# "Harmful and useful macromolecular methylations - formation and removal"

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MBV9100, 2 November 2011

**Lecture 1**

## Macromolecular methylation and its reversal

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## Methylation of macromolecules (DNA, RNA and protein)

1. Aberrant methylations (damage), induced by methylating agents
2. Targeted, enzyme mediated methylations

## Methylation of macromolecules (DNA, RNA and protein)

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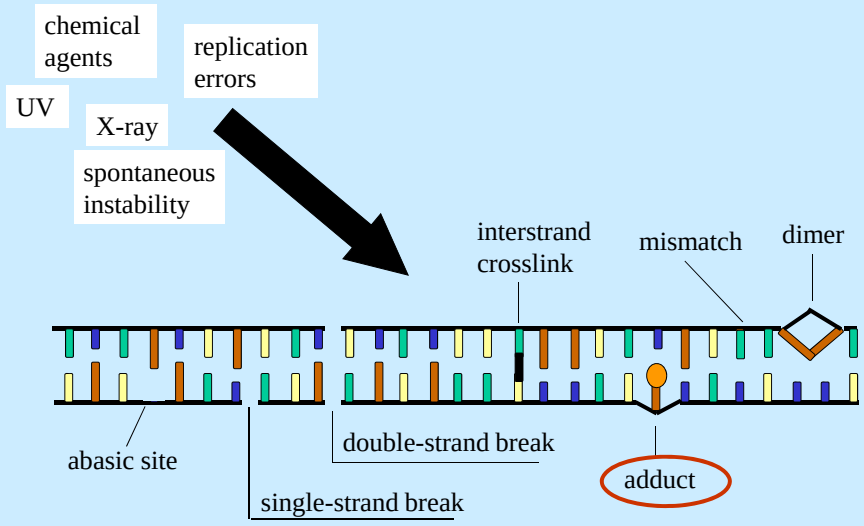
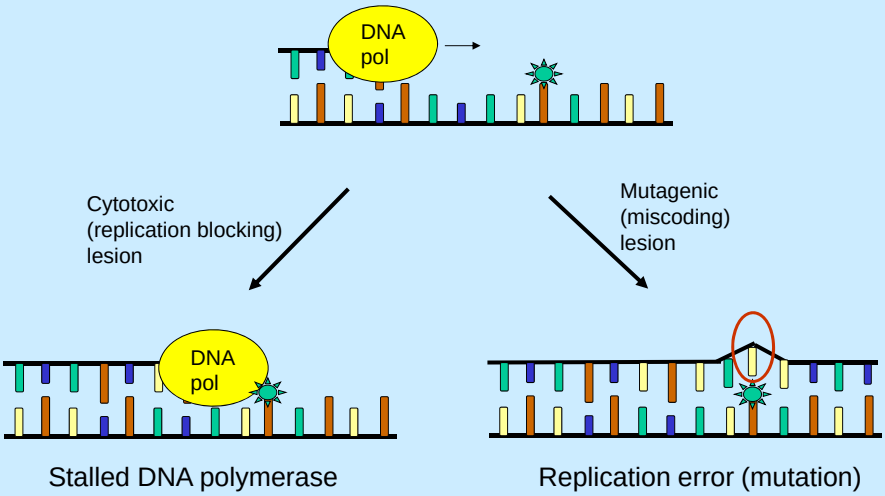
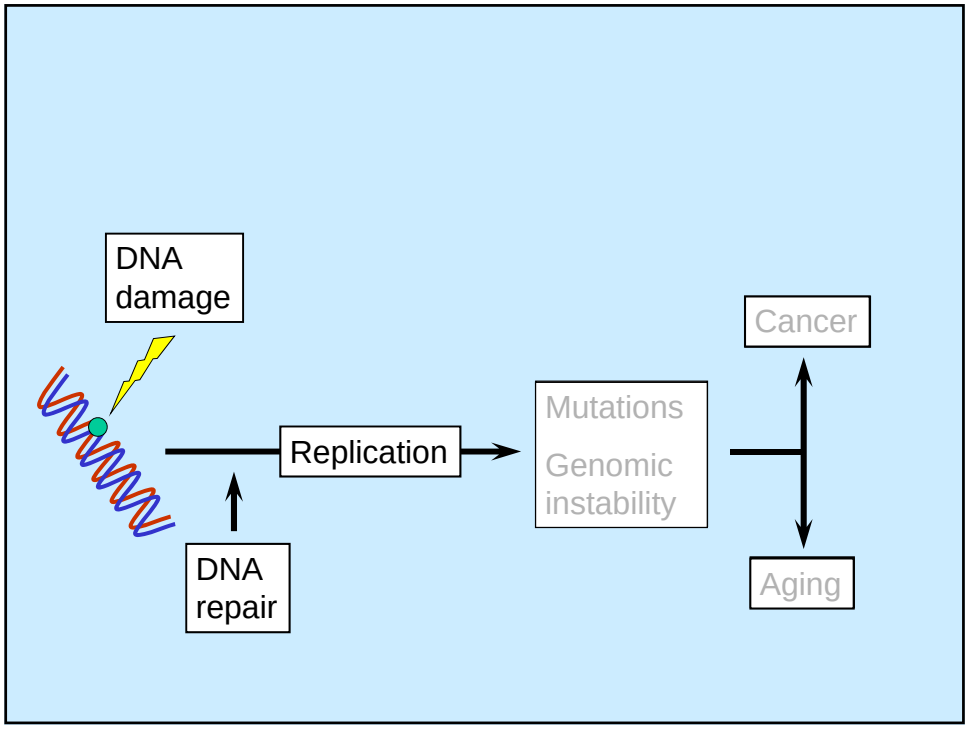
2. Targeted, enzyme mediated methylations

## Different types of DNA damage




## Lesions on DNA bases are cytotoxic or mutagenic



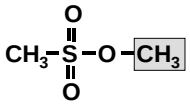



Chemical (aberrant) methylation of DNA and RNA

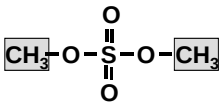
- Introduced at random positions by methylating agents
- Methylating agents are present in the environment
- Methylating agents are also generated intracellularly
- Most aberrant methylations of DNA are harmful (cytotoxic or mutagenic)
- Cells have multiple proteins dedicated to repairing alkylated DNA

## Methylating agents

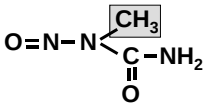
### Synthetic (lab chemicals)



Methyl methanesulfonate (MMS)



Dimethyl sulfate (DMS)



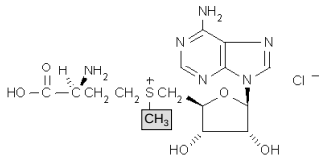
Methylnitrosourea (MNU)

### Environmental (natural)

$CH_3$- X

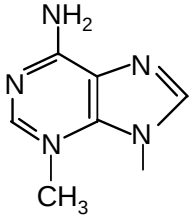
Methyl halides (X = Cl, I, Br)

### Intracellular

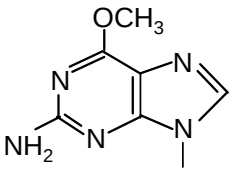


S-adenosylmethionine (SAM)

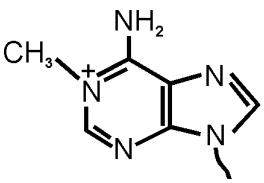
## Examples of methyl lesions on DNA bases



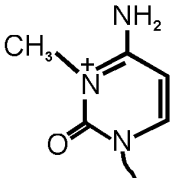
3-methyladenine



$O^6$-methylguanine



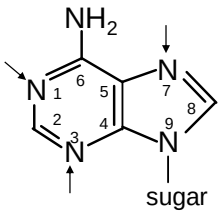
1-methyladenine



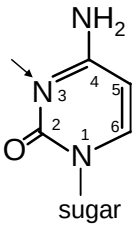
3-methylcytosine

## Targets for chemical alkylations in DNA bases

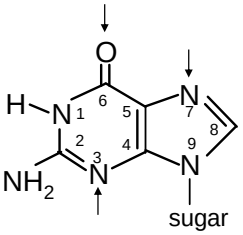
A



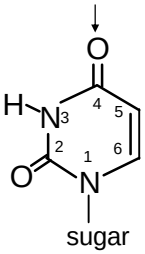

C




G



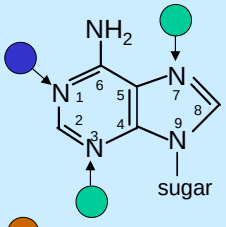

T




## Repair of alkylation damage by different proteins

- Alkylbase glycosylase
- Alkyl transferase
- Oxidative demethylase (AlkB)

A

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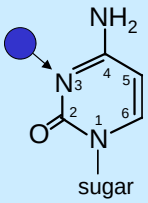


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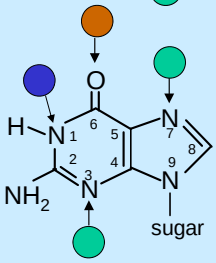


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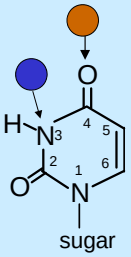
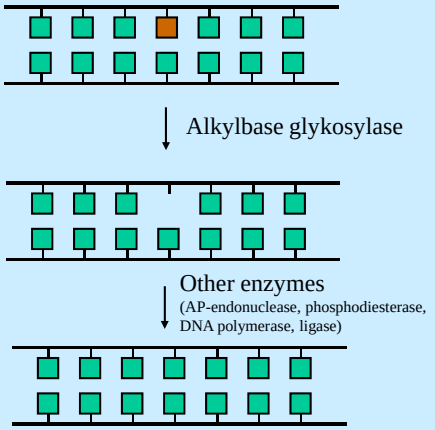
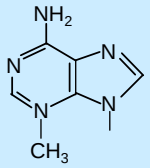


## Alkylbase glykosylases (base excision repair)

E. coli: Tag, AlkA
Human: MPG (methylpurine glycosylase)



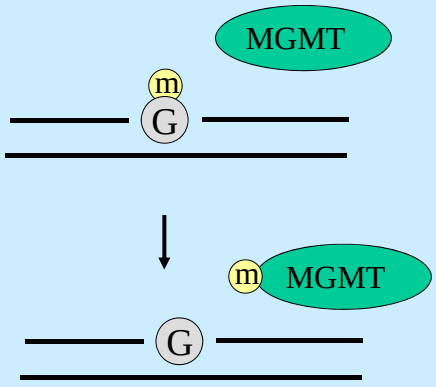

■ = alkylated base, e. g.

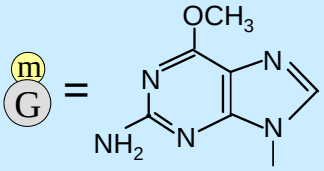



3-methyladenine

## Alkylbase transferases - suicide repair proteins

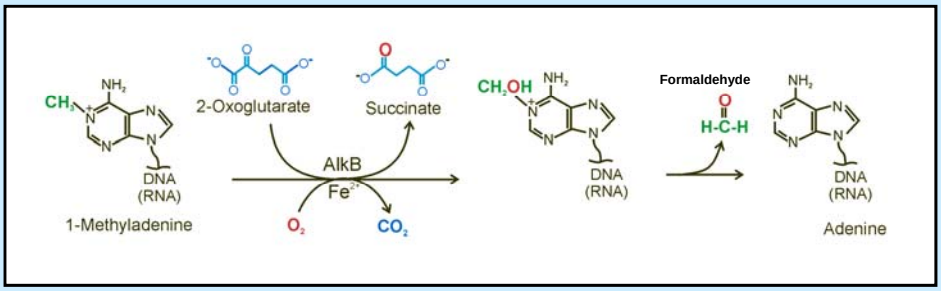
E. coli: Ogt, Ada
Humans: MGMT (methylguanine methyltransferase)




$O^6$-methylguanine

AlkB - DNA repair by oxidative demethylation




Methylation of macromolecules (DNA, RNA and protein)

1. Aberrant methylations (damage), induced by methylating agents

2. Targeted, enzyme mediated methylations

## Targeted, enzyme mediated methylations

1. DNA
2. RNA
3. Protein

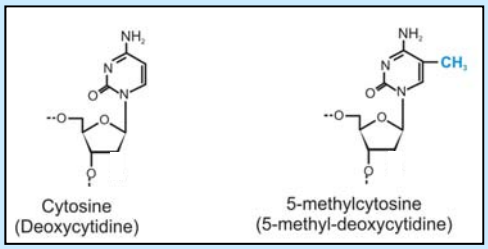
## Targeted, enzyme mediated methylations

1. DNA
2. RNA
3. Protein

## Targeted methylation of DNA – examples

- Restriction methylases (bacteria): Own DNA is methylated (e.g. N6-methyladenine) at specific sequences, thereby preventing cleavage by corresponding restriction endonucleases
- In bacteria: Dam methylation of GATC sequences is used to distinguish between ”old” and ”new” strand in DNA replication
- → Vertebrates and plants: Regulation of gene expression by cytosine methylation

## The modified base 5-methylcytosine




- Important mediator of gene regulation in vertebrates and flowering plants.
- Methylation is associated with repression of transcription.
- Cytosine methylation is important in mediating imprinting, i. e. the silencing of one of the two alleles of a gene.
- In mammals: Methylation at CG dinucleotides, referred to as CpG
- Plants: Methylation at CpG, but also at CpNpG.

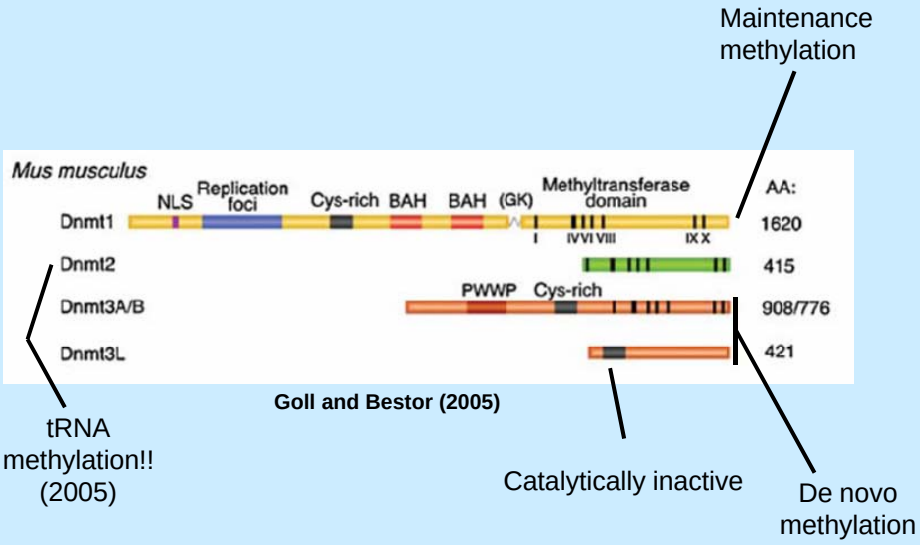
## CpG islands

- Found in the promoter region of most (76 %) human genes
- High frequency of CpGs (relative to rest of the genome)
- 0.4 – 3 kb long, rich in G + C (>55 %)
- Methylation of CpG islands is associated with (heritable) repression of transcription

## Two types of cytosine methylation of DNA

- De novo methylation. When an active, non-methylated gene becomes inactivated by methylation.
- Maintenance methylation. Mechanism responsible for maintaining methylation patterns as cells divide; the hemimethylated DNA resulting from DNA replication is converted to fully methylated DNA

## DNA cytosine methylases in mammalian cells



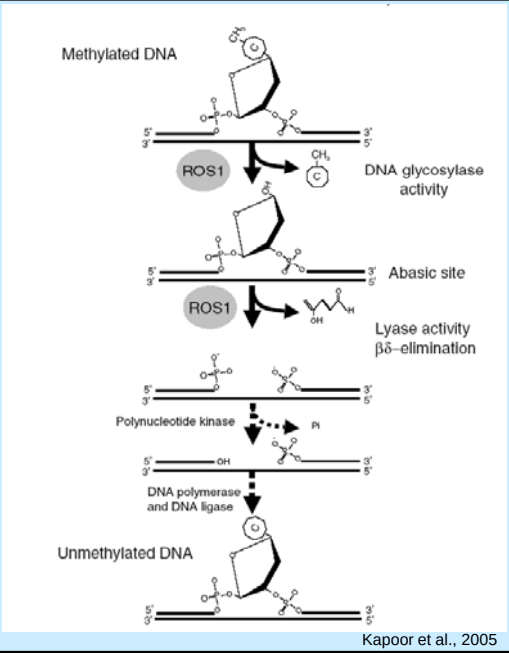

## Reversal of cytosine methylation

- Plants (Arabidopsis): Enzymes capable of demethylation have been characterized, and they have been shown to mediate gene activation.
- Vertebrates: Reversal appears to occur, but the mechanisms involved remain elusive.

