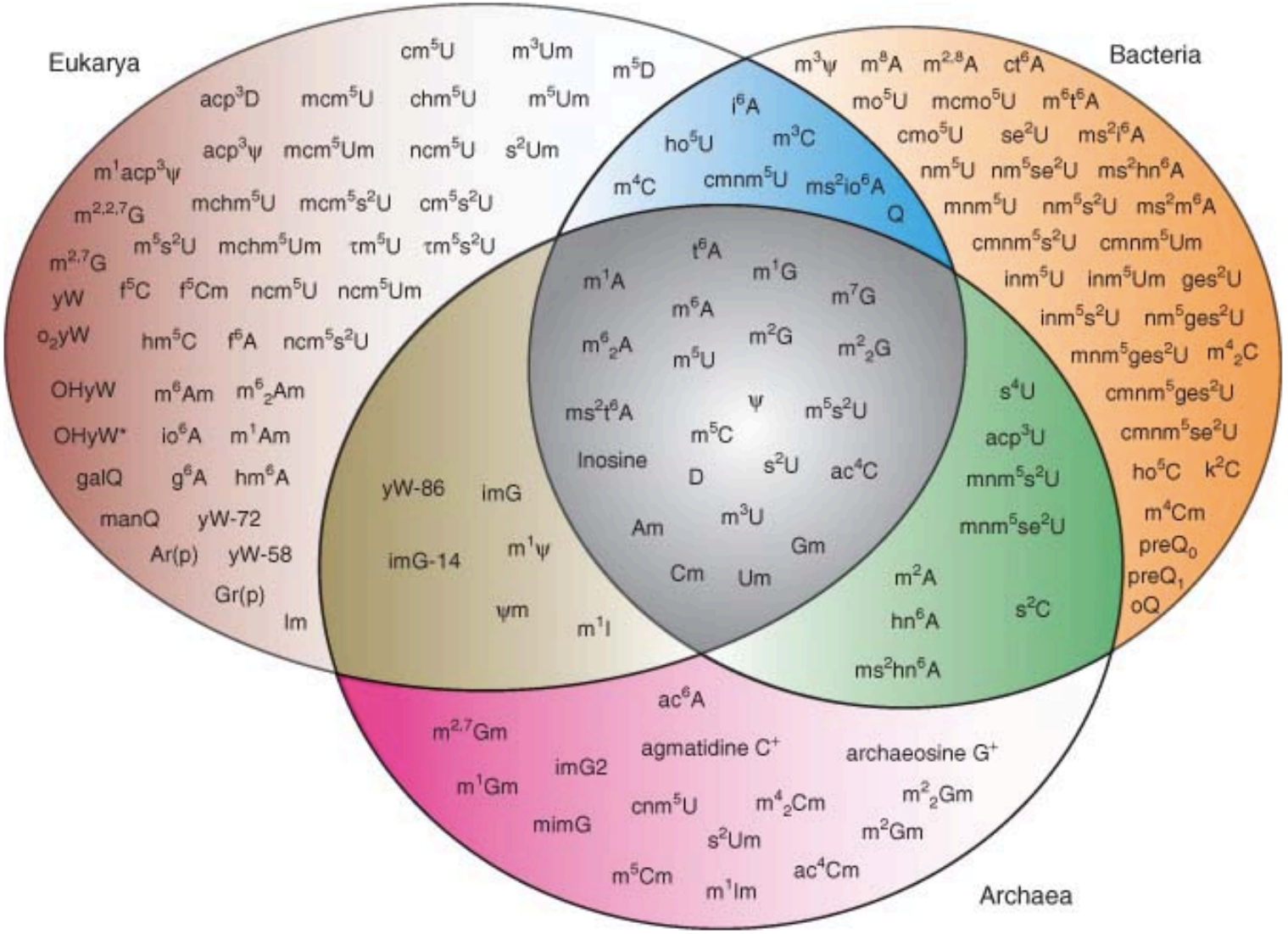
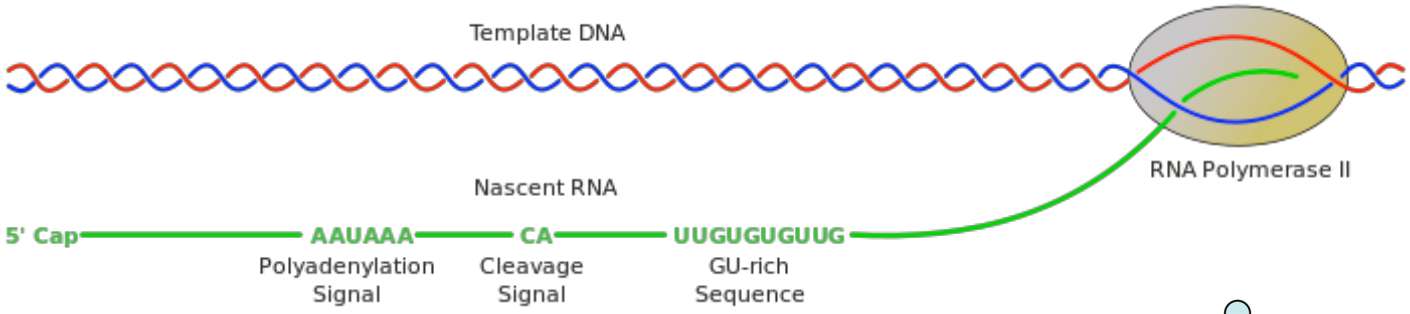
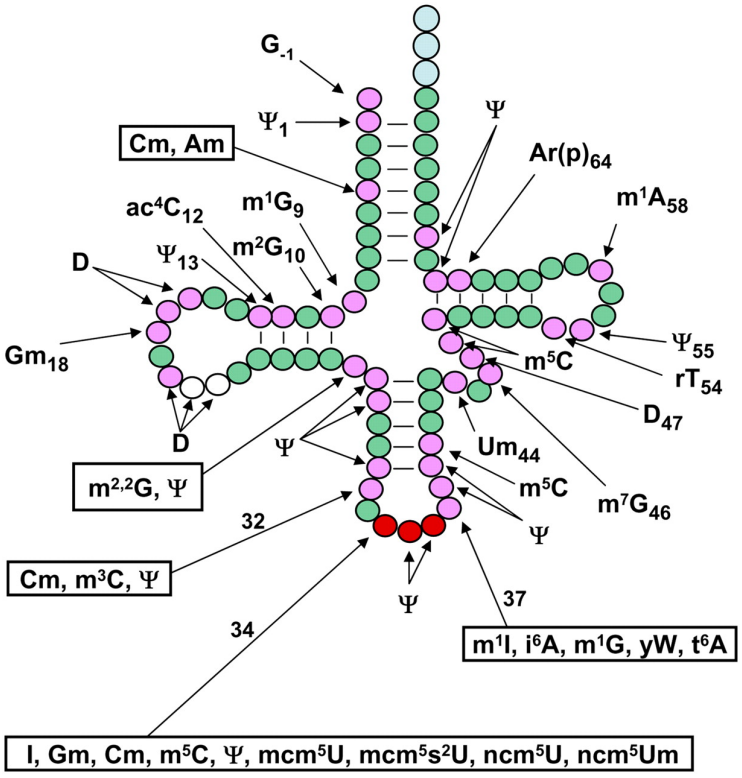
Phylogenetic distribution of modified nucleosides in RNA originating from the three domains of life



Motorin, Yuri(May 2015) RNA Modification. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net
[doi: 10.1002/9780470015902.a0000528.pub3]

