Année 2015-2016 :
“Epigénétique et Cancer”

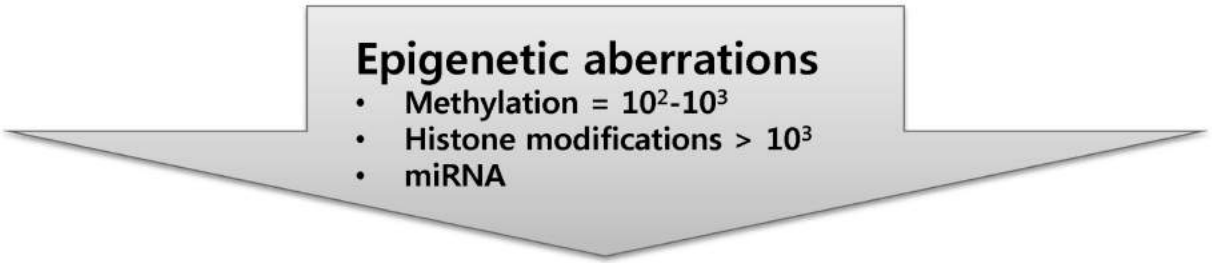
14 mars, 2016

Cours III

"Contrôle épigénétique des gènes et des génomes dans le cancer »

“Epigenetic control of genes and genomes in cancer”

Epigenetic control of Genes and Genomes in Cancer



- **Allow gene expression patterns to be reprogrammed**

Leading to changes in cell identity,
cell behavior (invasion, migration), generating cell diversity

- **Induce mutations**

Impact on DNA repair
Repeat mobility

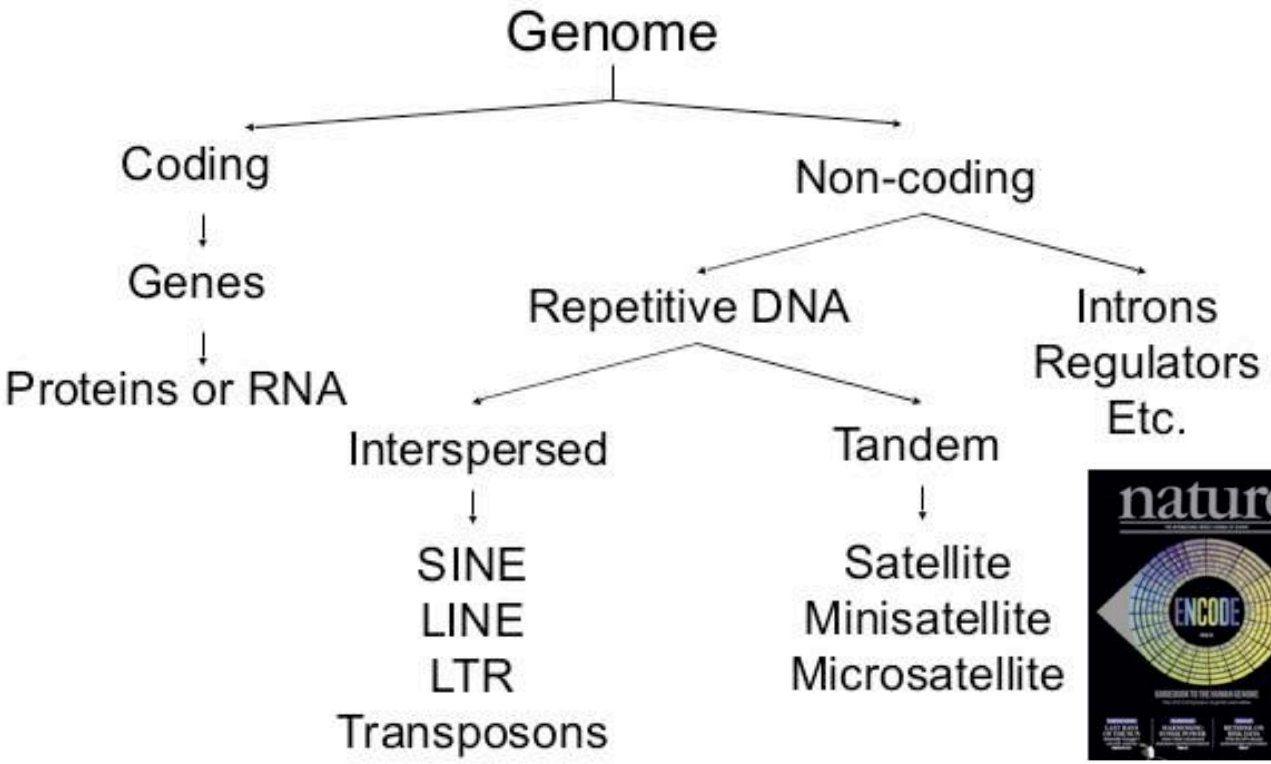
- **Activate oncogenes, silence tumor suppressors**

Epimutations – somatic and consitutional

How do Epigenetic Changes impact on Gene Expression and Genome Integrity in Cancer?

Influence of Epigenetic Changes on Gene Expression and Genome stability in Cancer?

The genome sequence can be classified into different groups based on the overall sequence composition and structure.



The ENCycleopedia Of DNA Elements

1. Transcripts
2. Regulatory elements
3. Enhancers
4. Silencers
5. Origins of replication
6. CpG islands
7. Histone modification sites
8. Open chromatin sites

Can epigenetic changes in genes, regulatory regions, repeats actually participate in cancer?

E. Heard, 2016

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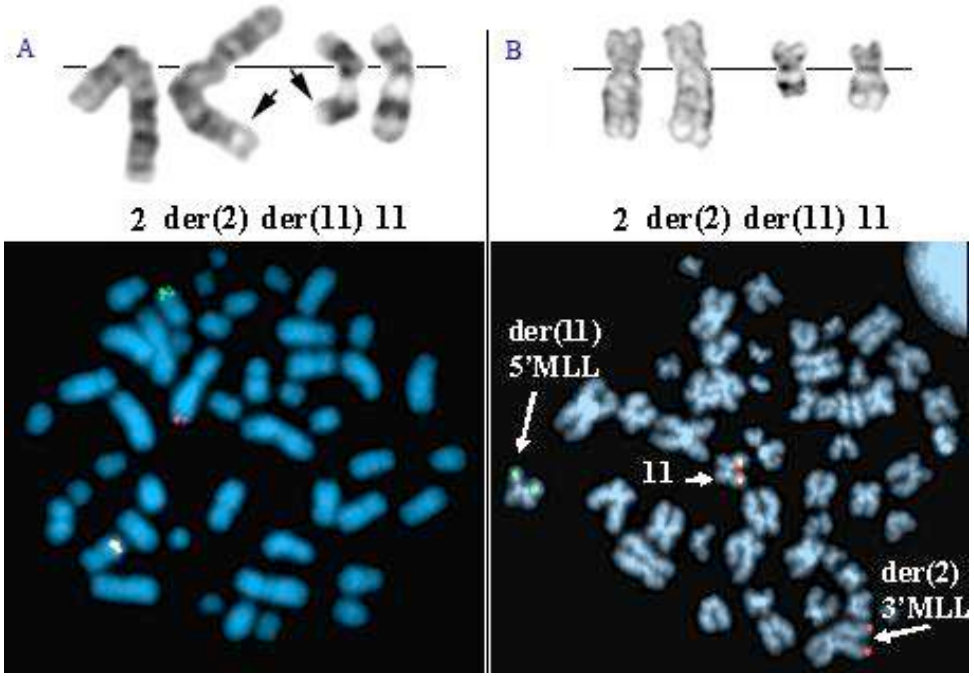
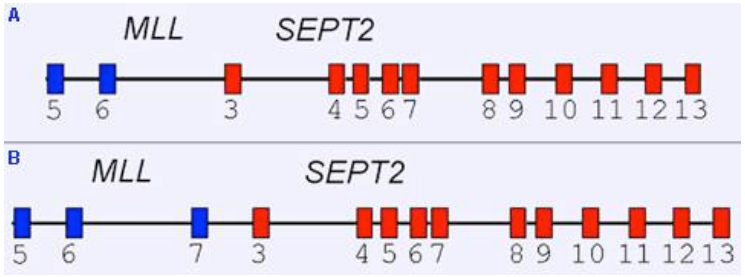
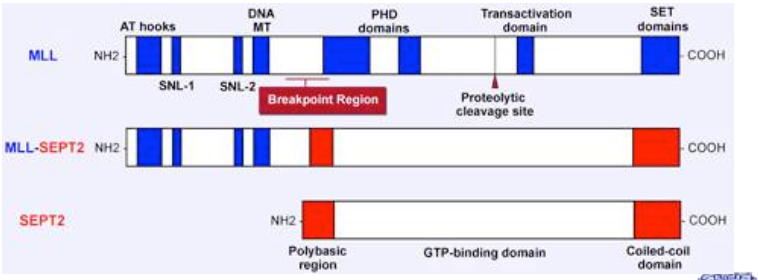
Aberrant Gene Expression by Mistargeting of Epigenetic Modifiers in Cancer