## Reversal of cytosine methylation in plants

- The proteins ROS1 (**R**epressor **o**f **s**ilencing) and Demeter have both been shown to be active 5-methylcytosine DNA glycosylases
- Both these proteins have been shown to prevent (or reverse) gene inactivation in plants

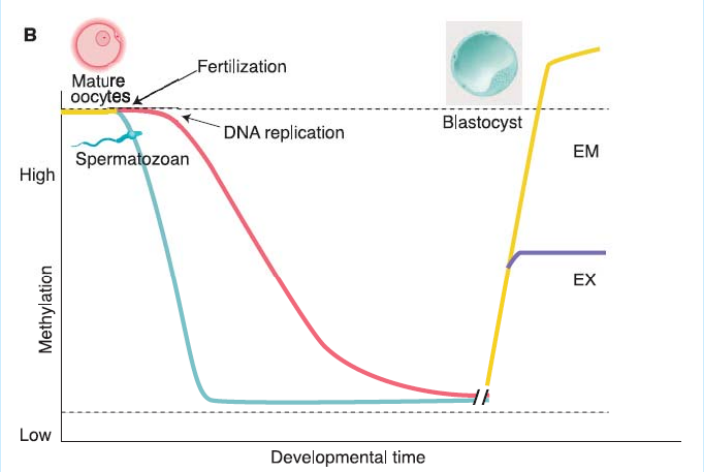
## Plants - demethylation by base excision repair (BER)




Kapoor et al., 2005

## Reversal of 5-meC methylation in mammalian cells - examples

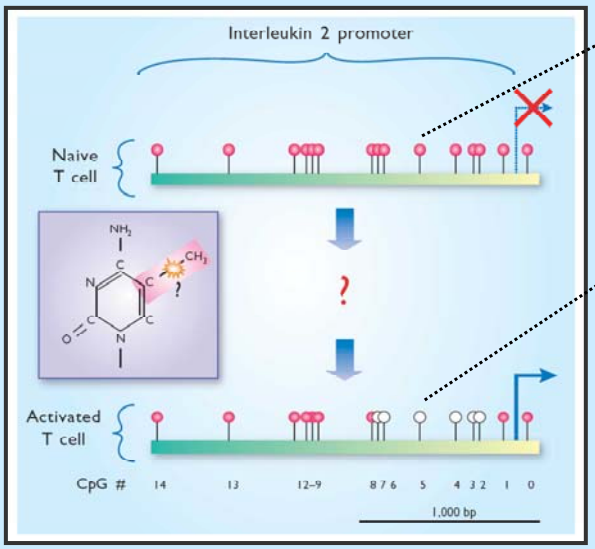
Rapid and active demethylation of sperm DNA upon fertilisation.
Demethylation of oocyte DNA involves slow, passive process (dilution by replication without remethylation)




**Reik et al., 2001**

## Reversal of 5-meC methylation in mammalian cells - examples

**Demethylation of the IL-2 promoter during T cell activation**




Methylated CpGs

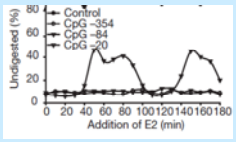
Unmethylated CpGs

**Bird, 2003**

## Reversal of 5-meC methylation in mammalian cells - examples

Cyclical demethylation at hormone driven promoters;
active demethylation is likely to be involved:

Methylation




**Cyclical DNA methylation of a transcriptionally active promoter**

Raphaël Métivier[1], Rozenn Gallais[1], Christophe Tiffoche[1], Christine Le Péron[1], Renata Z. Jurkowska[2], Richard P. Carmouche[3], David Ibberson[3], Peter Barath[1]†, Florence Demay[1], George Reid[1], Vladimir Benes[3], Albert Jeltsch[2], Frank Gannon[1]† & Gilles Salbert[1]

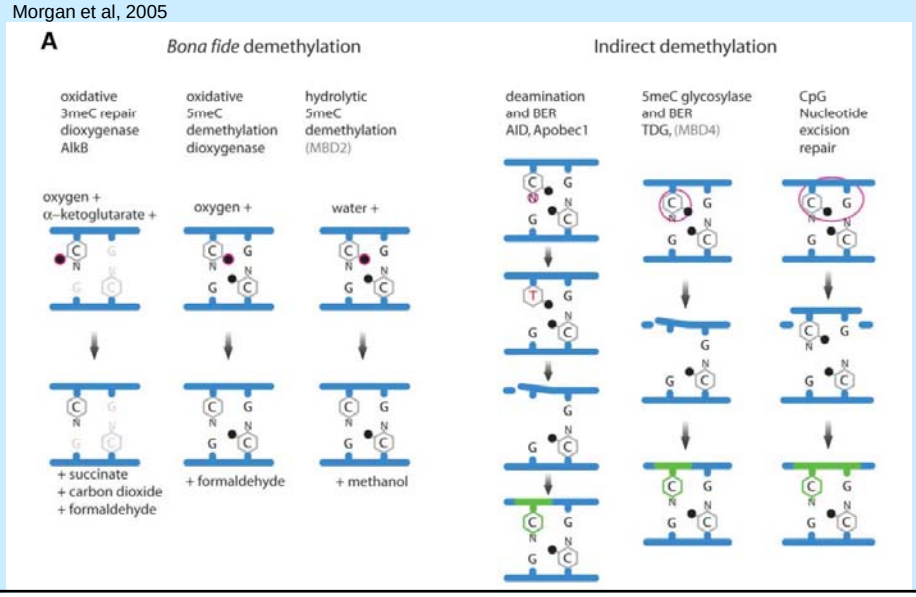
Nature 2008

**Transient cyclical methylation of promoter DNA**

Sara Kangaspeska[1]*, Brenda Stride[1]*†, Raphaël Métivier[2], Maria Polycarpou-Schwarz[2], David Ibberson[1], Richard Paul Carmouche[1], Vladimir Benes[1], Frank Gannon[1]† & George Reid[1]

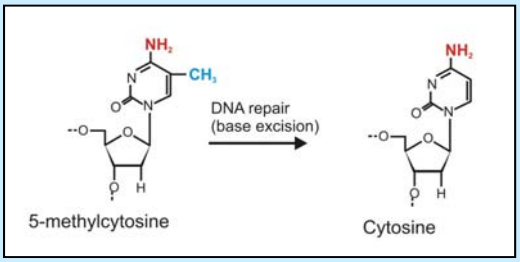
## Reversal of 5-meC methylation in mammalian cells - conceivable mechanisms

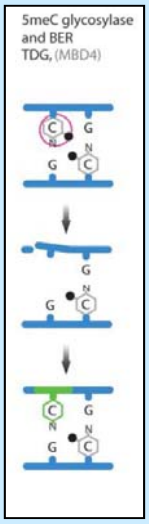
Morgan et al, 2005



## Active demethylation of mammalian DNA - some popular models (1:2)

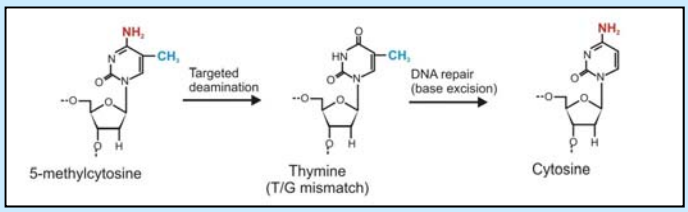
Base excision repair of 5meC by glycosylase (MBD4 or TDG)

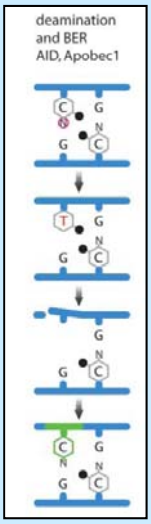



## Active demethylation of mammalian DNA - some popular models (2:2)

Deamination of 5meC by specific deaminase (AID, Apobec1), followed by base excision repair of resulting T/G mismatch by glycosylase (MBD4 or TDG)



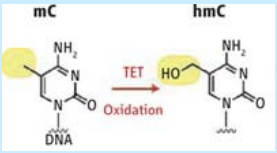
## A new player (2009): 5-hydroxymethylcytosine (5-hmC)

- A new enzyme, TET1, was found to use an AlkB-like mechanism to hydroxylate 5-meC to 5-hmC

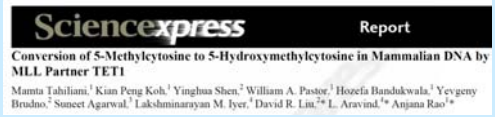
- Two additional related proteins exist, TET2 and TET3

- 5-hmC was found to be abundant in Purkinje neurons

- 5-meC and 5-hmC indistiguishable by most popular methods for studying DNA methylation




**Questions:**
- Is 5-hmC a new epigenetic mark?
- Is 5-hmC an intermediate in the demethylation of 5-meC?


Sciencexpress Report

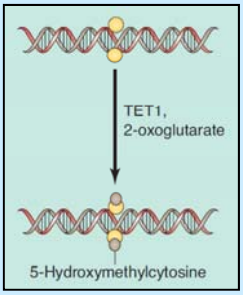
**Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1**

Mamta Tahiliani,[1] Kian Peng Koh,[1] Yinghua Shen,[2] William A. Pastor,[1] Hozefa Bandukwala,[1] Yevgeny Brudno,[2] Suneet Agarwal,[3] Lakshminarayan M. Iyer,[4] David R. Liu,[2]* L. Aravind,[4]* Anjana Rao[1]*


Sciencexpress Report

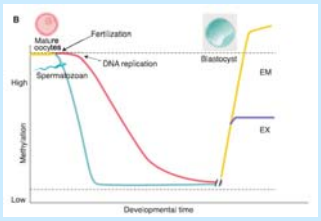
**The Nuclear DNA Base 5-Hydroxymethylcytosine Is Present in Purkinje Neurons and the Brain**

Skirmantas Kriaucionis and Nathaniel Heintz*




Ooi and Bestor,2009

## Involvement of 5meC hydroxylation in apparent demethylation in the zygote

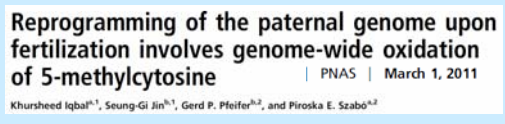




5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming

Mark Wossidlo[1], Toshinobu Nakamura[2], Konstantin Lepikhov[1], C. Joana Marques[3], Valeri Zakhartchenko[4], Michele Boiani[5], Julia Arand[1], Toru Nakano[2], Wolf Reik[3,6] & Jörn Walter[1]

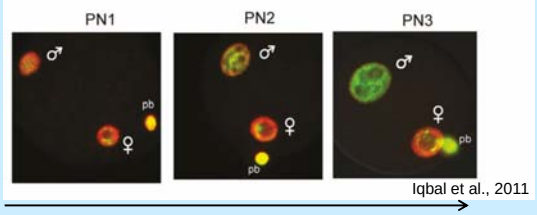
Published 15 Mar 2011


**Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine**

PNAS | March 1, 2011

Khursheed Iqbal[a,1], Seung-Gi Jin[b,1], Gerd P. Pfeifer[b,2], and Piroska E. Szabó[a,2]

Red: 5meC
Green: 5hmC




Iqbal et al., 2011

**Developmental time**

New knowledge: Apparent rapid demethylation of sperm DNA upon fertilisation really corresponds to hydroxylation of 5meC into 5hmC.

## Adding more complexity to active DNA demethylation
## Possible roles of Tet proteins and 5hmC

1. Tet proteins (Tet1, Tet2, Tet3) can further oxidize 5hmC into 5-formylcytosine (5fC) and 5-carboxycytosine (5caC)

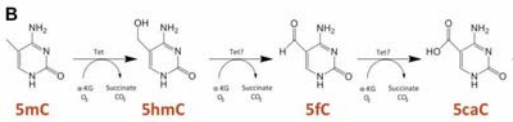
**Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine**

**Tet-Mediated Formation of 5-Carboxylcytosine and Its Excision by TDG in Mammalian DNA**

Shinsuke Ito,[1,2,3] Li Shen,[1,2,3] Qing Dai,[1] Susan C. Wu,[1,2] Leonard B. Collins,[4] James A. Swenberg,[4] Chuan He,[1] Yi Zhang[1,2,*]

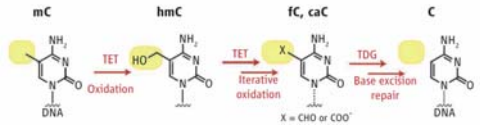
Yu-Fei He,[1,3] Bin-Zhong Li,[1,3] Zheng Li,[1] Peng Liu,[1] Yang Wang,[1] Qingyu Tang,[1] Jianping Ding,[2] Yinglong Jia,[2] Zhangcheng Chen,[2] Lin Li,[2] Yan Sun,[2] Xiaoxue Li,[2] Qing Dai,[1] Chun-Xiao Song,[1] Kangling Zhang,[1] Chuan He,[1] Guo-Liang Xu[2]

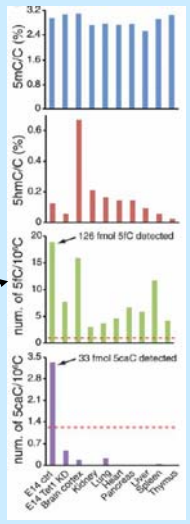
SCIENCE VOL 333 2 SEPTEMBER 2011




2. 5fC and 5caC are present in mammalian genomic DNA (especially in ES cells; E14)

3. Thymine DNA glycosylase (TDG) can excise 5caC from DNA




Ito et al., 2011

## Do Tet proteins and deaminases act together to achieve DNA demethylation?

**Thymine DNA Glycosylase Is Essential for Active DNA Demethylation by Linked Deamination-Base Excision Repair**

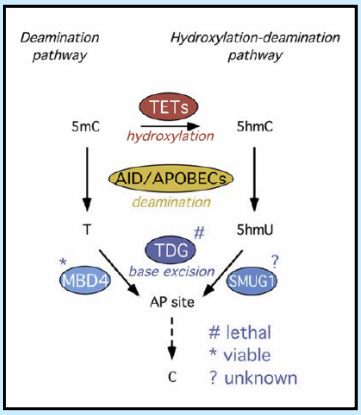
Salvatore Cortellino,[1] Jinfei Xu,[1] Mara Sannai,[1] Robert Moore,[1] Elena Caretti,[1] Antonio Cigliano,[1] Madeleine Le Coz,[1] Karthik Devarajan,[2] Andy Wessels,[4] Dianne Soprano,[5] Lara K. Abramowitz,[6] Marisa S. Bartolomei,[6] Florian Rambow,[7] Maria Rosaria Bassi,[1] Tiziana Bruno,[8] Maurizio Fanciulli,[8] Catherine Renner,[3] Andres J. Klein-Szanto,[3] Yoshihiro Matsumoto,[10,12] Dominique Kobi,[11] Irwin Davidson,[11] Christophe Alberti,[7,8] Lionel Larue,[7,8] and Alfonso Bellacosa[1,*]