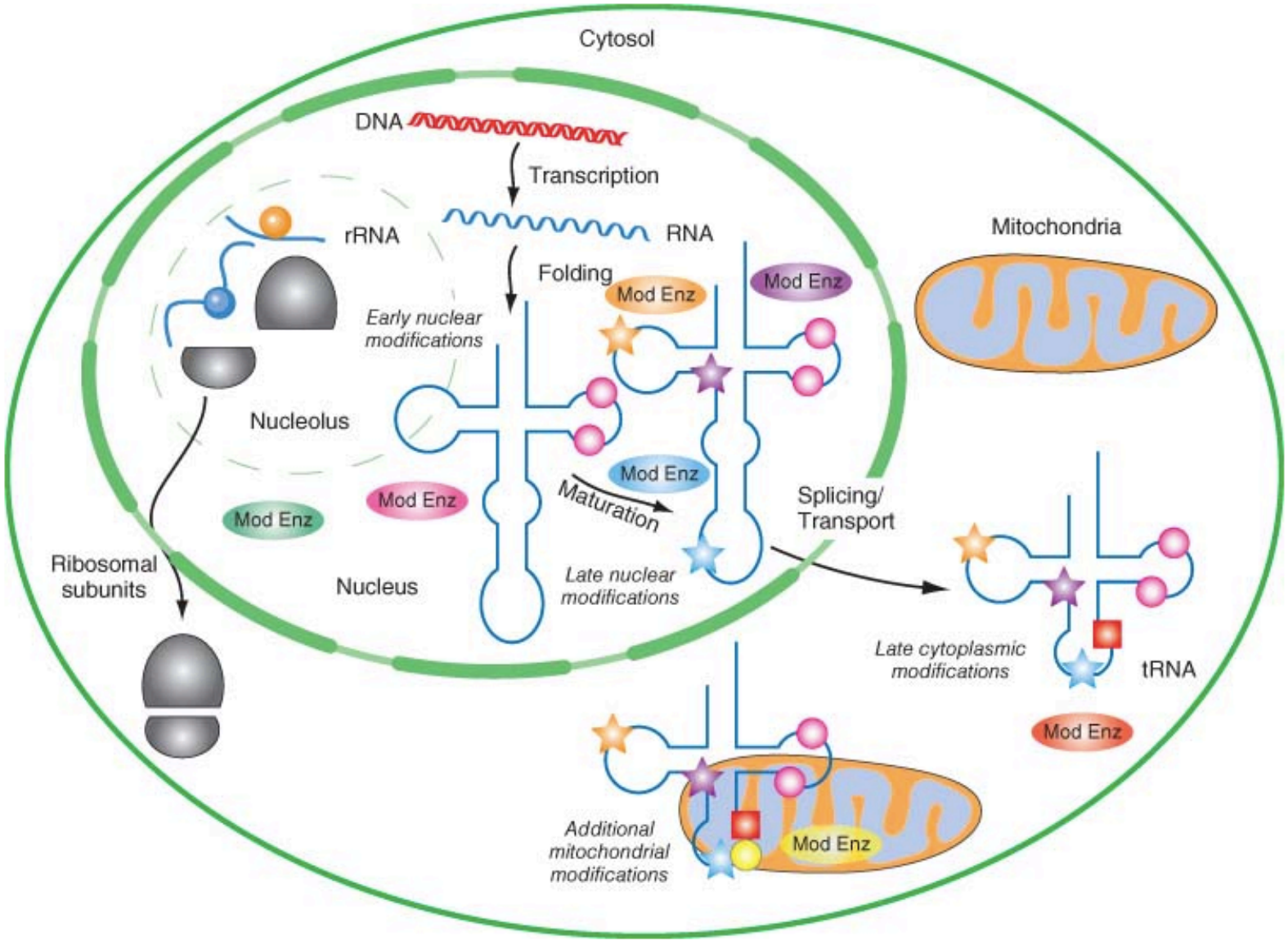
RNA modifications





Phizicky and Hopper, Genes Dev., 2010

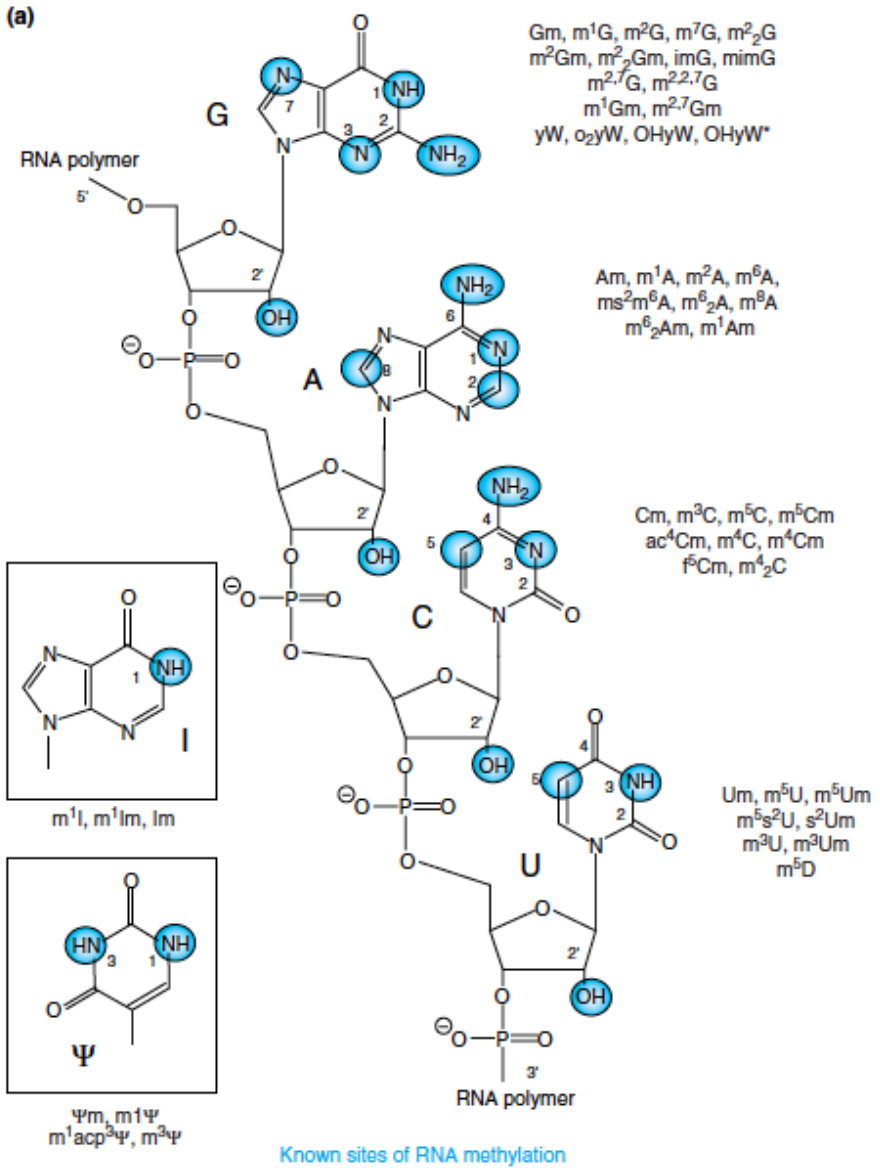
Cellular localisation of RNA:modification enzymes and coordination between RNA (tRNA) maturation and modification



Diversity of nucleotide methylation

Methylation sites on the chemical structures of the four major ribonucleotides, inosine, and pseudouridine.

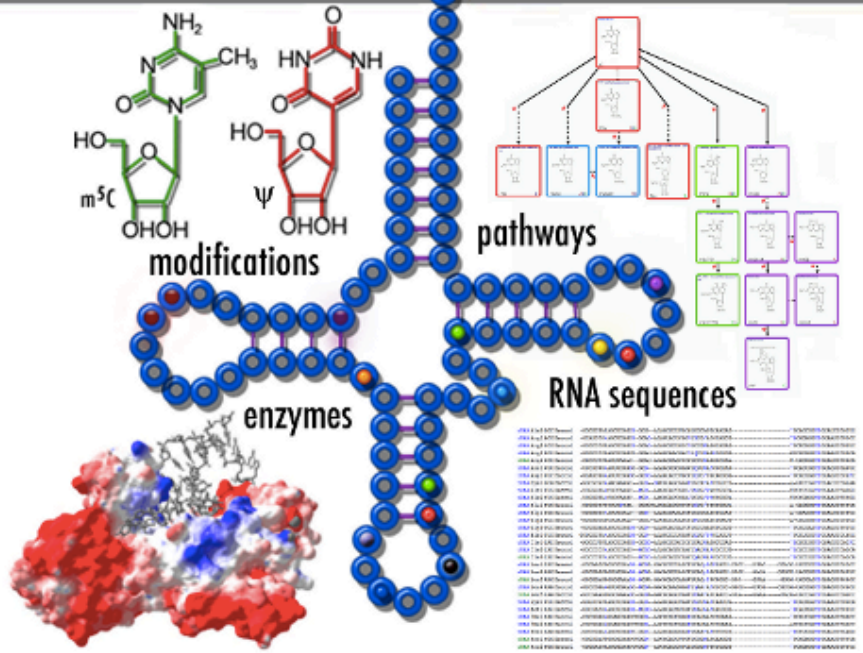
Multiple modifications may occur sequentially on a single nucleotide.



Motorin and Grosjean. tRNA modification. In: Encyclopedia of Life Sciences. 2005

Modomics

a database of RNA modification pathways



MODOMICS and **RNApathwaysDB** are two complementary resources whichpresent RNA metabolism at different levels. While MODOMICS presents RNA modification pathways on the level of nucleosides, RNApathwaysDB deals with RNA metabolism with respect to whole RNA molecules. Our ultimate goal is to integrate these databases, however at the moment the users are invited to consult both of these complementary resources, depending on their needs and interests.

Overview

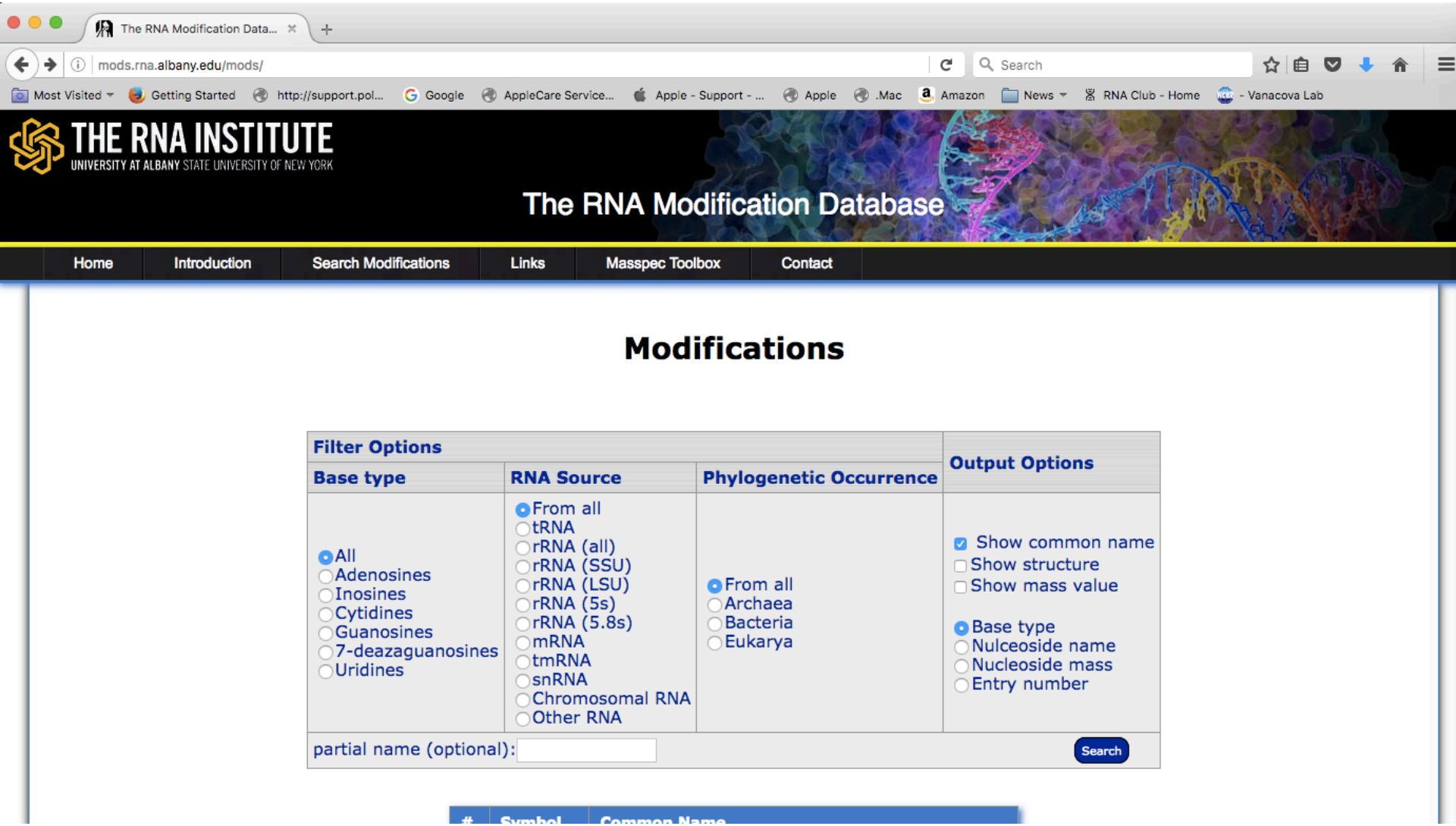
The RNA modification database provides a comprehensive listing of posttranscriptionally modified nucleosides from RNA and is maintained as an updated version of the initial printed report [1].