Chromosome translocations can produce oncogenic fusion proteins involving chromatin modifers such as MLL (Trithorax group protein)







http://atlasgeneticsoncology.org/Anomalies/t0211q37q23ANLLID1457.html

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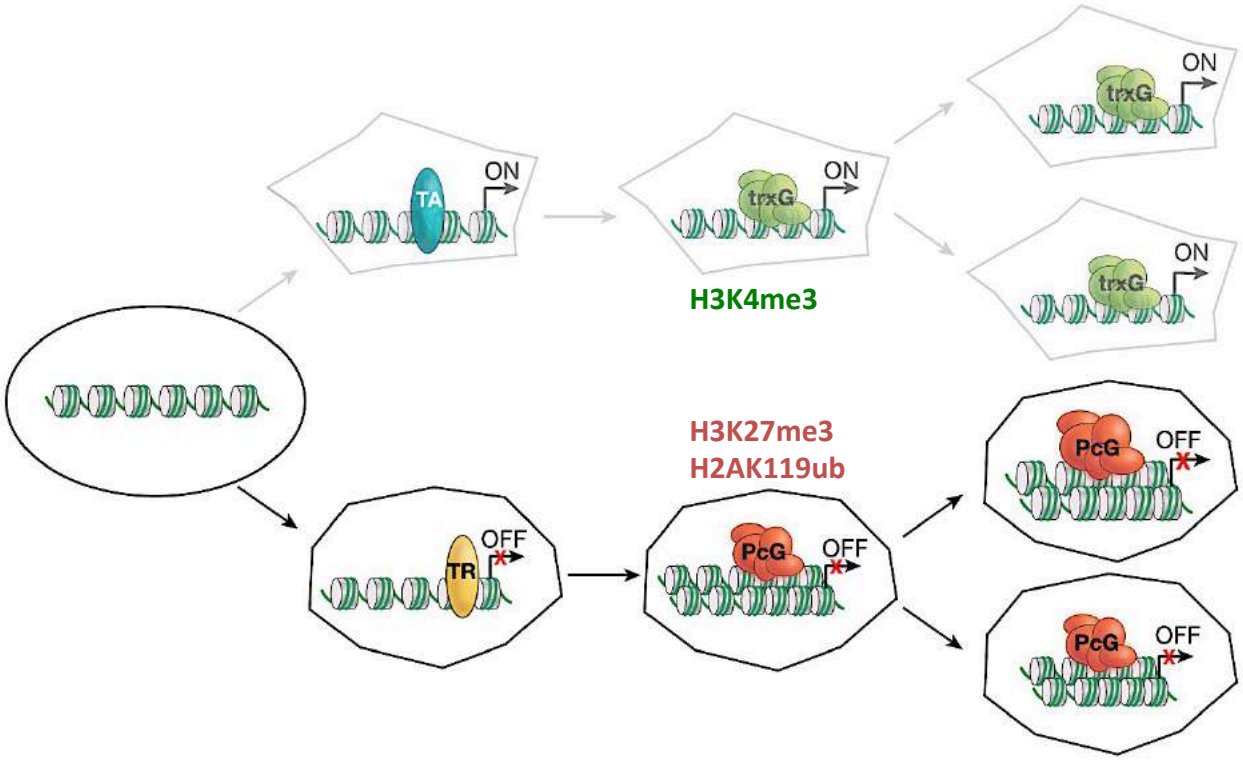
Aberrant Gene Expression by Mistargeting of Epigenetic Modifiers in Cancer

Active and inactive states of genes expression established by transcription factors are **maintained** during cellular differentiation by Polycomb (PcG) and trithorax (trxG) over multiple cell divisions



Maintenance of cell identity
Facultative heterochromatin
Stem cell plasticity

Disruption in development => **homeotic transformations, germ line defect**
Disruption in adults => **stem cell defects, cancer…**

In Drosophila studies show that several PcG and trxG components are required *throughout* development to maintain target gene activity.

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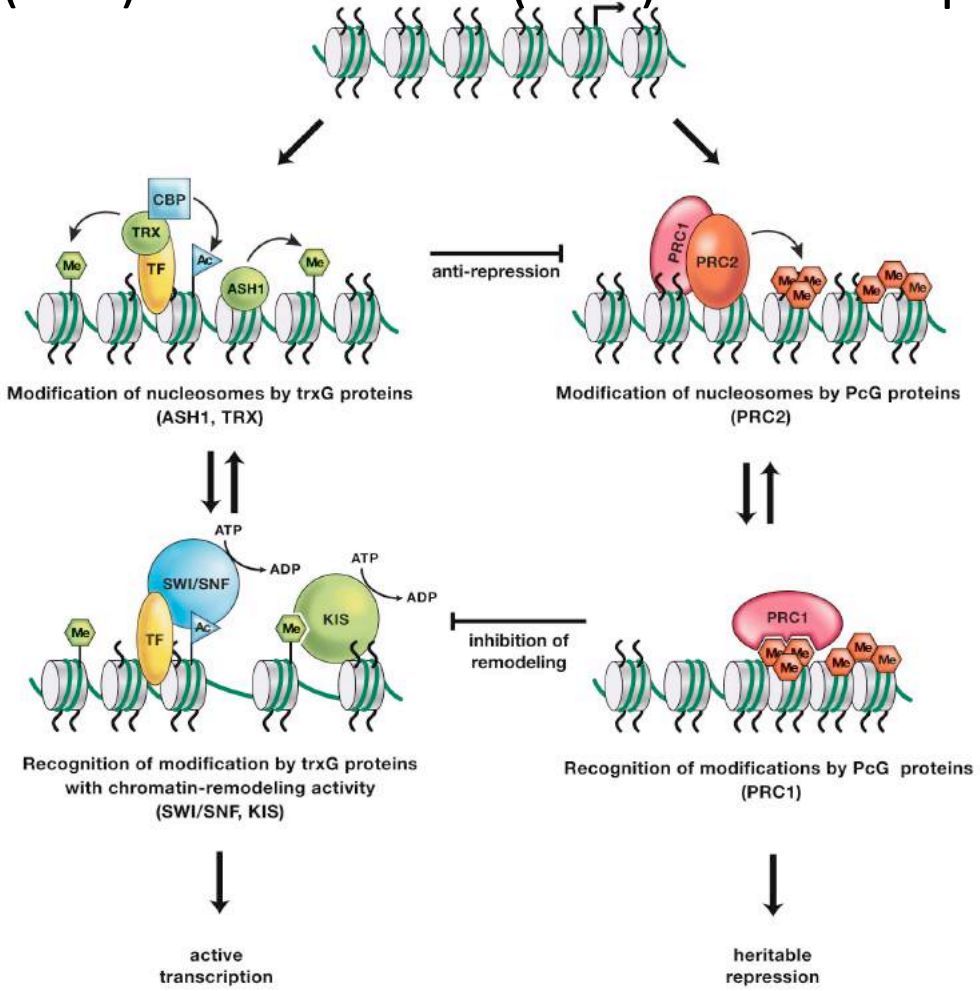

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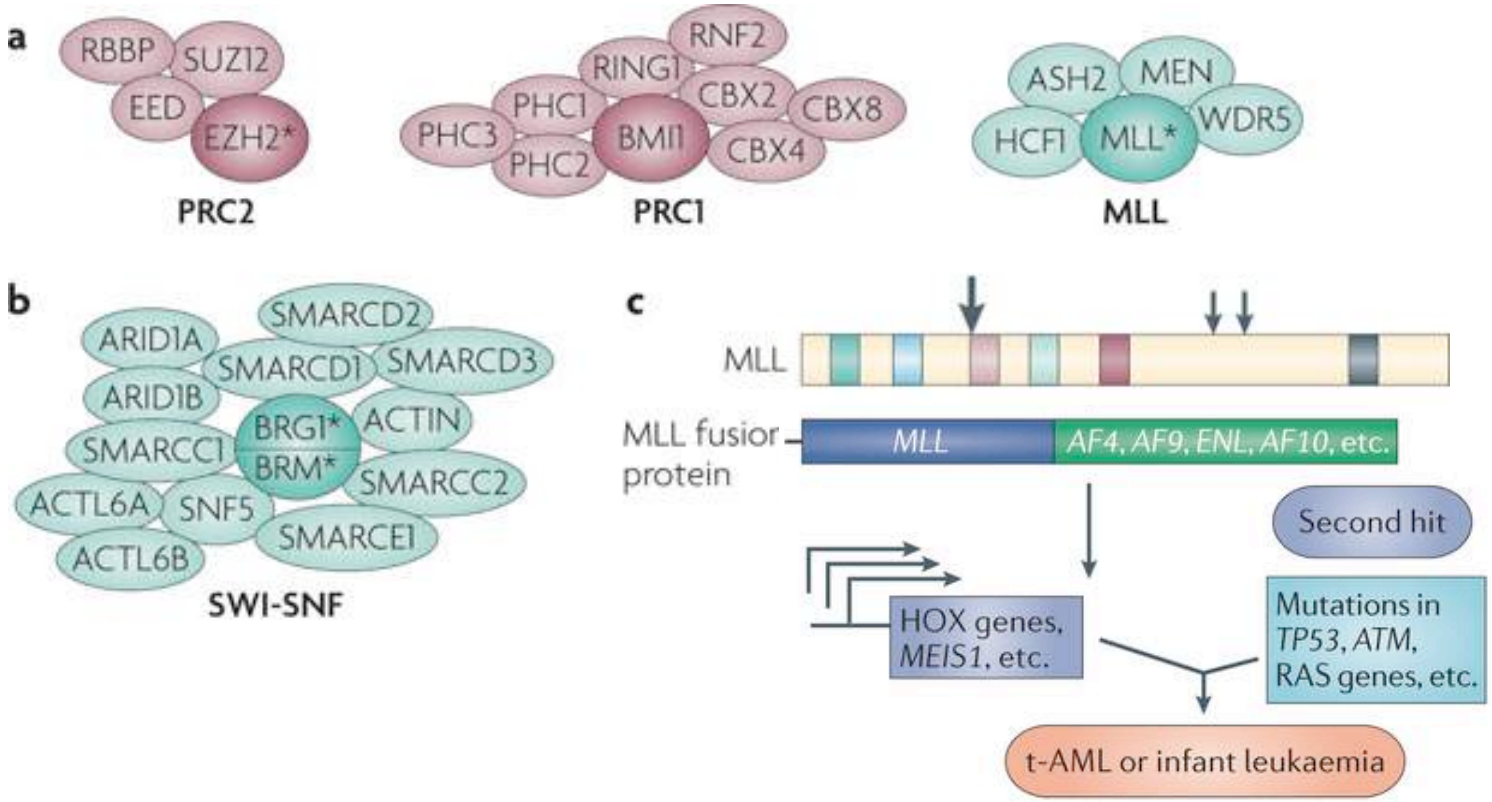
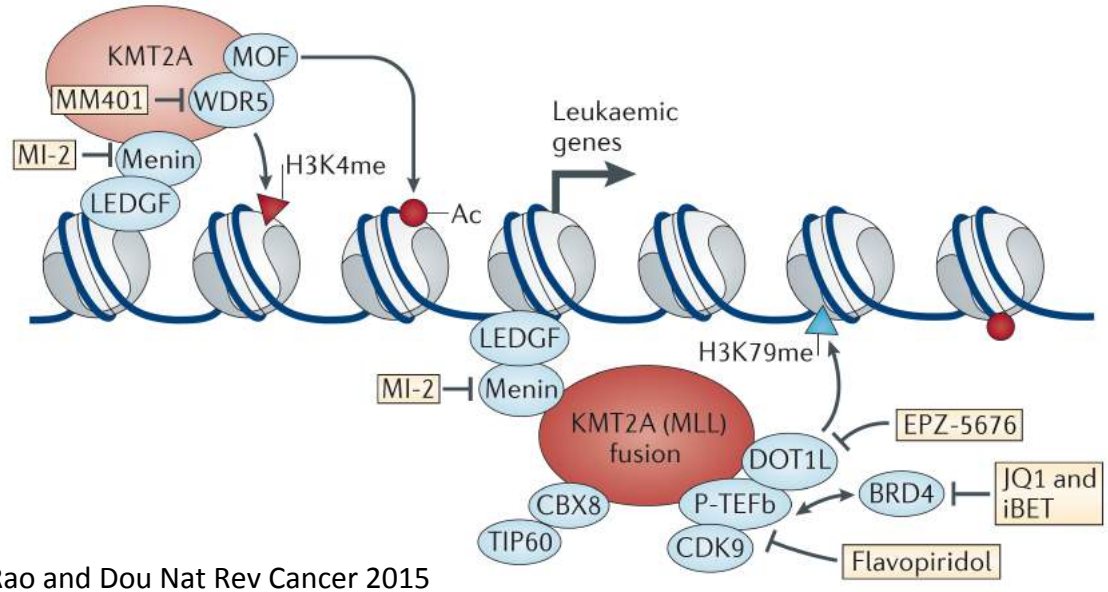