Cell *146*, 67–79, July 8, 2011

1. TDG knock-out mice displayed hypermethylation of certain genes

2. TDG associates with the cytosine deaminase AID

3. TDG displyed activity on 5-hydroxymethyluracil (product of 5-methylcytosine hydroxylation and deamination) in DNA



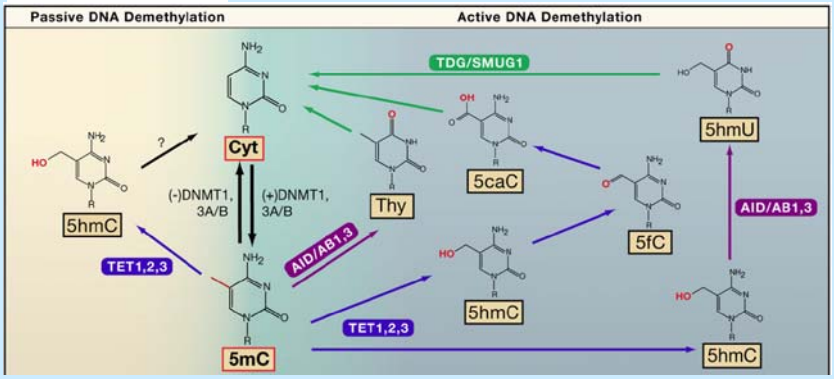
## Putting it all together into one (still messy) picture

**DNA Demethylation Dynamics**

Nidhi Bhutani,[1,2] David M. Burns,[1] and Helen M. Blau[1,*]

Cell *146*, September 16, 2011

Glycosylase
Oxygenase
Deaminase




Some possible pathways for active DNA demethylation:
1. Tet-mediated oxidation into 5caC, excision by TDG
2. Hydroxylation into 5hmC, deamination into 5hmU, excision by TDG
3. Deamination into T, excision by TDG

## A flurry of papers in Nature, Science and Cell on 5hmC and Tet proteins after their discovery in 2009. Hot stuff!

## Targeted, enzyme mediated methylations

1. DNA
2. RNA
3. Protein

## RNA methylation

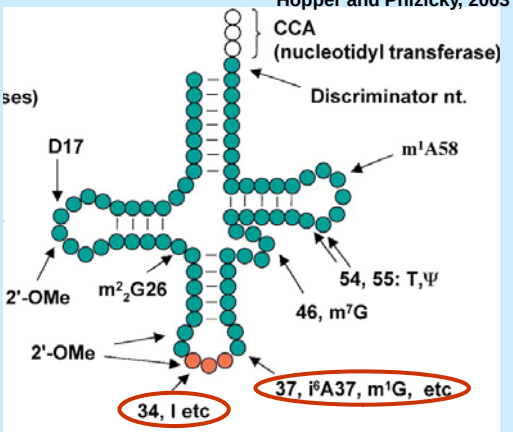
- RNA modifications: Nature's way of expanding the repertoire of building blocks
- ~100 different modified bases are found in RNAs, methylation is the most common modification
- tRNA is the most heavily modified RNA; each tRNA contains several modified nucleosides
- RNA modification is though to serve both structural and regulatory purposes
- Can such be modifications be reversed?

## tRNA methylation is important for proper translation

Positions 34 and 37 of particular importance

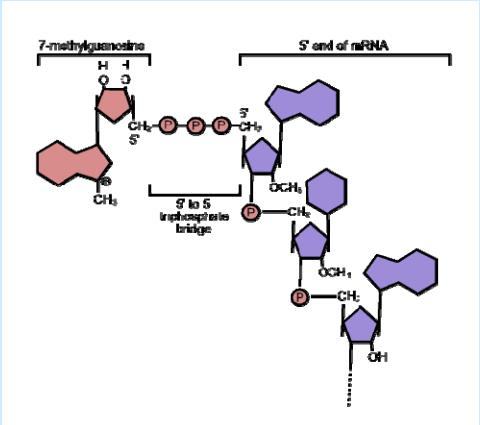
- Position 34 = wobble position; modification assures proper decoding during translation
- Position 37 = proper modification (often 1-methylguanosine) required for reading frame maintenance during translation

Hopper and Phizicky, 2003



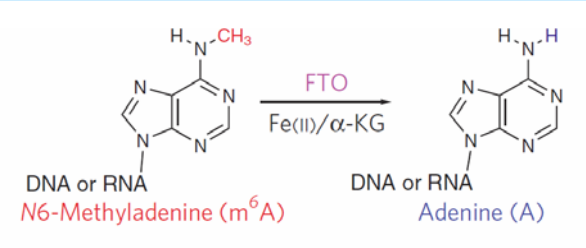

## mRNA methylation

- 7-methylguanine on 5' end (capping):
  - Promotes translation and nuclear export
  - Prevents degradation



## mRNA methylation

- *N*6-methyladenine
  - Dominating modified base in mRNA
  - Functional significance unknown, but may affect nuclear export or processing
- Recent article showed that the human enzyme FTO (fat mass and obesity protein), which is a human AlkB homologue, is able to efficiently demethylate *N*6-methyladenine



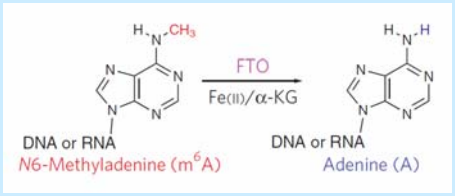

## FTO mediated demethylation of *N*6-methyladenine in RNA

Initially, FTO was shown to have weak activity on 3-methylthymine in DNA (Gerken et al., Science, 2007)

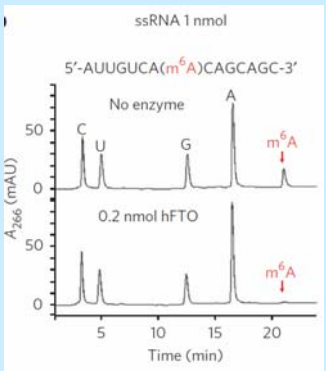

nature chemical biology
BRIEF COMMUNICATION
PUBLISHED ONLINE: 16 OCTOBER 2011 | DOI: 10.1038/NCHEMBIO.687

***N*6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO**

Guifang Jia[1,6], Ye Fu[1,6], Xu Zhao[2,6], Qing Dai[1], Guanqun Zheng[1], Ying Yang[2], Chengqi Yi[3], Tomas Lindahl[4], Tao Pan[3,5], Yun-Gui Yang[2] & Chuan He[1,5]*




HPLC-analysis of nucleosides from m$^6$A-containing RNA treated with hFTO



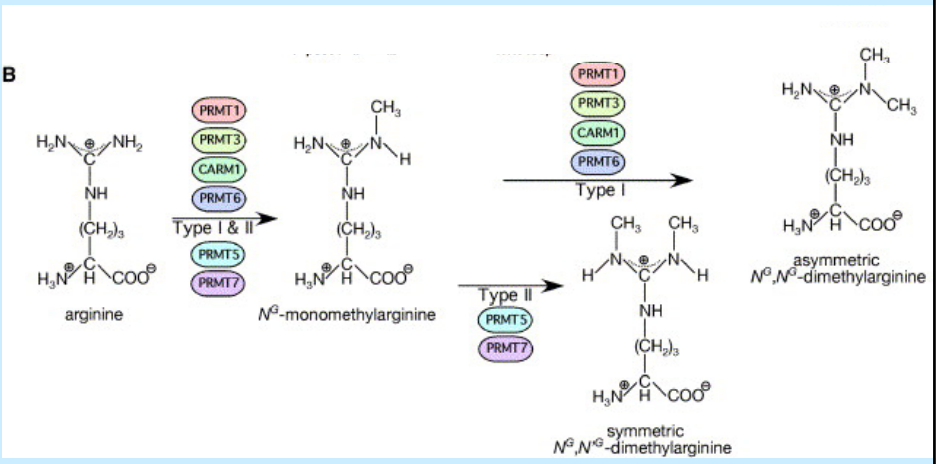
## Targeted, enzyme mediated methylations

1. DNA
2. RNA
3. Protein

## Protein methylation

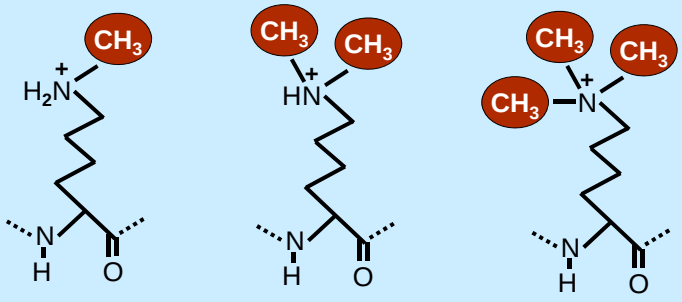
- Primarily Occurs on N-atoms of Lys and Arg residues
- Lys and Arg methylations are very frequent on histone proteins
- Arg (but not Lys) methylations are also found on other proteins, and has been shown to regulate several different processes

## Three different kinds of arginine methylation




Bedford and Richard, 2006

## Three different kinds of lysine methylation

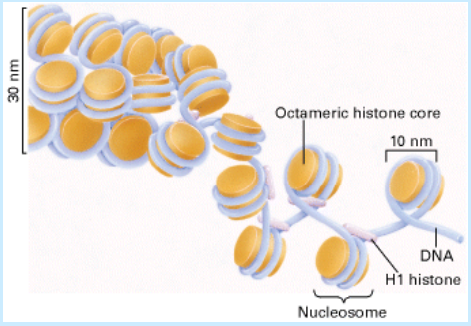


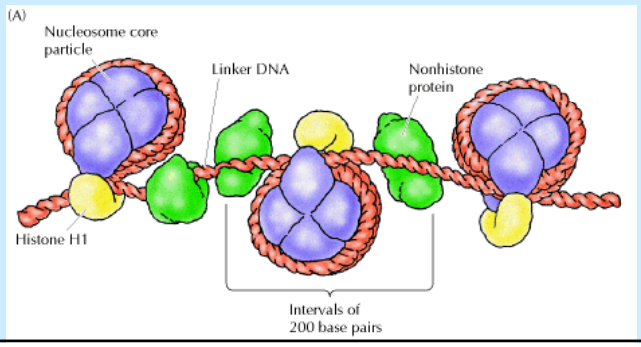
Monomethyl lysine

Dimethyl lysine

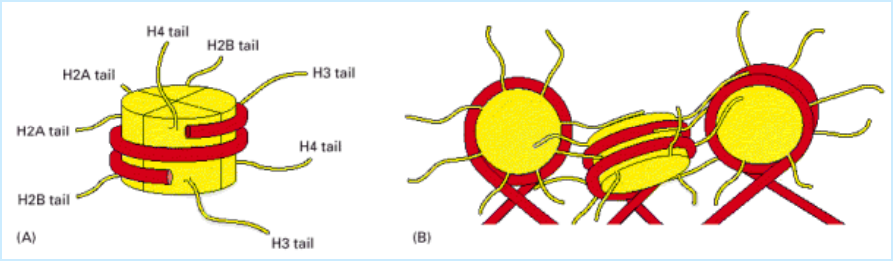
Trimethyl lysine

**Histones**
**in chromatin**

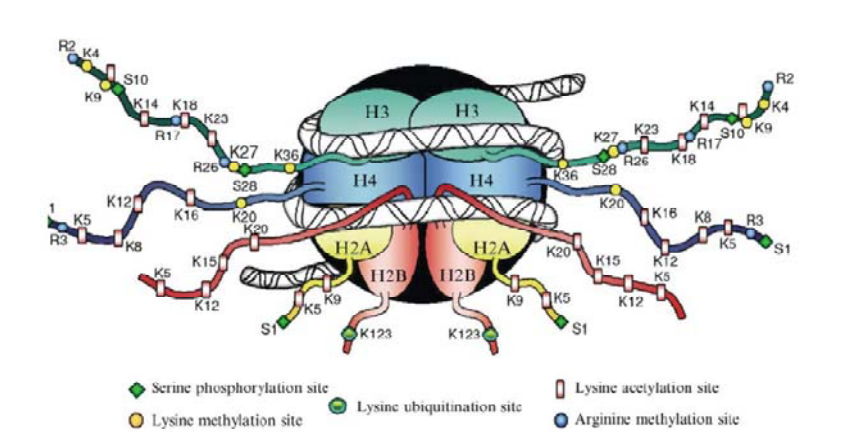



- Nucleosomes consist of histone octamers
- Protruding histone ”tails””
- Histones: Basic proteins, rich in Lys and Arg

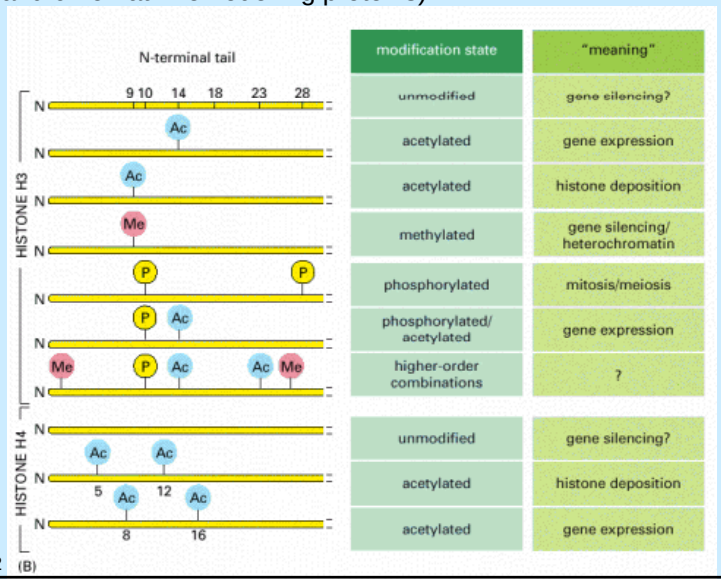


## Histone tails are heavily modified: Acetylation, metylation, phosphorylation



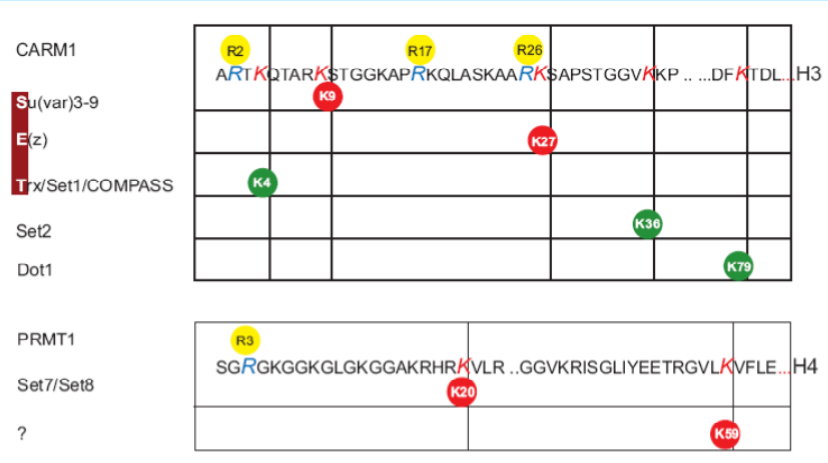

Wood and Shilatifard, 2006

<u>The histone code:</u> It has been postulated that the combination of various histone modifications constitute a code that regulates gene expression through recruitment of specific proteins (transcription factors and chromatin remodelling proteins)




Alberts et al, 2002

## Several different enzymes are involved in histone methylation




Schneider and Shilatifard, 2006

## Histone demethylation

Histone methylation was for a long time thought to be irreversible. During the last decade, this view has changed dramatically due to the discovery of three different classes of demethylases

Demethyliminases (PAD4)

Amine oxidases (LSD1)

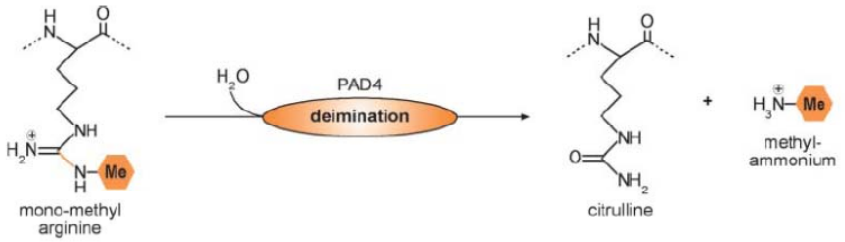
Oxidative demethylases (JmjC-containing proteins)

## Arginine demethylation by demethylimination

**Human PAD4 Regulates Histone Arginine Methylation Levels via Demethylimination**

SCIENCE VOL 306 8 OCTOBER 2004

Yanming Wang,[1,2] Joanna Wysocka,[1,2] Joyce Sayegh,[3] Young-Ho Lee,[4] Julie R. Perlin,[1] Lauriebeth Leonelli,[1] Lakshmi S. Sonbuchner,[1] Charles H. McDonald,[5] Richard G. Cook,[5] Yali Dou,[6] Robert G. Roeder,[6] Steven Clarke,[3] Michael R. Stallcup,[4] C. David Allis,[2]* Scott A. Coonrod[1]*



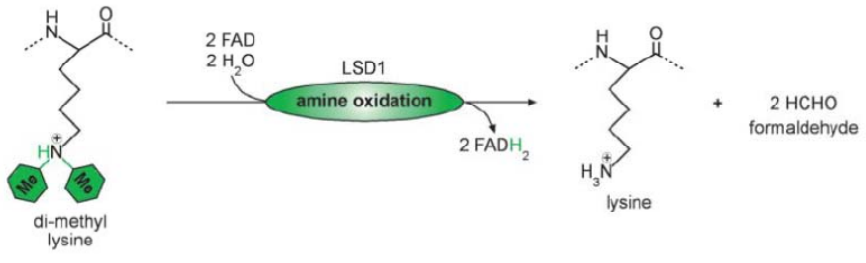

PAD4 acts on several methylated sites in histones H3 and h4
Not true demethylation (conversion to citrulline)

## Lysine demethylation by amine oxidation

**Histone Demethylation Mediated by the Nuclear Amine Oxidase Homolog LSD1**

Cell, Vol. 119, 941–953, December 29, 2004,

Yujiang Shi,[1] Fei Lan,[1] Caitlin Matson,[1] Peter Mulligan,[1] Johnathan R. Whetstine,[1] Philip A. Cole,[2] Robert A. Casero,[3] and Yang Shi[1,*]




LSD1 (lysine specific demethylase 1) demethylates lysine 4 of histone H3 (K4-H3)
First true histone demethylase to be discovered!!

