CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2015-2016 :
"Epigénétique et Cancer"

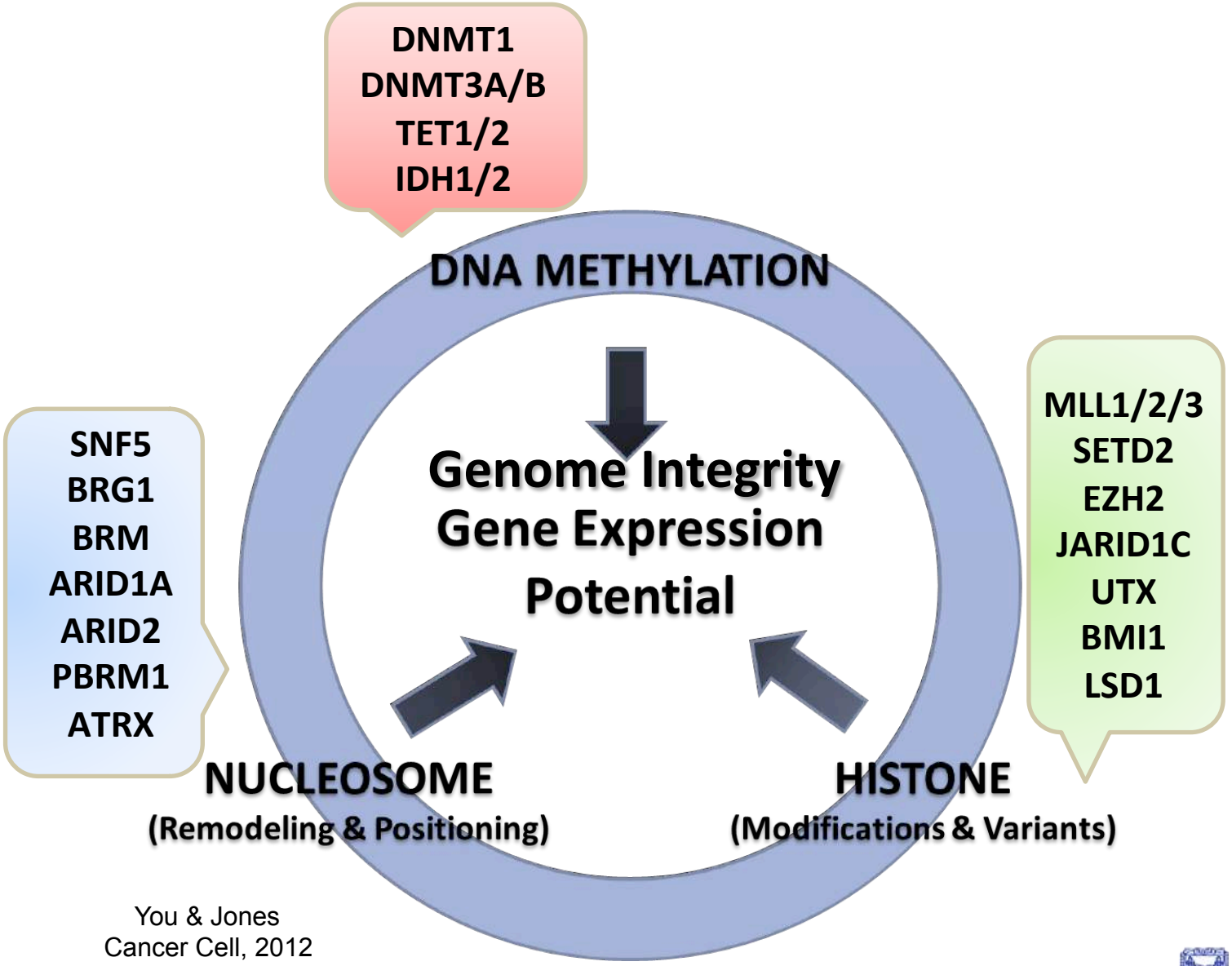
21 mars, 2016

Cours IV

"**Voies épigénétiques du cancer I**"

"*Epigenetic pathways in cancer I*"

Epigenetic Pathways in Cancer



You & Jones
Cancer Cell, 2012

New Lessons from Cancer Genomes

Published online 29 October 2014 *Nucleic Acids Research, 2015, Vol. 43, Database issue* **D805–D811**
doi: 10.1093/nar/gku1075

COSMIC: exploring the world's knowledge of somatic mutations in human cancer

Simon A. Forbes*, David Beare, Prasad Gunasekaran, Kenric Leung, Nidhi Bindal, Harry Boutselakis, Minjie Ding, Sally Bamford, Charlotte Cole, Sari Ward, Chai Yin Kok, Mingming Jia, Tisham De, Jon W. Teague, Michael R. Stratton, Ultan McDermott and Peter J. Campbell

Cancer Genome Project, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK, CB10 1SA

Point mutation

Gonzalez-Perez *et al. Genome Biology* 2013, **14**:r106
http://genomebiology.com/2013/14/9/r106

Genome Biology

RESEARCH Open Access

The mutational landscape of chromatin regulatory factors across 4,623 tumor samples

Abel Gonzalez-Perez[1†], Alba Jene-Sanz[1†] and Nuria Lopez-Bigas[1,2*]

9 | °
Copy-number change

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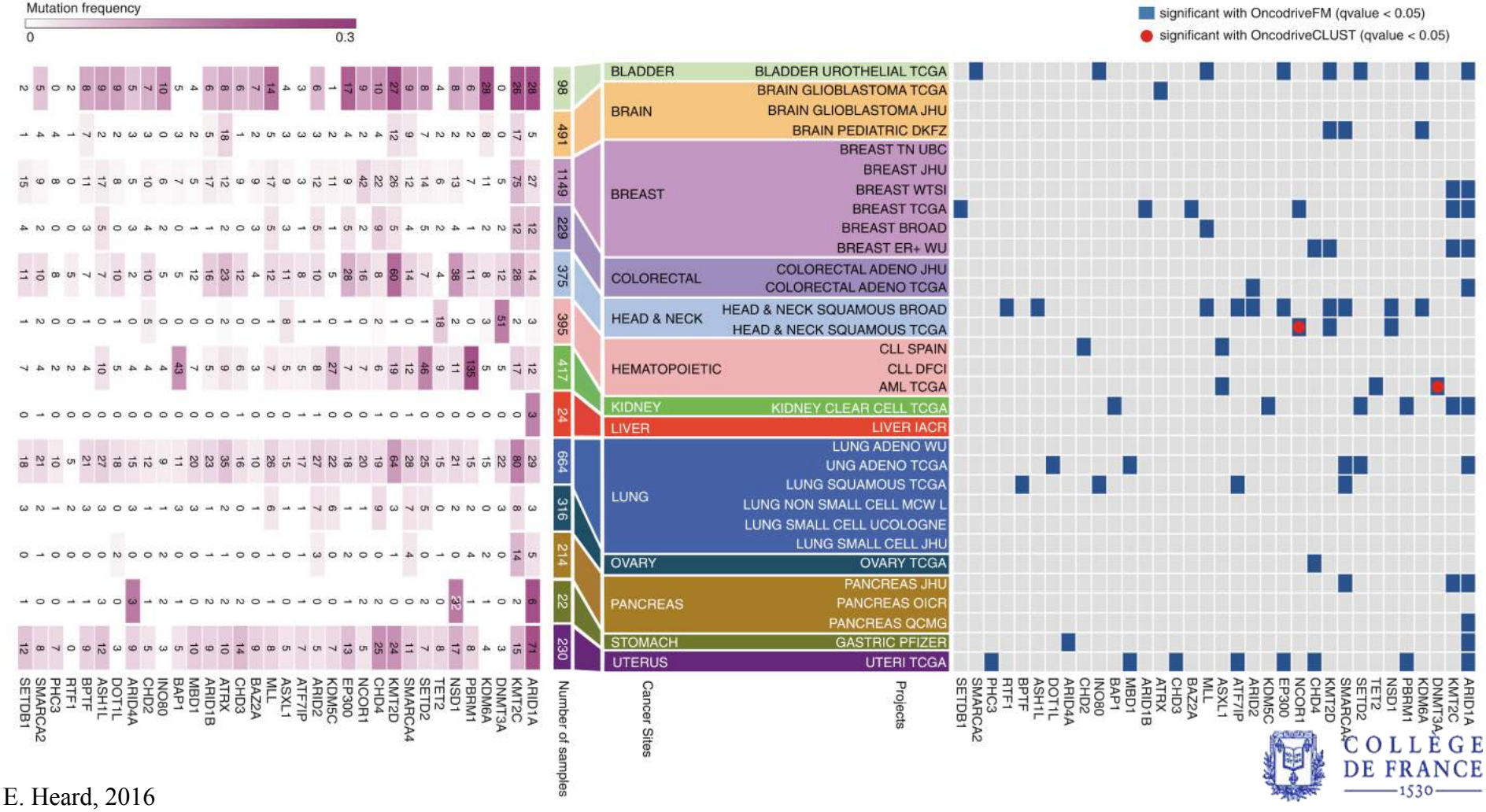
Identifying Chromatin Regulatory Factors as Putative Drivers in Cancer

4,623 tumor samples from thirteen anatomical sites to determine
Identify 34 chromatin regulatory factors that are likely **drivers** in tumors from at least one site
Tumors from all thirteen sites show mutations in likely driver chromatin regulatory factors
More prevalent in tumors arising from certain tissues: hematopoietic, liver and kidney tumors,



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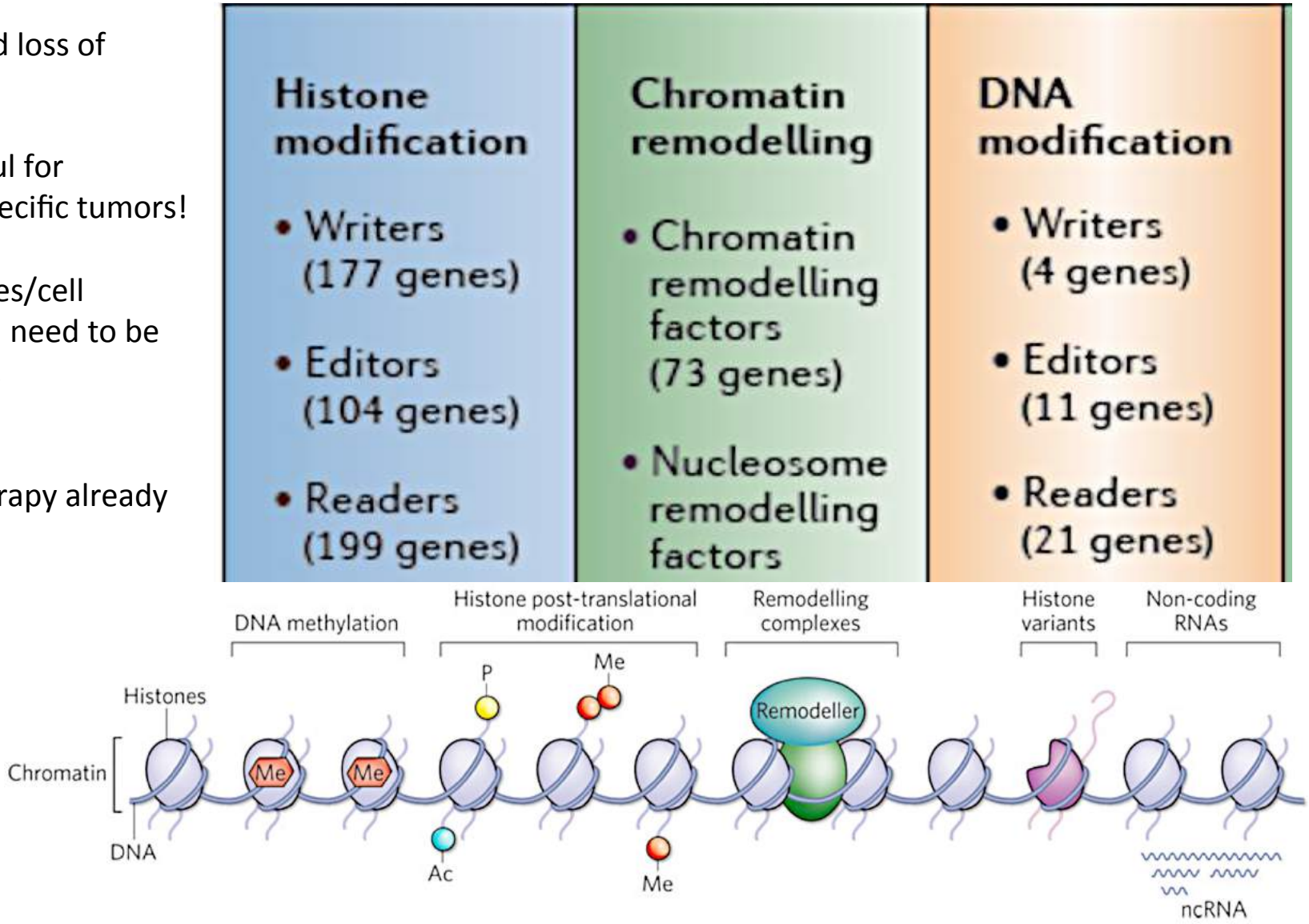
Mutated Cancer Genes affect a spectrum of Chromatin Modifers

Both gain and loss of function

Already useful for classifying specific tumors!

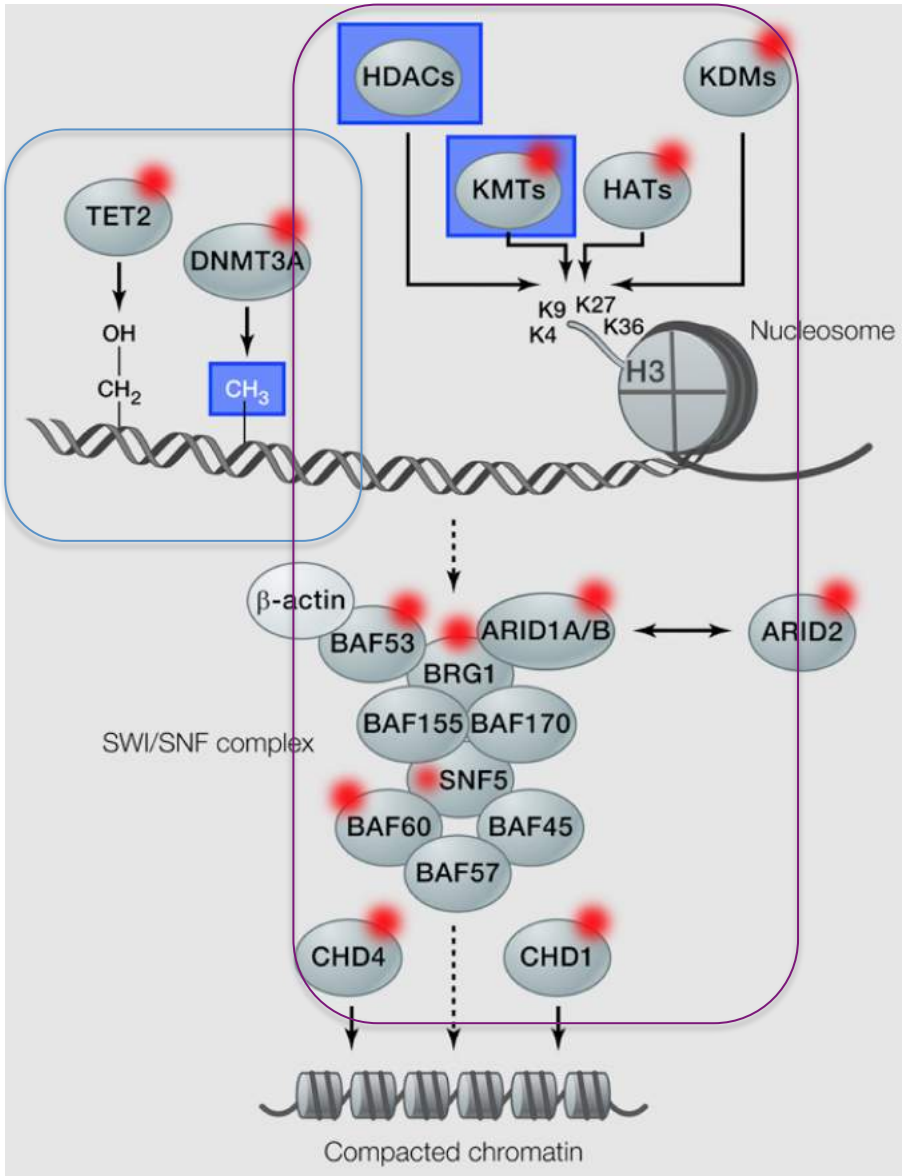
Affected genes/cell functions still need to be understood…

Targeted therapy already underway



 Shen et al, Cell 2013

Chromatin remodeling proteins, Histone Modifiers and DNA Methyltransferases/demethylases



COURS V and VI

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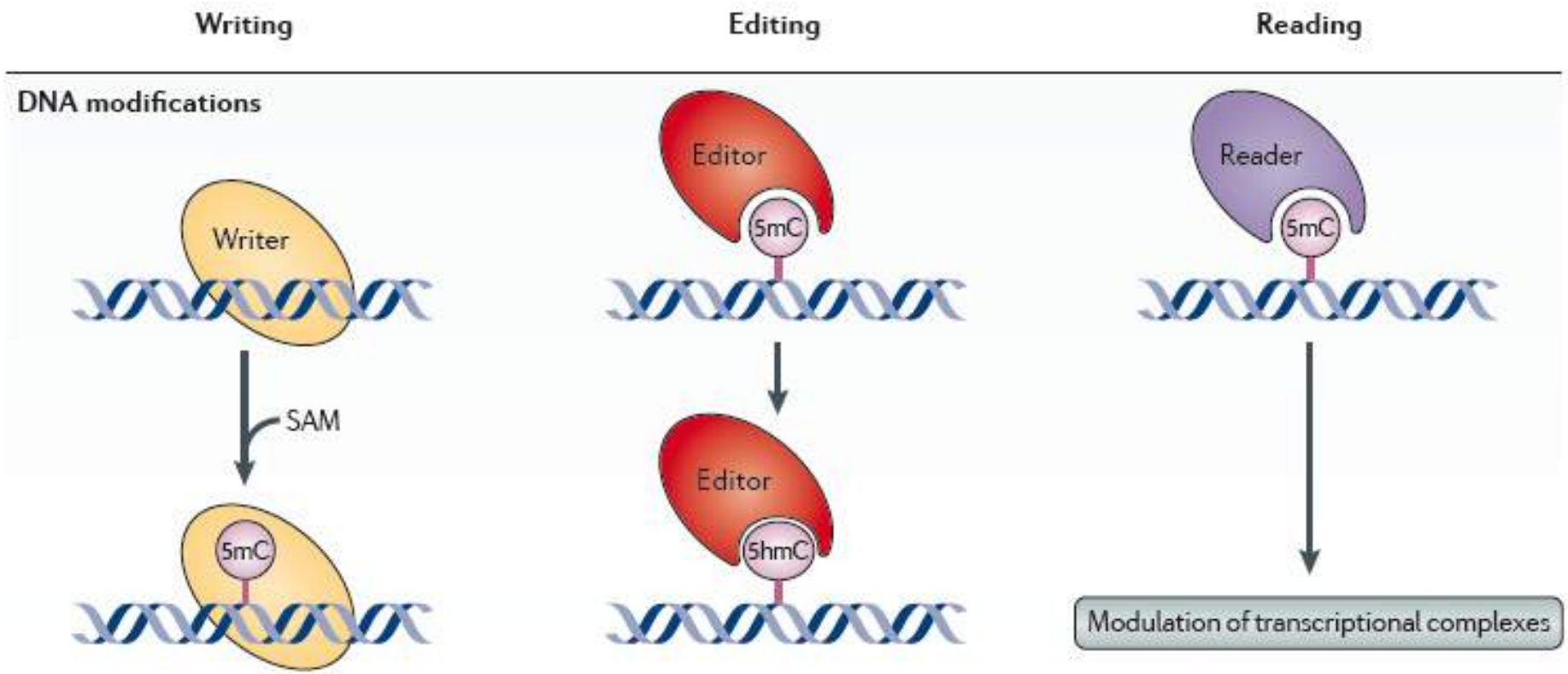
How do Epigenetic Changes Arise?
Mutations in DNA Methylation modifers



- *De novo* methylation of cytosine to 5mC: DNMT3A, DNMT3B and DNMT3L
- Maintenance methylation of cytosine to 5mC: DNMT1

- Cytosine demethylation through oxidation of 5mC to 5hmC: TET1, TET2 and TET3

- Reading of 5mC by proteins with methyl-binding domains (MBDs): MECP2

UHRF1 (DNA replication)
DNA Repair proteins?