Aberrant Gene Expression by Mistargeting of Epigenetic Modifiers in Cancer

The mixed lineage leukemia (MLL) gene:

- KMT2A is frequently rearranged in acute myeloid & lymphoblastic leukemias in adults and childrend (poor prognosis)
- Many types of leukemogenic MLL rearrangements (chromosomal translocations, partial tandem duplications of internal coding regions).
- MLL encodes a Trithorax complex histone methyltransferase (HMT) implicated in epigenetic regulation of transcription
- Critical for normal embryonic development and hematopoiesis (see COURS 2015)
- Amongst **target genes** of MLL transcriptional regulation are **HOX genes**, which themselves are implicated in the malignant transformation of hematopoietic progenitors.
- Chromosomal translocations fuse the amino-terminal part of MLL in-frame to one of more than 50 partner proteins
- KMT2 genes are frequently mutated, in a broad range of cancers in addition to haematological malignancies



Rao and Dou Nat Rev Cancer 2015

- Normal KMT2A function via its histone H3 lysine 4 (H3K4) methyltransferase activity in transcription initiation
- KMT2A fusion proteins function to recruit transcription cofactors such as menin, DOT1L, positive transcription elongation factor-b (P-TEFb), LEDGF (lens epithelium-derived growth factor and chromobox homologue 8 (CBX8)–TIP60 (Tat-interacting protein 60) to promote transcription elongation.
- Small molecules targeting each of these steps (shown in boxes) have shown efficacies in MLL cell lines and represent potential therapeutic strategies

E. Heard, 2016

Aberrant Gene Expression by Mistargeting of Epigenetic Modifiers in Cancer

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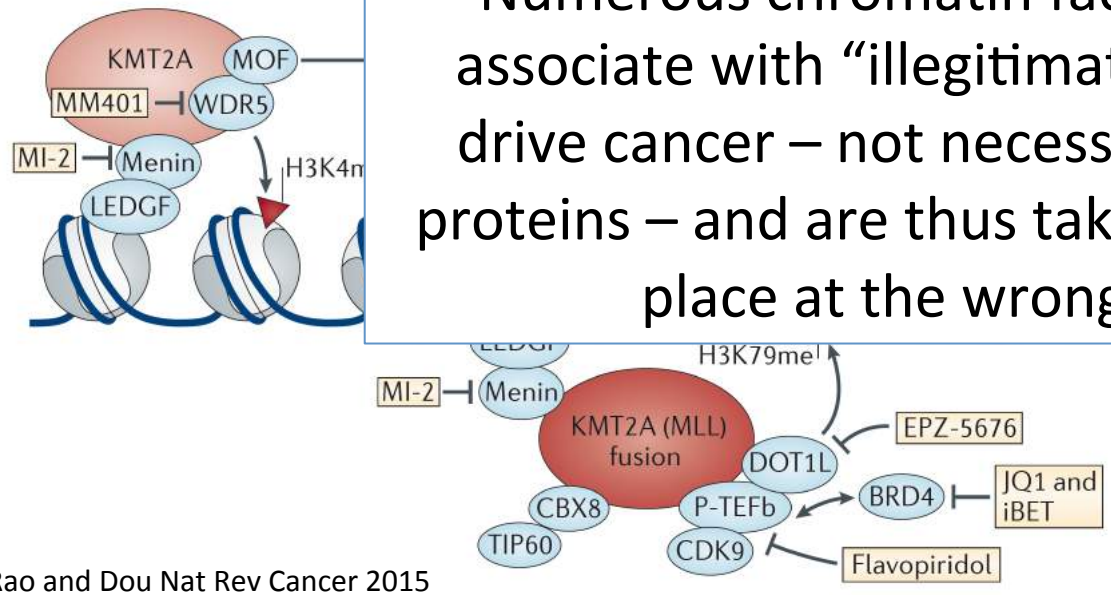
Numerous chromatin factors found to associate with "illegitimate" partners to drive cancer – not necessarily via fusion proteins – and are thus taken to the wrong place at the wrong time…



its histone H3
erase activity in

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as menin, DOT1L,
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ium-derived
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g protein 60) to
ion.

• Small molecules targeting each of these steps (shown in boxes) have shown efficacies in MLL cell lines and represent potential therapeutic strategies

Rao and Dou Nat Rev Cancer 2015

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Influence of Epigenetic Changes on Gene Expression and Genome stability in Cancer?

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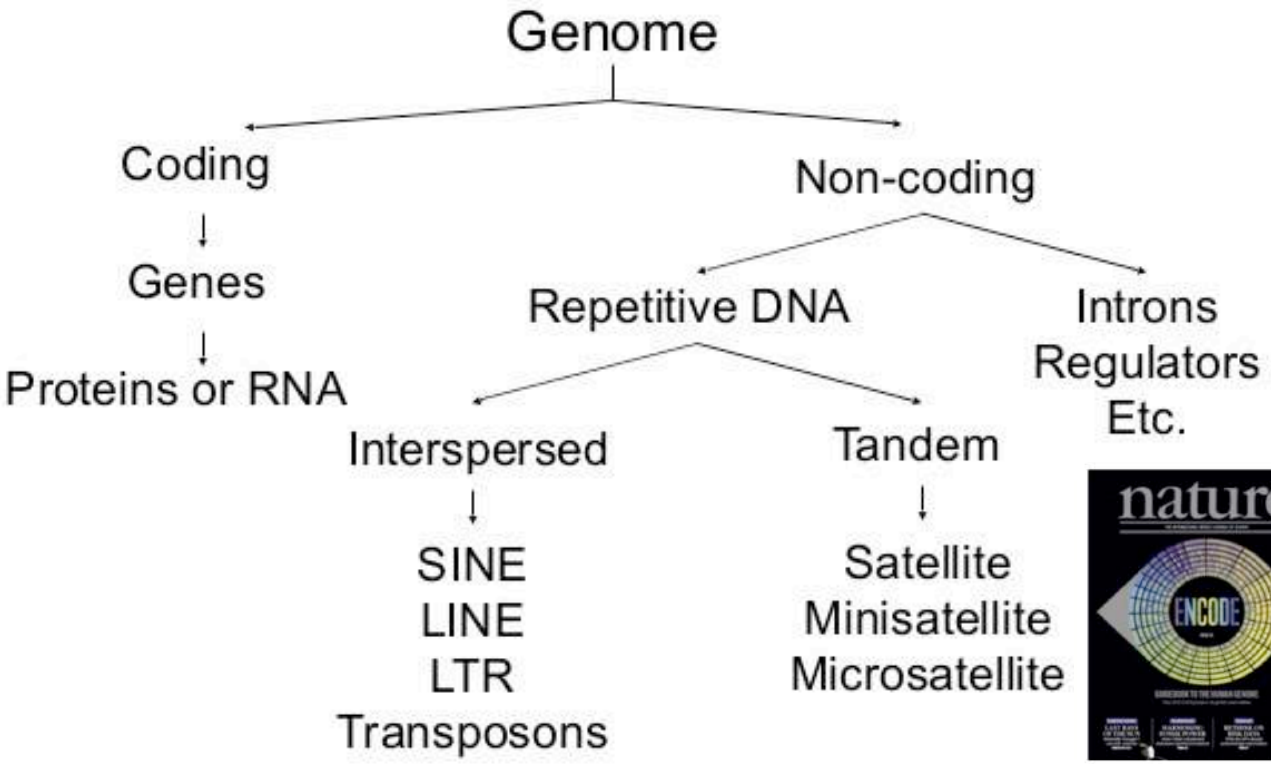

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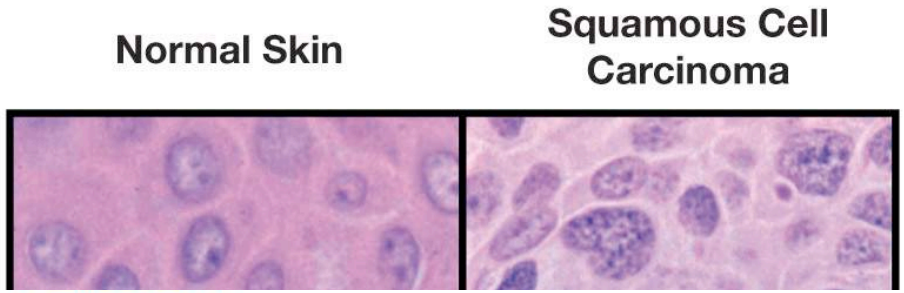
Can epigenetic changes or states participate in mutation generation in cancer?