The chemical composition of an RNA molecule allows for its inherent ability to play many roles within biological systems. This ability is further enhanced through the site selected addition of the 109 currently known post-transcriptional modifications catalyzed by specific RNA modification enzymes [2]. These naturally-occurring modifications are found in all three major RNA species (tRNA, mRNA and rRNA) in all three primary phylogenetic domains (archaea, bacteria and eukarya) as well as in a handful of other RNA species such as snRNA and miRNA [3,4,5,6]. Both the chemical and structural diversity and extent of posttranscriptional modification in RNA is remarkable [1,7,8,9], with 109 different modified nucleosides presently known. The modifications are one of the most evolutionarily conserved properties of RNAs. Due in large part to comprehensive investigations into the structural and functional roles of modified nucleosides in tRNA, significant advancements have been achieved in our understanding of the various roles played by these modifications [6,10,11,12,13,14]. The need to provide a comprehensive, searchable database to house this wealth of knowledge led to the first iteration of The RNA Modification Database (RNAMDB) in 1994 [1].

The current version of the database, now housed at The RNA Institute at the State University of New York at Albany, contains all naturally-occurring, RNA-derived modified ribonucleosides for which the chemical structures are known. They include those from established sequence positions, as well as those detected or characterized from hydrolysates of RNA. The RNAMDB provides a user-friendly, searchable interface that directs the user to a detailed information page for each database entry. The information provided permits access to the modified nucleoside literature through provision of both computer-searchable Chemical Abstracts registry numbers and key literature citations.

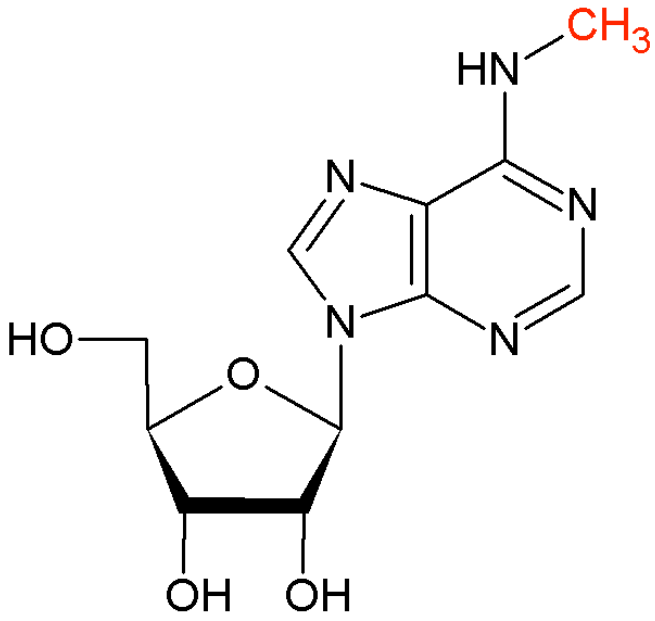
This database also provides an historical record of the initial reports of occurrence, characterization and chemical synthesis of modified nucleosides from RNA. The reader is referred to the earlier publication [1] and to paragraphs below for discussion of selected topics relevant to the database.

Users are invited to submit comments regarding existing entries, including errors and omissions, as well as suggestions for improvements to the following email address:

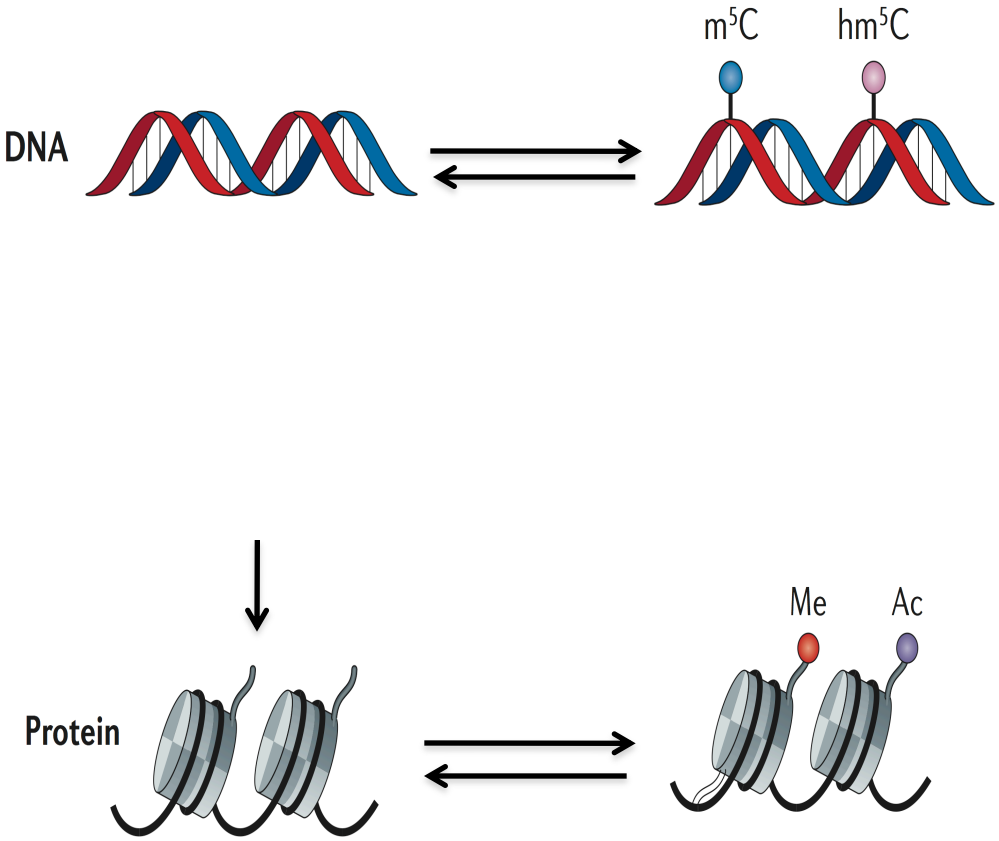

The RNA Modification Data...
mods.rna.albany.edu/mods/
Search
Most Visited
Getting Started
http://support.pol...
Google
AppleCare Service...
Apple - Support - ...
Apple
.Mac
Amazon
News
RNA Club - Home
- Vanacova Lab
THE RNA INSTITUTE
UNIVERSITY AT ALBANY STATE UNIVERSITY OF NEW YORK
The RNA Modification Database
Home
Introduction
Search Modifications
Links
Masspec Toolbox
Contact
Modifications
Filter Options
Base type
All
Adenosines
Inosines
Cytidines
Guanosines
7-deazaguanosines
Uridines
RNA Source
From all
tRNA
rRNA (all)
rRNA (SSU)
rRNA (LSU)
rRNA (5s)
rRNA (5.8s)
mRNA
tmRNA
snRNA
Chromosomal RNA
Other RNA
Phylogenetic Occurrence
From all
Archaea
Bacteria
Eukarya
Output Options
Show common name
Show structure
Show mass value
Base type
Nulceoside name
Nucleoside mass
Entry number
partial name (optional):
Search
#
Symbol
Common Name

#	Symbol	Common Name
1	m^1A	1-methyladenosine
2	m^2A	2-methyladenosine
3	m^6A	*N*^{6}-methyladenosine
4	Am	2'-O-methyladenosine
5	ms^2m^6A	2-methylthio-*N*^{6}-methyladenosine
6	i^6A	*N*^{6}-isopentenyladenosine
7	ms^2i^6A	2-methylthio-*N*^{6}-isopentenyladenosine
8	io^6A	*N*^{6}-(*cis*-hydroxyisopentenyl)adenosine
9	ms^2io^6A	2-methylthio-*N*^{6}-(cis-hydroxyisopentenyl) adenosine
10	g^6A	*N*^{6}-glycinylcarbamoyladenosine
11	t^6A	*N*^{6}-threonylcarbamoyladenosine
12	ms^2t^6A	2-methylthio-*N*^{6}-threonyl carbamoyladenosine
13	m^6t^6A	*N*^{6}-methyl-*N*^{6}-threonylcarbamoyladenosine
14	hn^6A	*N*^{6}-hydroxynorvalylcarbamoyladenosine
15	ms^2hn^6A	2-methylthio-*N*^{6}-hydroxynorvalyl carbamoyladenosine
16	Ar(p)	2'-O-ribosyladenosine (phosphate)
17	I	inosine
18	m^1I	1-methylinosine
19	m^1Im	1,2'-O-dimethylinosine
20	m^3C	3-methylcytidine
21	m^5C	5-methylcytidine
22	Cm	2'-O-methylcytidine
23	s^2C	2-thiocytidine
24	ac^4C	*N*^{4}-acetylcytidine
25	f^5C	5-formylcytidine
26	m^5Cm	5,2'-O-dimethylcytidine
27	ac^4Cm	*N*^{4}-acetyl-2'-O-methylcytidine
28	k^2C	lysidine
29	m^1G	1-methylguanosine
30	m^2G	*N*^{2}-methylguanosine
31	m^7G	7-methylguanosine
32	Gm	2'-O-methylguanosine
33	$m^2{}_2G$	*N*^{2},*N*^{2}-dimethylguanosine
34	m^2Gm	*N*^{2},2'-O-dimethylguanosine
35	$m^2{}_2Gm$	*N*^{2},*N*^{2},2'-O-trimethylguanosine
36	Gr(p)	2'-O-ribosylguanosine (phosphate)
37	yW	wybutosine
38	o_2yW	peroxywybutosine
39	OHyW	hydroxywybutosine
40	OHyW*	undermodified hydroxywybutosine
41	imG	wyosine
42	mimG	methylwyosine
43	Q	queuosine
44	oQ	epoxyqueuosine
45	galQ	galactosyl-queuosine