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Mutated Cancer Genes are Involved in DNA Methylation

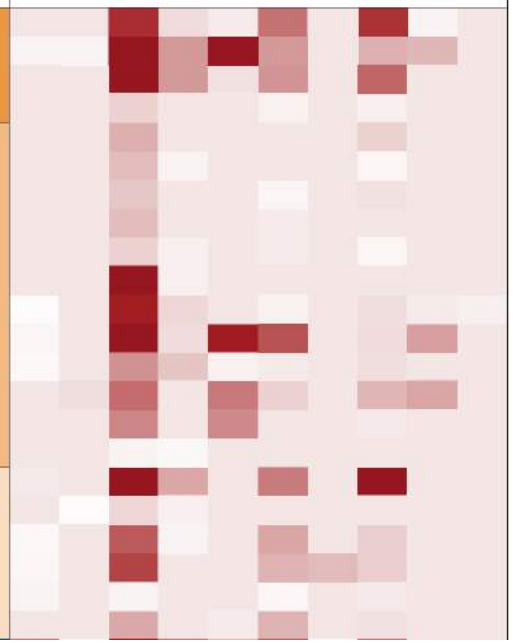
Eg high frequency of DNA methylation associated mutations in hematopoietic malignancies:

DNMT3A mutations are found in:
AML (30%)
Myeloproliferative neoplasia (MPN) (7–15%)
Myelodysplastic syndrome (MDS) (8%)

TET2 is frequently mutated in myeloid disease:
AML (7–23%),
Chronic myelomonocytic leukemia (CMML) (50%),
MDS (10–20%)

IDH1/2 mutations found in:
AML (16-19%),
MPN (2-9%)
MDS (3%)

Group	Subgroup	Modifications	Mutated genes	Tissue type (number of donors)
				Breast (1,030) / Brain (947) / Lung (760) / Ovarian (576) / Blood (512) / Kidney (502) / Colon (460) / Uterus (451) / Liver (390) / Pancreas (330)
DNA modification	Writers	5mC	*DNMT1* *DNMT3A* *DNMT3B* *DNMT3L*	
	Editors	5hmC, 5caC and 5fC	*AICDA* *ALKBH1* *ALKBH3* *APOBEC1* *FTO* *TDG* *TET1* *TET2* *TET3* *IDH1* *IDH2* *MGMT*	
	Readers	5mC	*MBD1* *MBD3* *MBD4* *MECP2* *PCNA* *UHRF1*	



See also: “dbEM: A database of epigenetic modifiers curated from cancerous and normal genomes”. Nanda et al, *Scientific Reports* 2016

 Shen et al, Cell 2013

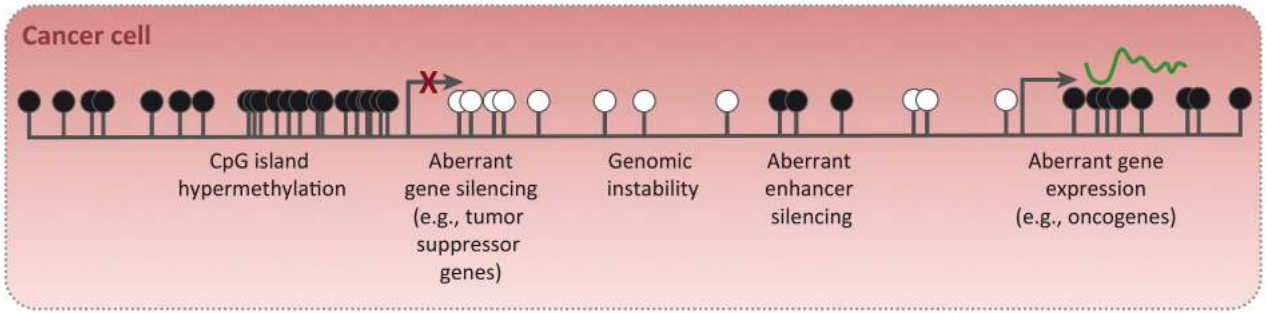
Aberrant DNA Methylation profiles in Cancer

1. **Genes:** **gain/loss of promoter DNA me - > epimutations (eg aberrant silencing of tumor suppressors eg *MLH1*; aberrant "locking in" of bivalent promoters)**
2. **Repeats:** **loss of DNA me, TE activation, genome instability (See last week, COURS III)**
3. **Non-coding Regulatory Regions:** **CGI shores, enhancers, insulators**

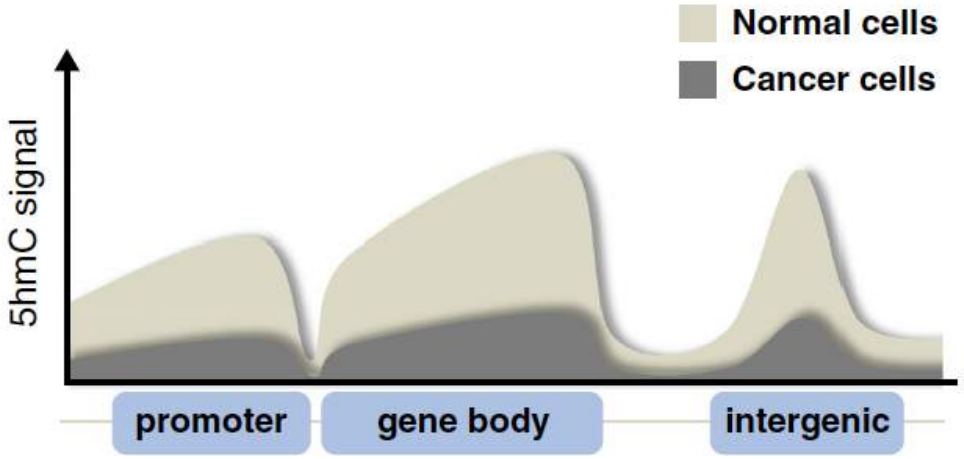
DNA methylation profiling studies:

2000-3000 aberrantly methylated gene promoters / cancer genome
mostly associated with gene silencing
The number of mutations in protein coding genes is 2-3 orders of magnitude less.
⇒ Although some genes are mutated in cancer
the majority are inactivated by *epigenetic alterations*

How many of these involved in cancer?
Do these DNA methylate states promote noisy expression that allows sampling of different cell states by the tumor?
How do they arise?
("errors" due to stress, ageing - or mutated epigenetic machinery?)



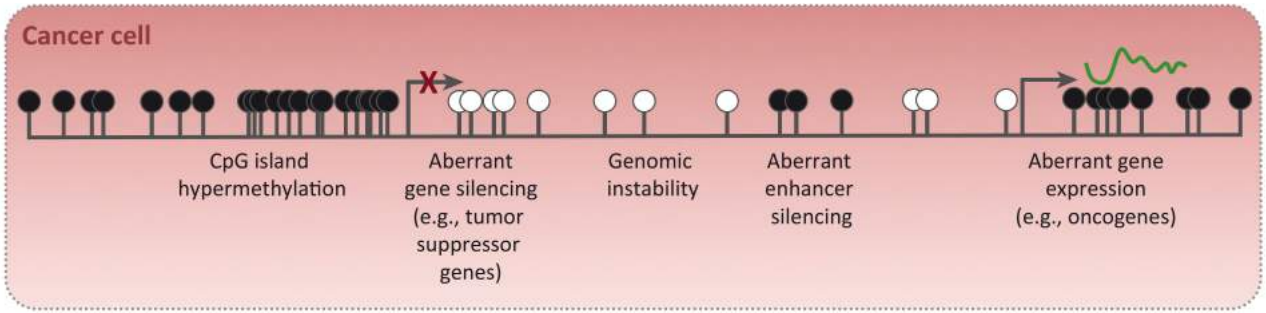
DNA Methylation profiles in Cancer



- Genome wide DNA methylation patterns in different tumors
- Specific methylation events seen in different tumors: CIMPs, LRES, PMDs
- Non-random patterns of aberrant CpG island methylation are often found

  => Cell of origin of a given tumor type?

  => Specific molecular mechanisms involved?



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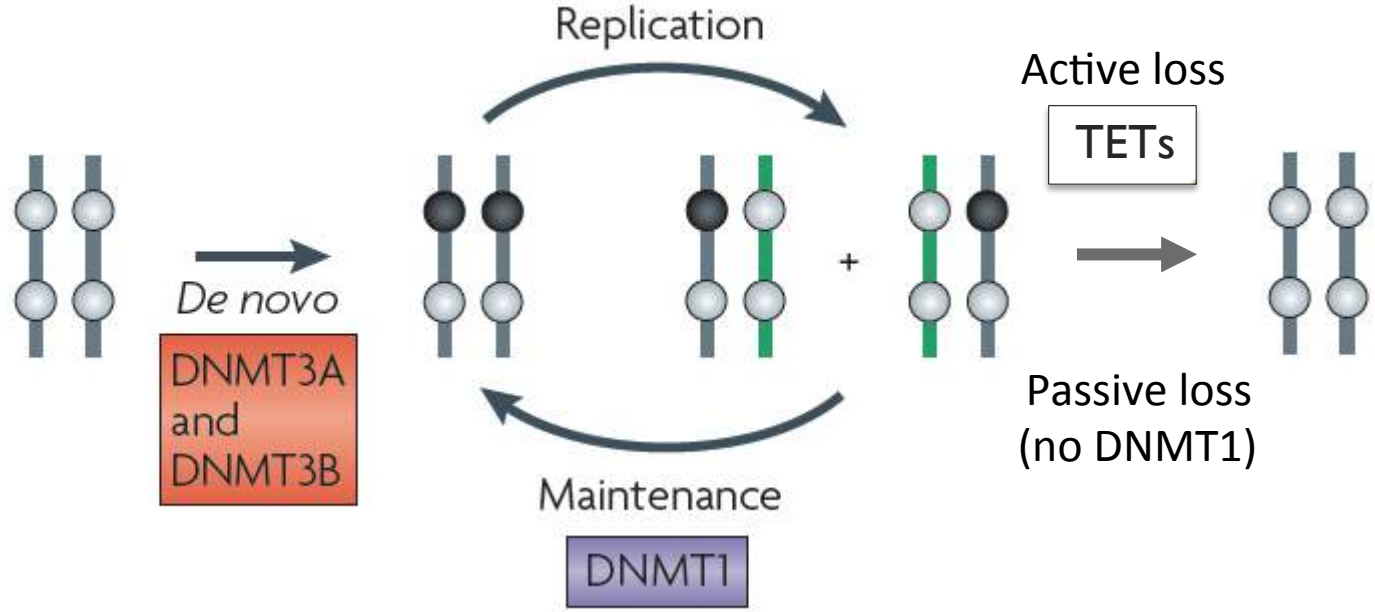
DNA Methyltransferases: Orchestrators of DNA Methylation

Is the DNA Methylation machinery a driver in cancer?



Jones P.A. *et. al.* 2009. *Nat Rev Genet.*

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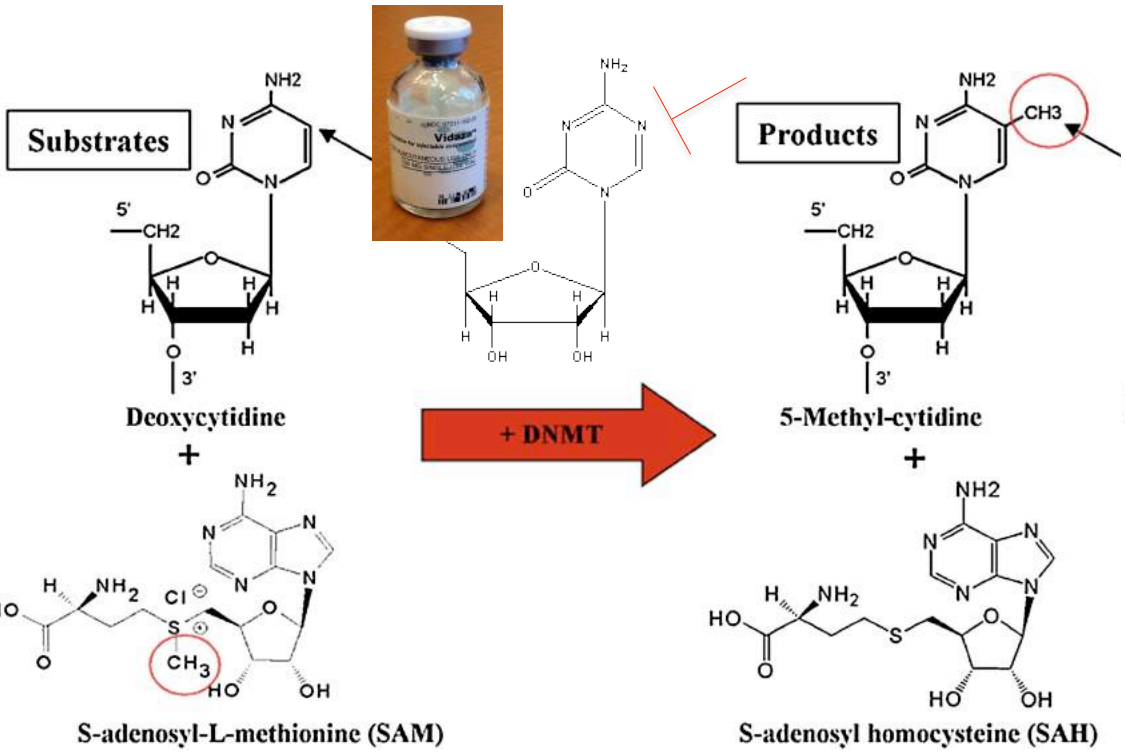
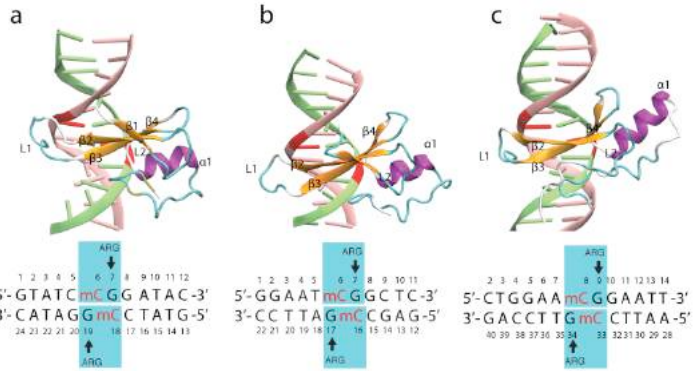
- **DNMT3A/3B *de novo* methylate both unmethylated and hemimethylated DNA**
- **DNMT3L stimulates DNMT3A/3B activity in ES cells**
- **TET enzymes result in loss of 5mC through oxidation**
- **DNA methylation can be passively lost in absence of DNMT1 or actively lost via TETs**

DNA Methyltransferases: Orchestrators of DNA Methylation

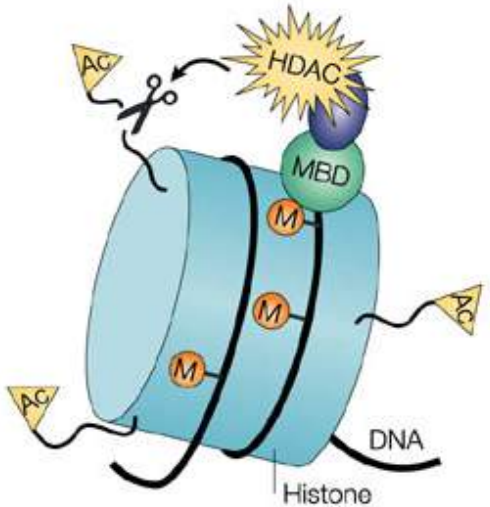
Therapy: inhibitors



Methyl Binding Domain (MBD) proteins ("Readers") orchestrate cytosine methylation functions in gene expression, DNA replication, repair…



Nutrition can influence the availability of methyl-donors to a cell



E. Heard,

Can DNMT1 disruption lead to Cancer?