Chromatin organisation influences mutation rates in cancer

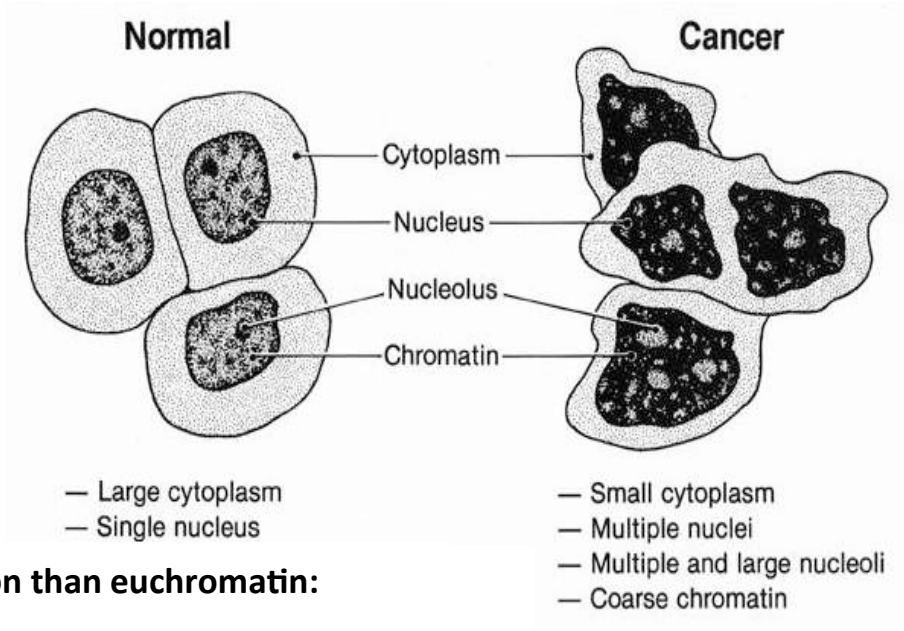


LETTER

doi:10.1038/nature11273

Chromatin organization is a major influence on regional mutation rates in human cancer cells

Benjamin Schuster-Böckler[1,2] & Ben Lehner[1,3]



Heterochromatin is more prone to mutation than euchromatin:

- Repeat element environment?
- Replication timing?
- Accessibility to DNA Repair machinery?
- Exposure to different environments in nucleoplasm?

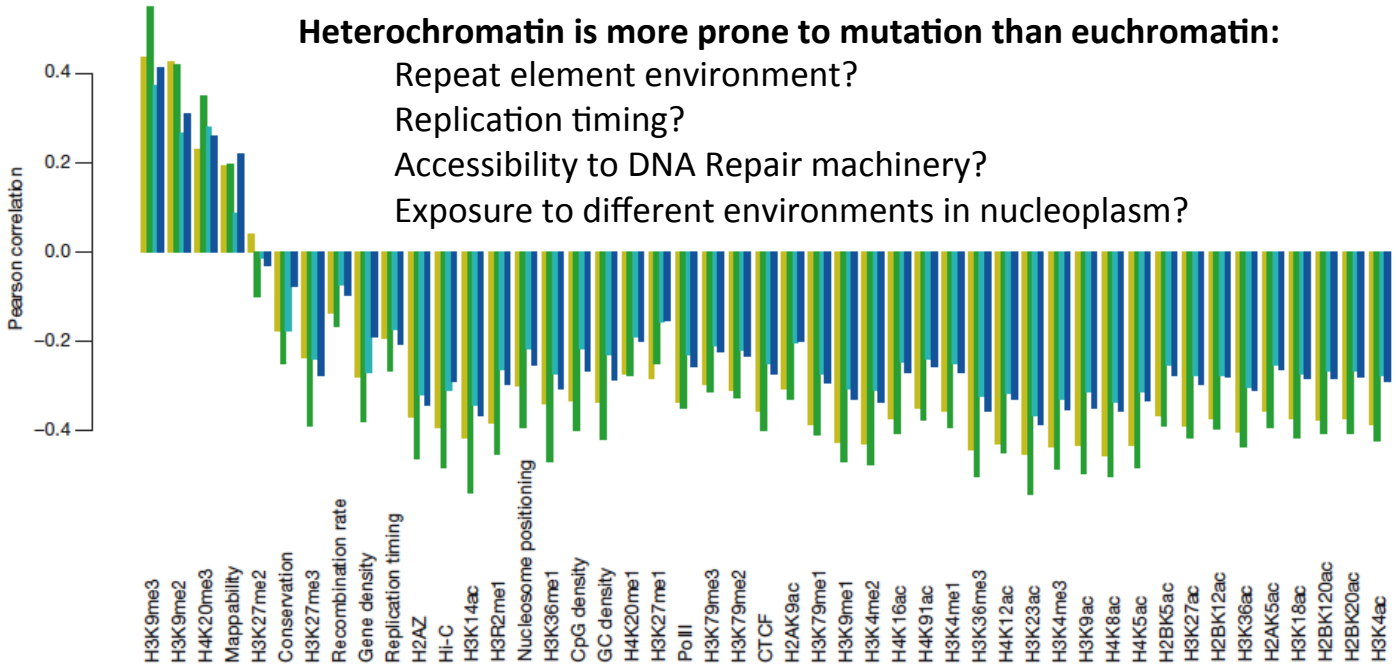


Figure 2 | Correlation coefficients of SNV density from individual cancer genomes at 1-Mb resolution with diverse genetic and epigenetic features.

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Chromatin organisation influences both mutationand epimutation rates in cancer

Hypermutation of the Inactive X Chromosome Is a Frequent Event in Cancer

Natalie Jäger,[1] Matthias Schlesner,[1] David T.W. Jones,[2] Simon Raffel,[3,4] Jan-Philipp Mallm,[5] Kristin M. Junge,[6] Dieter Weichenhan,[7] Tobias Bauer,[1] Naveed Ishaque,[1,21] Marcel Kool,[2] Paul A. Northcott,[2] Andrey Korshunov,[8,9] Ruben M. Drews,[1] Jan Koster,[10] Rogier Versteeg,[10] Julia Richter,[11] Michael Hummel,[12] Stephen C. Mack,[13] Michael D. Taylor,[13] Hendrik Witt,[2,14] Benedict Swartman,[15] Dietrich Schulte-Bockholt,[15] Marc Sultan,[16] Marie-Laure Yaspo,[16] Hans Lehrach,[16] Barbara Hutter,[1] Benedikt Brors,[1] Stephan Wolf,[17] Christoph Plass,[7] Reiner Siebert,[11] Andreas Trumpp,[3,4,18] Karsten Rippe,[5] Irina Lehmann,[6] Peter Lichter,[18,19,21] Stefan M. Pfister,[2,14,18] and Roland Eils[1,20,21,*]

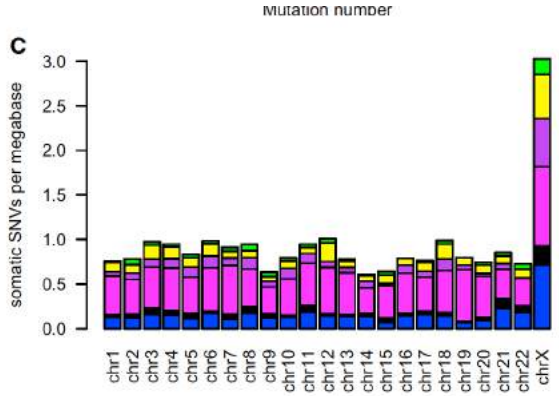
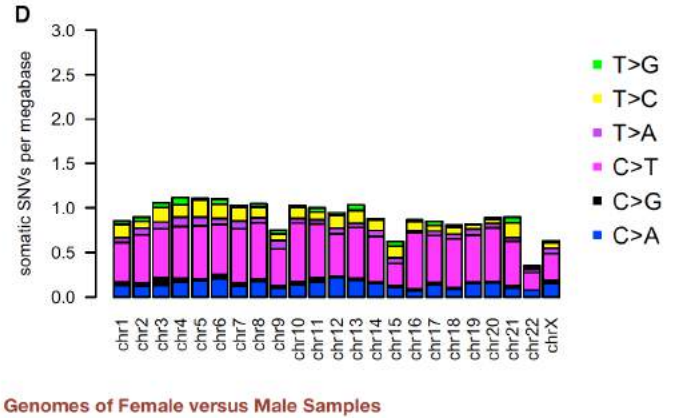


Figure 1. Distribution of Somatic Mutations in Medulloblastoma



Genomes of Female versus Male Samples

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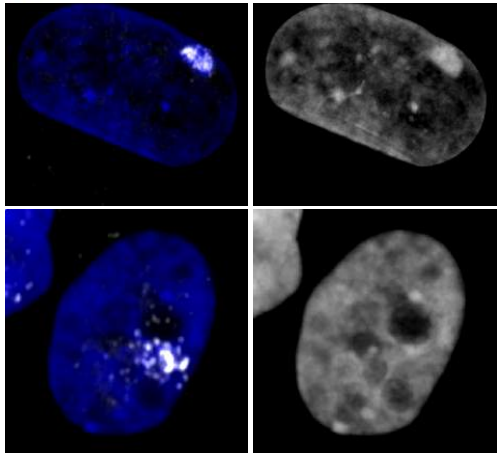
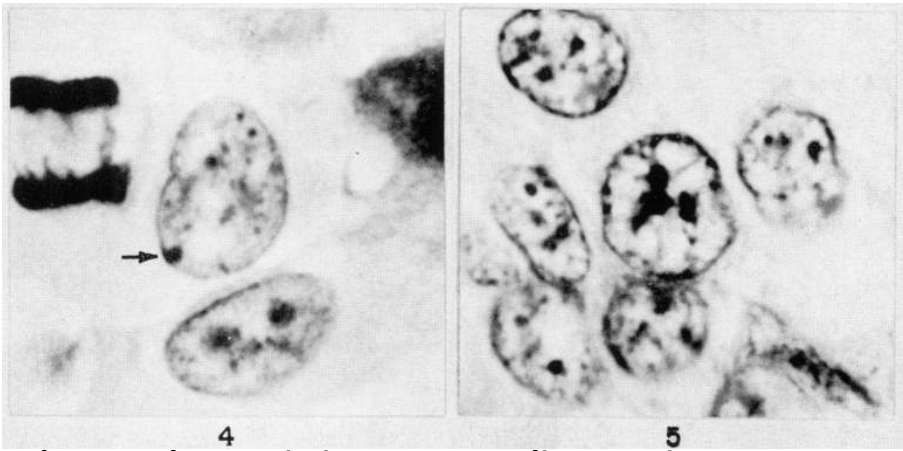
The inactive X chromosome is epigenetically unstable and transcriptionally labile in breast cancer

Ronan Chaligné,[1,2,3,4] Tatiana Popova,[1,5] Marco-Antonio Mendoza-Parra,[6] Mohamed-Ashick M. Saleem,[6] David Gentien,[1,7] Kristen Ban,[1,2,3,4] Tristan Piolot,[1,8] Olivier Leroy,[1,8] Odette Mariani,[7] Hinrich Gronemeyer,[6] Anne Vincent-Salomon,[1,4,5,7] Marc-Henri Stern,[1,5,7] and Edith Heard[1,2,3,4]