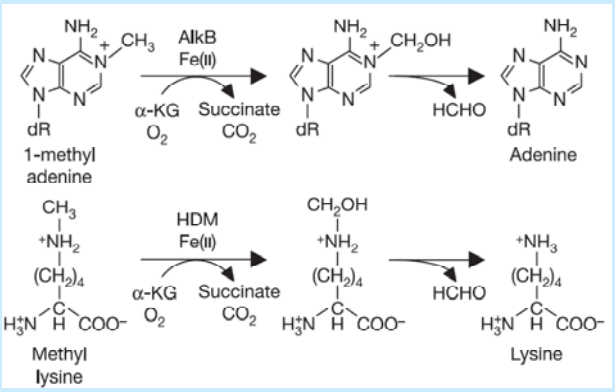
# Lysine demethylation by JmjC-containing proteins

## Histone demethylation by a family of JmjC domain-containing proteins

Yu-ichi Tsukada[1,2], Jia Fang[1,2], Hediye Erdjument-Bromage[3], Maria E. Warren[2], Christoph H. Borchers[2], Paul Tempst[3] & Yi Zhang[1,2]

nature

Vol 439|16 February 2006|d




Reversal of methylation at H3-K9, H3K27, and H3-K36.

Reverses both mono-, di-, and trimethylated lysines.

Identical to the AlkB mechanism for DNA/RNA repair.

Several different proteins have been identified

**The initial discovery of histone demethylation by JmjC proteins followed by numerous articles in Nature, Science and Cell**

10: Klose RJ, Yan Q, Tothova Z, Yamane K, Erdjument-Bromage H, Tempst P, Gilliland DG, Zhang Y, Kaelin WG Jr.
The retinoblastoma binding protein RBP2 is an H3K4 demethylase.
Cell. 2007 Mar 9;128(5):889-900. Epub 2007 Feb 22.
PMID: 17320163 [PubMed - indexed for MEDLINE]

11: Lee MG, Norman J, Shilatifard A, Shiekhattar R.
Physical and functional association of a trimethyl H3K4 demethylase and Ring6a/MBLR, a polycomb-like protein.
Cell. 2007 Mar 9;128(5):877-87. Epub 2007 Feb 22.
PMID: 17320162 [PubMed - indexed for MEDLINE]

12: Iwase S, Lan F, Bayliss P, de la Torre-Ubieta L, Huarte M, Qi HH, Whetstine JR, Bonni A, Roberts TM, Shi Y.
The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases.
Cell. 2007 Mar 23;128(6):1077-88. Epub 2007 Feb 22.
PMID: 17320160 [PubMed - indexed for MEDLINE]

13: Klose RJ, Yamane K, Bae Y, Zhang D, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y.
The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36.
Nature. 2006 Jul 20;442(7100):312-6. Epub 2006 May 28.
PMID: 16732292 [PubMed - indexed for MEDLINE]

14: Chen Z, Zang J, Whetstine J, Hong X, Davrazou F, Kutateladze TG, Simpson M, Mao Q, Pan CH, Dai S, Hagman J, Hansen K, Shi Y, Zhang G.
Structural insights into histone demethylation by JMJD2 family members.
Cell. 2006 May 19;125(4):691-702. Epub 2006 May 4.
PMID: 16677698 [PubMed - indexed for MEDLINE]

15: Whetstine JR, Nottke A, Lan F, Huarte M, Smolikov S, Chen Z, Spooner E, Li E, Zhang G, Colaiacovo M, Shi Y.
Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases.
Cell. 2006 May 5;125(3):467-81. Epub 2006 Apr 6.
PMID: 16603238 [PubMed - indexed for MEDLINE]

16: Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y.
JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor.
Cell. 2006 May 5;125(3):483-95. Epub 2006 Apr 6.
PMID: 16603237 [PubMed - indexed for MEDLINE]

17: Huang Y, Fang J, Bedford MT, Zhang Y, Xu RM.
Recognition of histone H3 lysine-4 methylation by the double tudor domain of JMJD2A.
Science. 2006 May 5;312(5774):748-51. Epub 2006 Apr 6.
PMID: 16601153 [PubMed - indexed for MEDLINE]

18: Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, Zhang Y.
Histone demethylation by a family of JmjC domain-containing proteins.
Nature. 2006 Feb 16;439(7078):811-6. Epub 2005 Dec 18.
PMID: 16362057 [PubMed - indexed for MEDLINE]

**- New enzymes**
**(27 human JmjC proteins exist)**

**- New specificities**

**- Processes**

1: Saze H, Shiraishi A, Miura A, Kakutani T.
Control of genic DNA methylation by a jmjC domain-containing protein in Arabidopsis thaliana.
Science. 2008 Jan 25;319(5862):462-5.
PMID: 18218897 [PubMed - indexed for MEDLINE]

2: Frescas D, Guardavaccaro D, Bassermann F, Koyama-Nasu R, Pagano M.
JHDM1B/FBXL10 is a nucleolar protein that represses transcription of ribosomal RNA genes.
Nature. 2007 Nov 8;450(7167):309-13.
PMID: 17994099 [PubMed - indexed for MEDLINE]

3: Chang B, Chen Y, Zhao Y, Bruick RK.
JMJD6 is a histone arginine demethylase.
Science. 2007 Oct 19;318(5849):444-7.
PMID: 17947579 [PubMed - indexed for MEDLINE]

4: Okada Y, Scott G, Ray MK, Mishina Y, Zhang Y.
Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis.
Nature. 2007 Nov 1;450(7166):119-23. Epub 2007 Oct 17.
PMID: 17943087 [PubMed - indexed for MEDLINE]

5: Swigut T, Wysocka J.
H3K27 demethylases, at long last.
Cell. 2007 Oct 5;131(1):29-32. Review.
PMID: 17923085 [PubMed - indexed for MEDLINE]

6: Lan F, Bayliss PE, Rinn JL, Whetstine JR, Wang JK, Chen S, Iwase S, Alpatov R, Issaeva I, Canaani E, Roberts TM, Chang HY, Shi Y.
A histone H3 lysine 27 demethylase regulates animal posterior development.
Nature. 2007 Oct 11;449(7163):689-94. Epub 2007 Sep 12.
PMID: 17851529 [PubMed - indexed for MEDLINE]

7: De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G.
The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing.
Cell. 2007 Sep 21;130(6):1083-94. Epub 2007 Sep 6.
PMID: 17825402 [PubMed - indexed for MEDLINE]

8: Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J, Issaeva I, Canaani E, Salcini AE, Helin K.
UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development.
Nature. 2007 Oct 11;449(7163):731-4. Epub 2007 Aug 22.
PMID: 17713478 [PubMed - indexed for MEDLINE]

9: Tahiliani M, Mei P, Fang R, Leonor T, Rutenberg M, Shimizu F, Li J, Rao A, Shi Y.
The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation.
Nature. 2007 May 31;447(7144):601-5. Epub 2007 Apr 29.
PMID: 17468742 [PubMed - indexed for MEDLINE]

MBV9100, 2 November 2011

**Lecture 2**

# Current research in our group - methylation, demethylation and hydroxylation

Pål Ø. Falnes

*Department of Molecular Biosciences, University of Oslo*

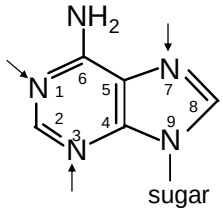
## Outline

- The discovery of a new repair mechanism: AlkB mediated oxidative demethylation
- Unravelling the function of the human AlkB homologue ALKBH8
- (Novel protein methyltransferases - another time..)

- The discovery of a new repair mechanism: AlkB mediated oxidative demethylation
- Unravelling the function of the human AlkB homologue ALKBH8

Targets for chemical alkylations in DNA bases

A

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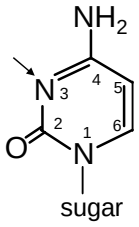


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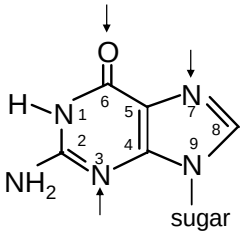


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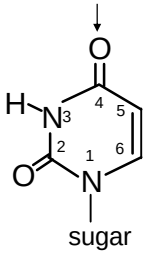
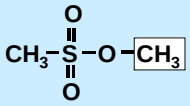
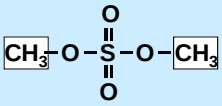


## The two types of methylating agents

$S_N2$-type
(N-methylations)



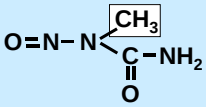
Methyl methanesulfonate (MMS)



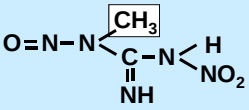
Dimethyl sulfate (DMS)

Methyl halides (X = Cl, I, Br)

$S_N1$-type
(N- and O-methylations)



Methylnitrosourea (MNU)

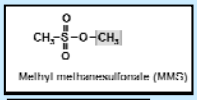


*N*-methyl-*N*´-nitro-*N*-nitrosoguanidine (MNNG)

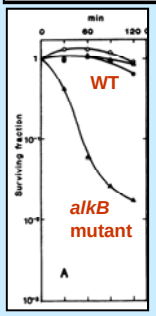
## Repair of alkylation damage in *E. coli*

- Repair genes were identified by isolating mutant strains that were hypersensitive towards alkylating agents
- Repair proteins
  - Alkylbase glycosylases (Tag, AlkA)
  - Alkyl transferases (Ada, Ogt)
  - AlkB??

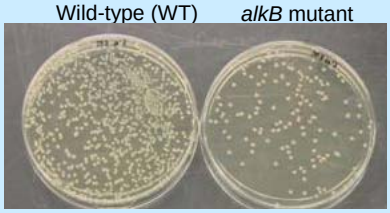
# AlkB




- alkylation (methylation) sensitive mutant of *E. coli* (1983)
- sensitive to MMS and DMS ($S_N2$-type), but not MNU and MNNG ($S_N1$-type) methylating agents



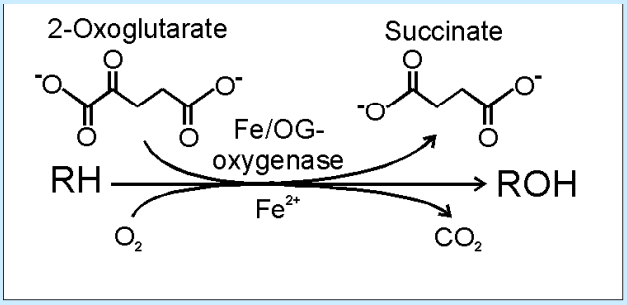

Wild-type (WT) *alkB* mutant



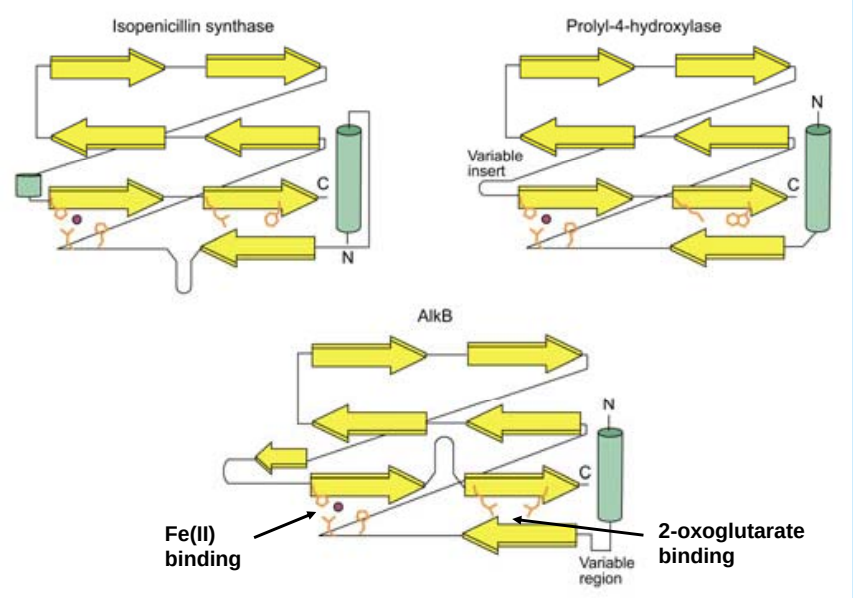
- gene cloned and protein expressed (1985)
- for many years, no *in vitro* DNA repair activity of AlkB detected
- homologues found in most organisms, *e.g*. humans, *C. elegans*, drosophila, and yeast (*S. pombe*)

# Aravind and Koonin (2001)

Sequence profile searches showed that AlkB may belong to the family of 2-oxoglutarate- and iron-dependent oxygenases.