Induction of Tumors in Mice by Genomic Hypomethylation

François Gaudet,[1,2,3] J. Graeme Hodgson,[4] Amir Eden,[1] Laurie Jackson-Grusby,[1] Jessica Dausman,[1] Joe W. Gray,[4] Heinrich Leonhardt,[2,3] Rudolf Jaenisch[1]*

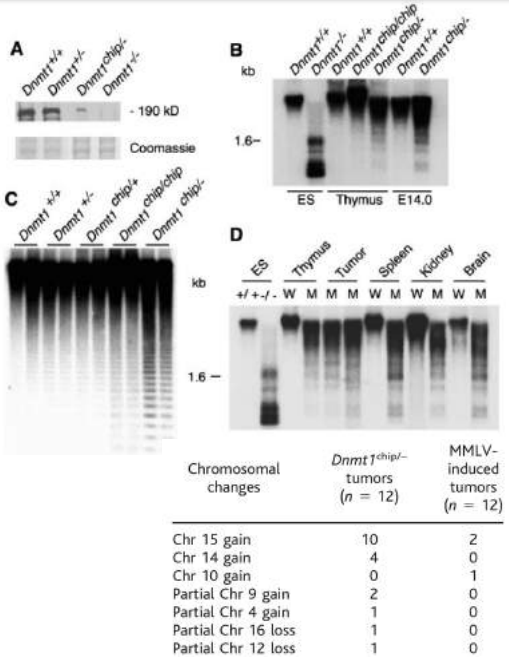
Chromosomal Instability and Tumors Promoted by DNA Hypomethylation

Amir Eden,[1] François Gaudet,[1,3] Alpana Waghmare,[1,2] Rudolf Jaenisch[1,2]*

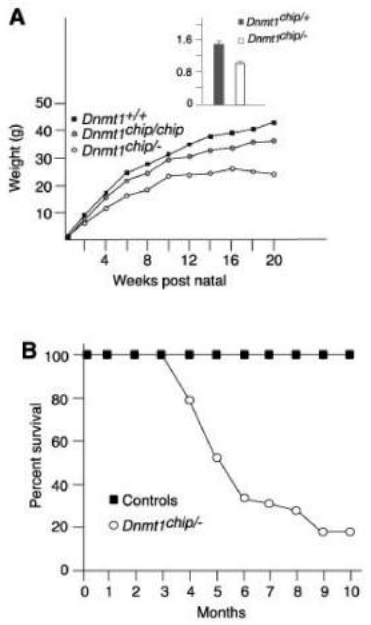
SHORT COMMUNICATION

Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice

G Howard[1], R Eiges[1], F Gaudet[2,3,4], R Jaenisch[2,3] and A Eden[1]



Chromosomal changes	*Dnmt1*$^{chip/-}$ tumors (n = 12)	MMLV-induced tumors (n = 12)
Chr 15 gain	10	2
Chr 14 gain	4	0
Chr 10 gain	0	1
Partial Chr 9 gain	2	0
Partial Chr 4 gain	1	0
Partial Chr 16 loss	1	0
Partial Chr 12 loss	1	0



Dnmt1 hypomorphic mice develop aggressive T cell lymphomas:

Genomic instability and gene rearrangements observed in these mice

Chromosome segregation problems?

DNA hypomethylation at centromeric satellites?

Repeat element transcription / reactivation?

Defects in DNA Damage Repair ?
DNMT1 is normally recruited to repair sites…

Can DNMT1 disruption lead to Cancer?

DNMT1 deficiency triggers mismatch repair defects in human cells through depletion of repair protein levels in a process involving the DNA damage response

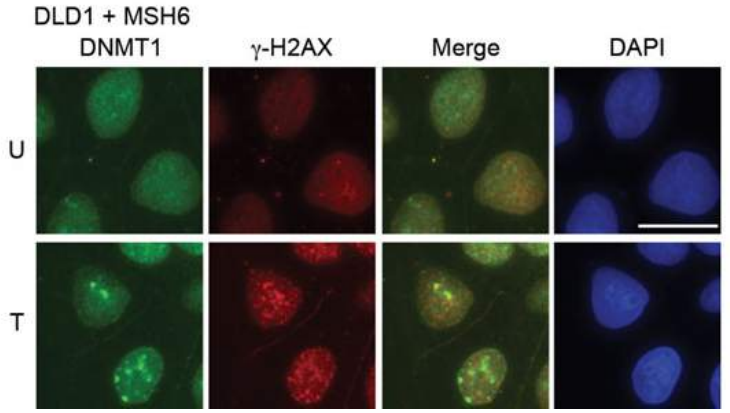
Jayne E.P. Loughery[1,†,‡], Philip D. Dunne[1,†,¶], Karla M. O'Neill[1], Richard R. Meehan[2], Jennifer R. McDaid[3] and Colum P. Walsh[1,*]

Mismatch repair proteins recruit DNA methyltransferase 1 to sites of oxidative DNA damage

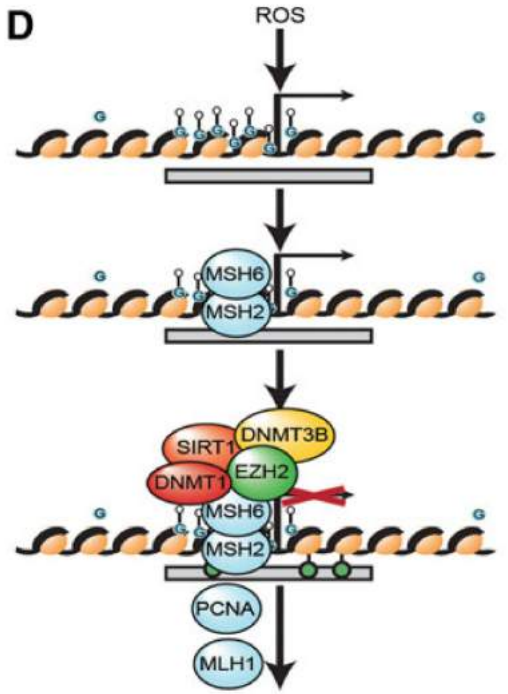
Ning Ding[1], Emily M. Bonham[1], Brooke E. Hannon[1], Thomas R. Amick[1], Stephen B. Baylin[2], and Heather M. O'Hagan[1,3,*]

Journal of Molecular Cell Biology (2015),

Possible role of DNMT1 at sites of oxidative damage to reduce transcription, in order to prevent transcription from interfering with DNA repair?



MSH6 recruits DNMT1 to damaged chromatin treated with 4 mM H2O2 for 30 min



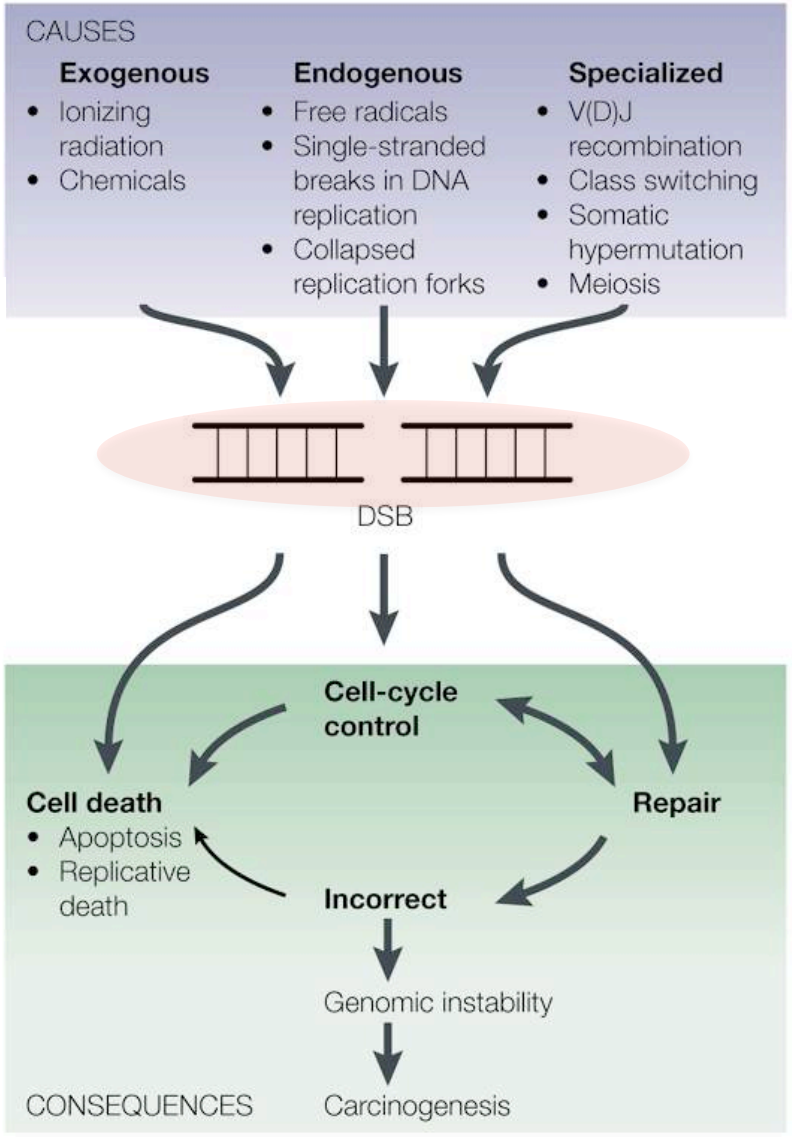
Completion of repair and resumption of transcription

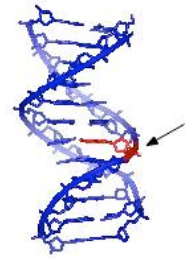
Genotoxic Stress and the Epigenetic Machinery

DNA damage repair may require epigenetic proteins such as DNMT1 to ensure efficient repair

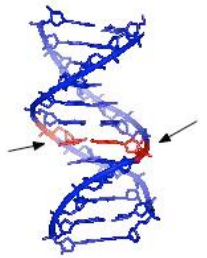
Genotoxic stress can induce genome damage & chromatin memory loss: both at the site of damage and elsewhere by redeployment of chromatin proteins

(see last week's lecture And also COURS V and VI)





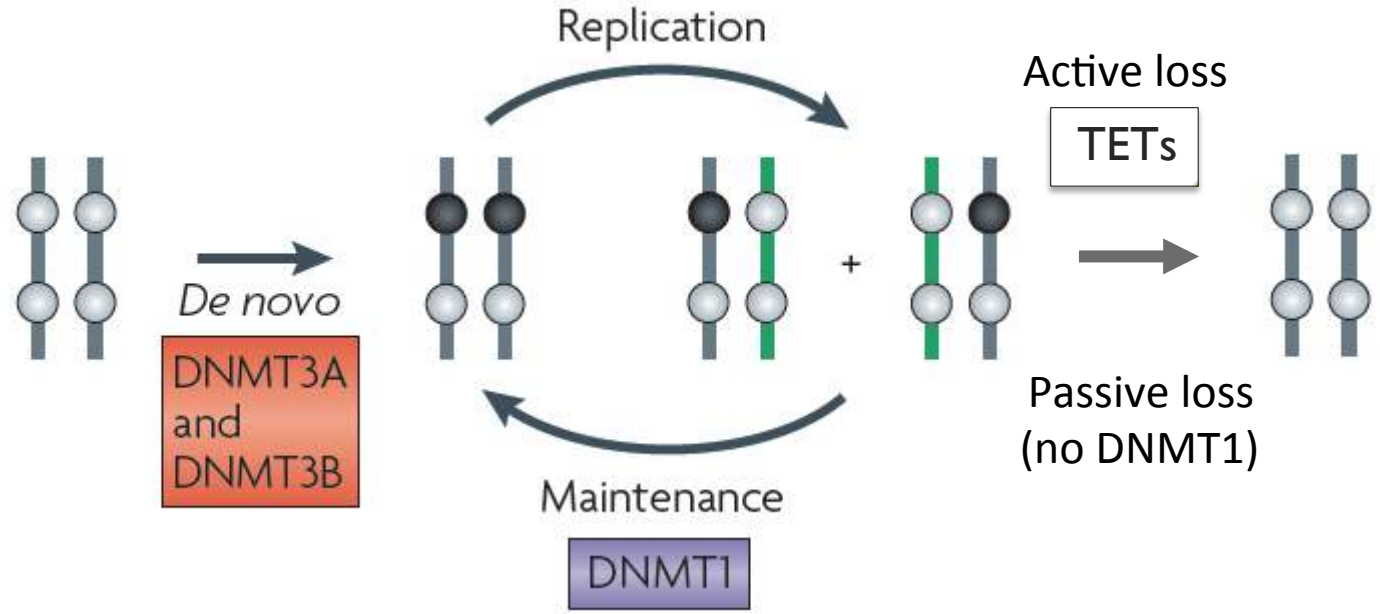
Single - strand break



DSB

DNA Methyltransferases: Orchestrators of DNA Methylation

Is the DNA Methylation machinery a driver in cancer?



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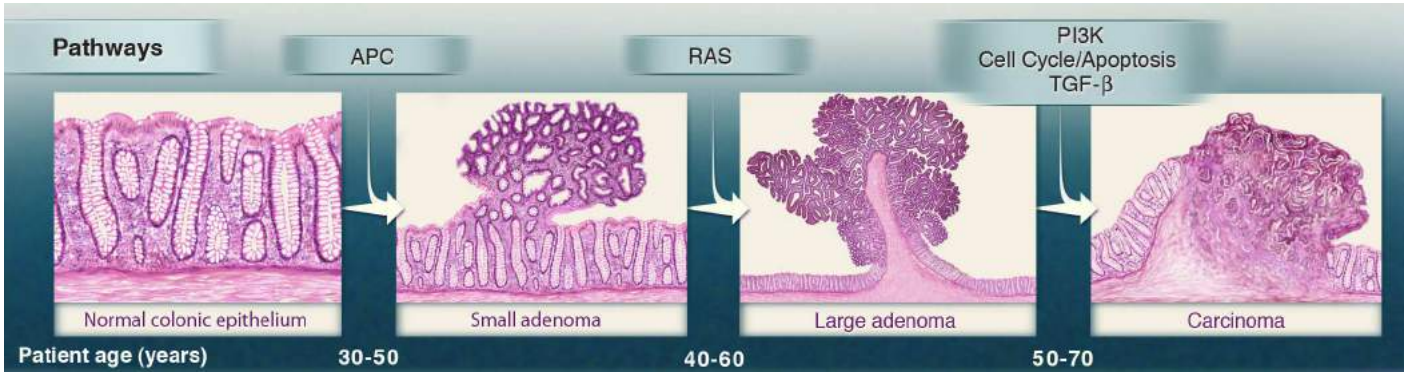
Dnmt3b overexpression promotes tumorigenesis

Dnmt3b promotes tumorigenesis in vivo by gene-specific de novo methylation and transcriptional silencing

Heinz G. Linhart,[1] Haijiang Lin,[1,6] Yasuhiro Yamada,[2] Eva Moran,[1] Eveline J. Steine,[1] Sumita Gokhale,[1] Grace Lo,[3] Erika Cantu,[3] Mathias Ehrich,[4] Timothy He,[5] Alex Meissner,[1] and Rudolf Jaenisch[1,3,7]

- **Overexpressed de novo Dnmt3a and Dnmt3b in *Apc*Min/+ mice.**

Colorectal cancer: *APC* 1st mutation "flowchart" of events (Fearon,Vogelstein, 1990, 1991)
Mutations of the *APC* (adenomatous polyposis coli) gene are strongly associated with both inherited and sporadic cases of colon cancer. APC, like many tumor suppressors, functions to control the expression of genes critical in the cell division process



Apc mutation alone does not lead to cancer – need subsequent events… mutations or epimutations?

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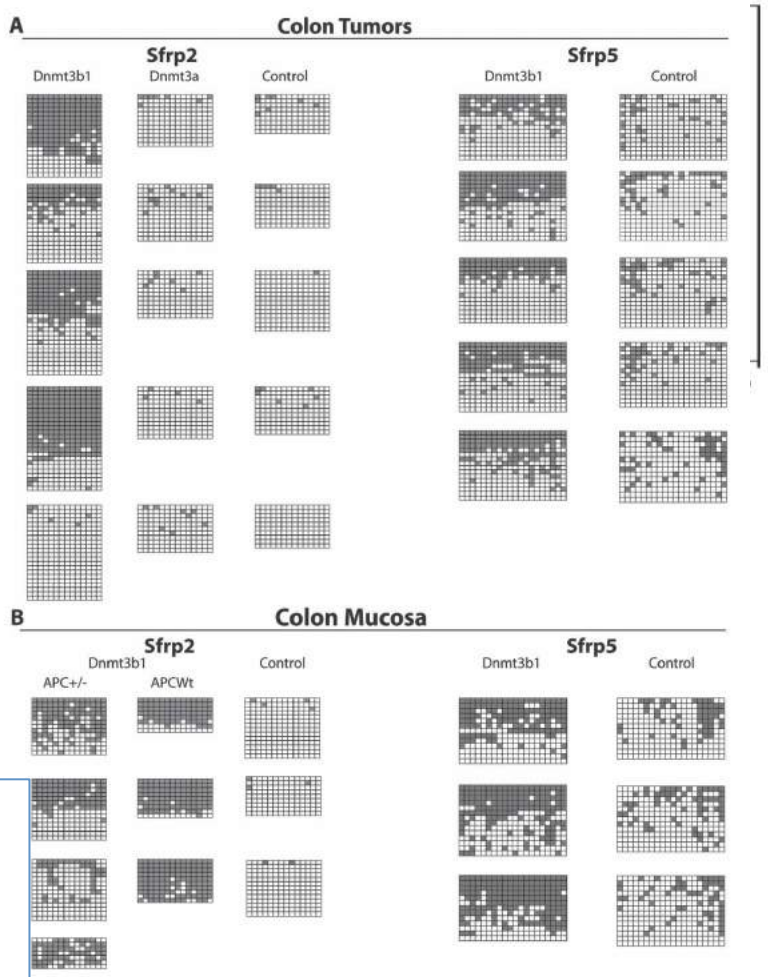
- **Overexpressed de novo Dnmt3a and Dnmt3b in *Apc*Min/+ mice.**
- Dnmt3b enhanced the number of colon tumors in *Apc*Min/+ mice approximately two-fold and increased size of colonic microadenomas
- Dnmt3a had no such effect
- Overexpression of Dnmt3b caused loss of imprinting, increased expression of *Igf2* as well as methylation and transcriptional silencing of tumor suppressor genes *Sfrp2*, *Sfrp4*, and *Sfrp5*.
- Dnmt3b but not Dnmt3a efficiently methylates the same set of genes in tumors and in non-tumor tissues



DNA methylation patterns in cancer are the result of <u>specific targeting</u> of at least some tumor suppressor genes rather than of random, stochastic methylation followed by clonal selection due to a proliferative advantage caused by tumor suppressor gene silencing.