Normal cells

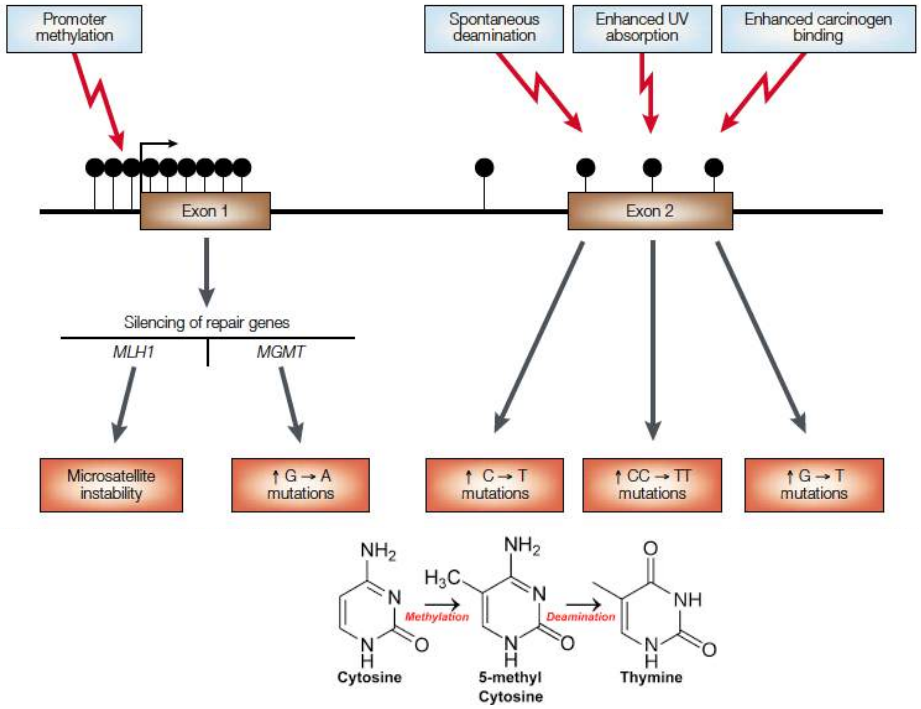
Basal-like breast cancer cells





The Sex Chromatin in Human Malignant Tissues
K. L. Moore and M. L. Barr, **1957**

Impact of DNA Methylation on Genes and Genomes



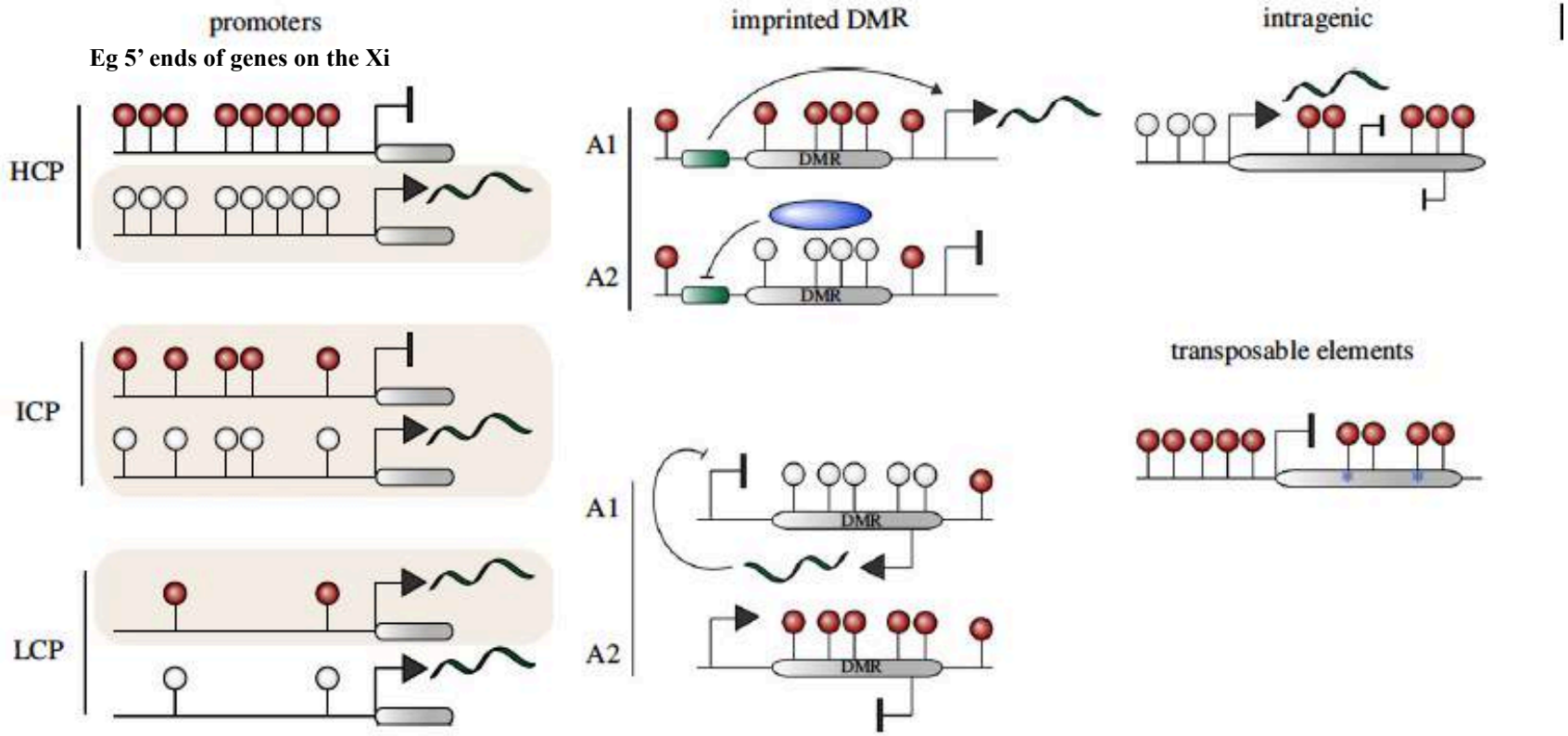
- DNA methylation can lead to DNA sequence mutations
- It can also influence gene and repeat element expression and this in turn can lead to mutation and genetic instability…

Epigenetic changes can influence the rates at which mutations appear and the rates at which they are repaired

Where is DNA Methylation in the genome?

What does it do there?

- Prevent binding of factors (CTCF, transcription machinery, some repressive complexes…?)
- Facilitate recruitment of factors (MBD proteins, co-repressor complexes…?)
- Accompany transcription (gene bodies)



Reviewed by Hackett J. and Surani, A. "DNA Methylation dynamics during the mammalian life cycle" *Phil.Trans. Of the Royal Soc*. (2013)

Altered DNA Methylation patterns in Cancer

WGBS
Sonication of DNA
Library preparation
Gel-size selection
Bisulfite treatment
Library amplification

Input requirement
Genomic DNA: 1–5 µg
FFPE DNA: 1–5 µg
>500 million reads per sample
Coverage ~95%

MBDCap-Seq
Sonication of DNA
capture 5mC by MBD
Library preparation
Gel-size selection
Library amplification

Input requirement
Genomic DNA: 0.2–1 µg
FFPE DNA: 0.5–1 µg
30 million reads per sample
Coverage 17.8%

RRBS
Digestion with *MspI*
Library preparation
Gel-size selection
Bisulfite treatment
Library generation

Input requirement
Genomic DNA: 0.01–0.03 µg
FFPE DNA: 1 µg
10 million reads per sample
Coverage 3.7%

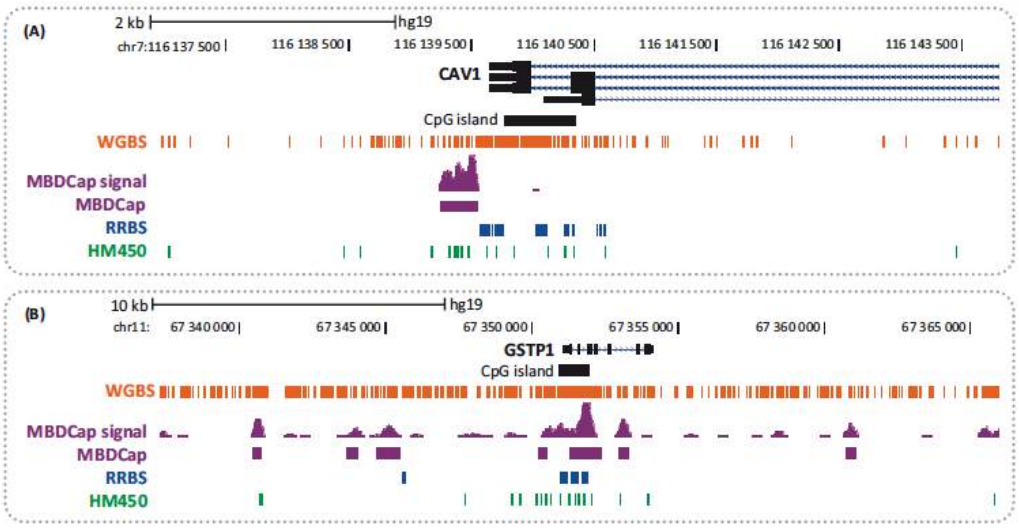
High-throughput illumina sequencing

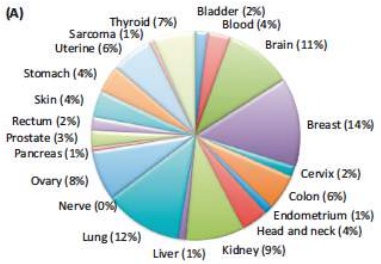
Array

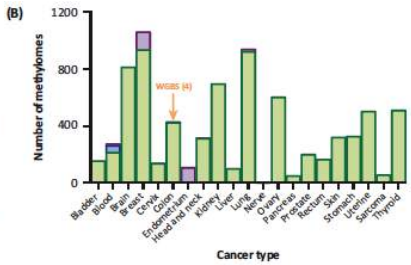
HM450
Bisulfite treatment
Hybridization
Single-base extension
Stain BeadChip
HiScan scan BeadChip

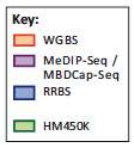
Input requirement
Genomic DNA: 0.5–1 µg
FFPE DNA: 0.5–1 µg
Array
Coverage 1.7%

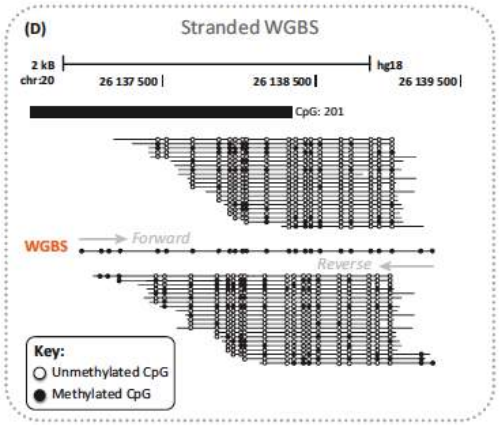
Increasing coverage











(C)