The role of m6A

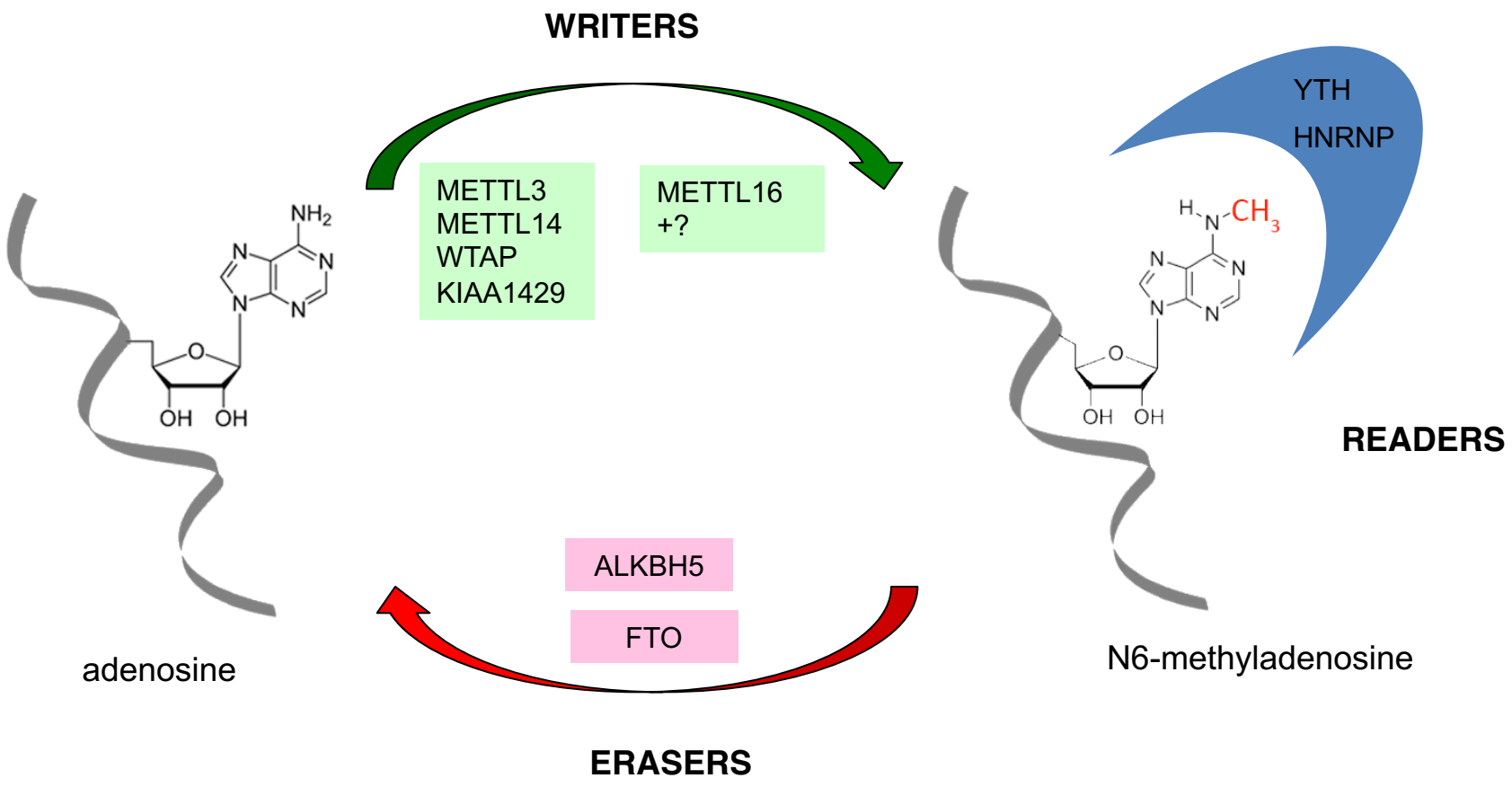


Epigenetic modifications



Adapted from *Fu, Y. et al, Nature Rev. Genet. (2014)*

The players in the m^{6}A pathway



Gerken et Al., *Science*, 2007; Dominissimi et Al., *Nature*, 2012; Meyer et Al., *Cell*, 2012;, Liu et Al., *Nat. Chem. Biol.* , 2014; Wang et Al., *Nature*, 2014, Pendleton et al., Cell 2017, Warda et al., EMBO Rep 2017

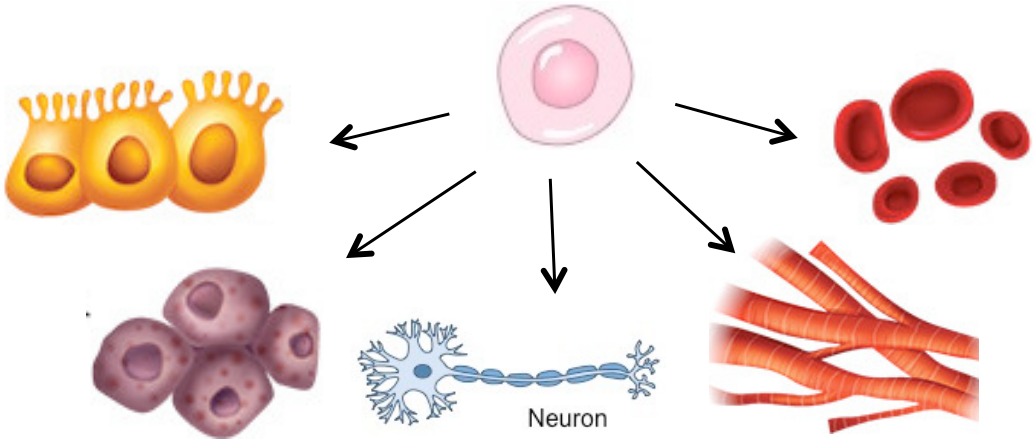
The m^6A and the related factors affect numerous physiological functions

Translation regulation and mRNA decay:

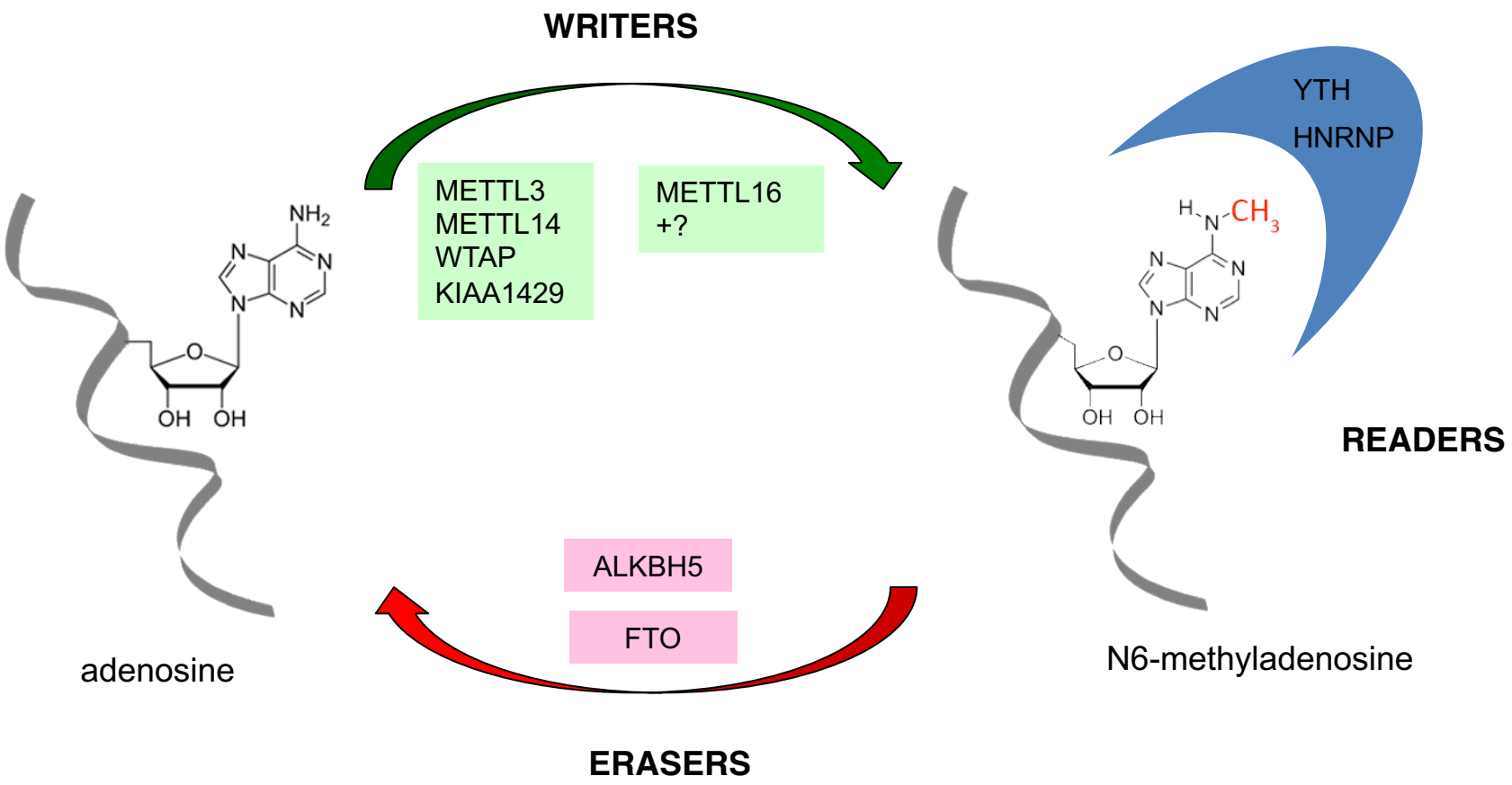
Germ cell maturation, cell differentiation and development

Stress response

May contribute to cancer, infections and other diseases



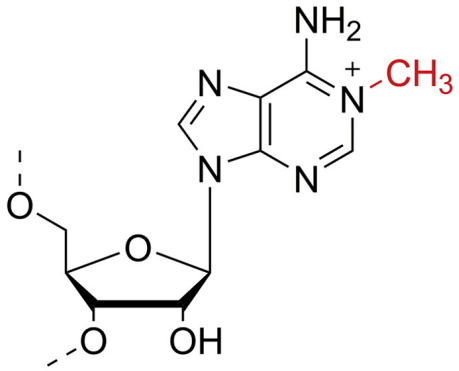
m6A deposition is a reversible modification



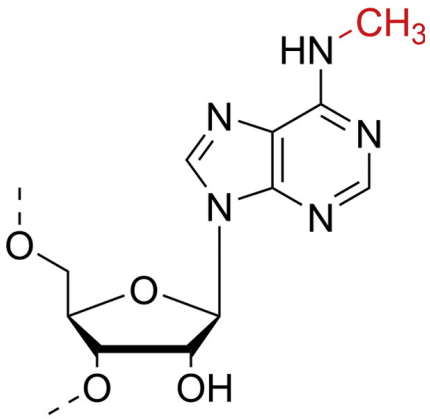
Gerken et Al., *Science*, 2007; Dominissimi et Al., *Nature*, 2012; Meyer et Al., *Cell*, 2012; Liu et Al., *Nat. Chem. Biol.* , 2014; Wang et Al., *Nature*, 2014