Genes & Development 21:3110–3122 (2007)

Deletion of Dnmt3a and Dnmt3b in tumor formation

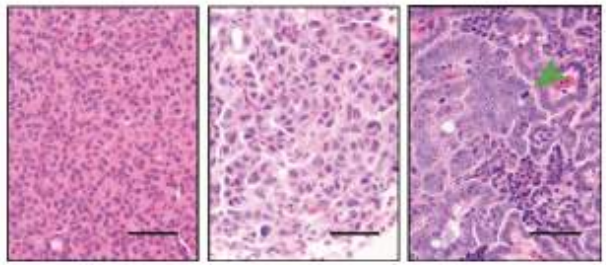
Deletion of the de novo DNA methyltransferase *Dnmt3a* promotes lung tumor progression

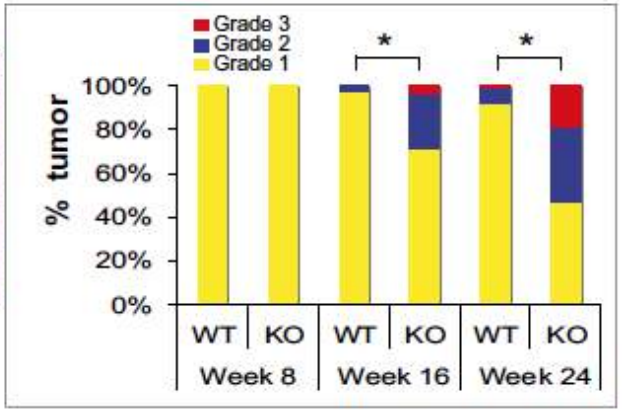
Qing Gao[a], Eveline J. Steine[a], M. Inmaculada Barrasa[a], Dirk Hockemeyer[a], Mathias Pawlak[a], Dongdong Fu[a], Seshamma Reddy[a,1], George W. Bell[a], and Rudolf Jaenisch[a,b,2]

Dnmt3a conditional KO mouse tumor model:
K-Ras - oncogene activation and Dnmt3a deletion induced together

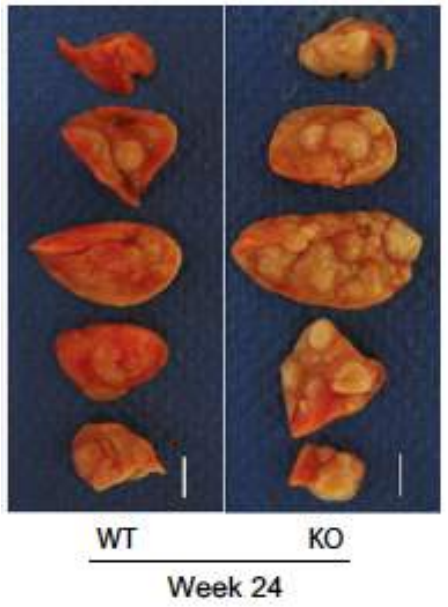
- Dnmt3a deficiency promotes tumor growth & progression but *not* initiation.
- Gene expression changes show that Dnmt3a deficiency affects key steps in cancer progression, such as angiogenesis, cell adhesion, and cell motion, consistent with accelerated and more malignant growth.
- Dnmt3a may act like a tumor-suppressor gene in lung tumor progression and may be a critical determinant of lung cancer malignancy.
- NO effect seen when Dnmt3b is deleted

Dnmt3a deficiency leads to more advanced tumors:





Oncogenic mutation of K-ras is one of the most common genetic lesions and can be found in a large fraction of lung cancers.



Deletion of Dnmt3a and Dnmt3b in tumor formation

Tumor suppressor functions of Dnmt3a and Dnmt3b in the prevention of malignant mouse lymphopoiesis

Leukemia (2014) **28,** 1138–1142; doi:10.1038/leu.2013.364

Thy1.1^{lo},Sca-1+,CD11b+) in fetal liver cells from E15.5 embryos

- Mutations in DNMT3A frequently found in human myeloid and lymphoid malignancies.
- Allelic losses reported in 48% non-Hodgkin lymphomas

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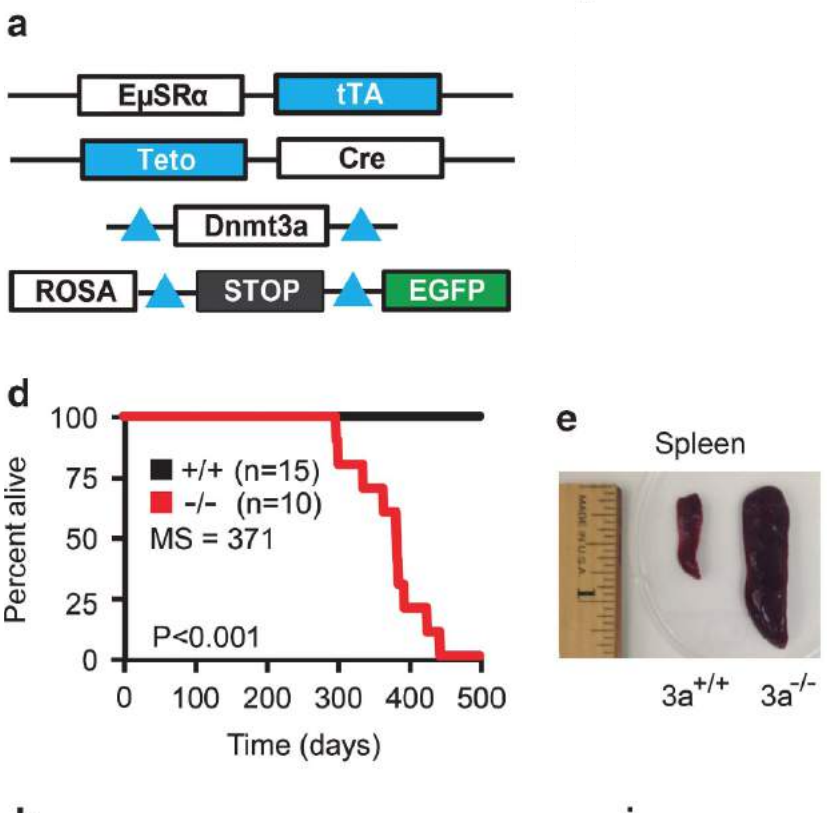
- Mutations in DNMT3A frequently found in human myeloid and lymphoid malignancies.
- Allelic losses reported in 48% non-Hodgkin lymphomas
- Long-term DNMT3A inactivation in mice leads to impaired differentiation of hematopoietic stem cells (HSCs) resulting in accumulation of undifferentiated cells.
- DNMT3A loss may promote tumorigenesis in multiple hematopoietic lineages.
- DNMT3B rarely mutated in human hematologic malignancies.



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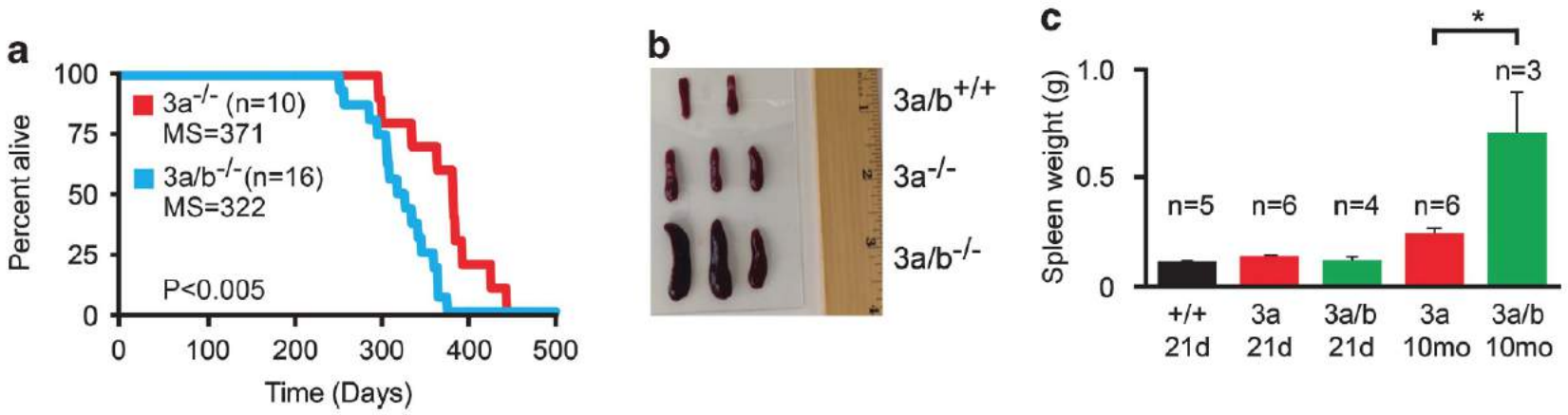
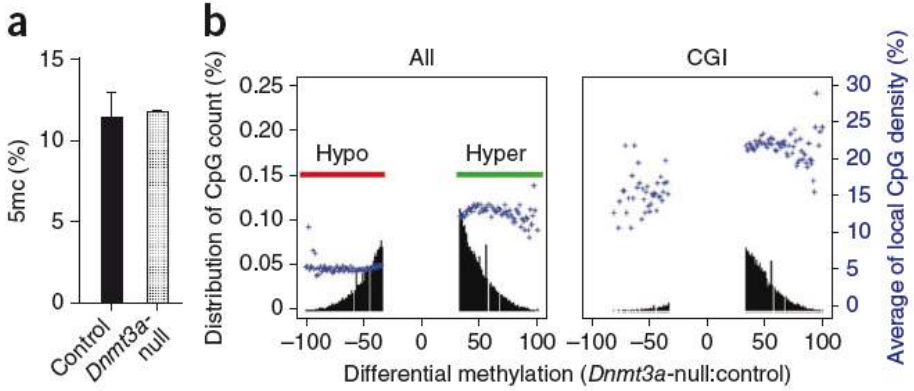


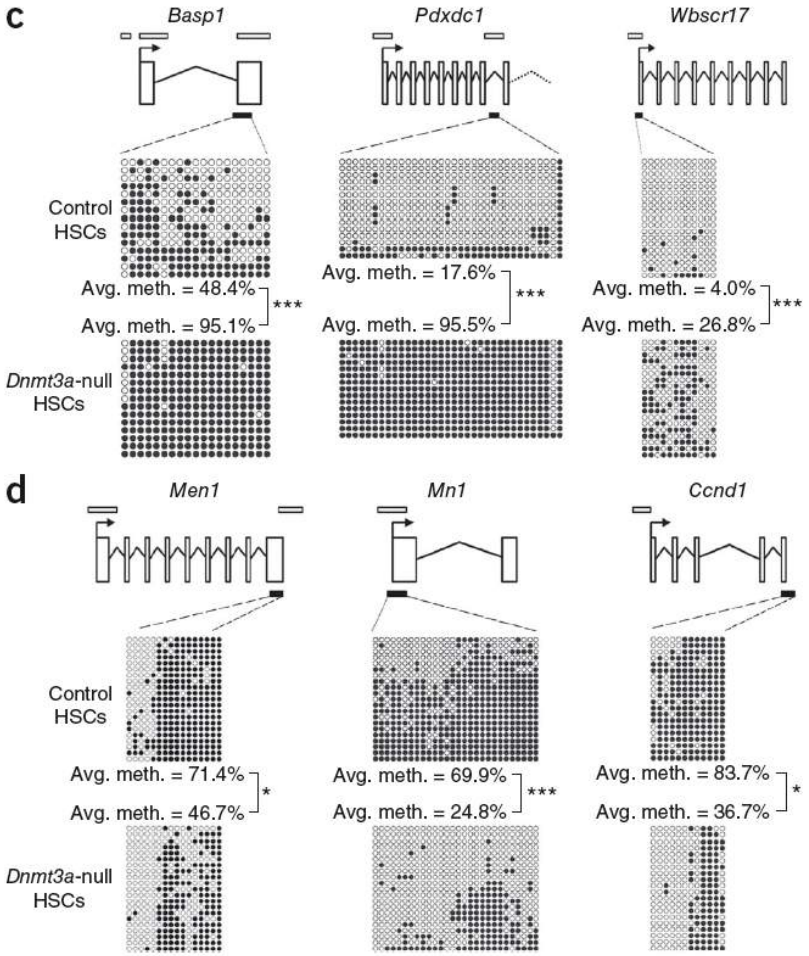
Loss of Dnmt3b accelerates lymphoid tumor development in Dnmt3a-/- mice

Roles of Dnmt3a and Dnmt3b in HSCs?

Dnmt3a is essential for hematopoietic stem cell differentiation

Grant A Challen[1–3], Deqiang Sun[4,5,15], Mira Jeong[1,2,6,15], Min Luo[6,15], Jaroslav Jelinek[7,15], Jonathan S Berg[8,9,15], Christoph Bock[10,11], Aparna Vasanthakumar[12], Hongcang Gu[7], Yuanxin Xi[4,5], Shoudan Liang[13], Yue Lu[7], Gretchen J Darlington[6], Alexander Meissner[10,11], Jean-Pierre J Issa[7], Lucy A Godley[12], Wei Li[4,5] & Margaret A Goodell[1,2,14]





***Dnmt3a* loss in HSCs results in both CpG Island hyper- and hypomethylation**

Roles of Dnmt3a and Dnmt3b in HSCs?

Dnmt3a is essential for hematopoietic stem cell differentiation

Grant A Challen[1–3], Deqiang Sun[4,5,15], Mira Jeong[1,2,6,15], Min Luo[6,15], Jaroslav Jelinek[7,15], Jonathan S Berg[8,9,15], Christoph Bock[10,11], Aparna Vasanthakumar[12], Hongcang Gu[7], Yuanxin Xi[4,5], Shoudan Liang[13], Yue Lu[7], Gretchen J Darlington[6], Alexander Meissner[10,11], Jean-Pierre J Issa[7], Lucy A Godley[12], Wei Li[4,5] & Margaret A Goodell[1,2,14]

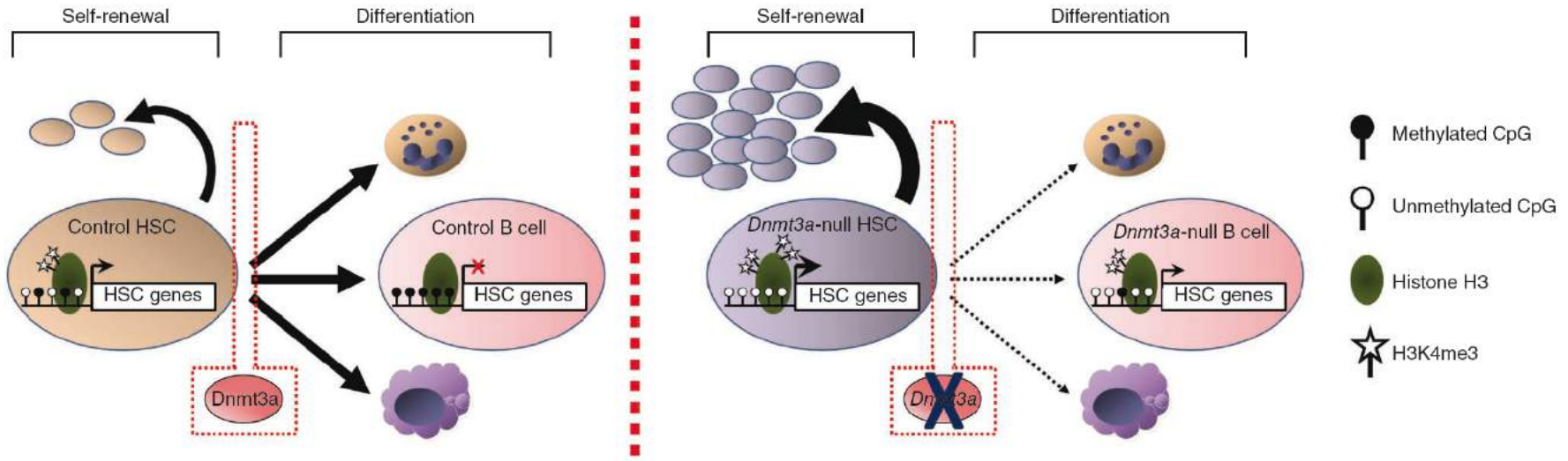


Figure 8 Model for Dnmt… (left). Upon receiving a signal to differentiate, Dnmt3a … oss of H3K4me3 and gene repression in B cells. *Dnm…* HSC-specific genes remain expressed due to a lack o… s active in *Dnmt3a*-null HSCs, resulting in their accumu… w incomplete methylation and partial repression of HSC…

HSC self-renewal promoting genes are normally silenced by Dnmt3a upon differentiation

Loss of Dnmt3a function promotes a progressive expansion of long term HSCs probably due to inability to adequately repress genes involved in self renewal?