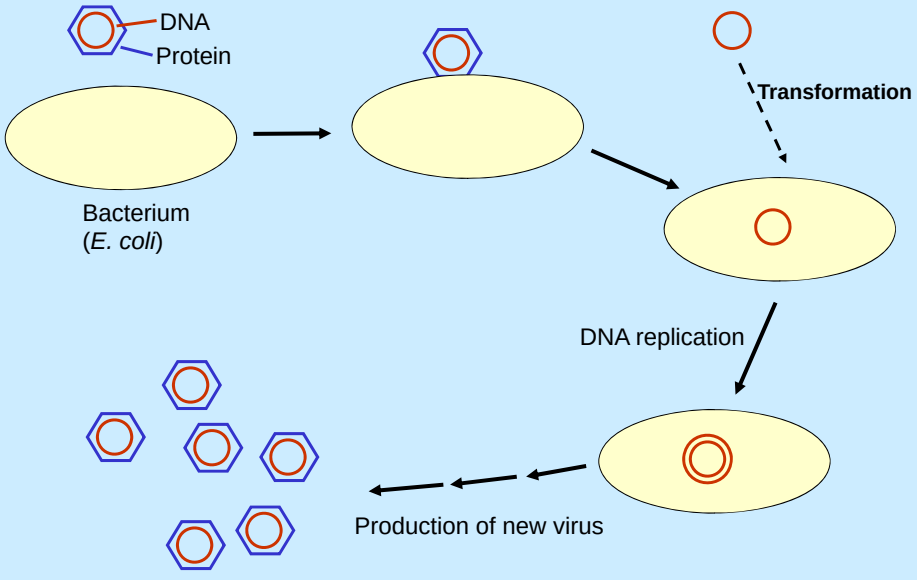


## Suggested common fold for 2-OG/Fe(II)-oxygenases

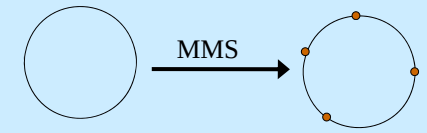



Aravind and Koonin (2001)

## Bacteriophage M13 (single-stranded DNA virus)

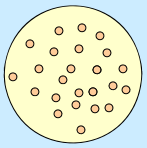


Dinglay et al. (2000):
AlkB mutants are defective in reactivation of MMS treated single-strand (but not double-stranded) phage DNA

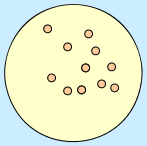



M13 ssDNA

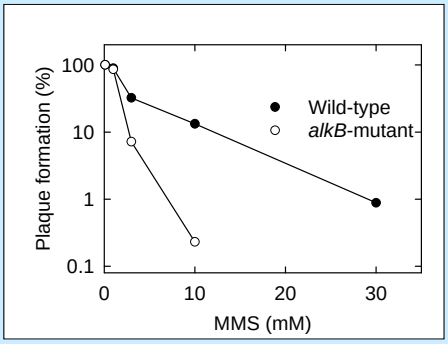
Transform *E. coli*



Wild-type

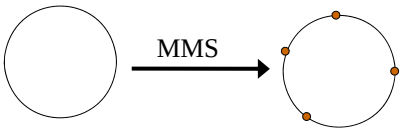


*AlkB* mutant




Conclusion:
AlkB may be involved in processing replication blocking lesions introduced into single-stranded DNA

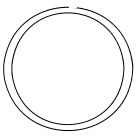
Question: Does AlkB enhance the replication of methylated ssDNA in an *E. coli* protein extract?



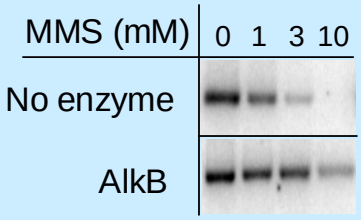

M13 ssDNA

*E. coli* prot. extr.
dNTP, NTP,
[α-$^{32}$P]dCTP

2OG/$Fe^{2+}$
+/- AlkB

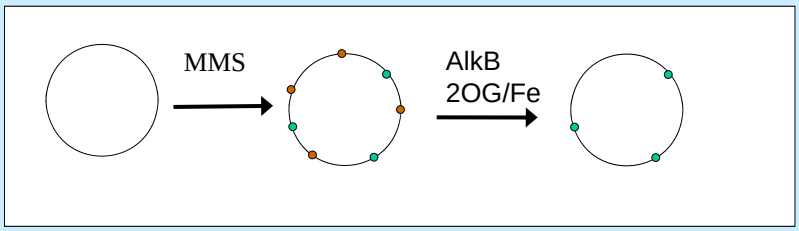


M13 RF DNA
(double-stranded)



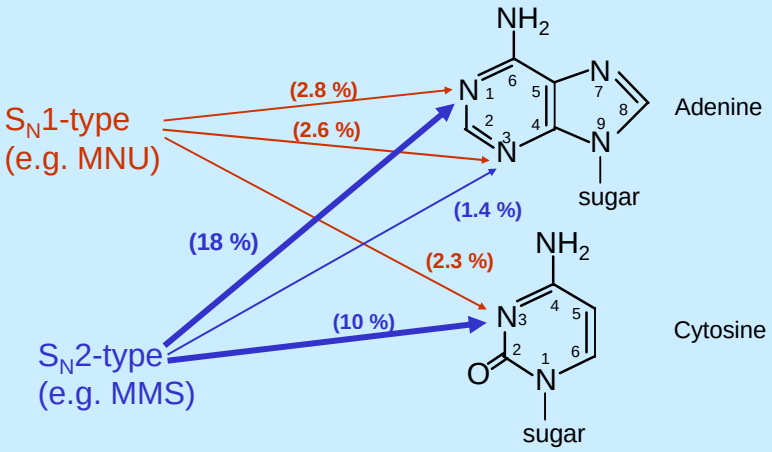

Yes!

Conclusion 1
AlkB removes (or modifies) replication blocking methyl lesions from single-stranded DNA



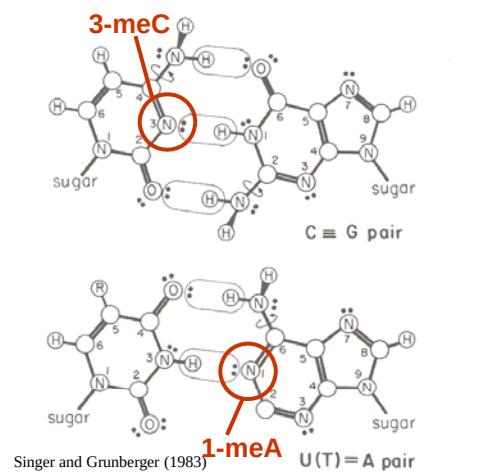

Question:
What lesions are repaired by AlkB?

Introduction of cytotoxic N-alkylations in single-stranded DNA - differences between $S_N1$- and $S_N2$-type alkylating agents




$S_N2$ agents generate much more of 1-meA and 3-meC
(The *E. coli alkB* mutant is hypersensitive towards $S_N2$, but not $S_N1$ alkylating agents)

1-methyladenine and 3-methylcytosine are primarily introduced into single-stranded (and not double-stranded) DNA

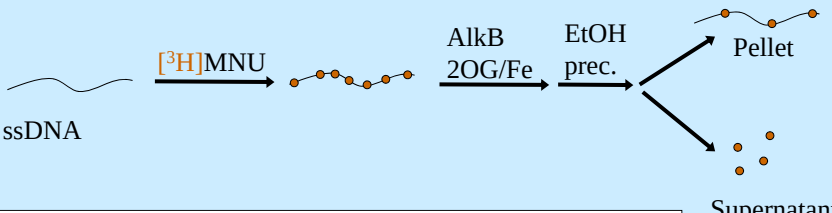


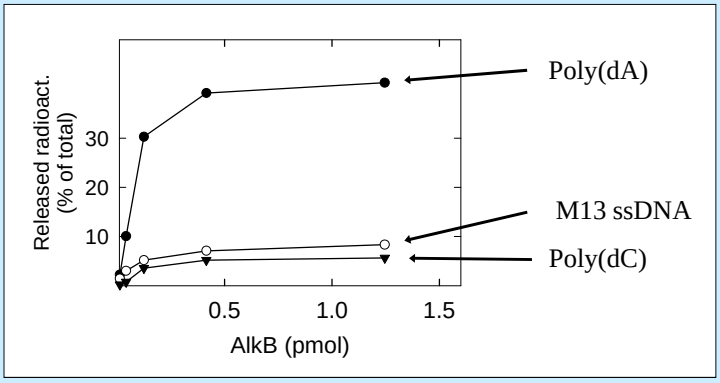

Singer and Grunberger (1983)

N3 of adenosine and N3 of cytosine are shielded from methylation in dsDNA.

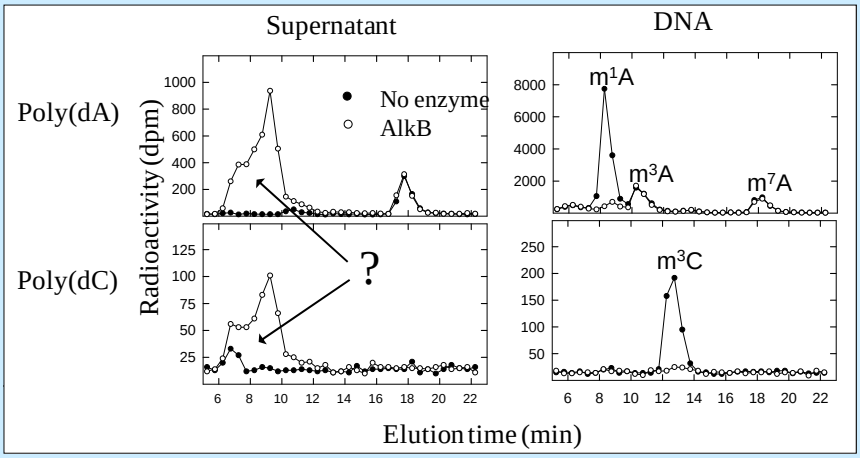
Could it be that 1-meA and/or 3-meC are repaired by AlkB?
(AlkB relevant lesions seem to be formed primarily in dsDNA)

Release of radioactivity from [$^3$H]methylated ssDNA by AlkB



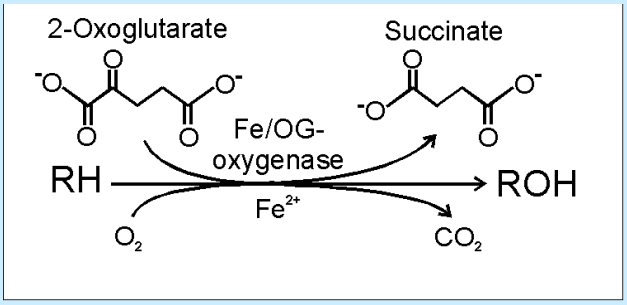



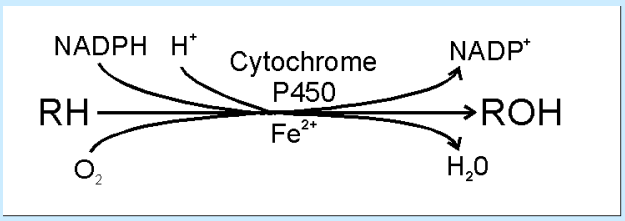
HPLC analysis of released material (supernatant) and remaining DNA



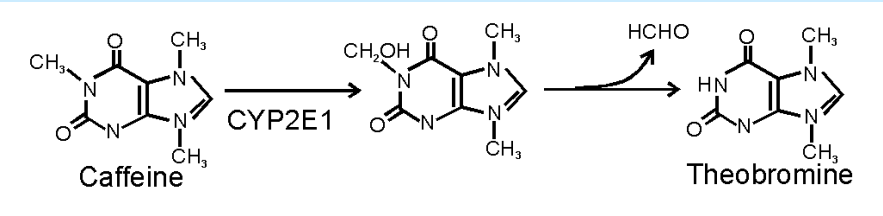

AlkB removes 1-meA and 3-meC from DNA. But how??

Mechanistic similarity between 2OG/Fe-oxygenases and cytochrome P450 (CYP) enzymes



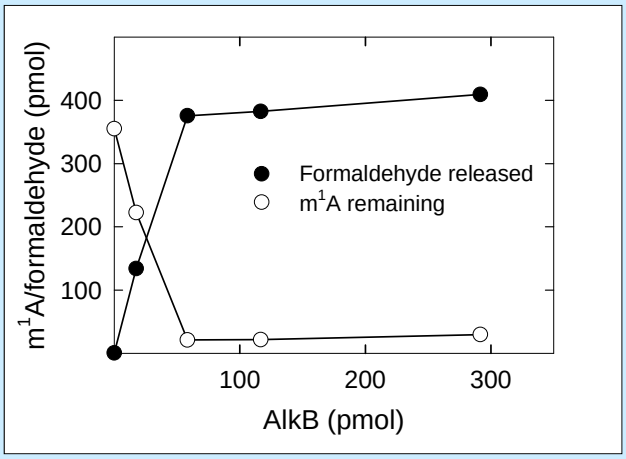
Cytochrome P450 enzymes catalyse dealkylation reactions



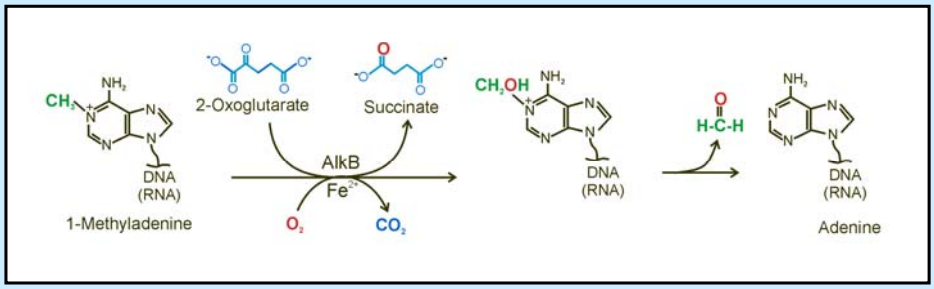

Methyl group is hydroxylated,
then spontaneously released as formaldehyde (HCHO)

Does AlkB work by a similar mechanism?

Formaldehyde generation after incubation of methylated poly(dA) with AlkB

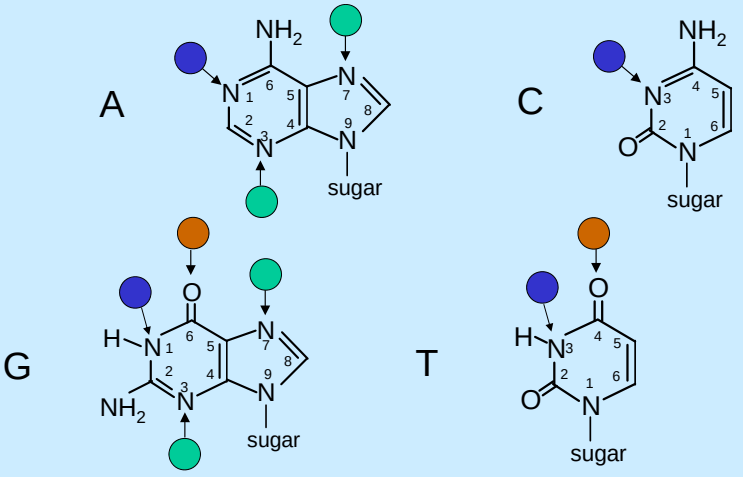


## Mechanism for DNA damage reversal by AlkB




## Repair of alkylation damage by different proteins

- Alkylbase glycosylase
- Alkyl transferase
- Oxidative demethylase (AlkB)



NATURE | VOL 419 | 12 SEPTEMBER 2002

## AlkB-mediated oxidative demethylation reverses DNA damage in *Escherichia coli*

**Pål Ø. Falnes, Rune F. Johansen & Erling Seeberg**

*Centre for Molecular Biology and Neuroscience, and Institute of Medical Microbiology, University of Oslo, National Hospital, 0027 Oslo, Norway*

Received 27 May; accepted 30 July 2002;

## Oxidative demethylation by *Escherichia coli* AlkB directly reverts DNA base damage

**Sarah C. Trewick*†, Timothy F. Henshaw†‡, Robert P. Hausinger‡, Tomas Lindahl* & Barbara Sedgwick***

** Cancer Research UK London Research Institute, Clare Hall Laboratories, South Mimms, Hertfordshire EN6 3LD, UK*
*‡ Departments of Chemistry or Microbiology & Molecular Genetics, Michigan State University, East Lansing, Michigan 48824, USA*
*† These authors contributed equally to this work*

Received 7 March; accepted 6 June 2002;

## VG, 15. october 2002

**Molekyl-funn kan hjelpe kreftsyke**

Av TORE JOHANSEN

LONDON (VG) Britiske forskere har oppdaget en my metode for å reparere ødelagte DNA-molekyler. Oppdagelsen kan få stor betydning for kreftsyke som behandles med cellegift.

Ifølge en artikkel i det medisinske tidsskriftet Nature har forskerne oppdaget at et molekyl som kalles AlkB, fungerer på en helt spesiell måte, som hjelper til med å reparere skadede gener på en unik måte.

Prosessen kalles på fagspråket for «oksydativ avmetallisering» fordi AlkB benytter seg av en kjemisk prosess som er avhengig av jern og andre metaller.