METHYLATION:
the most prevalent mRNA modification

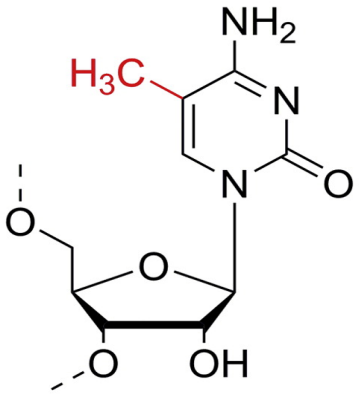
m1A



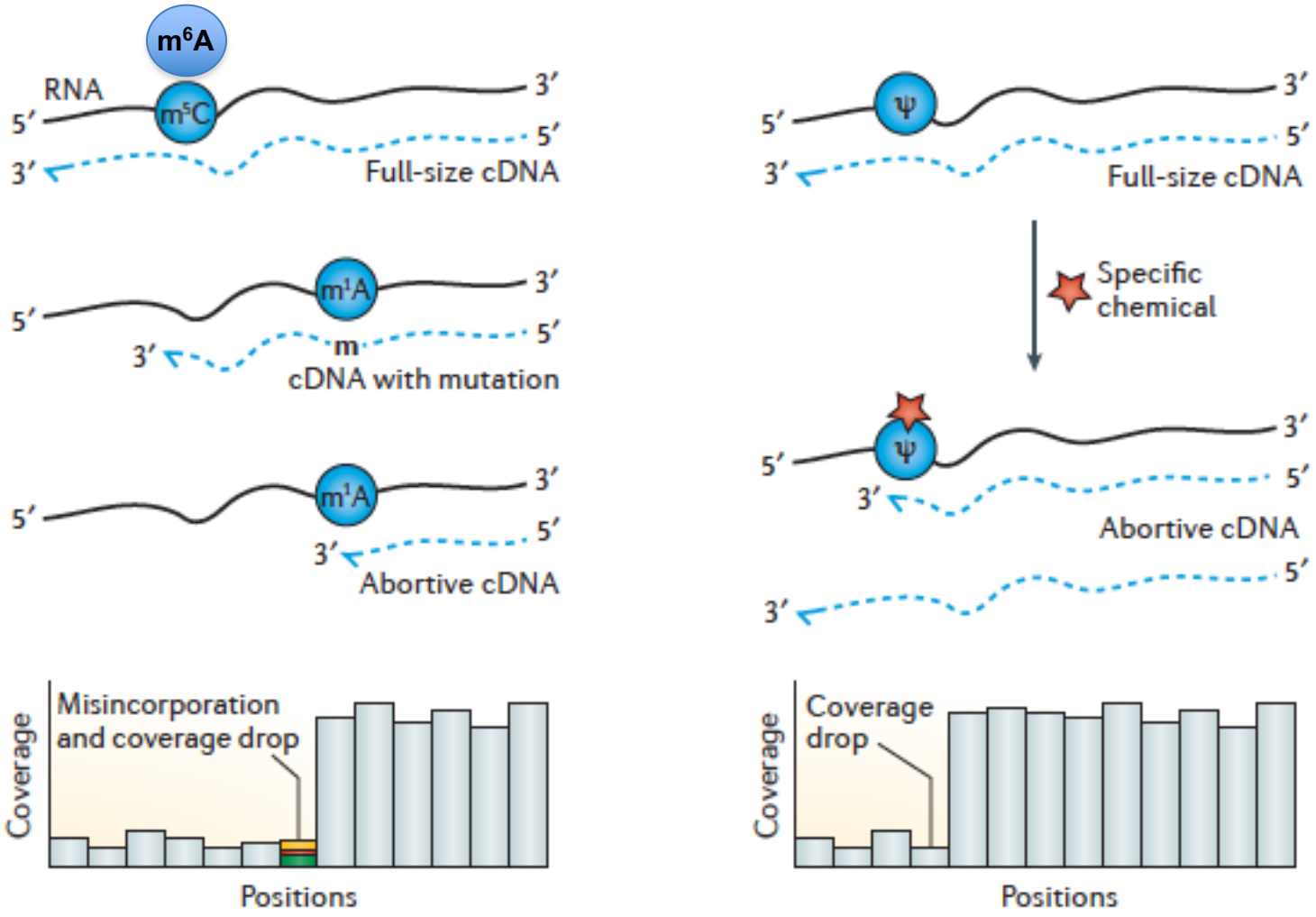
m6A



m5C

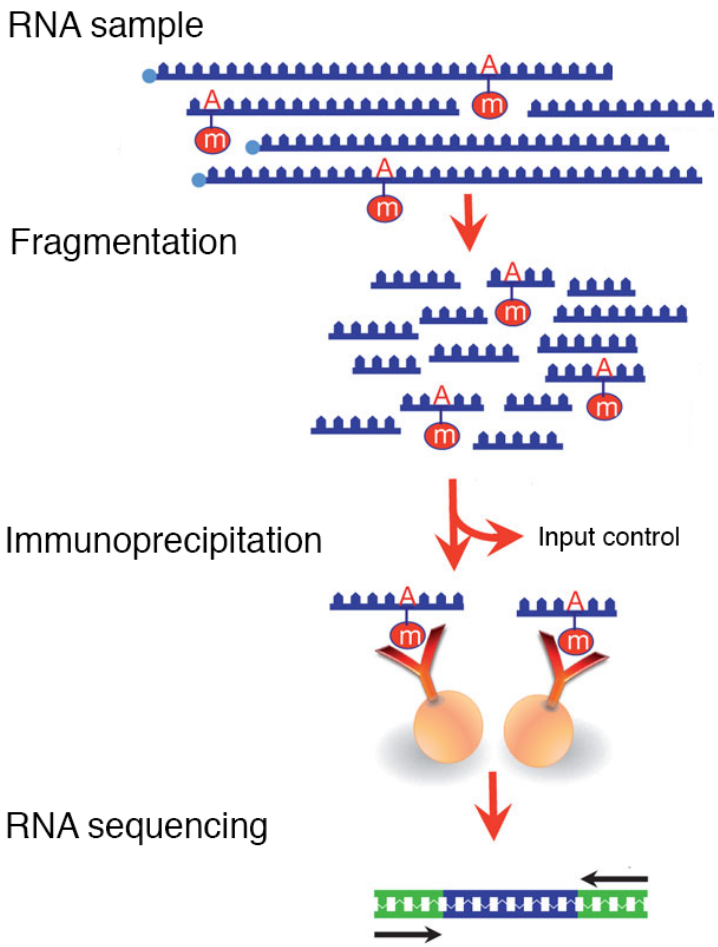


Reverse transcription-based techniques for detection of modified nucleotides

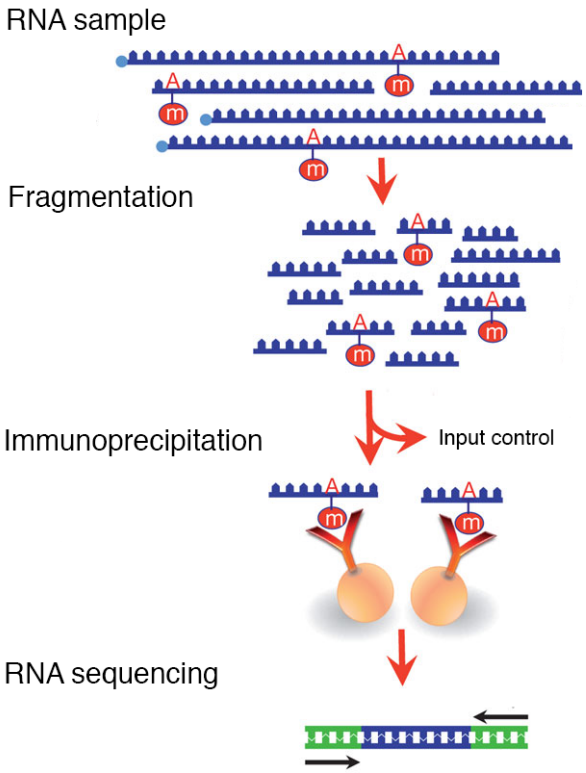


Helm, Motorin, Nat Rev Gen 2017

Antibody-based detection of m6A



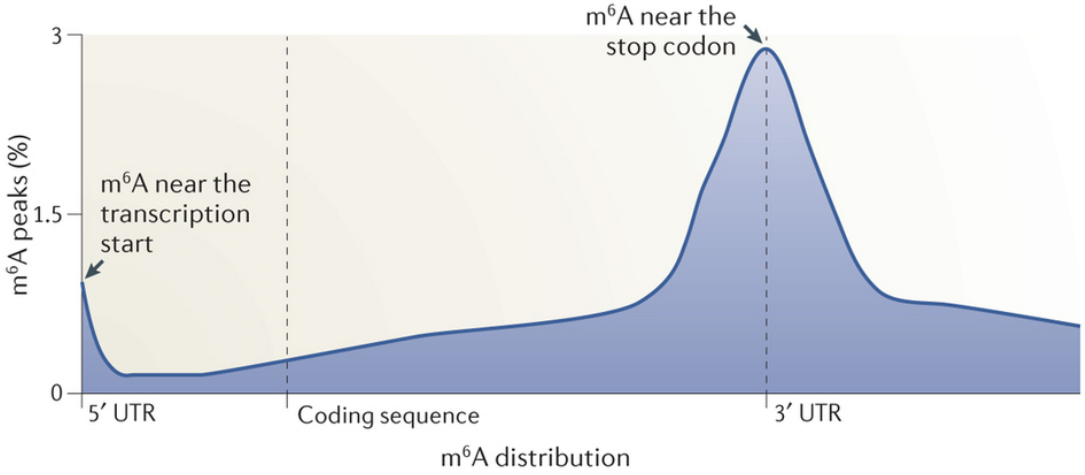
N6-methyladenosine is enriched at stop codon at DRACH motif