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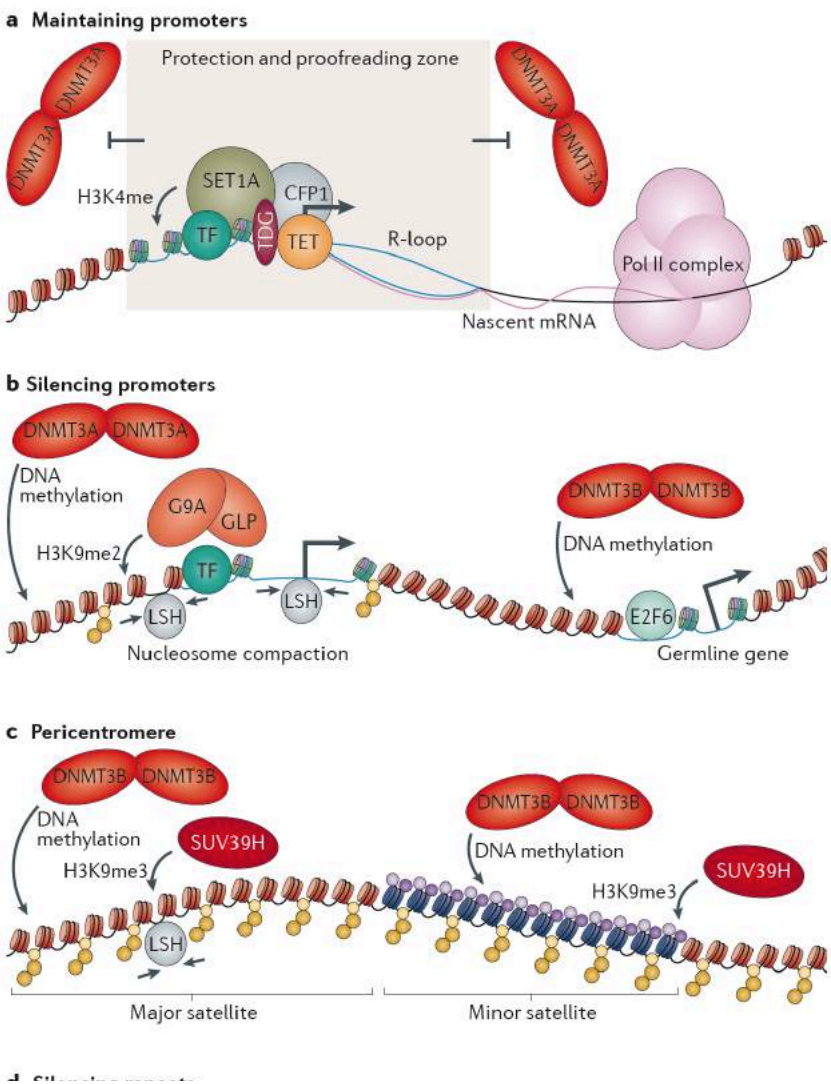
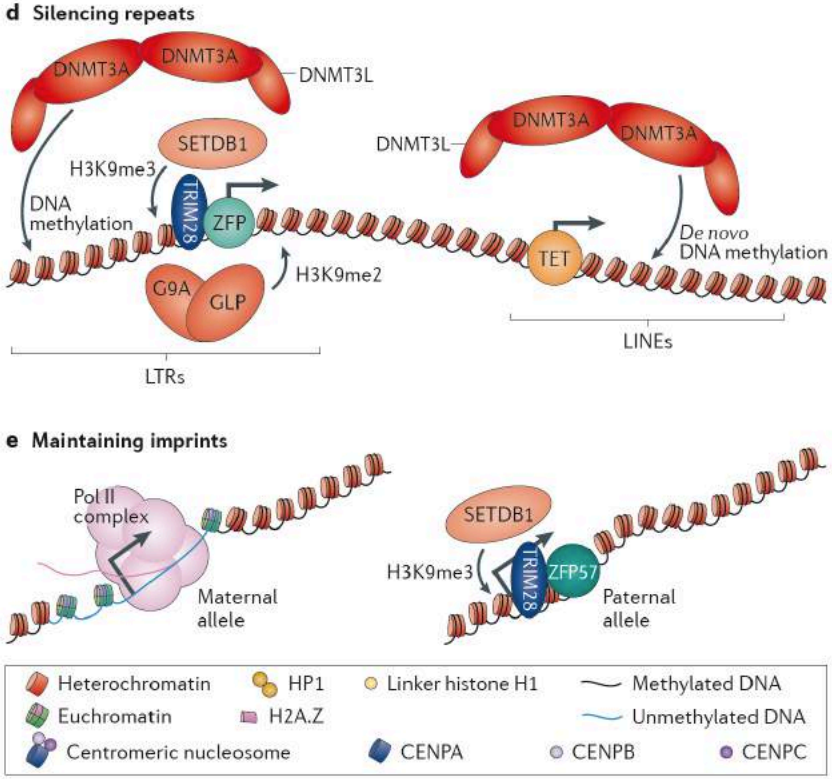
Multiple Roles of DNMT3A/3B





***De novo* DNMTs 3A/3B have multiple possible roles**

Implication in cancer - still very much work in progress…

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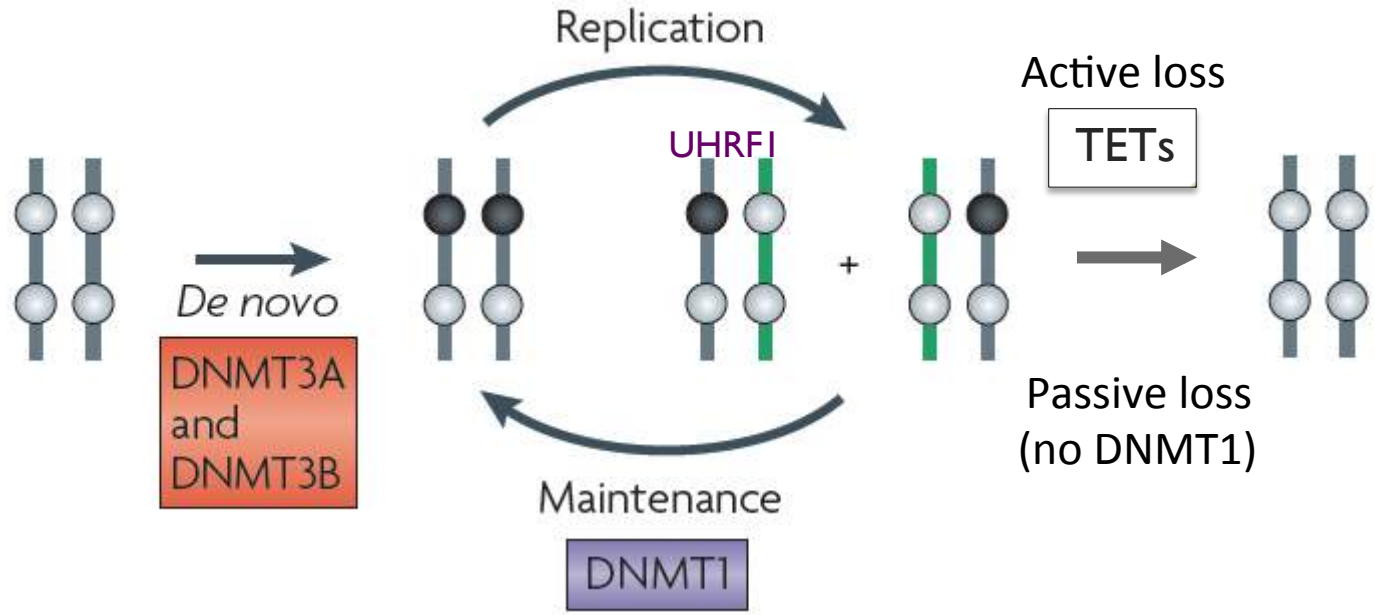
DNA Methyltransferases: Orchestrators of DNA Methylation



Jones P.A. *et. al.* 2009. *Nat Rev Genet.*

- **DNMT1 preferentially methylates hemimethylated DNA**
- **DNMT3A/3B *de novo* methylate both unmethylated and hemimethylated DNA**
- **DNMT3L stimulates DNMT3A/3B activity in ES cells**
- **TET enzymes result in loss of 5mC through oxidation**
- **DNA methylation can be passively lost in absence of DNMT1 or actively lost via TETs**

Cytosine demethylation may play and important tumor suppressive role:
In its absence, get CpG methylation eg at promoters of tumor suppressors?

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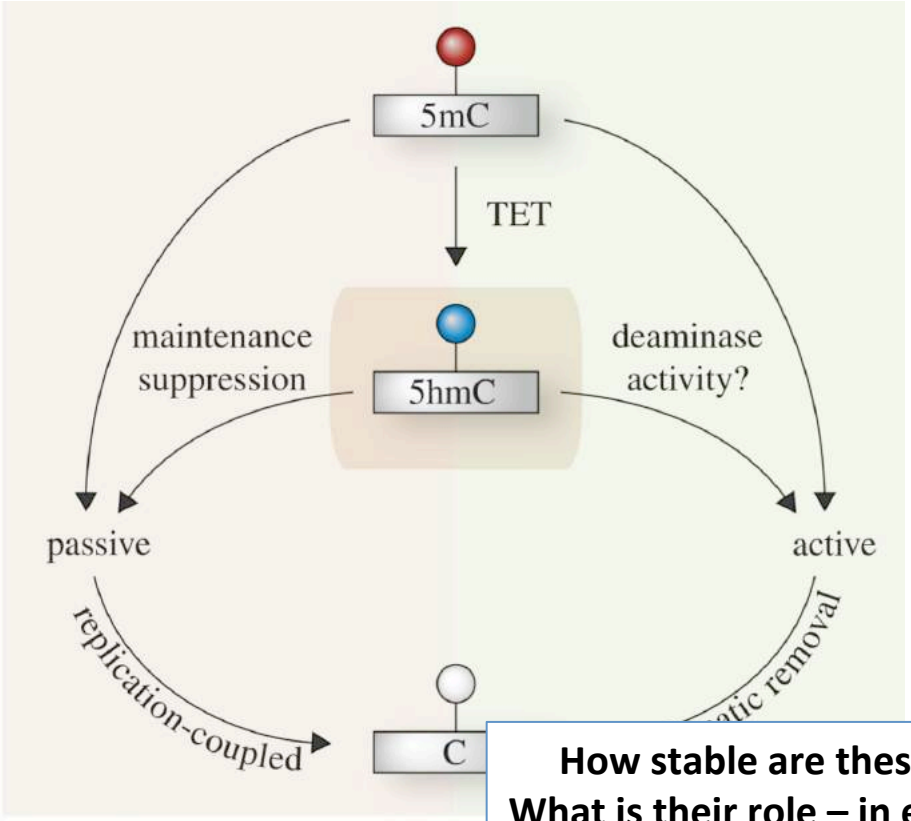
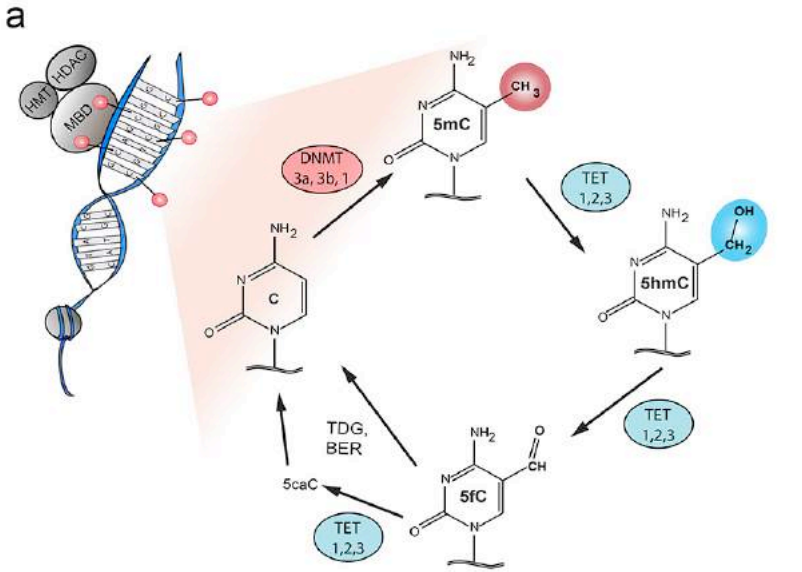
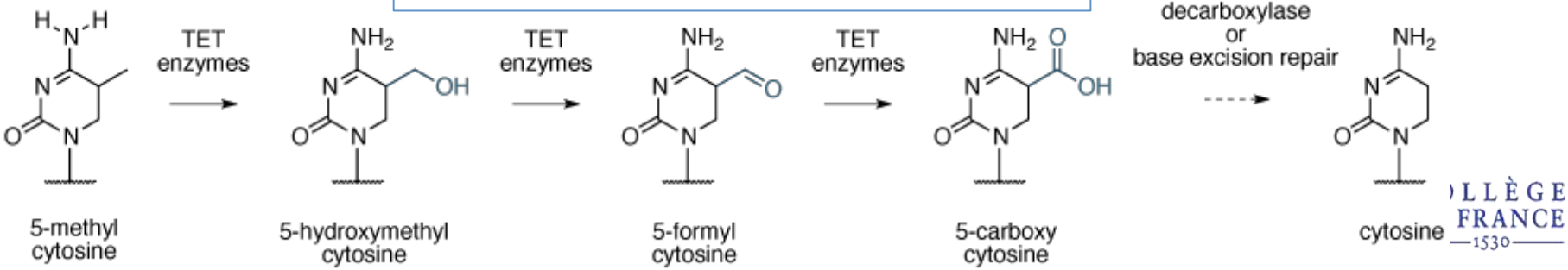
DNA Methylation can be removed either passively or actively via the TET enzymes



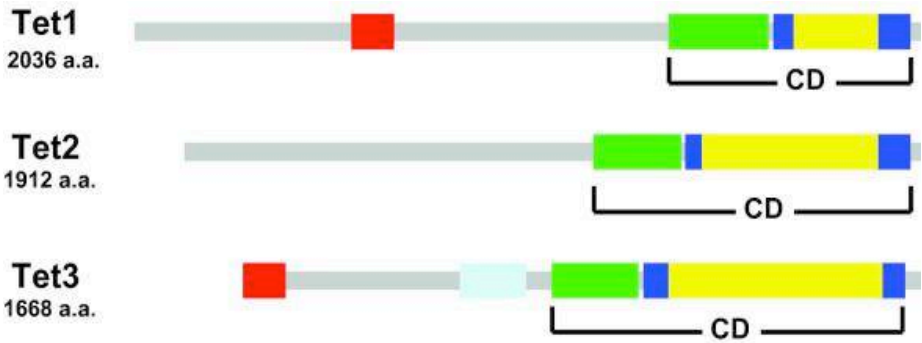


How stable are these oxidised forms?
What is their role – in expression, repair…?
Can they be read by specific "Readers"?

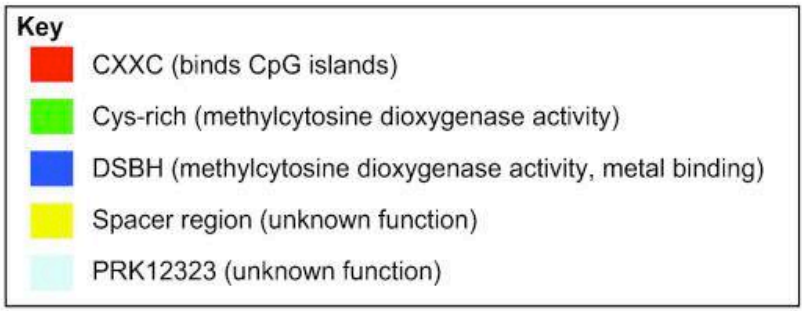


The TET Enzymes

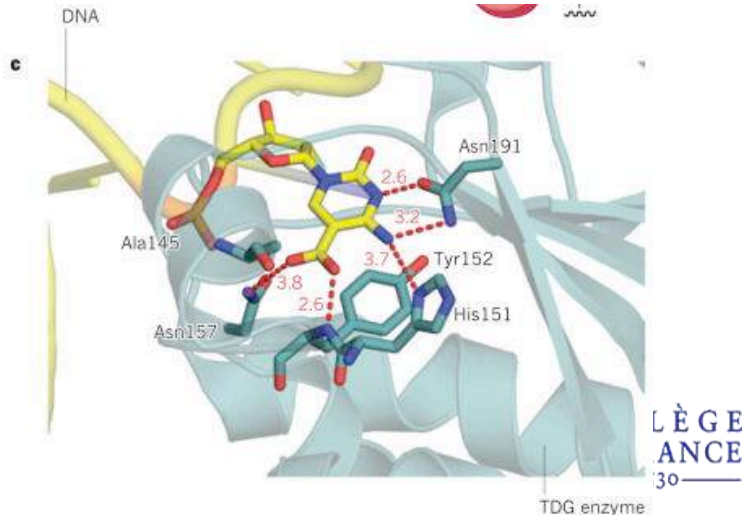
TET1, 2 and 3 each have a core catalytic domain with a double-stranded β-helix fold that contains the crucial metal-binding residues found in the family of Fe(II)/α-ketoglutarate (αKG) -dependent oxygenases.



TET uses molecular oxygen as a substrate to catalyse oxidative decarboxylation of α-KG, thereby generating a reactive high-valent enzyme-bound Fe(IV)-oxo intermediate that converts 5mC to 5hmC



TET1 and TET3 also contain a chromatin-associated CXXC domain that is known to bind CpG sequences, whereas TET2 partners with IDAX, an independent CXXC-containing protein.