Det har vært forsket i mange år for å kunne reparere ødelagt DNA. A oppdage en helt ny metode å gjøre det på var derfor overraskende og veldig spennende, sier doktor Barbara Sedgwick ved London-avdelingen av det britiske kreftforskningsinstituttet.

I behandlingen av kreftsyke dreper cellegiften kreftcellene ved å angripe DNA-molekyler. AlkB hjelper kreftcellene til å motstå cellegiften, derfor har det vært viktig å finne ut hvordan man kan redusere virkningen av den.

– Vi mener at AlkB-molekylet er en av de viktigste årsakene til cellegiftens manglende effekt på noen pasienter. Nå når vi vet hvordan den virker, tror vi det er mulig å finne en måte å løse dette problemet på, sier Sedgwick.

E-post: tore.johansen@vg.no

LONDON (VG) Britiske forskere har oppdaget en my metode for å reparere ødelagte DNA-molekyler.

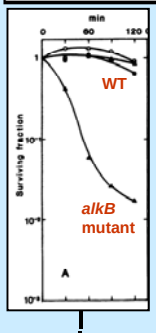
**"British scientists discover new mechanism for DNA repair"**

Prosessen kalles på fagspråket for «oksydativ avmetallisering» fordi AlkB benytter seg av en kjemisk prosess som er avhengig av jern og andre metaller.

# The discovery of the AlkB function - a timeline

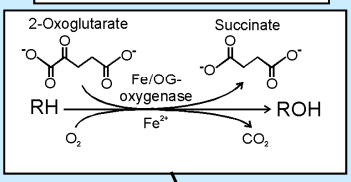
A methylation (MMS) sensitive mutant, *alkB*, of *E. coli* was isolated
(Kataoka et al., J. Bact., 1983)
A new DNA repair enzyme?

Methyl methanesulfonate (MMS)




*AlkB* gene cloned, protein expressed
(Kataoka et al., Mol Gen Genet. 1985)

Bioinformatics:
AlkB is a member of the superfamily of Fe/OG oxygenases
(Aravind and Koonin, Genome Biol., 2001)




AlkB important for protection against methylation damage in ssDNA (rather than dsDNA) (Dinglay et al., Genes Dev., 2000)

AlkB mechanism unraveled

1983 1985 2000 2001 2002

18 papers ("AlkB" + "repair" in title/abstract)

104 papers

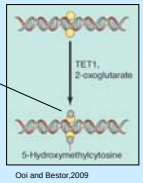
# Unraveling the AlkB function has stimulated discoveries within epigenetics

AlkB mechanism (2002):
- enzymatic, macromolecular demethylation
- DNA hydroxylation

**The protein that binds to DNA base J in trypanosomatids has features of a thymidine hydroxylase** Yu et al. *Nucleic Acids Research, 2007.*

JBP-1 protein in trypanosomes

A novel epigenetic mark?




Ooi and Bestor,2009

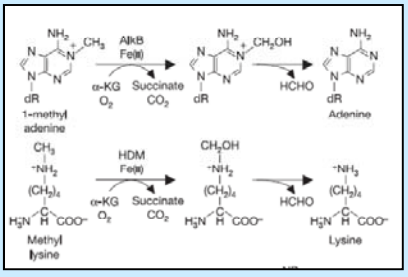
**Human Tet1 (JBP-1 homolog)**

**Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1**

Mamta Tahiliani,[1] Kian Peng Koh,[1] Yinghua Shen,[2] William A. Pastor,[1] Hozefa Bandukwala,[1] Yevgeny Brudno,[2] Suneet Agarwal,[3] Lakshminarayan M. Iyer,[4] David R. Liu,[2]* L. Aravind,[4]* Anjana Rao[1]*

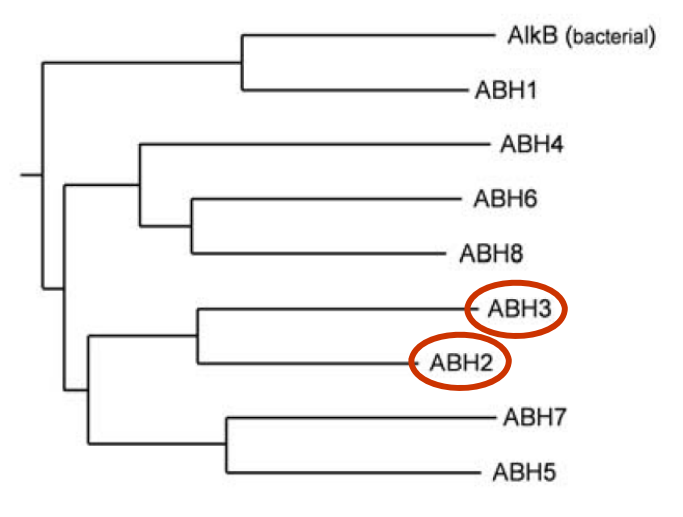
**Science, 2009**

**Histone demethylation by a family of JmjC domain-containing proteins**




**Nature, 2006**

# Putative human AlkB homologues



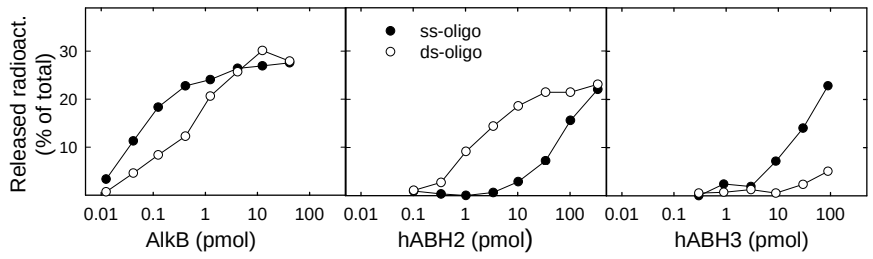

Kurowski et al. (2003) *BMC Genomics*, **4**:48

## *E. coli* AlkB and human AlkB homologs: Different preferences for single-stranded vs double-stranded DNA

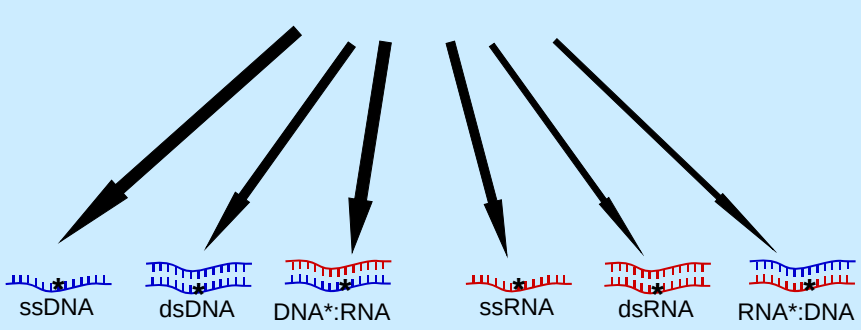
ds-oligo: TTTCGTTCTTTGCTTTTTCGCTTT
AAAGCAAGAAACGAAAAAGCGAAA

ss-oligo: AAAGCAAGAAACGAAAAAGCGAAA

Radiolabel

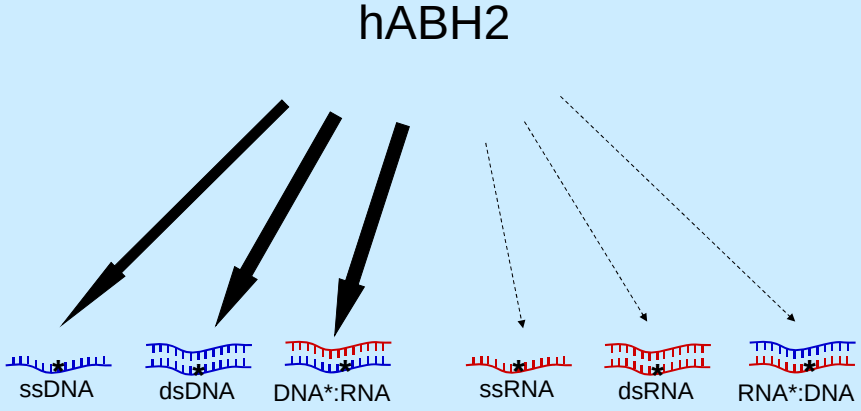


## Extended substrate specificity of AlkB



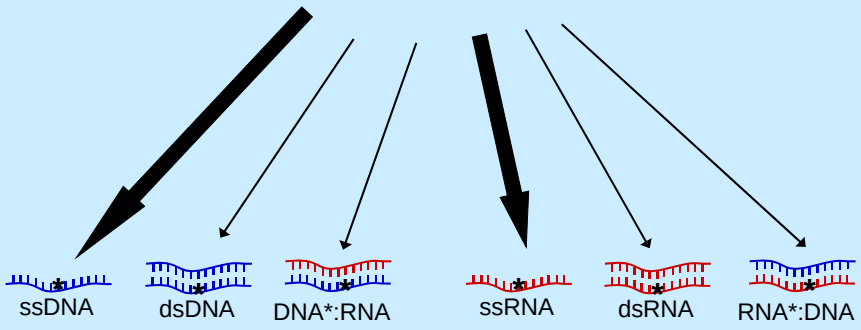

Falnes et al., (2002) *Nature*, **419,** 178-182
Aas et al., (2003) *Nature*, **421,** 859-863
Falnes et al., (2004) *Nucl. Acids Res.*, **32,** 3456-61

## Substrate specificity of hABH2

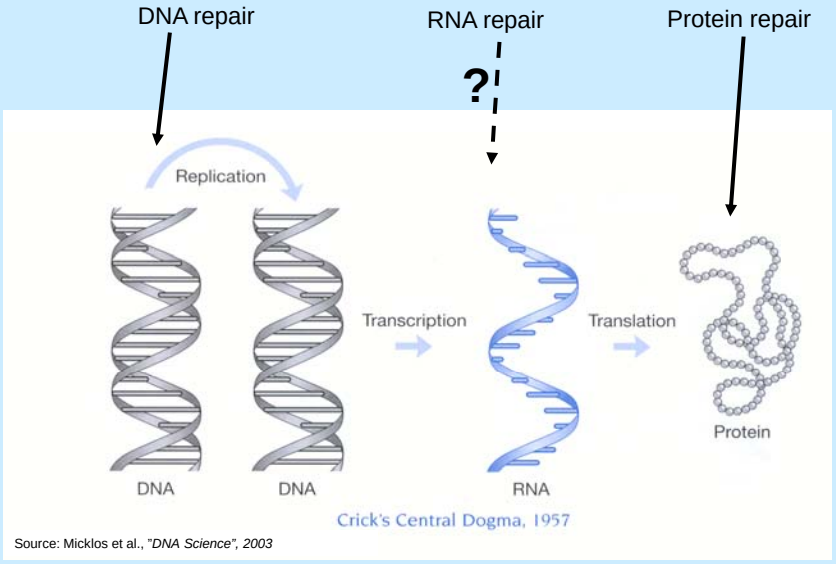



Aas et al., (2003) *Nature*, **421,** 859-863
Falnes et al., (2004) *Nucl. Acids Res.*, **32,** 3456-61

## Substrate specificity of hABH3




Aas et al., (2002) *Nature*, **421,** 859-863
Falnes et al., (2004) *Nucl. Acids Res.*, **32,** 3456-61

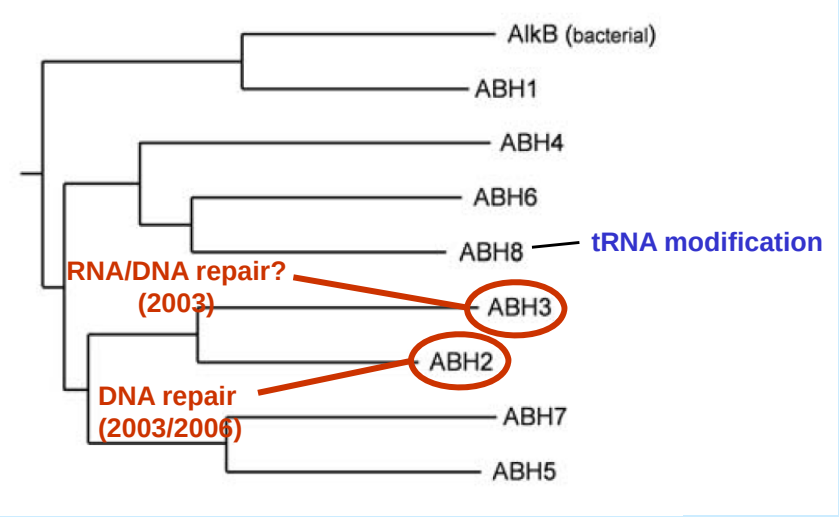



Source: Micklos et al., "*DNA Science*", *2003*

- The discovery of a new repair mechanism: AlkB mediated oxidative demethylation

- Unravelling the function of the human AlkB homologue ALKBH8

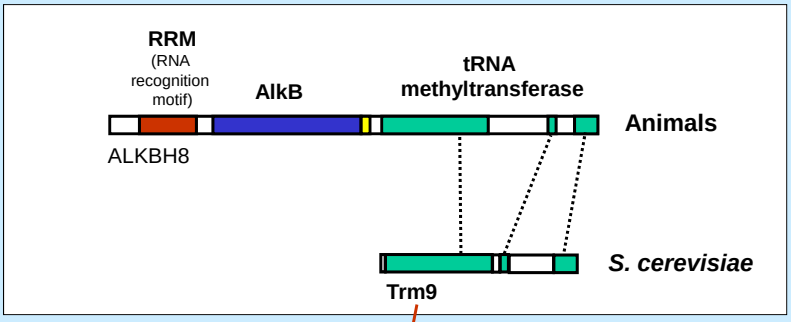
Putative human AlkB homologues (ABH or ALKBH)
- very little is still known




Kurowski et al. (2003) *BMC Genomics*, **4**:48

FTO (ninth member of family): Role in mRNA demethylation (previous lecture; Nat.Chem. Biol., 2011)

Bioinformatics gave clues about a role for ALKBH8 in tRNA modification.




Novel Methyltransferase for Modified Uridine Residues at the Wobble Position of tRNA

Hamid R. Kalhor and Steven Clarke*

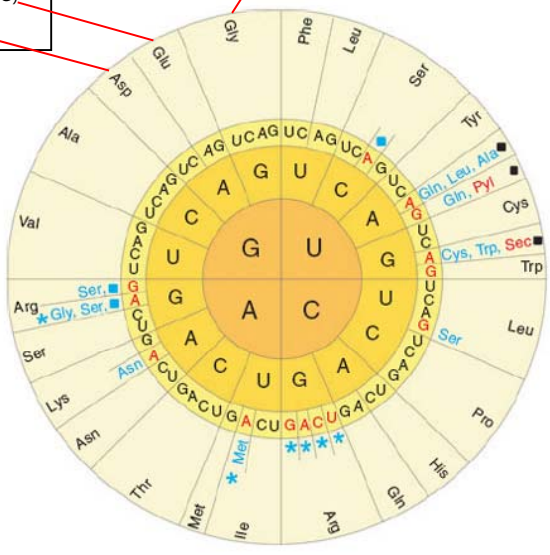
*Department of Chemistry and Biochemistry and Molecular Biology Institute, University of California, Los Angeles, California 90095*

MOLECULAR AND CELLULAR BIOLOGY, Dec. 2003,

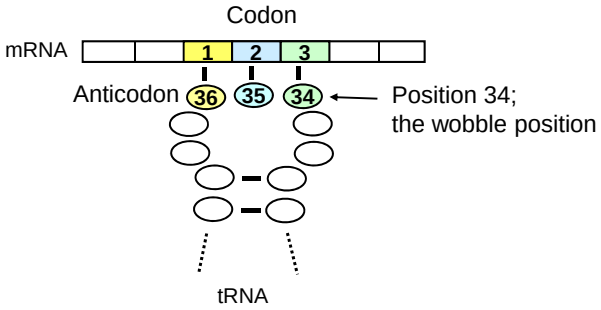
## The genetic code

Family codon box:
Purine (A, G) and pyrimidine (C,T) ending codons encode the same amino acid

Split codon box:
Pyrimidine (C,T) and purine (A, G) ending codons encode different amino acids

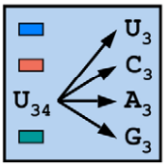


## Reading the genetic code



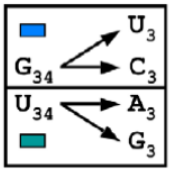

## The challenging case of wobble uridine

Family codon boxes:
- A single tRNA with U in wobble position can read all four codons (bacteria)




Gly

Split codon boxes:
- The tRNA with U in wobble position only reads the purine (A, G) ending codons

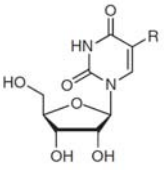



Asp

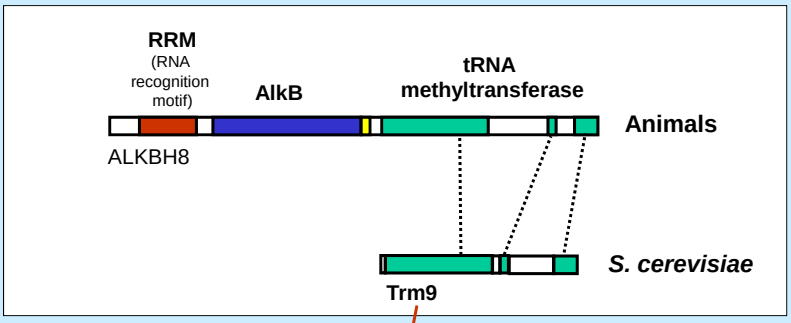
Glu

Two conflicting requirements
- Promiscuity (wobbling)
- Restriction

Solution: Wobble uridine modification
(wobble uridines are usually modified)



Bioinformatics gave clues about a role for ALKBH8 in tRNA modification.