Methylation data portal	Online resource
Cancer methylome system	http://cbbiweb.uthscsa.edu/KMethylomes/
Encyclopedia of DNA elements (ENCODE)	http://genome.ucsc.edu/ENCODE/
Gene expression omnibus (GEO)	http://www.ncbi.nlm.nih.gov/geo/
The cancer genome atlas (TCGA)	http://cancergenome.nih.gov/
International cancer genome consortium (ICGC)	http://icgc.org/
Array express	http://www.ebi.ac.uk/arrayexpress/

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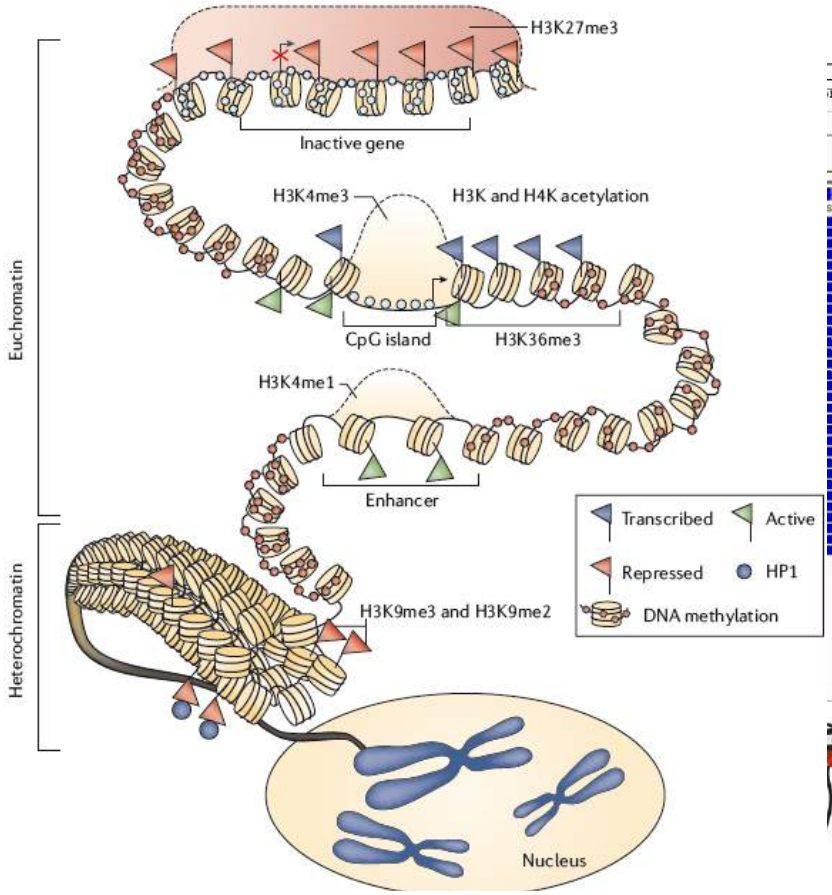
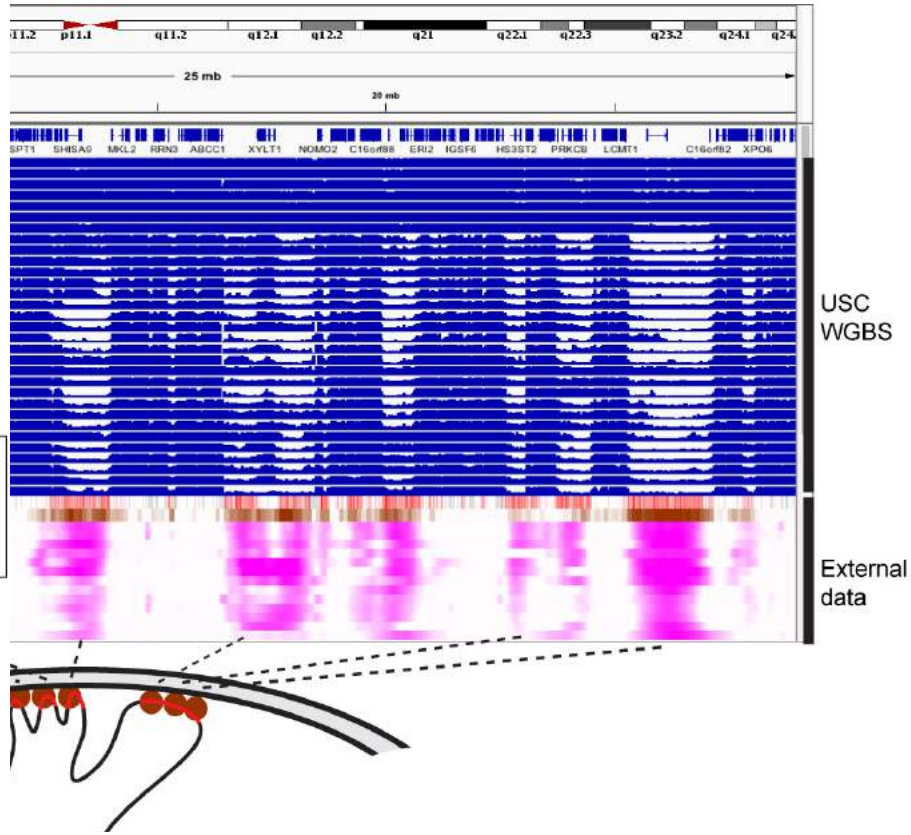
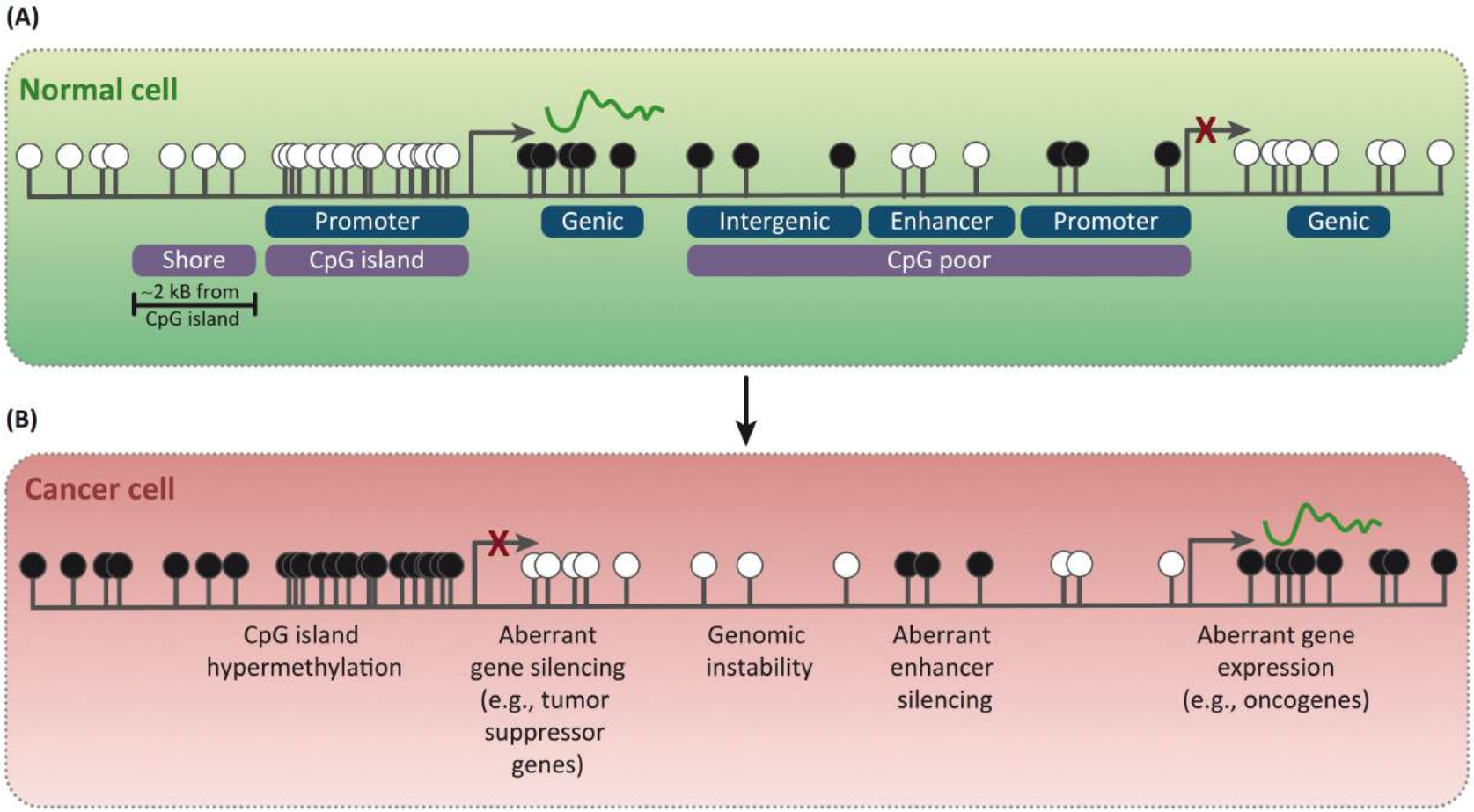
Altered DNA Methylation patterns in Cancer





- Frequent global loss of 5mC (based on HPLC measures of DNA digested to mononucleotides)
- Long partially methylated domains (PMDs) (Berman et al, 2012)
- Loss of DNAme correlates with increase of repressive chromatin (large organised chromatin K9 regions - LOCKs) (Hansen et al 2012) that coincide with LADs
- Coordinated genomic blocks of repressed (LRES) or active (LREA) (Clark, 2007; Stransky, Vallot 2006)
- Some cancers show a CpG island methylator phenotype (CIMP) (Toyota et al., 1999) – linked to IDH1 mutations (Turcan et al 2012)

Altered DNA Methylation patterns in Cancer



The majority of methylated CpG dinucleotides are in fact found within repetitive elements, comprising approximately 45% of the human genome.
DNA methylation is believed to be an essential mechanism in silencing the transcription of these elements to prevent their movement and expansion throughout the genome [Bourc'his and Bestor, 2007]

Altered DNA Methylation patterns in Cancer

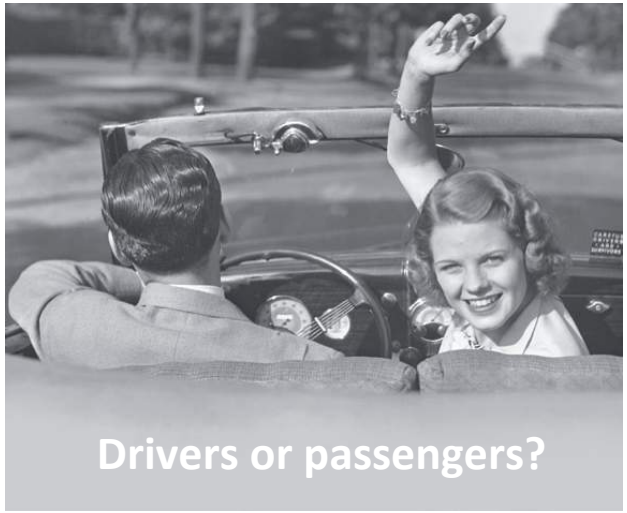
Altered methylation patterns in cancer cell genomes: Cause or consequence?