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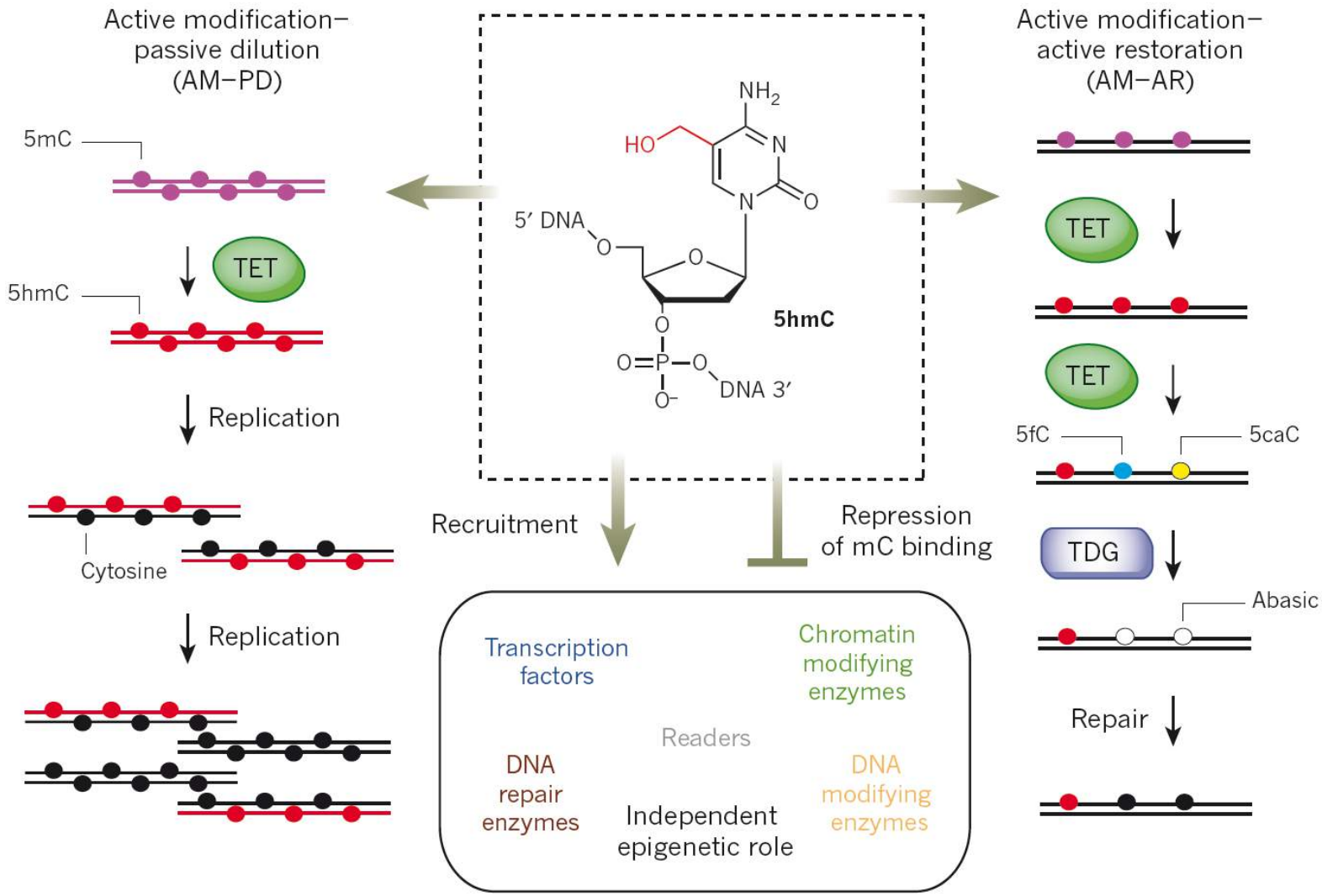
Roles of TET induced oxidised derivatives?



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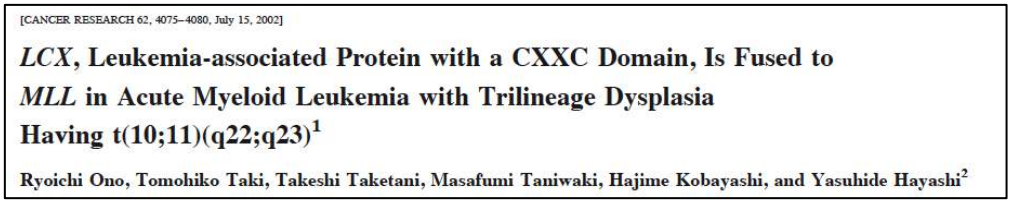
TET discovery and implication in cancer

• *TET1* was initially identified through fusion to *MLL* (*KMT2A*) in patients with acute myeloid leukaemia
(*NB TET1-fusion may have lost its 5mC oxidase activity but recruit unknown factors aberrantly targeted to MLL genes)*



[CANCER RESEARCH 62, 4075–4080, July 15, 2002]

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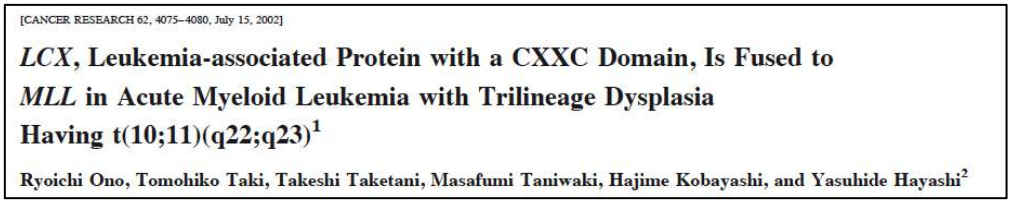

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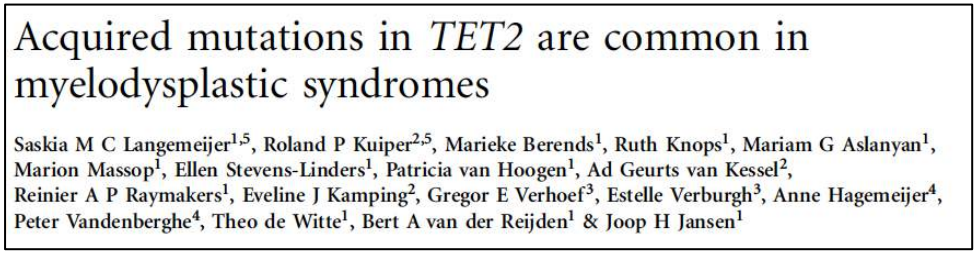
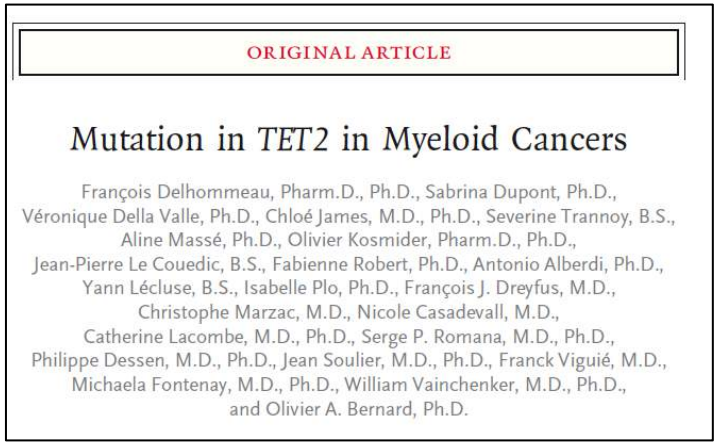
• *TET2* mutations were since demonstrated to be one of most frequent lesions in myeloid lineage malignancies
(AML: 7-23%; CMML: 50%; MDS: 10-20%)

• Importantly, these myeloid-lineage conditions are susceptible to therapy aimed at inhibiting DNA methylation



Acquired mutations in *TET2* are common in myelodysplastic syndromes

Saskia M C Langemeijer[1,5], Roland P Kuiper[2,5], Marieke Berends[1], Ruth Knops[1], Mariam G Aslanyan[1], Marion Massop[1], Ellen Stevens-Linders[1], Patricia van Hoogen[1], Ad Geurts van Kessel[2], Reinier A P Raymakers[1], Eveline J Kamping[2], Gregor E Verhoef[3], Estelle Verburgh[3], Anne Hagemeijer[4], Peter Vandenberghe[4], Theo de Witte[1], Bert A van der Reijden[1] & Joop H Jansen[1]



ORIGINAL ARTICLE

Mutation in *TET2* in Myeloid Cancers

François Delhommeau, Pharm.D., Ph.D., Sabrina Dupont, Ph.D., Véronique Della Valle, Ph.D., Chloé James, M.D., Ph.D., Severine Trannoy, B.S., Aline Massé, Ph.D., Olivier Kosmider, Pharm.D., Ph.D., Jean-Pierre Le Couedic, B.S., Fabienne Robert, Ph.D., Antonio Alberdi, Ph.D., Yann Lécluse, B.S., Isabelle Plo, Ph.D., François J. Dreyfus, M.D., Christophe Marzac, M.D., Nicole Casadevall, M.D., Catherine Lacombe, M.D., Ph.D., Serge P. Romana, M.D., Ph.D., Philippe Dessen, M.D., Ph.D., Jean Soulier, M.D., Ph.D., Franck Viguié, M.D., Michaela Fontenay, M.D., Ph.D., William Vainchenker, M.D., Ph.D., and Olivier A. Bernard, Ph.D.

Delhommeau, F. *et al*. Mutation in TET2 in myeloid cancers. *N. Engl. J. Med*. **360,** 2289–2301 (2009).
Langemeijer, S. M. *et al*. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nature Genet.* **41**, 838–842 (2009).

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(AML: 7-23%; CMML: 50%; MDS: 10-20%)

• Importantly, these myeloid-lineage conditions are susceptible to therapy aimed at inhibiting DNA methylation

• Mouse models have shown that Tet2 is a crucial regulator of self-renewal and differentiation in HSCs => supporting a role for Tet2 in normal haematopoiesis

• Downregulation of TET expression also seen in human breast, liver, lung, pancreatic and prostate cancers

• TET mutations are consistently associated with a decrease in 5hmC; the relevance of 5fC and 5caC in cancer has not yet been explored

• TET mutations may affect DNA methylation or hydroxymethylation of gene regulatory elements?

TET2 Mutations Affect Non-CpG Island DNA Methylation at Enhancers and Transcription Factor–Binding Sites in Chronic Myelomonocytic Leukemia

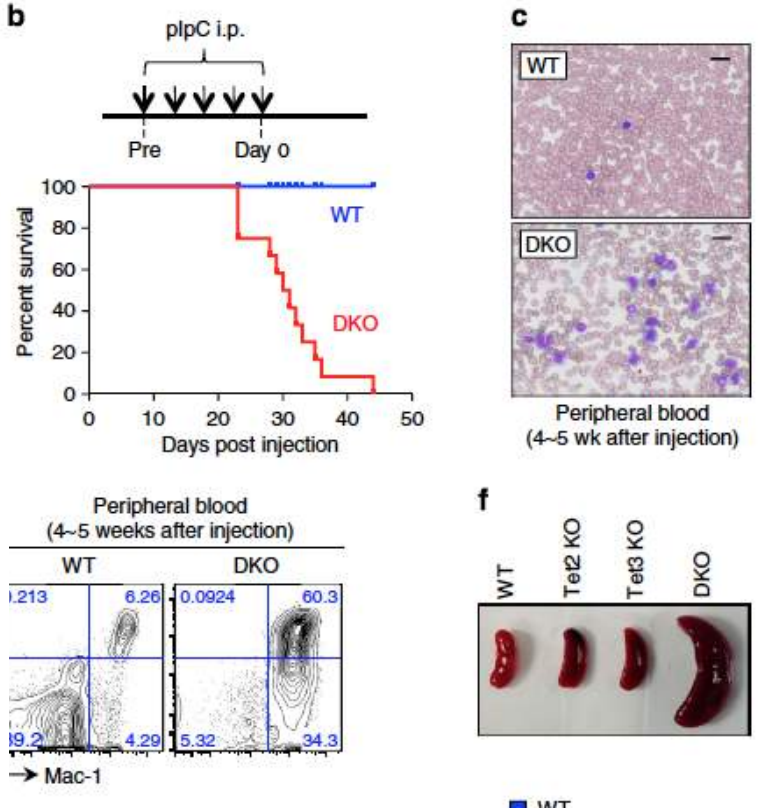
Jumpei Yamazaki[1,2], Jaroslav Jelinek[1,2], Yue Lu[3], Matteo Cesaroni[1], Jozef Madzo[1,4], Frank Neumann[2], Rong He[2], Rodolphe Taby[2], Aparna Vasanthakumar[4], Trisha Macrae[4], Kelly R. Ostler[4], Hagop M. Kantarjian[2], Shoudan Liang[5], Marcos R. Estecio[2,3], Lucy A. Godley[4], and Jean-Pierre J. Issa[1,2]

Loss of TET activity leads to aggressive myeloid cancer in mice

Received 10 Sep 2015 | Accepted 29 Oct 2015 | Published 26 Nov 2015 DOI: 10.1038/ncomms10071 OPEN

Acute loss of *TET* function results in aggressive myeloid cancer in mice

Jungeun An[1,†], Edahí González-Avalos[1], Ashu Chawla[1], Mira Jeong[2], Isaac F. López-Moyado[1], Wei Li[3], Margaret A. Goodell[2,3], Lukas Chavez[1,4], Myunggon Ko[1,5] & Anjana Rao[1,6,7]



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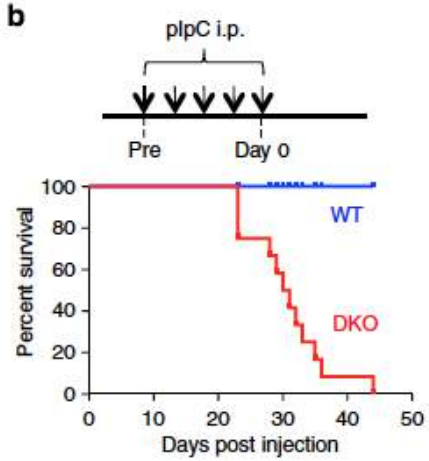

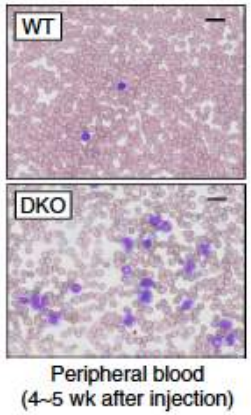

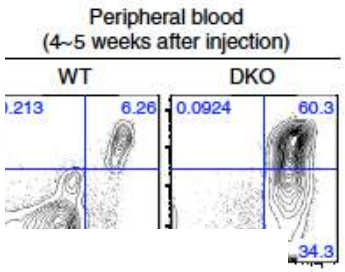

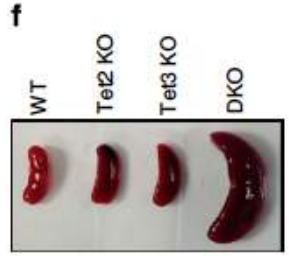

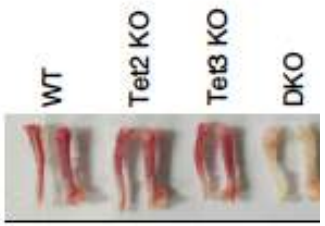

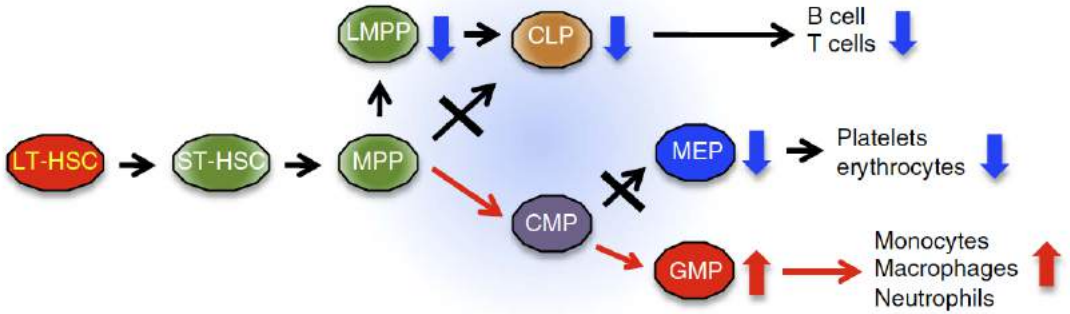

Myeloid expansion and impaired lymphoid and erythroid development upon loss of Tet2 +Tet3

Maintain HSC pool size but becomes skewed towards Myeloid progenitor cells.