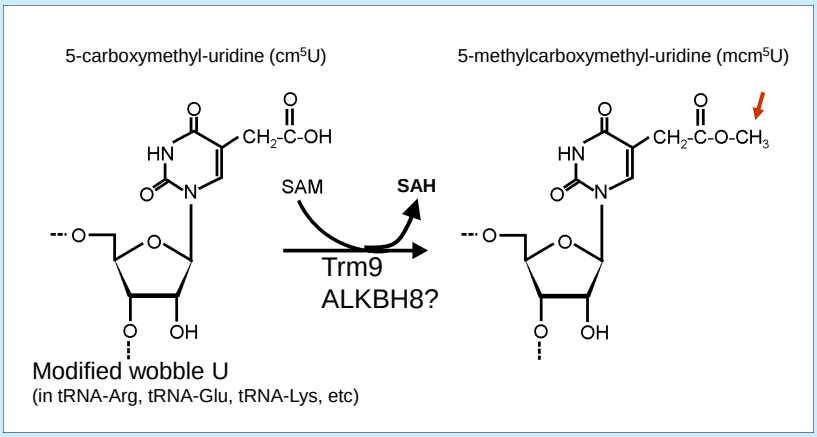

Novel Methyltransferase for Modified Uridine Residues at the Wobble Position of tRNA

Hamid R. Kalhor and Steven Clarke*

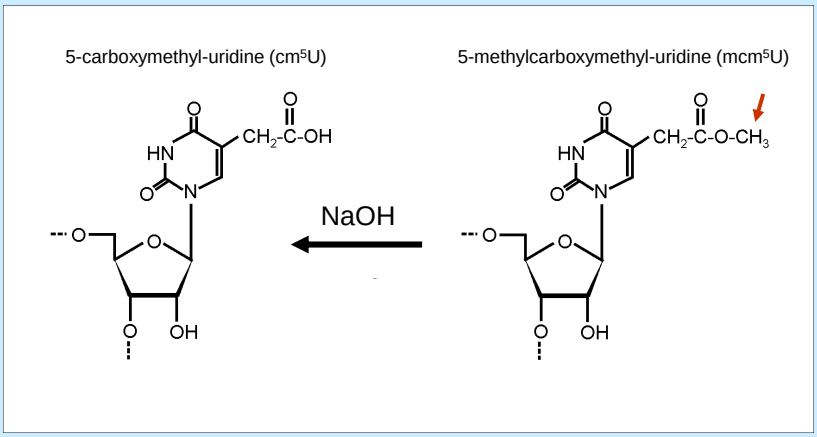
*Department of Chemistry and Biochemistry and Molecular Biology Institute, University of California, Los Angeles, California 90095*

MOLECULAR AND CELLULAR BIOLOGY, Dec. 2003,

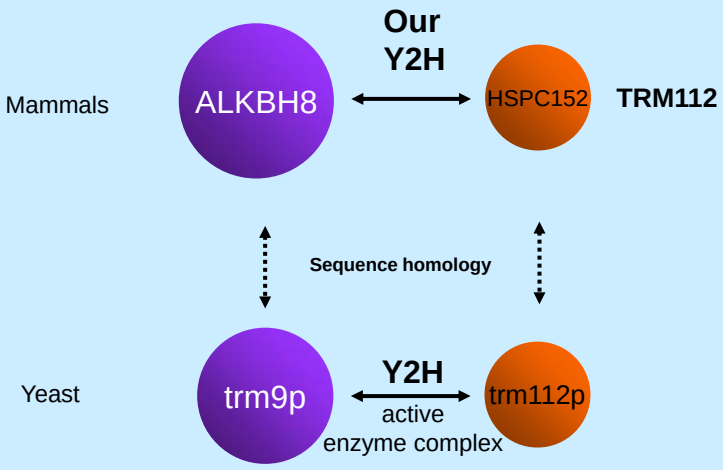
The reaction catalysed by Trm9 (on the wobble nucleoside in some tRNAs)



## Saponification of tRNA
## (generating substrate for metyltransferase (MT) assay)



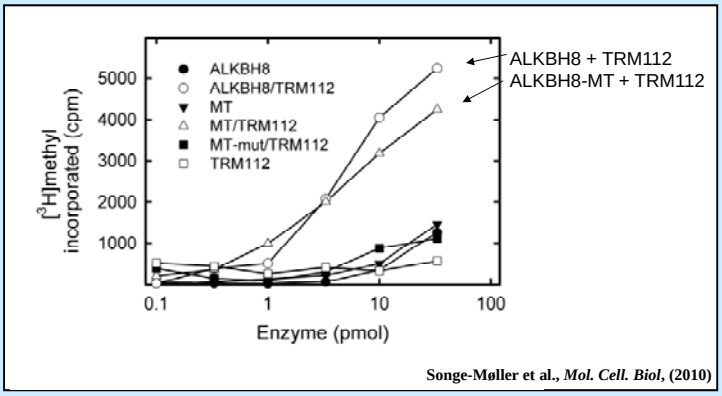

## TRM112 (HSPC152) - a partner of ALKBH8




Studte et al. (2008) Mol Microbiol

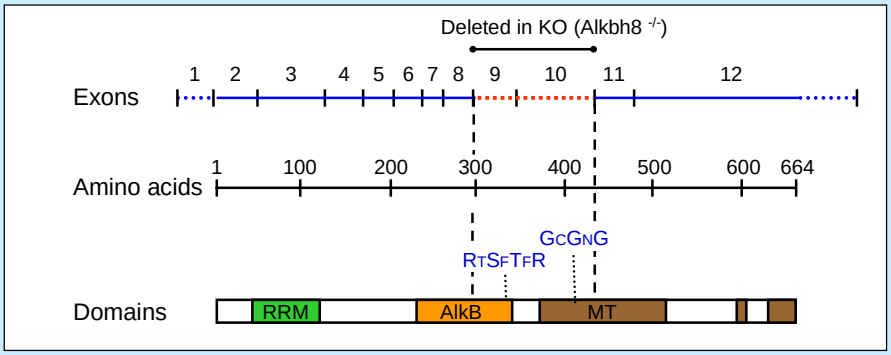
## Methyltransferase activity of a ALKBH8/TRM112 complex
(Erwin van den Born)

- Coexpression of 6xHis-tagged ALKBH8 (or individual domains) with untagged (co-purifying) TRM112 in E. coli
- Purification of proteins from *E. coli* lysate on Talon beads
- Incubation of recombinant enzyme with saponified calf tRNA in the presence of [$^3$H]SAM

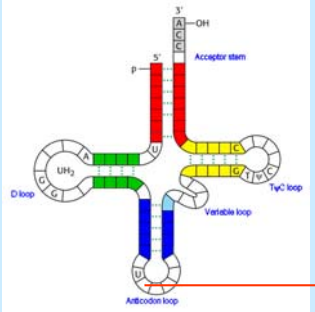


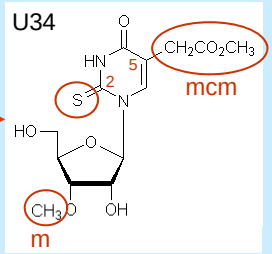

Songe-Møller et al., *Mol. Cell. Biol*, (2010)

## Generating a ALKBH8 knock-out mouse (Klungland group)



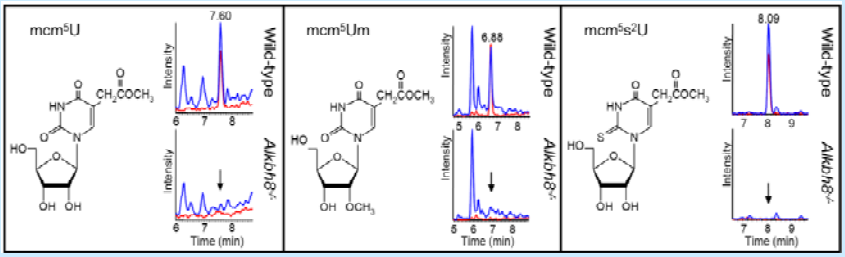
## Mammalian tRNAs containing mcm$^5$U and derivatives in the wobble position



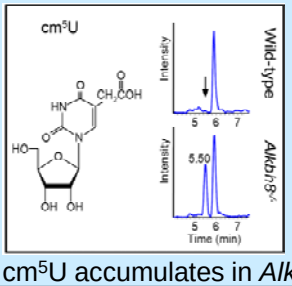




| Modifications found in: | | |
|---|---|---|
| | Lys tRNA | (mcm$^5$s$^2$U) |
| | Arg tRNA | (mcm$^5$s$^2$U) |
| | Glu tRNA | (mcm$^5$s$^2$U) |
| | Sec tRNA | (mcm$^5$U/mcm$^5$Um) |

## LC/MS/MS analysis of nucleosides from *Alkbh8*$^{-/-}$ tRNA (Cathrine Vågbø)

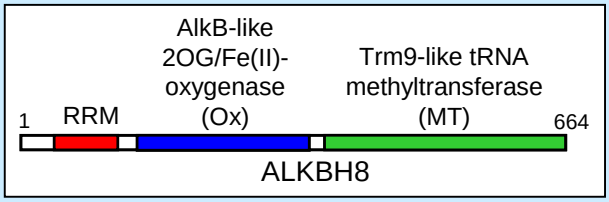



mcm$^5$U,mcm$^5$s$^2$U, and mcm$^5$Um are absent from *Alkbh8*$^{-/-}$ tRNA




cm$^5$U accumulates in *Alkbh8*$^{-/-}$ tRNA

Songe-Møller et al., *Mol. Cell. Biol*, 2010



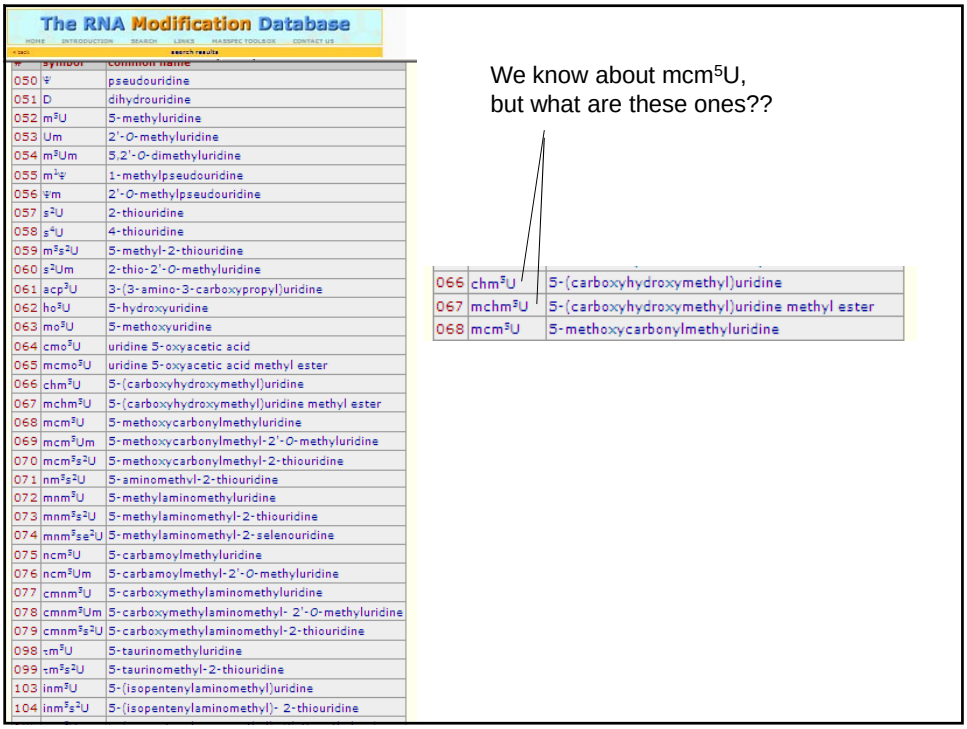

So, the MT domain of ALKBH8 has the expected Trm9-like MT activity - what about the AlkB (oxygenase) domain?

Several possibilities

- A demethylase for the methylation introduced by the MT domain, converting mcm$^5$U to cm$^5$U; possibly to regulate protein translation? No

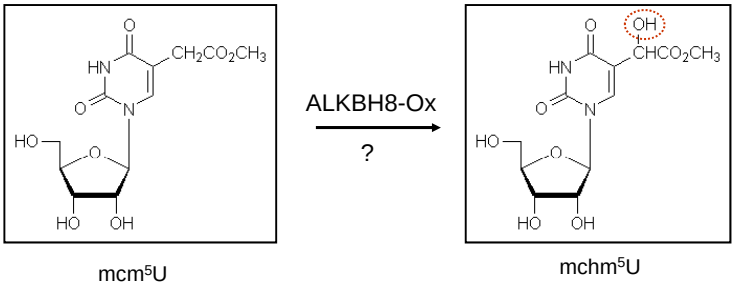
- A demethylase for the ribose methylation of mcm$^5$Um in tRNA-Sec (regulating selenoprotein expression)? No

- Not a demethylase, but a hydroxylase (unlike N-linked methyl groups, C-linked methyl groups will not spontaneously rearrange to formaldehyde upon hydroxylation)? ????