Stephen Baylin[1,3] and Timothy H. Bestor[2,3]

[1]The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21231
[2]Department of Genetics and Development, College of Physicians and Surgeons of Columbia University, 701 West 168th Street, New York, New York 10032
[3]Correspondence: sbaylin@jhmi.edu (S.B.); thb12@columbia.edu (T.B.)

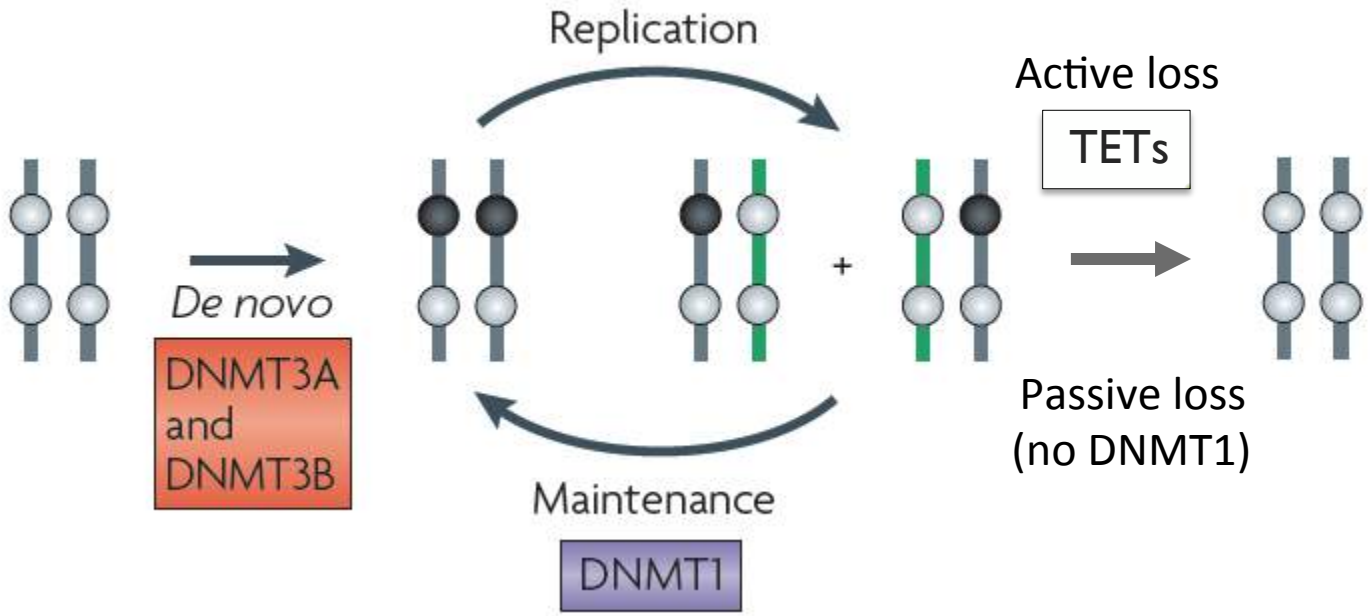
CpG islands are associated with at least half of all cellular genes and are normally methylation-free. Dense methylation of cytosine residues within islands causes strong and heritable transcriptional silencing. Such silencing normally occurs almost solely at genes subject to genomic imprinting or to X chromosome inactivation. Aberrant methylation of CpG islands associated with tumor suppressor genes has been proposed to contribute to carcinogenesis. However, questions of mechanisms underlying the cancer changes and the precise consequences for tumorigenesis exist in the field, and must continue to be addressed before the importance of abnormalities in genomic methylation patterns in carcinogenesis can be fully understood. In this article, two workers in DNA methylation, one concentrating on cancer biology and the other on developmental biology, address recurrent questions about cancer epigenetics from different perspectives. The goal is to highlight important controversies in the field which can be productive targets of ongoing and future research.

Baylin and Bestor, 2002, *Cancer Cell* 1:299-305.



Courtesy of Peter Jones

DNA Methyltransferases: Orchestrators of DNA Methylation



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

- **DNMT1 preferentially methylates hemimethylated DNA**
- **DNMT3A/3B show equal preference for hemimethylated and unmethylated DNA**
- **DNMT3L stimulates DNMT3A/3B activity in ES cells**

Inducing DNA Hypomethylation can 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]*

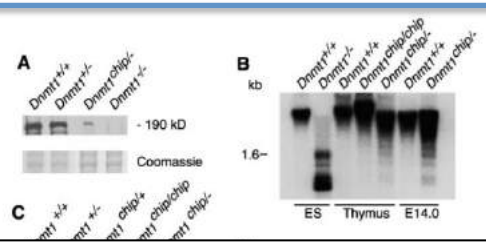
Chromosomal Instability and Tumors Promoted by DNA Hypomethylation

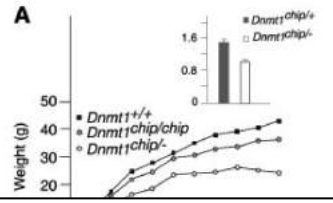
Amir Eden,[1] François Gaudet,[1,3] Alpana Waghmare,[1,2] Rudolf Jaenisch[1,2]*

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]

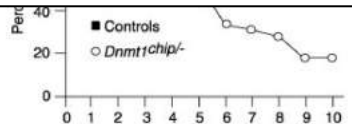




DNMT1 deficiency triggers mismatch repair defects in human cells through depletion of repair protein levels in a process involving the DNA damage response

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,*]

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



Defects in DNA methylation have been linked to genome instability in studies of colorectal tumor cell lines, mouse tumor models, and patients with immunodeficiency–centromeric instability–facial anomalies (ICF) syndrome. Dnmt1 hypomorphic mice develop aggressive T cell lymphomas:

(i) Hypomethylation may induce endogenous retroviral elements, leading in turn to insertional activation of proto-oncogenes? *No obvious sign of retroviral insertion in c-myc – but later in Notch*

(ii) Hypomethylation may activate protooncogenes through epigenetic effects? c-*myc* was overexpressed in most hypomethylated tumors - but unlikely that activation of c-*myc* is direct consequence of promoter demethylation because the gene is expressed at normal levels in thymuses from 2- and 4-week-old mice that show a level of hypomethylation identical to that of the tumors

(iii) Hypomethylation may induce genomic Instability? *Modest but significant chromosome instability – chromosome segregation problems due to DNA hypomethylation at centromeric satellite DNA*? *Modest increase in LOH (2.2 fold)*

(iv) The transcription of repetitive elements is increased following genomic hypomethylation in Dnmt1 knockout mice which may underpin the genomic instability and frequent gene rearrangements observed in these mice

F

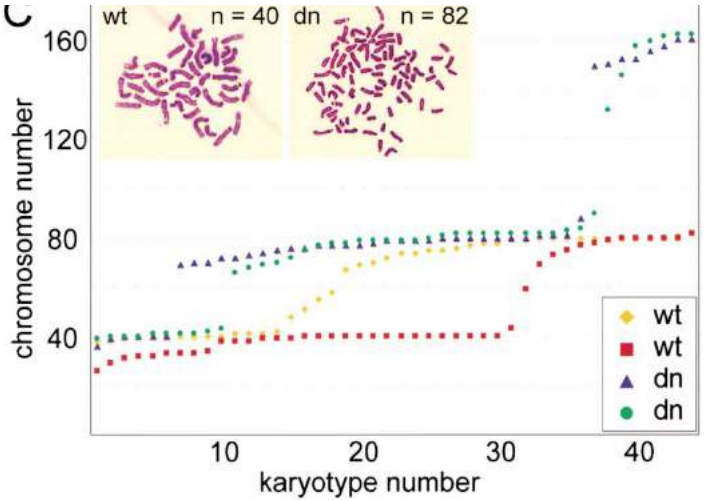
DNA and H3K9 Hypomethylation both lead to Cancer via genetic instability?

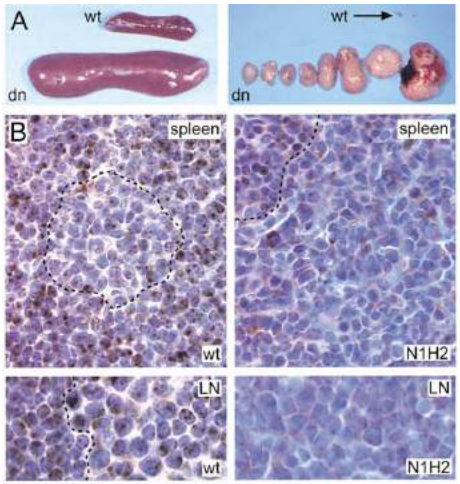
Cell, Vol. 107, 323–337, November 2, 2001, Copyright ©2001 by Cell Press

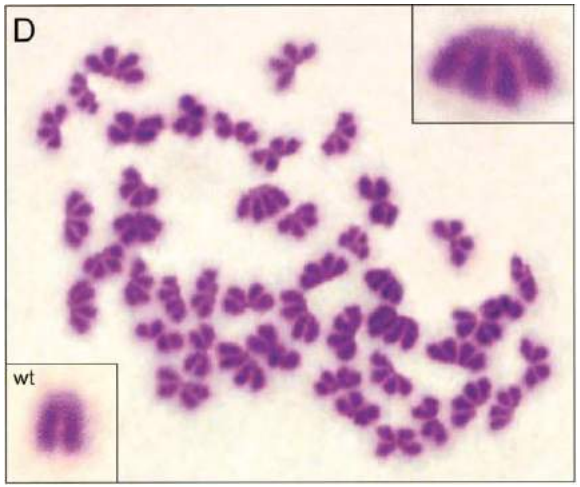
Loss of the *Suv39h* Histone Methyltransferases Impairs Mammalian Heterochromatin and Genome Stability

Antoine H.F.M. Peters,[1,3] Dónal O'Carroll,[1,3,6] Harry Scherthan,[2,6] Karl Mechtler,[1] Stephan Sauer,[1] Christian Schöfer,[3] Klara Weipoltshammer,[3] Michaela Pagani,[1] Monika Lachner,[1] Alexander Kohlmaier,[1] Susanne Opravil,[1] Michael Doyle,[1,5] Maria Sibilia,[1,4] and Thomas Jenuwein[1,4]

Histone H3 lysine 9 methylation has been proposed to provide a major "switch" for the functional organization of chromosomal subdomains. Here, we show that the murine Suv39h histone methyltransferases (HMTases) govern H3-K9 methylation at pericentric heterochromatin and induce a specialized histone methylation pattern that differs from the broad H3-K9 methylation present at other chromosomal regions. *Suv39h*-deficient mice display severely impaired viability and chromosomal instabilities that are associated with an increased tumor risk and perturbed chromosome interactions during male meiosis. These in vivo data assign a crucial role for pericentric H3-K9 methylation in protecting genome stability, and define the *Suv39h* HMTases as important epigenetic regulators for mammalian development.