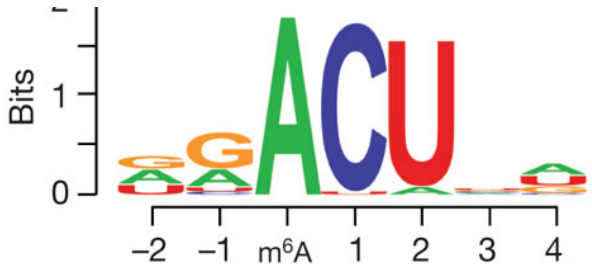
>18 000 methylation sites

3-5 per mRNA

appear in clusters

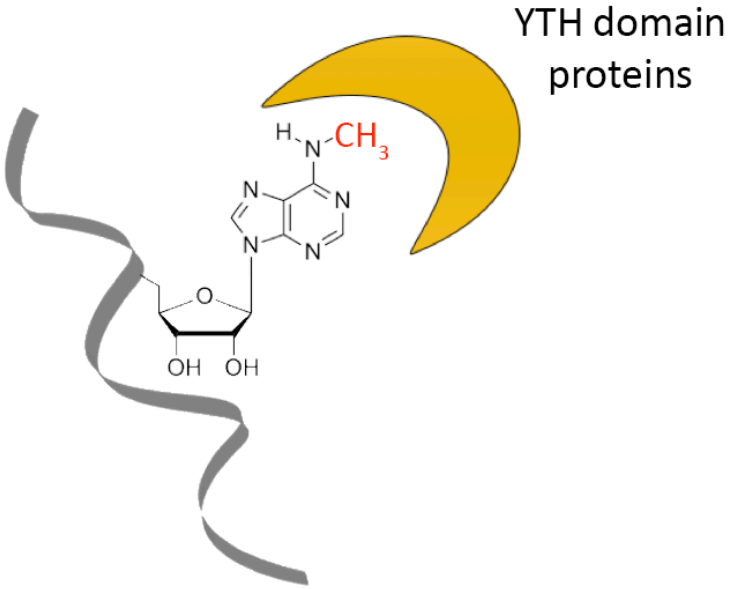


Nature Reviews | Molecular Cell Biology

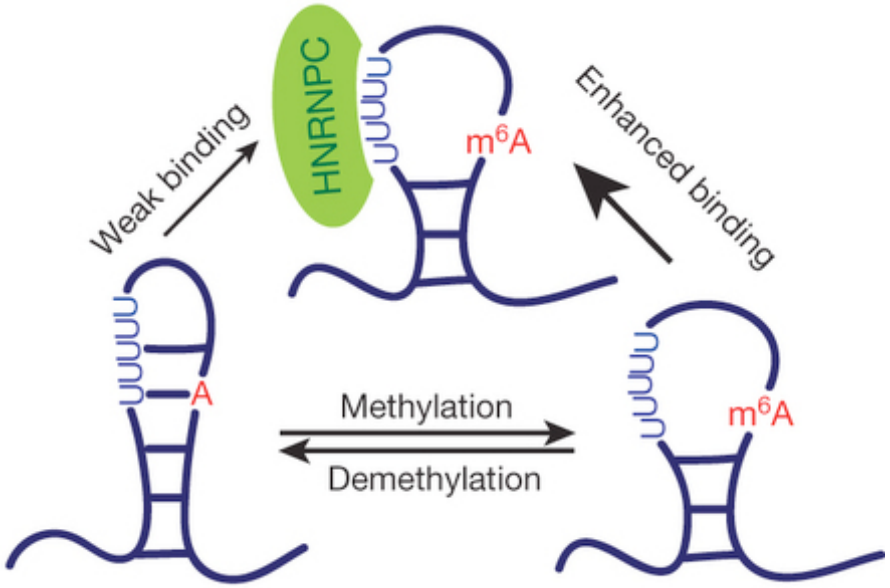


N6-methyladenosine is recognized by sensing proteins in two modes

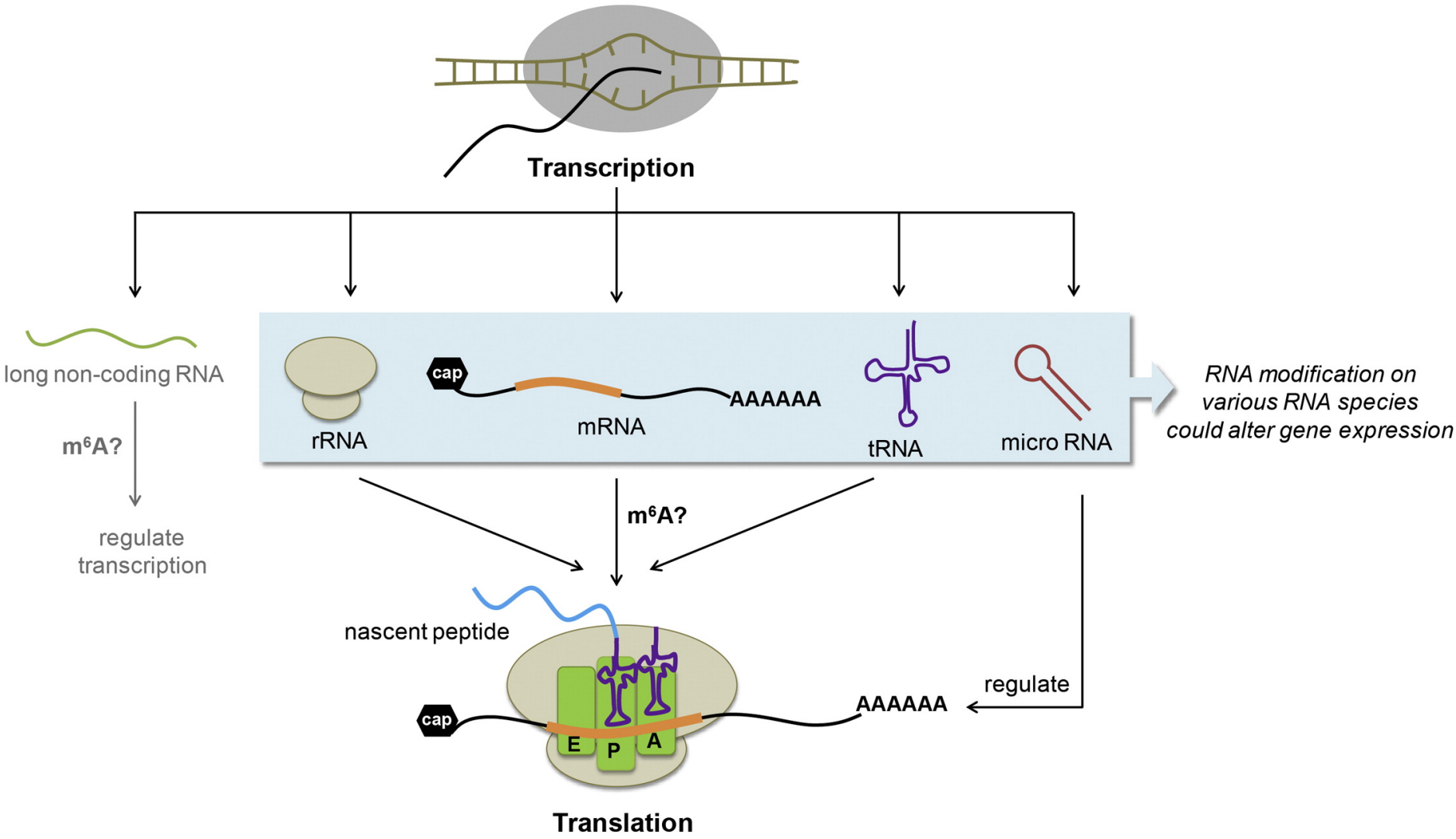
Direct recognition



Structural switch

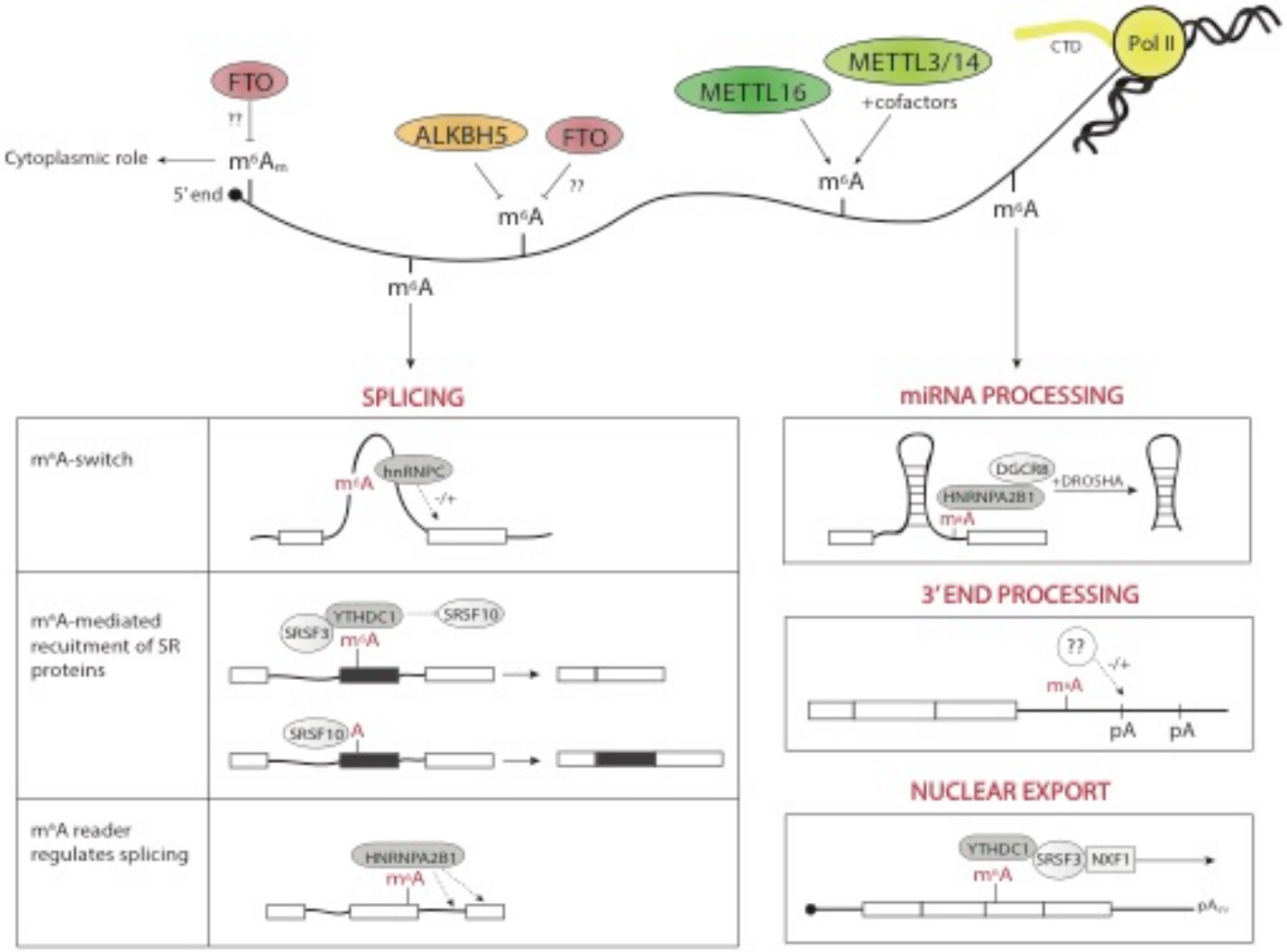


Dynamic RNA Modifications in Gene Expression Regulation



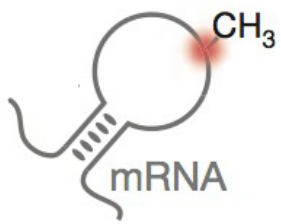
http://dx.doi.org/10.1016/j.molcel.2014.09.001

The nuclear roles of m^{6}A



Covelo-Molares et al., 2018

N6-methyladenosine modification serves multiple functions



RNA stability and sequestering to P-bodies
RNA export
alternative splicing
miRNA processing
translation efficiency

How is methylation at stop codon achieved ?

Why are some DRACH sites methylated and other not ?

Which factors are able to distinguish methylated and non-methylated RNA ?

What is the function of demethylases ?