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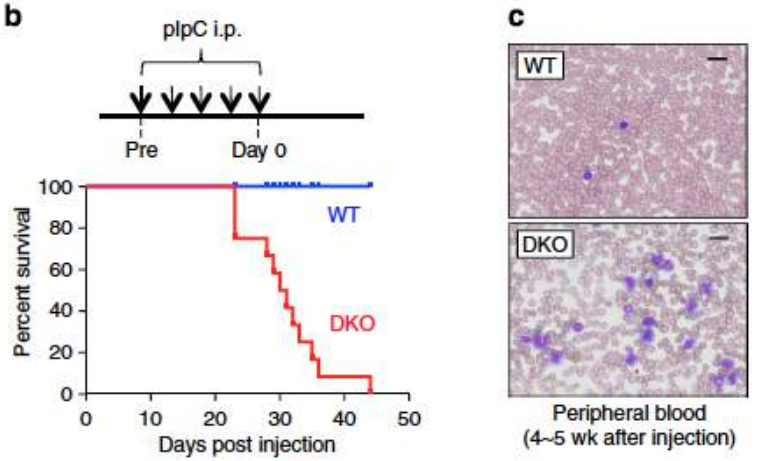

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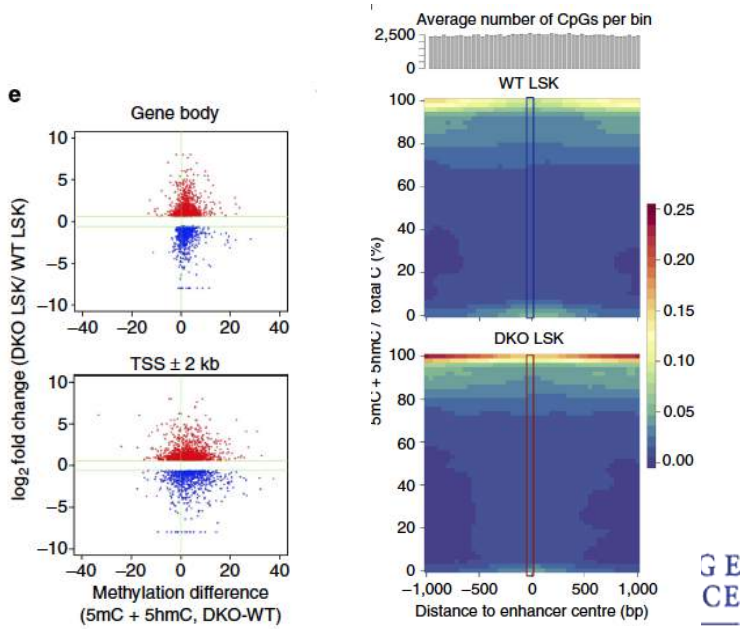

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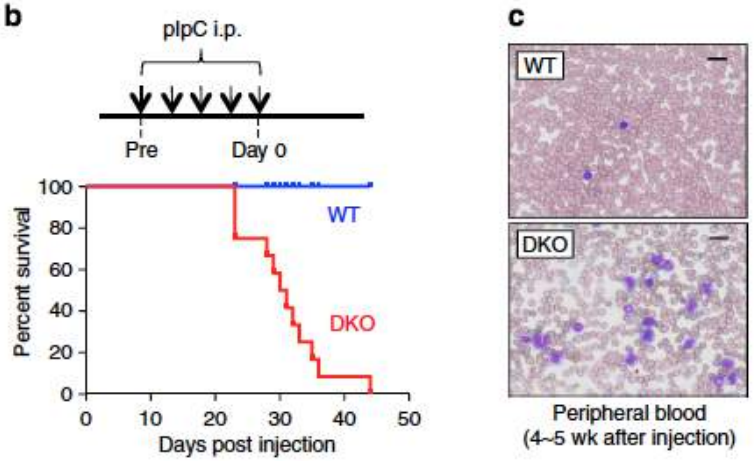

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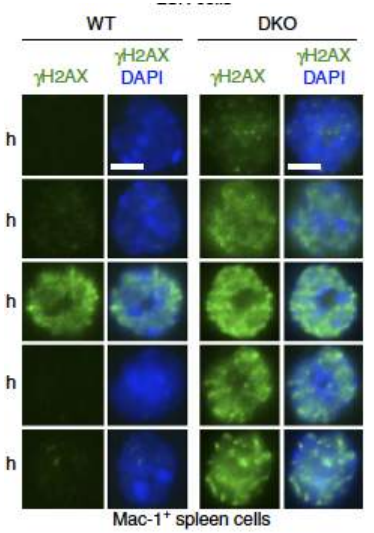
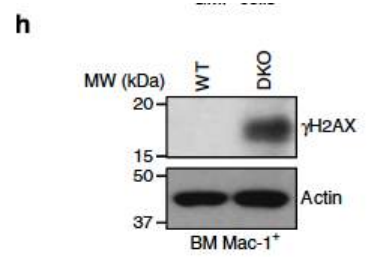
- Progressive accumulation of phospho-H2AX and strong impairment of DNA damage repair pathways

=> **key role for TET proteins in maintaining genome integrity?**





After irradiation – induced DNA damage, gH2Ax foci are resolved with hours in normal cells but linger for days in Tet2/3 KO bone marrow and spleen

Loss of TET activity leads to aggressive myeloid cancer in mice

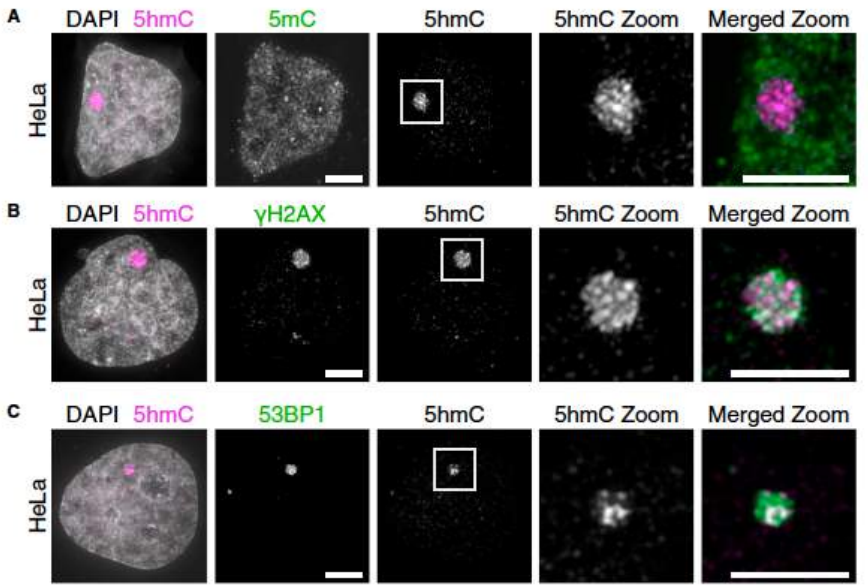
Aberrant methylomes associated with Tet mutant induced leukemia not easily connected to changes in gene expression…

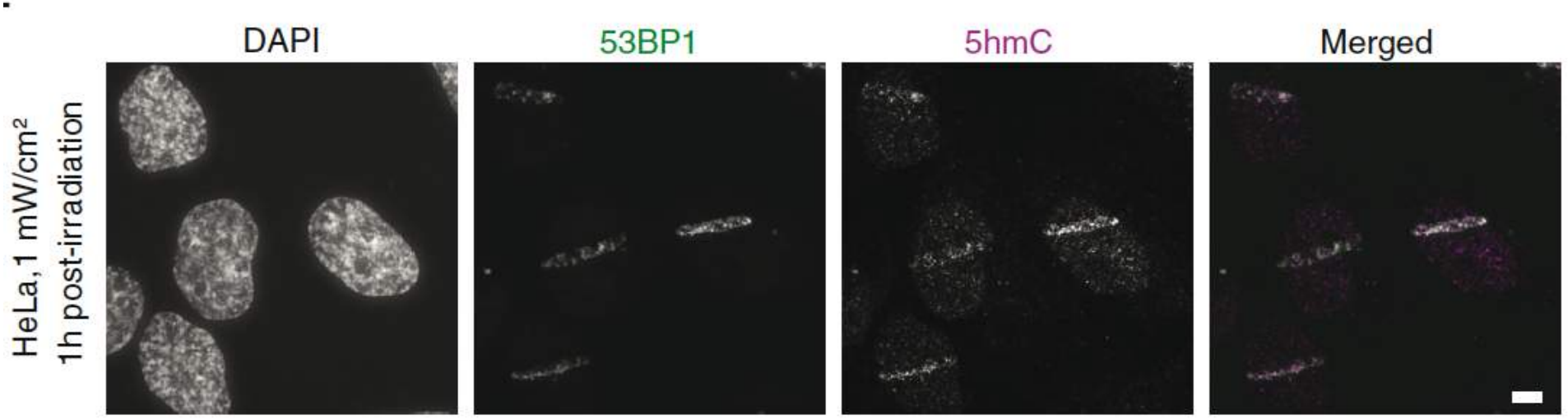
Could be due to effects on enhancers, insulators, repetitive elements? (need whole genome analyses)

Could be due to DNA damage repair and genome integrity…?

5-Hydroxymethylcytosine marks sites of DNA damage and promotes genome stability

- 5hmC is actively enriched at endogenous DNA damage sites in cancer cell lines
- DNA damage induced by aphidicolin or microirradiation increases 5hmC locally
- TET2 is required for damage-associated 5hmC foci (but not to recruit DDR proteins to damage)
- TET enzymes promote genome integrity under replication stress in mouse ES cells





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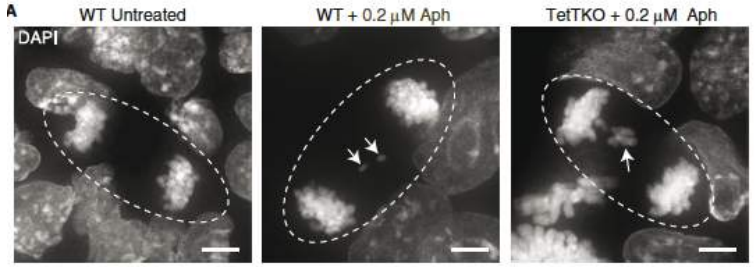

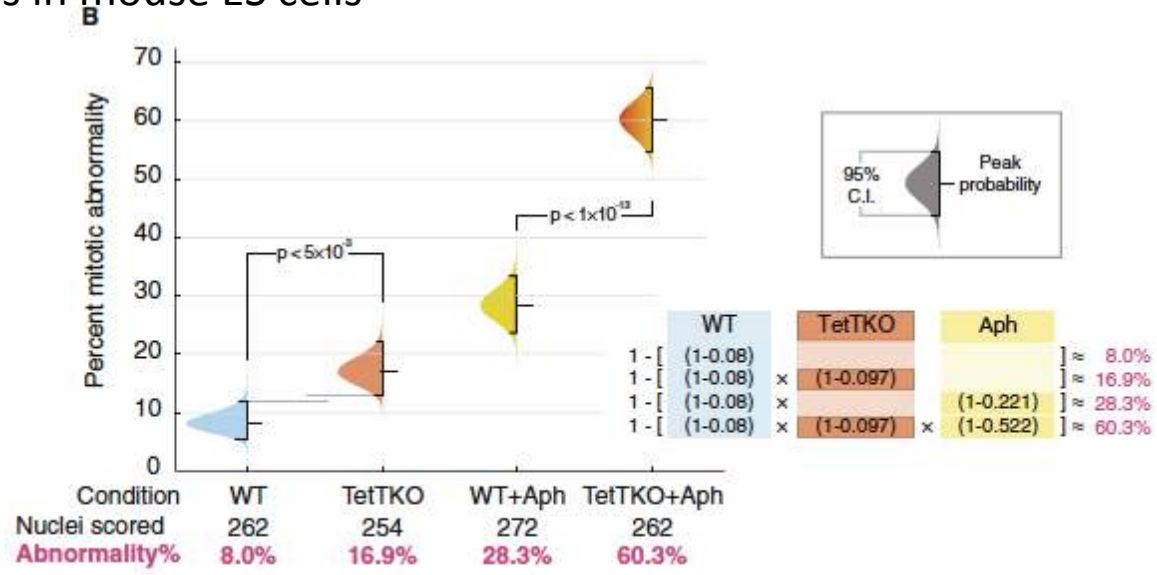

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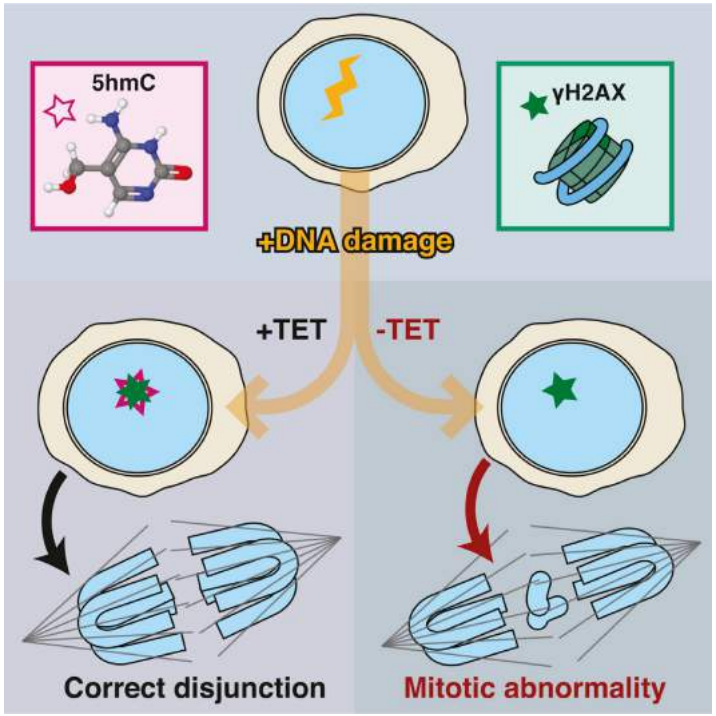
5-Hydroxymethylcytosine marks sites of DNA damage and promotes genome stability

- 5hmC is actively enriched at endogenous DNA damage sites in cancer cell lines
- DNA damage induced by aphidicolin or microirradiation increases 5hmC locally
- TET2 is required for damage-associated 5hmC foci (but not to recruit DDR proteins to damage)
- TET enzymes promote genome integrity under replication stress in mouse ES cells



TET2 induced 5hmC important for both genome integrity and gene regulation?

Both Dnmt3a and Tet2 Lead to similar defects in hematopoetic system that can result in cancer – could this be linked to aberrant DNA Damage repair?

IDH1/2 Dysfunction in Cancer

- IDH1 and IDH2 genes encoding isocitrate dehydrogenases

- Mutations frequently found in human glioblastomas and cytogenetically normal acute myeloid leukaemias (AML)

- Gain-of-function mutations – drive the synthesis of the 'oncometabolite' R-2-hydroxyglutarate (2HG) instead of α-ketoglutarate (αKG)

- How do IDH1 andIDH2 mutations modify myeloid cell development and promote leukaemogenesis?

Parsons, D. W. *et al.* An integrated genomic analysis of human glioblastoma multiforme. *Science* **321,** 1807–1812 (2008).
The Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N. Engl. J. Med.* **372,**
2481–2498 (2015).
Dang, L. *et al.* Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **462,** 739–744 (2009).

IDH1/2 Dysfunction in Cancer

Leukemic IDH1 and IDH2 Mutations Result in a Hypermethylation Phenotype, Disrupt TET2 Function, and Impair Hematopoietic Differentiation

Table 1. Clinical and Genetic Parameters of *IDH1/2*- and *TET2*-Wild-Type and -Mutant AML Samples from the ECOG E1900 Cohort

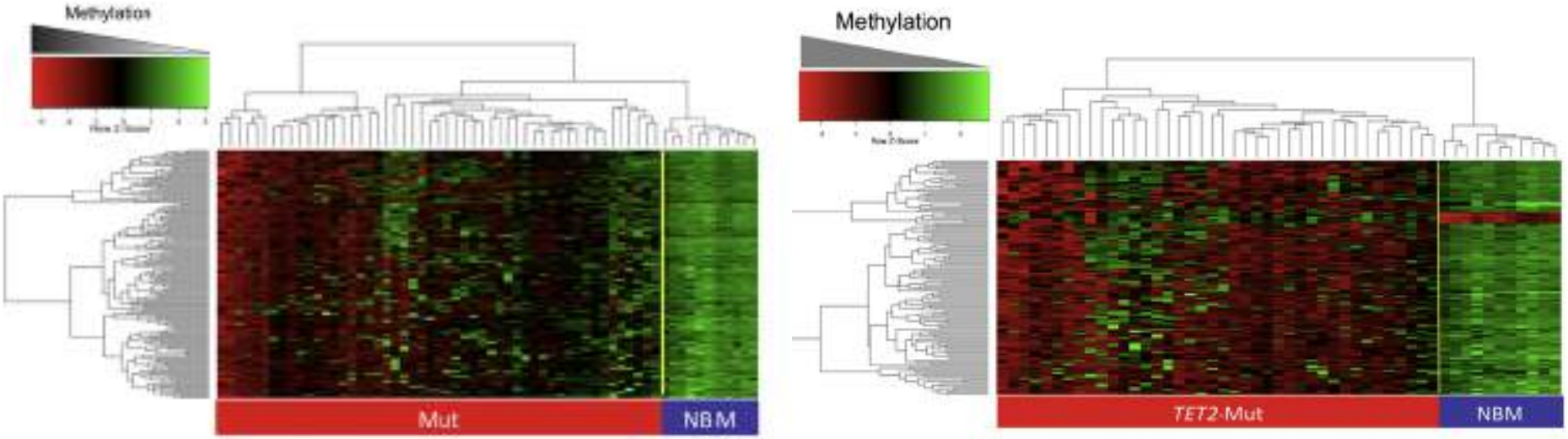
IDH and *TET2* Status	Median Age (Range)	Gender (M/F)	Cytogenetic Risk Class (Favorable/ Intermediate/Unfavorable/ Indeterminate)	FLT3 Mutant (%/%) (ITD/TKD)	NPM1 Mutant (%)	CEBPA Mutant (%)	Bone Marrow Blast at Sample Acquisition Median (%) (Range)
TET2 and *IDH1/2* wild-type (n = 300)	45.5 (18–60)	160/140	61/133/51/55	32/8	11	11	65 (3–100)
TET2 mutant (n = 28)	55 (30–60)	17/11	2/10/4/12	35.7/3.6	21.4	10.7	69.5 (20–99)
IDH1 or *IDH2* mutant (n = 57)	46.5 (18–60)	25/32	1/35/5/15	19.3/3.5	24.6	1.8	79 (11–100)
IDH1 mutant (n = 24)	46 (18–58)	10/14	1/18/0/5	16.7/0	25	4.2	79 (30–96)
IDH2 mutant (n = 33)	46.5 (24–60)	15/18	0/18/5/10	21.2/6.1	24.2	0	78 (11–100)
All patients (n = 385)	46.5 (18–60)	202/183	64/179/60/82	31.7/7	14	9.9	68 (3–100)

ITD/TKD, internal tandem duplication/tyrosine kinase domain.

- Examine a large cohort of AML paties for mutations and DNA Methylomes
- *IDH1/2* mutations associated with a specific DNA **hyper**methylation profile in AML
- Expression of mutant IDH1/2 induces an **increase in global 5-methylcytosine levels**
- IDH1/2 mutations inhibit the hydroxylation reaction of methylcytosine by TET2
- Expression of IDH2 mutants or loss of TET2 impair myeloid differentiation, with increased stem/progenitor cell marker expression,

=> shared proleukemogenic effects?

IDH1/2 Dysfunction in Cancer



Heatmap representation of genes identified as **differentially methylated** between IDH1/2-mutant or TET2-mutant primary AML cases (indicated by the red bar) and wild-type cases
Each row represents a probe set and each column represents a patient.

IDH1/2- and TET2-mutant leukemias are a biologically distinct disease subtype

Link between cancer metabolism with epigenetic control of gene expression.