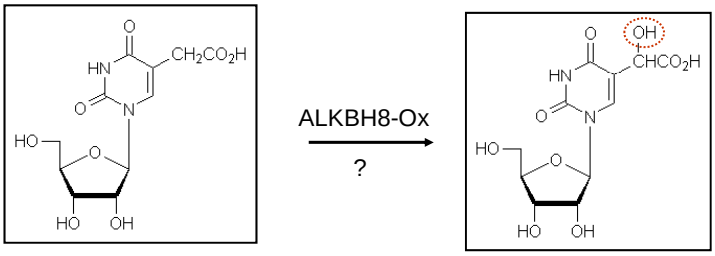



| # | symbol | common name |
|---|---|---|
| 050 | Ψ | pseudouridine |
| 051 | D | dihydrouridine |
| 052 | m$^5$U | 5-methyluridine |
| 053 | Um | 2'-*O*-methyluridine |
| 054 | m$^5$Um | 5,2'-*O*-dimethyluridine |
| 055 | m$^1$Ψ | 1-methylpseudouridine |
| 056 | Ψm | 2'-*O*-methylpseudouridine |
| 057 | s$^2$U | 2-thiouridine |
| 058 | s$^4$U | 4-thiouridine |
| 059 | m$^5$s$^2$U | 5-methyl-2-thiouridine |
| 060 | s$^2$Um | 2-thio-2'-*O*-methyluridine |
| 061 | acp$^3$U | 3-(3-amino-3-carboxypropyl)uridine |
| 062 | ho$^5$U | 5-hydroxyuridine |
| 063 | mo$^5$U | 5-methoxyuridine |
| 064 | cmo$^5$U | uridine 5-oxyacetic acid |
| 065 | mcmo$^5$U | uridine 5-oxyacetic acid methyl ester |
| 066 | chm$^5$U | 5-(carboxyhydroxymethyl)uridine |
| 067 | mchm$^5$U | 5-(carboxyhydroxymethyl)uridine methyl ester |
| 068 | mcm$^5$U | 5-methoxycarbonylmethyluridine |
| 069 | mcm$^5$Um | 5-methoxycarbonylmethyl-2'-*O*-methyluridine |
| 070 | mcm$^5$s$^2$U | 5-methoxycarbonylmethyl-2-thiouridine |
| 071 | nm$^5$s$^2$U | 5-aminomethyl-2-thiouridine |
| 072 | mnm$^5$U | 5-methylaminomethyluridine |
| 073 | mnm$^5$s$^2$U | 5-methylaminomethyl-2-thiouridine |
| 074 | mnm$^5$se$^2$U | 5-methylaminomethyl-2-selenouridine |
| 075 | ncm$^5$U | 5-carbamoylmethyluridine |
| 076 | ncm$^5$Um | 5-carbamoylmethyl-2'-*O*-methyluridine |
| 077 | cmnm$^5$U | 5-carboxymethylaminomethyluridine |
| 078 | cmnm$^5$Um | 5-carboxymethylaminomethyl- 2'-*O*-methyluridine |
| 079 | cmnm$^5$s$^2$U | 5-carboxymethylaminomethyl-2-thiouridine |
| 098 | τm$^5$U | 5-taurinomethyluridine |
| 099 | τm$^5$s$^2$U | 5-taurinomethyl-2-thiouridine |
| 103 | inm$^5$U | 5-(isopentenylaminomethyl)uridine |
| 104 | inm$^5$s$^2$U | 5-(isopentenylaminomethyl)- 2-thiouridine |

## mchm$^5$U and chm$^5$U - hydroxylated forms of mcm$^5$U and cm$^5$U, respectively




mcm$^5$U mchm$^5$U




cm$^5$U chm$^5$U

## Where are mchm$^5$U an chm$^5$U found - and what is known about these modifications?

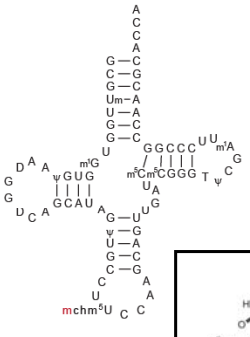
**1979:** chm$^5$U was reported in the wobble position of tRNA2Gly from silk worm (*B. mori*)
(the silk glands of *B. mori* contain a lot of the very Gly-rich silk fibroin protein, making is easy to isolate large amounts of tRNA2Gly from this tissue)

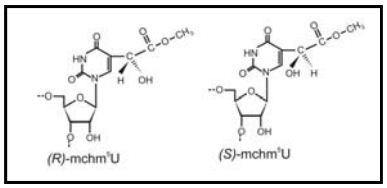
5-(Carboxy-hydroxymethyl)uridine, a new modified nucleoside located in the anticodon of tRNA2Gly from the posterior silk glands of Bombyx mori.
2. Kawakami M, Nishio K, Takemura S, Kondo T, Goto T.
Nucleic Acids Symp Ser. 1979;(6):s53-5.
PMID: 547240 [PubMed - indexed for MEDLINE]
Related articles

**1988:** The nucleoside in the wobble position was found to be mchm$^5$U, not chm$^5$U, (mchm$^5$U is readily demethylated to chm$^5$U under weakly alkaline conditions).

It was found that tRNA2Gly contains the *S*-stereoisomer of mchm$^5$U

No further studies on this nucleoside

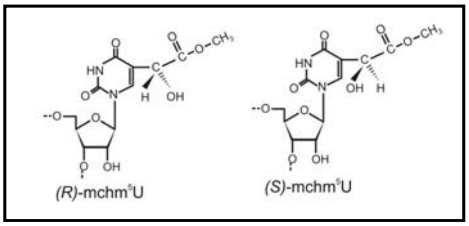


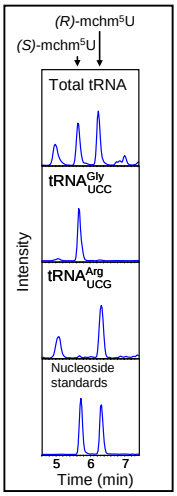



## Investigating the presence of (*S*)-mchm$^5$U in mammalian tRNA

Nucleoside standards for (*S*)-mchm$^5$U and (*R*)-mchm$^5$U were obtained (Andrzej Malkiewicz and Grazyna Leszczynska)

The presence of these nucleosides in calf liver tRNA was investigated by LC-MS/MS



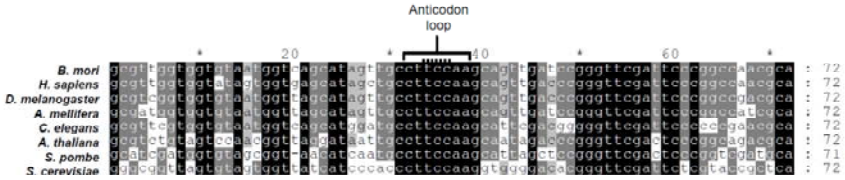

- Both *R*- and *S*-diastereromers of mchm$^5$U are present i calf liver tRNA

- (*S*)-mchm$^5$U is found in tRNA-Gly(UCC)

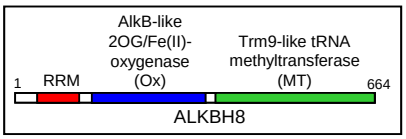
- (*R*)-mchm$^5$U is found in tRNA-Arg(UCG)

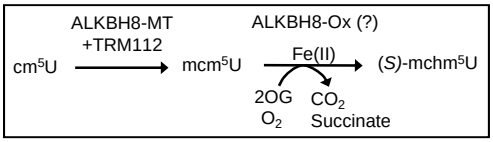
- ALKBH8 is found in all multicellular eukaryotes

- Strong sequence similarity (identical anticodon loop) between tRNA-Gly(UCC) from such organisms

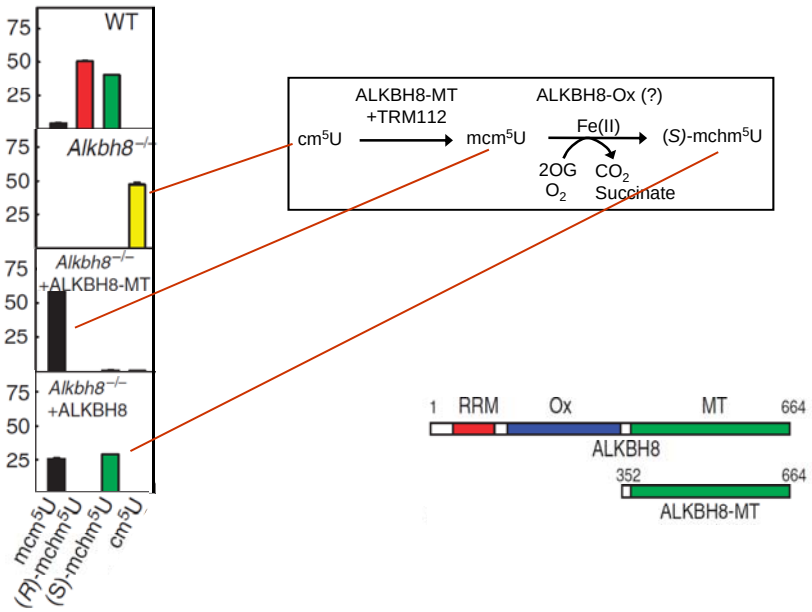



Could ALKBH8 be the hydroxylase responsible for generating (*S*)-mchm$^5$U in tRNA-Gly(UCC)?



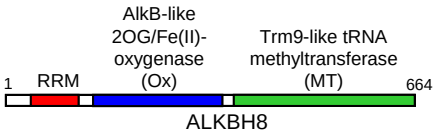



## Incubation of Alkbh8$^{-/-}$ tRNA with recombinant enzymes reveals the biochemical activity of the ALKBH8 oxygenase




## Using mouse models to further investigate the role ALKBH8 in mchm$^5$U formation in mammals

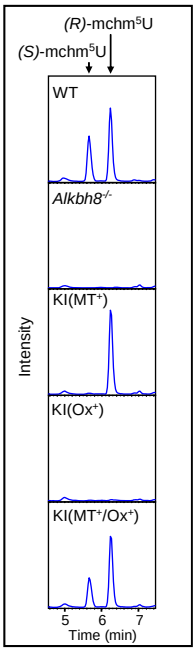
The Klungland lab generated additional mouse models in the *Alkbh8*$^{-/-}$ background, containing transgenes where one of the two domains of ALKBH8 are inactivated (*).




| Mouse model | Abbreviation | Expressed ALKBH8 protein(s) |
|---|---|---|
| Wild-type | WT | |
| *Alkbh8*$^{-/-}$ | *Alkbh8*$^{-/-}$ | None |
| *Alkbh8*$^{-/-}$*/KI(MT*$^{+}$*)* | *KI(MT*$^{+}$*)* | |
| *Alkbh8*$^{-/-}$*/KI(Ox*$^{+}$*)* | *KI(Ox*$^{+}$*)* | |
| *Alkbh8*$^{-/-}$*/KI(MT*$^{+}$*)/KI(Ox*$^{+}$*)* | *KI(MT*$^{+}$*/Ox*$^{+}$*)* | + |

## LC-MS/MS analysis of mchm$^5$U in total tRNA from the various mouse models

| Mouse model | Abbreviation | Expressed ALKBH8 protein(s) |
|---|---|---|
| Wild-type | WT | |
| *Alkbh8*$^{-/-}$ | *Alkbh8*$^{-/-}$ | None |
| *Alkbh8*$^{-/-}$/KI(MT$^+$) | KI(MT$^+$) | * |
| *Alkbh8*$^{-/-}$/KI(Ox$^+$) | KI(Ox$^+$) | * |
| *Alkbh8*$^{-/-}$/KI(MT$^+$)/KI(Ox$^+$) | KI(MT$^+$/Ox$^+$) | * + * |



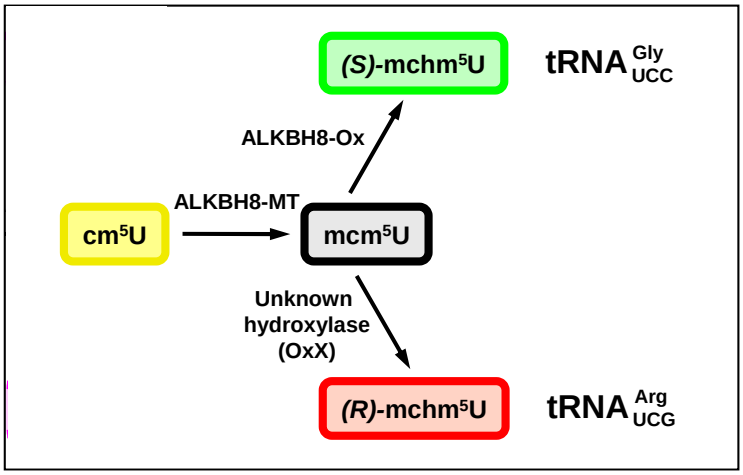

- (*S*)-mchm$^5$U formation requires both the methyltransferase (MT) and oxygenase (Ox) activities of ALKBH8

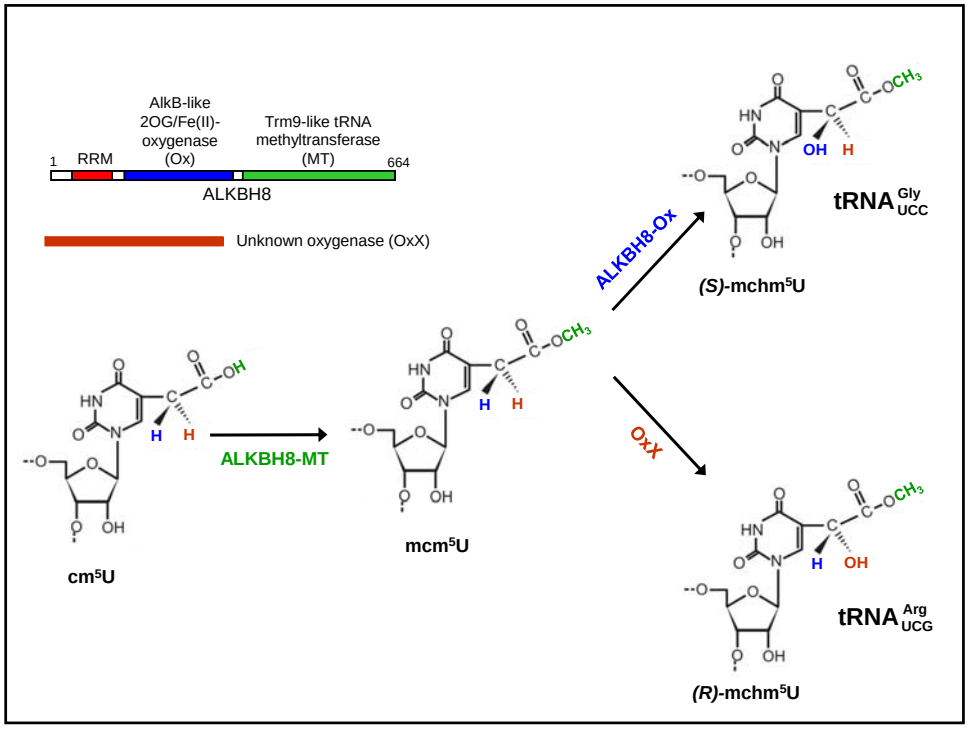
- (*R*)-mchm$^5$U formation requires the methyltransferase activity, but not the oxygenase activity of ALKBH8

**van den Born et al., *Nat. Commun.* (in press)**

# Model

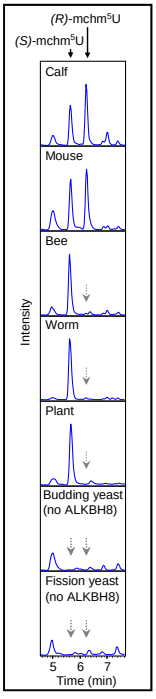
**- based on data from mice and in vitro enzymology**






## Presence of the mchm$^5$U diastereomers in various organisms

LC-MS/MS analysis of total tRNA
(Cathrine Vågbø)




**_S_-mchm$^5$U shows co-occurence with ALKBH8**

**_R_-mchm$^5$U observed only in mammals**

van den Born et al., *Nat. Commun.* (2011)

## Conclusions - ALKBH8

- mchm$^5$U is a novel wobble uridine modification in mammalian tRNA

- mchm$^5$U exists in two diastereomeric forms, *R*- and *S*-, found in tRNA-Arg(UCG) and tRNA-Gly(UCC), respectively

- (*R*)- and (*S*)-mchm$^5$U represents the first example of a isostereomeric pair of RNA modifications

- Mammalian ALKBH8 is a bifunctional tRNA modification enzyme with both methyltransferase and oxygenase activities

- The methyltransferase activity of ALKBH8 is required for the formation of a number of modified wobble uridines, including (*R*)- and (*S*)-mchm$^5$U

- The oxygenase activity of ALKBH8 hydroxylates mcm$^5$U to *S*-mchm$^5$U in tRNA-Gly(UCC)

## Collaborators on ALKBH8

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**Erwin van den Born**
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- Guro Flor Lien

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- Cathrine Vågbø

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