Wang et al., *Cell,*2013; Fustin et al., *Cell*, 2013, Dominissini et al., *Nature*,2012; Meyer et al., *Cell* 2012

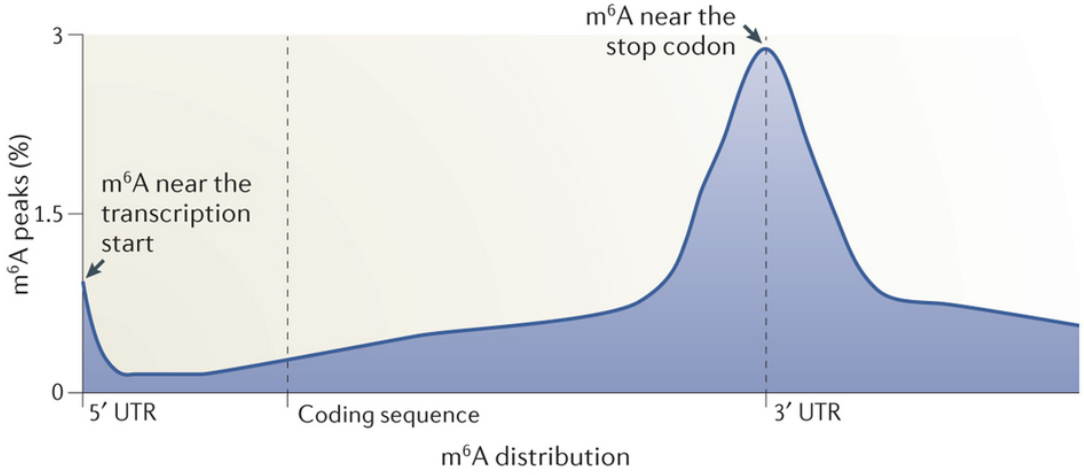
m6A is enriched at stop codon at DRACH motif

>18 000 methylation sites

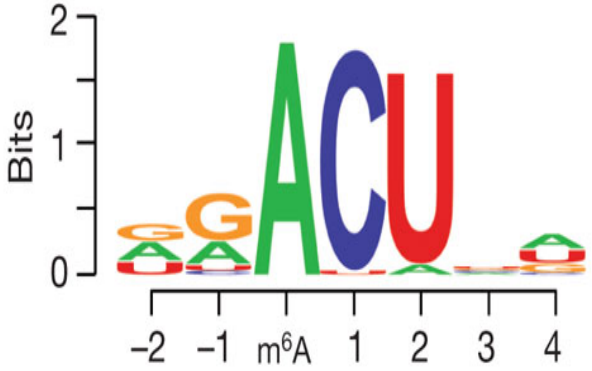
1-3 per mRNA

appear in clusters

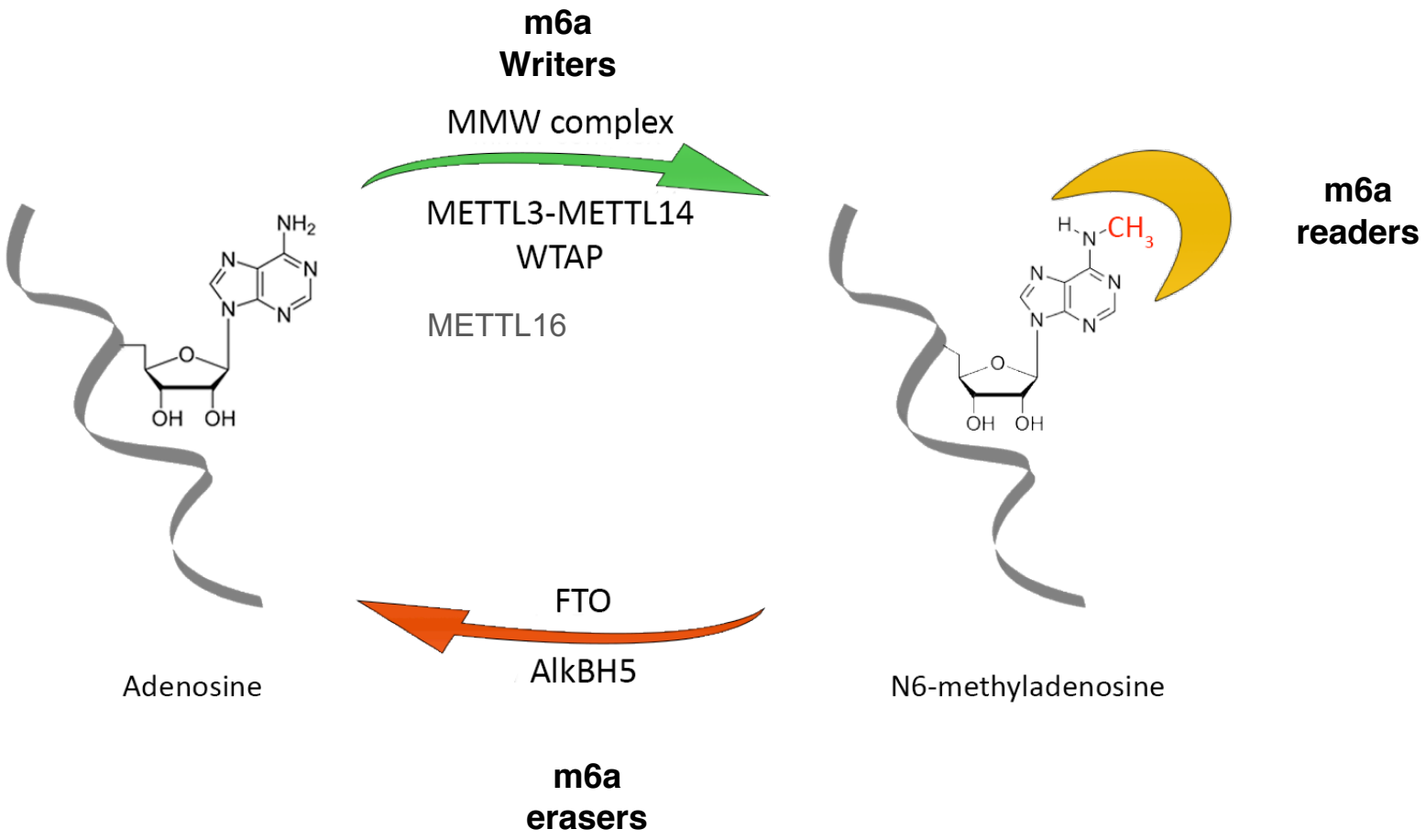
Conserved motif **DRACH**
D=A/G/U; R=G/A, H=A/C/U



Nature Reviews | Molecular Cell Biology

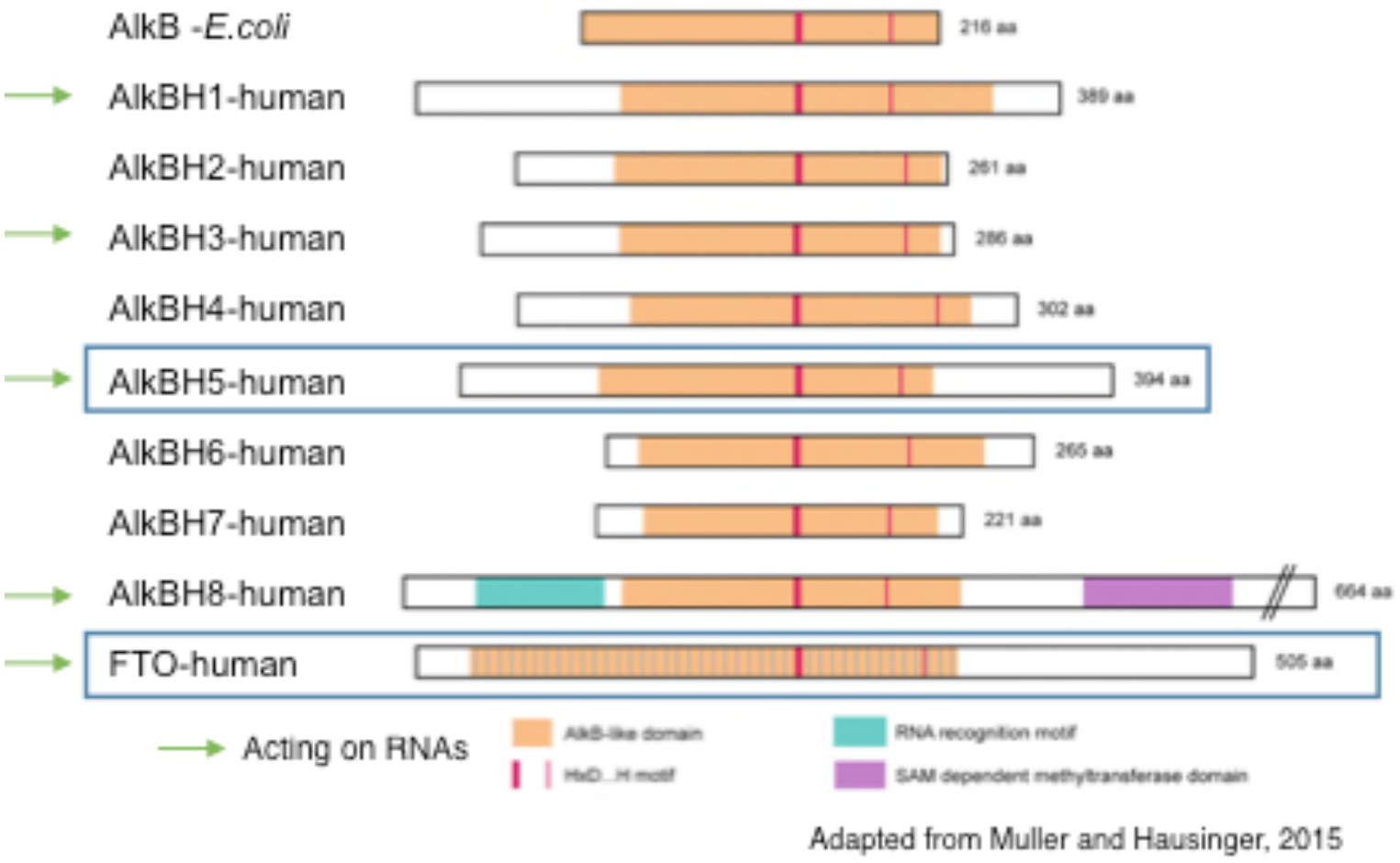


mRNA methylation is a reversible process



Gerken et Al., *Science*, 2007; Dominissimi et Al., *Nature*, 2012; Meyer et Al., *Cell*, 2012; Liu et Al., *Nat. Chem. Biol.* , 2014; Wang et Al., *Nature*, 2014

AlkBH family of Fe(II)/α-ketoglutarate-dependent dioxygenases



Adapted from Muller and Hausinger, 2015

m^6A/m^6A_m demethylases: FTO and AlkBH5

	FTO	AlkBH5
Substrate	m^6A and m^6A_m	m^6A
Oxidative demethylation	With 2 detectable intermediates	Without stable intermediates
Highest expression	Brain	Testis
K.O. mouse model phenotype	Reduce adipose tissue	Aberrant spermatogenesis

Fu Y, et al. *Nature Com.,* 2013; Jia et al., *Nat. Chem. Biol.,* 2011; Mauer, J. et al., *Nature,* 2017; Zheng, et al., *Mol. Cell,* 2013; Fsicher, et al., *Nature*, 2009

FTO (Fat mass and Obesity associated) phenotypes associated with obesity

SNPs in human:

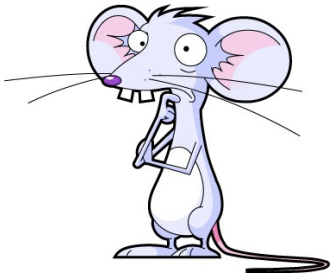
SNP hotspot in FTO intron correlates with diabetes and obesity

1 in 6 adults are homozygous for the risk allele (Frayling et al., 2007, Science)

higher weight (≥3 kg compared to average) and 1,67-fold increased odds of developing obesity (Frayling et al., 2007, Science)

Mouse models:

FTO over-expression
(Church et al.,2010, *Nature Genetics*)



FTO knockout
(Fischer et al., 2009,*Nature*)

FTO catalytical mutant
(Church et al., 2009, *PLoS Genetics*)

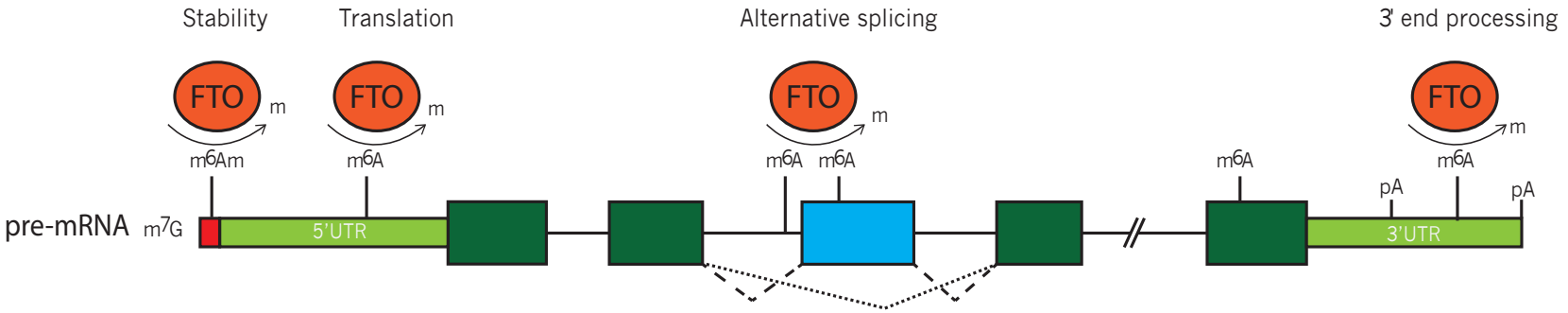
Our FTO results

1. FTO binds pre-mRNAs, it likely functions co-transcriptionally.

2. FTO binding is not enriched around the RRACH motif and correlates with adenosine methylation positions at TSS.

3. FTO appears to play a role in 3’ end processing.

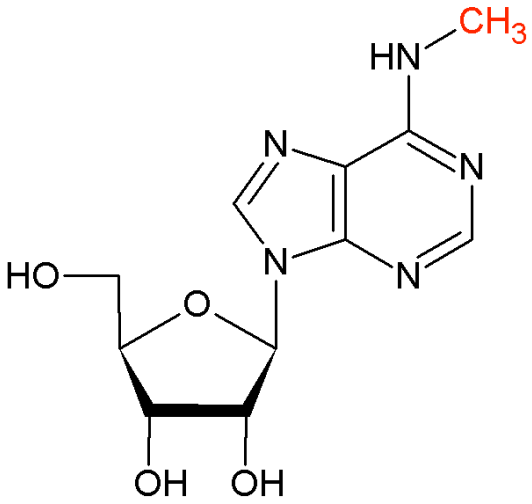
4. FTO demethylation activity facilitates exon inclusion in a subset of mRNAs.



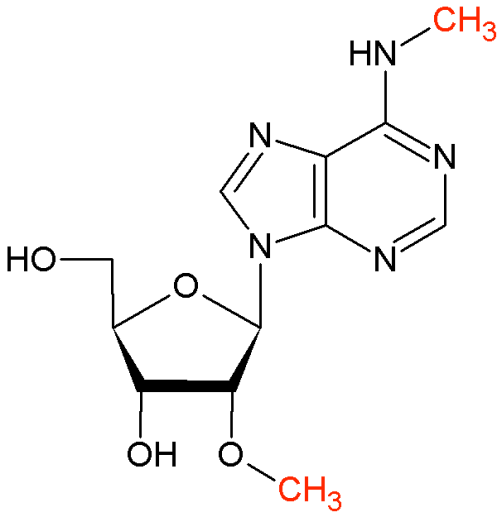
Bartosovic et al., 2017

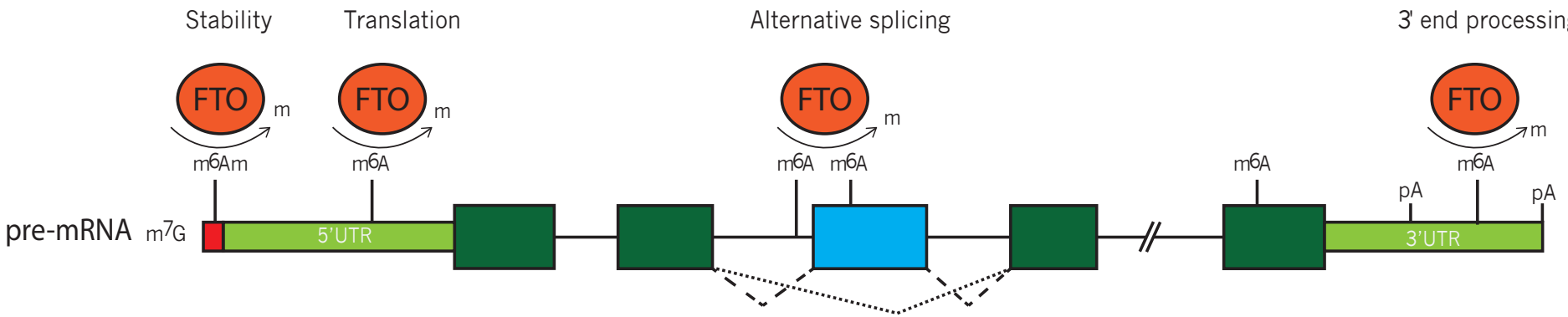
FTO specificity for m^6A versus m^6A_m

m6A



m6Am





RESEARCH ARTICLE SUMMARY

MOLECULAR BIOLOGY

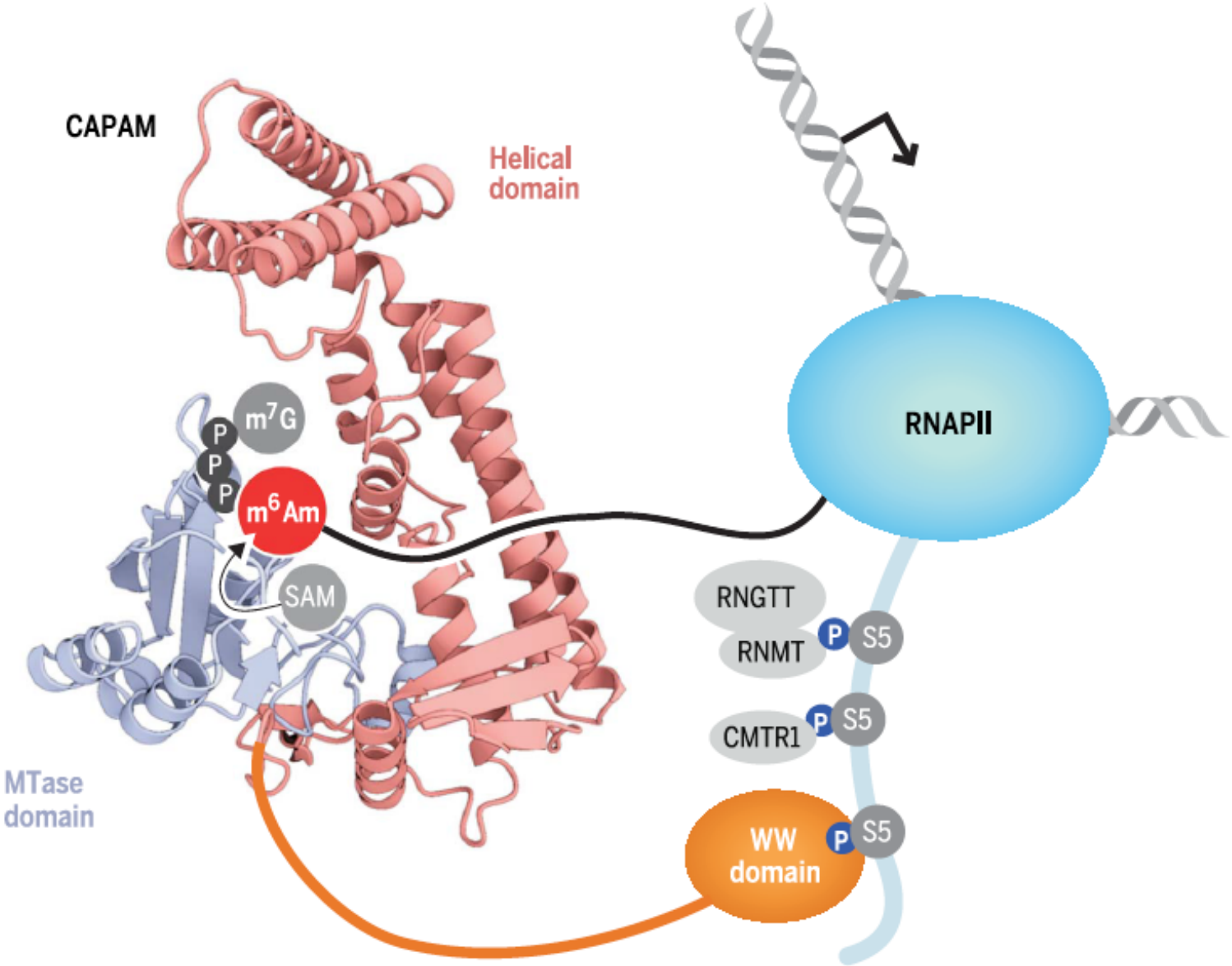
Cap-specific terminal N^6-methylation of RNA by an RNA polymerase II–associated methyltransferase

Shinichiro Akichika*, Seiichi Hirano*, Yuichi Shichino, Takeo Suzuki, Hiroshi Nishimasu, Ryuichiro Ishitani, Ai Sugita, Yutaka Hirose, Shintaro Iwasaki, Osamu Nureki†, Tsutomu Suzuki†

Resource

Molecular Cell

Identification of the m^{6}Am Methyltransferase PCIF1 Reveals the Location and Functions of m^{6}Am in the Transcriptome


CAPAM
Helical domain
RNAPII
m7G
P
P
P
m6Am
SAM
MTase domain
RNGTT
RNMT
P
S5
CMTR1
P
S5
WW domain
P
S5