More in COURS V

IDH1/2 Dysfunction in Cancer

IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics

Masato Sasaki[1]*, Christiane B. Knobbe[1,2]*, Joshua C. Munger[3], Evan F. Lind[1], Dirk Brenner[1], Anne Brüstle[1], Isaac S. Harris[1,4], Roxanne Holmes[5], Andrew Wakeham[1], Jillian Haight[1], Annick You-Ten[1], Wanda Y. Li[1], Stefanie Schalm[9], Shinsan M. Su[9], Carl Virtanen[6], Guido Reifenberger[2], Pamela S. Ohashi[1], Dwayne L. Barber[4], Maria E. Figueroa[7], Ari Melnick[8], Juan-Carlos Zúñiga-Pflücker[5] & Tak W. Mak[1,4]

- IDH1(R132H) inserted into endogenous murine *Idh1* locus

- Mutants show increased early haematopoietic progenitors, develop splenomegaly and anaemia with extramedullary haematopoiesis
=> dysfunctional bone marrow niche.

- Mice have hypermethylated histones and changes to DNA methylation similar to those observed in human IDH1- or IDH2-mutant AML.

=> IDH1 single amino acid change induces a leukaemic DNA methylation signature in a mouse model of human AML.

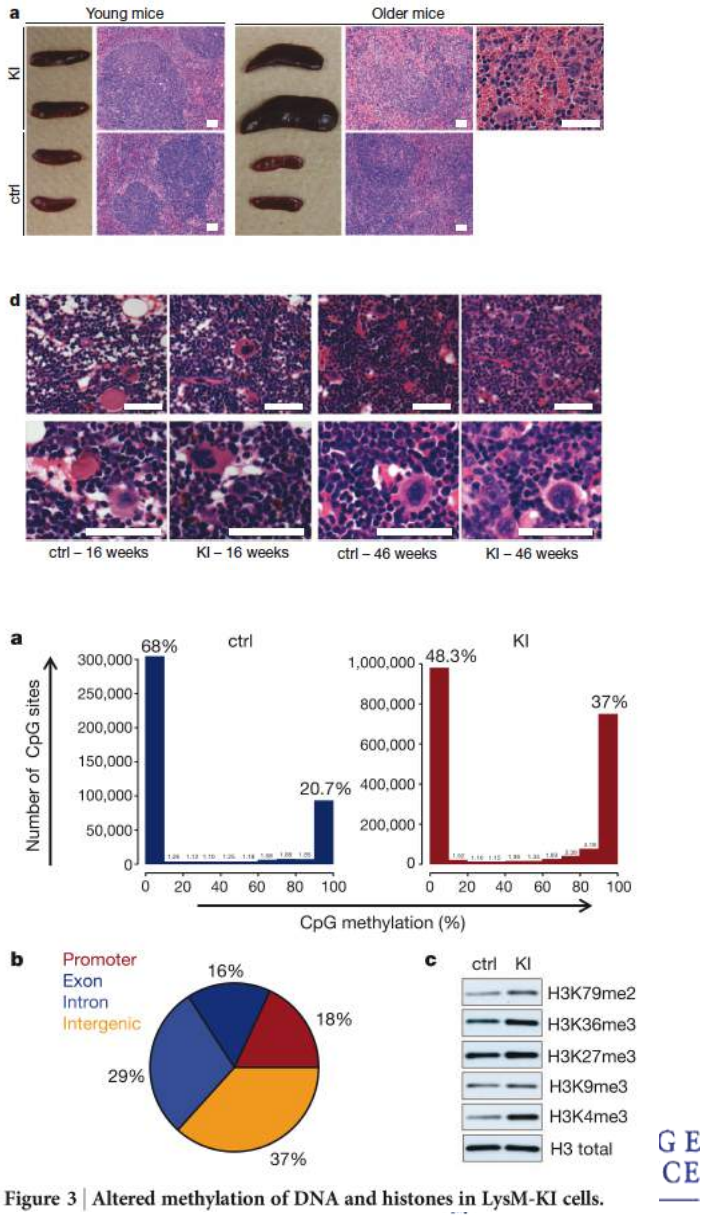
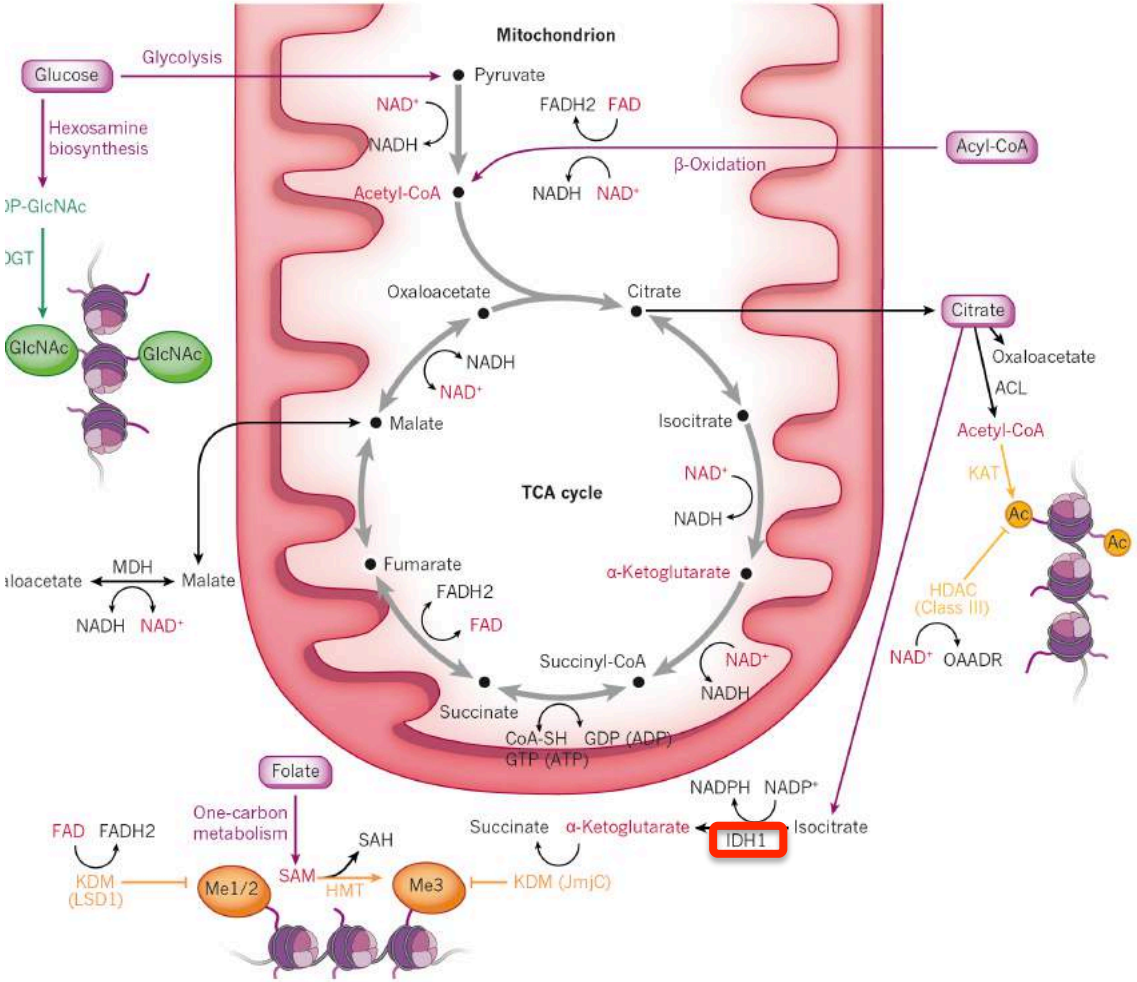


Figure 3 | Altered methylation of DNA and histones in LysM-KI cells.

Chromatin regulation and metabolism



From Gut and Verdin, Nature 2013

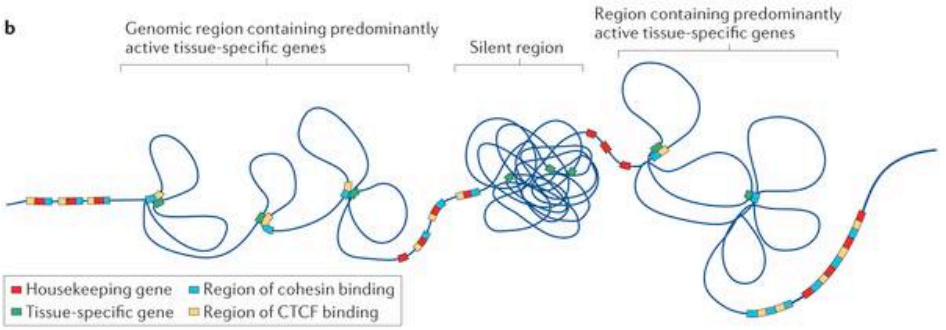
IDH1/2 Dysfunction in Cancer

LETTER

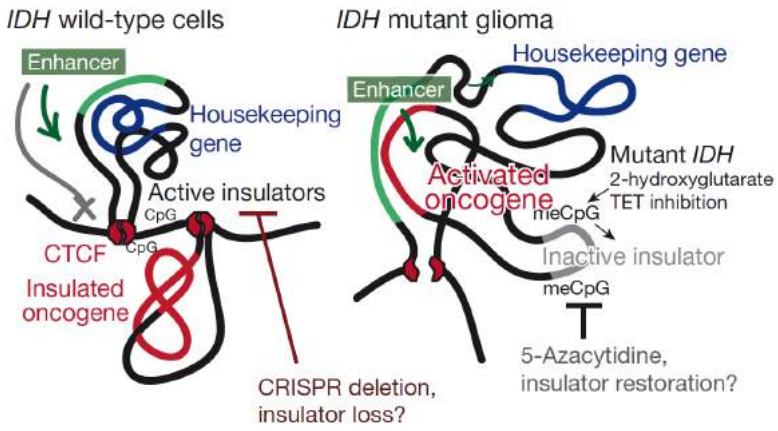
doi:10.1038/nature16490

Insulator dysfunction and oncogene activation in *IDH* mutant gliomas

William A. Flavahan[1,2,3*], Yotam Drier[1,2,3*], Brian B. Liau[1,2,3], Shawn M. Gillespie[1,2,3], Andrew S. Venteicher[1,2,4], Anat O. Stemmer-Rachamimov[1], Mario L. Suvà[1,2] & Bradley E. Bernstein[1,2,3]



- *IDH* mutant gliomas manifest a CpG island methylator phenotyp
- Human *IDH* mutant gliomas exhibit hypermethylation at cohesin and CCCTCbinding factor (CTCF)-binding sites, compromising binding of this methylation-sensitive insulator protein.
- Reduced CTCF binding => loss of insulation between topological domains and aberrant gene activation.
- Loss of CTCF at a domain boundary permits a constitutive enhancer to interact aberrantly with the receptor tyrosine kinase gene *PDGFRA*, a prominent glioma oncogene.
- CRISPR-mediated disruption of the CTCF motif in *IDH* wild-type gliomaspheres upregulates *PDGFRA* and increases proliferation.
- Treatment of *IDH* mutant glio restores insulator function and



IDH mutations promote tumor formation (gliomas) by disrupting chromosomal topology and allowing aberrant regulatory interactions that induce oncogene expression

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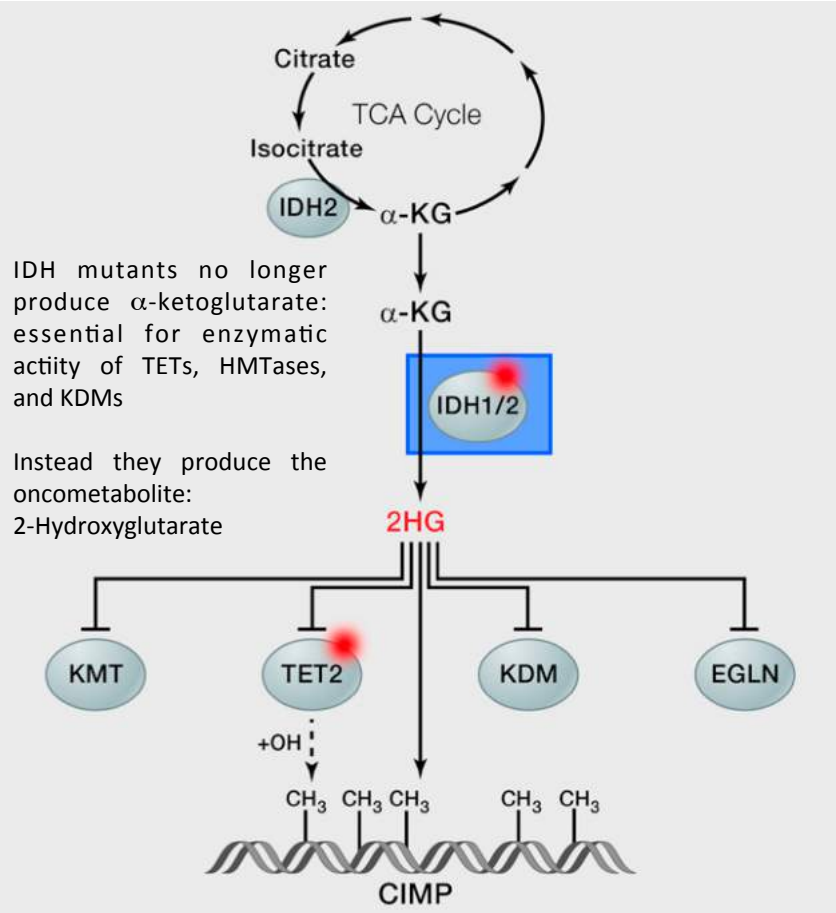
IDH1/2 mutations inhibit Tet2 (and other enzymes) and affect DNA methylation patterns



Somatic IDH1/2 Mutations Produce the Oncometabolite 2HG

Oncogenic effects of 2HG:
- generation of a CIMP-like(CpG island methylator) phenotype
- inhibition of a-ketoglutarate-dependent enzymes such as histone methyltransferases (KMT), histone demethylases (KDM), and prolyl hydroxylases (EGLN).

TET2 mutations are mutually exclusive with IDH1/2 mutations in leukemias and may exert common downstream effects on DNA methylation.

Mutant IDH1/2 proteins are the targets of emerging drug discovery effort

Balance of *de novo* DNA methyltransferase and DNA demethylase seems to be critical
Absence of either one leads to widespread changes in the epigenome, its overall organisation and at gene regulatory elements and repeats…

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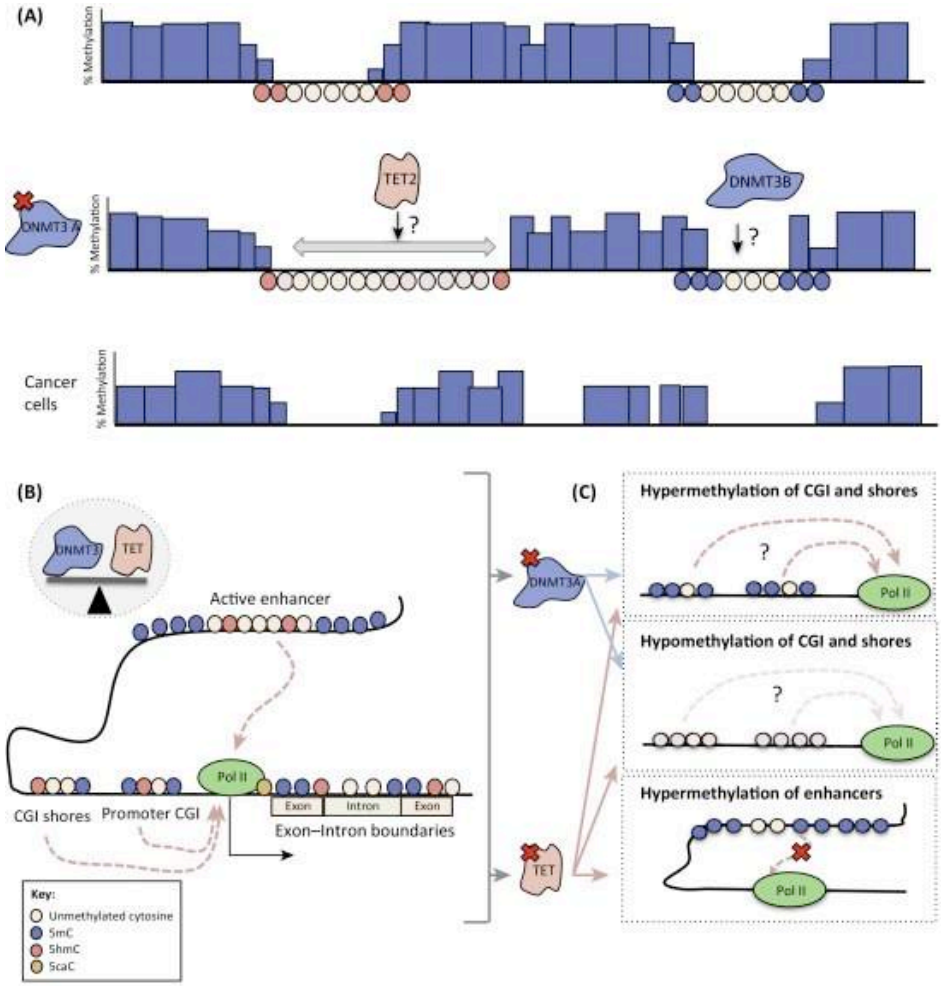
IDH1/2 mutations inhibit Tet2 (and other enzymes) and affect DNA methylation patterns



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How does the DNA methylation machinery impact on Cancer?

• Mutations in DNA Methylation enzymes (DNMT3A, TET1/2 and IDH1/2) are frequent in some cancers (leukemia and lymphoma)

• Dynamic DNA methylation patterns in coding and non-coding regions are found during hematopoietic transformation (tumor formation)

• Similar phenotypes are found in Dnmt3a KO and Tet2/3 KO mice (ie increased HSC self renewal, myeloid skewing and transformation)

• Yet loss of Dnmt3a should lead to *decreased* 5mC, while loss of Tet enzymes should lead to *increased* 5mC?

⇒ Effects in all cases may be due to **decreased 5hmC products**

• **Roles? Gene regulation (enhancers, insulators) and DNA repair…**

Whatever its functions, aberrant DNA methylation can define leukemia and lymphoma subtypes

⇒ Powerful prognostic value and key therapeutic target

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"Epigénétique et Cancer"

4 avril, 2016

Cours V

"**Voies épigénétiques du cancer II**"

"*Epigenetic pathways in cancer II*"