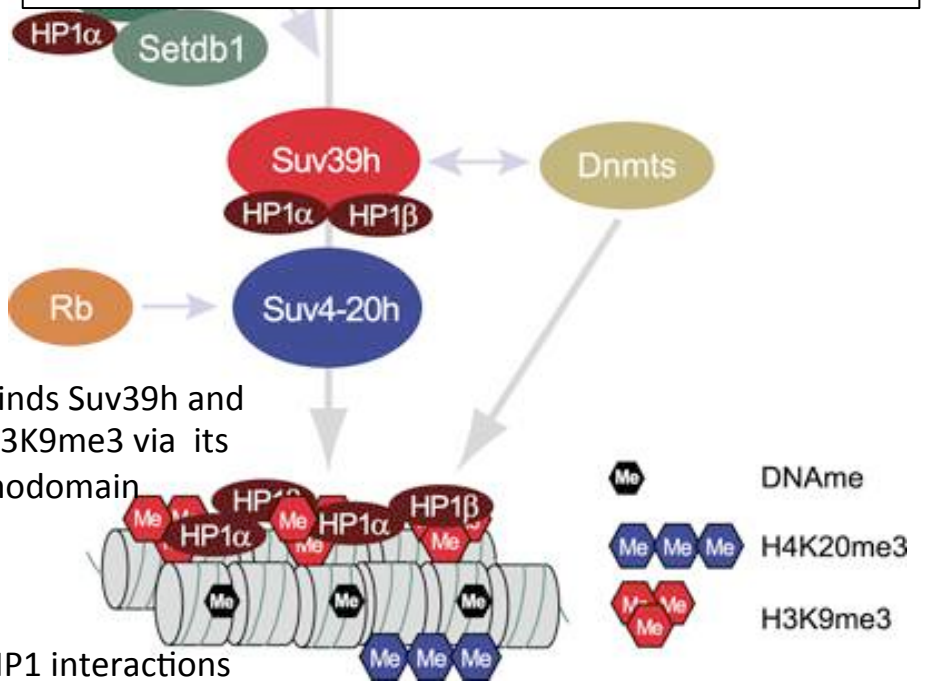




Maintenance of Pericentric Heterochromatin is required to prevent chromosome segregation defects

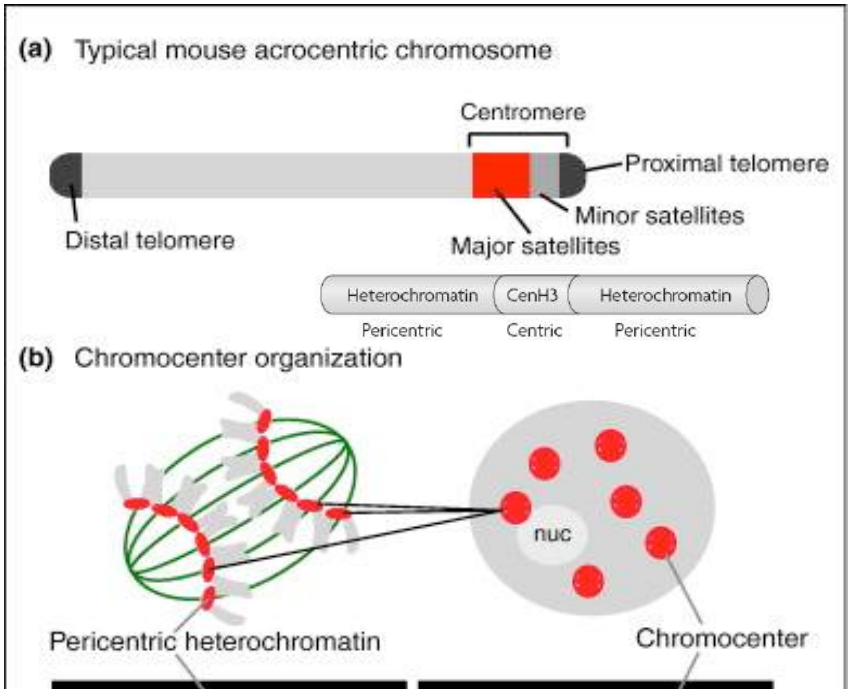
In mammalian cells, H3K9 trimethylation (H3K9me3) and DNA methylation are hallmarks of constitutive heterochromatin and are also required for transcriptional silencing of genes and retroviral elements (Magklara et al.,2011; Matsui et al., 2010; Nielsen et al., 2001).

Pericentric heterochromatin (pHC) contributes to centromere function by ensuring sister chromatid cohesion. Pericentric heterochromatin remains condensed throughout the cell cycle (in mouse, cluster together to form "chromocenters")



HP1 binds Suv39h and also H3K9me3 via its chromodomain

HP1-HP1 interactions favour compaction?



Inner core: Centric domain (CenH3 histone vrariant) = basis of kinetochore formation and and essential for chromosome segregation

Adjacent to this is pericentric heterochromatin (pHC) which contributes to centromere function by ensuring sister chromatid cohesion

Probst and Almouzni, TIGs 2011 TRENDS in Genetics

DNA Hypomethylation and Genetic instability

Defects in heterochromatin can promote genome instability and carcinogenesis:

Patients with ICF (immunodeficiency, centromeric instability, and facial anomalies) syndrome, which is caused by mutation in DNMT3B

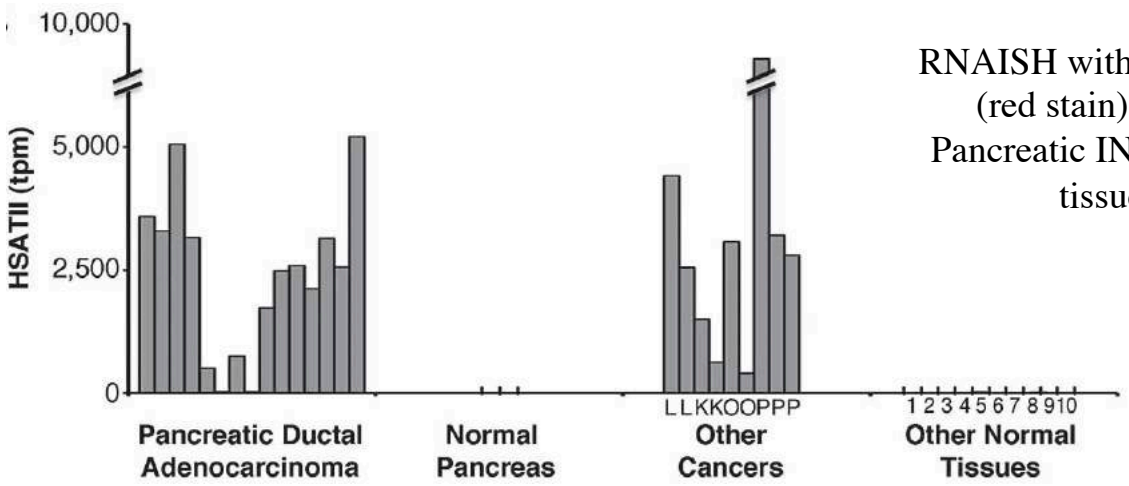
Mice lacking DNMT1 or SUV39H form tumors and display genome instability

Failure to restore constitutive heterochromatin domains after replication can lead to chromosome breakages and aberrant chromosome segregation in mitosis.

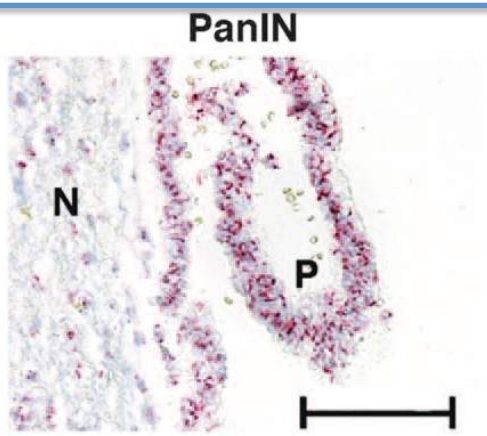
Heterochromatin instability can also lead to aberrant repeat expression…

Xu, G. L. *et al*. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* **402**, 187–191 (1999).
Peters, A. H. *et al*. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107**, 323–337 (2001).
Gaudet, F. *et al*. Induction of tumors in mice by genomic hypomethylation. *Science* **300**, 489–492 (2003).
Hansen, K. D. *et al*. Increased methylation variation in epigenetic domains across cancer types. *Nature Genet*. **43**, 768–775 (2011).
Fraga, M. F. *et al*. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nature Genet*. **37**, 391–400 (2005).
Wen, B., Wu, H., Shinkai, Y., Irizarry, R. A. & Feinberg, A. P. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nature Genet*. **41**, 246–250 (2009).

Aberrant Satellite Repeat Expression in Cancer



RNAISH with HSATII probe (red stain): in human Pancreatic IN (P) & normal tissue (N).



Satellite transcripts are greatly overexpressed in mouse and human epithelial cancers. (~40-fold increase in pancreatic cancer over that in normal tissue).

Derepression of satellite transcripts correlated with overexpression of LINE-1 retrotransposons and with aberrant expression of nearby neuroendocrine-associated genes.

Accumulation of satellite transcripts in mouse and human cell lines can arise from DNA demethylation, heat shock, or the induction of apoptosis, and their overexpression has been associated with genomic instability.

The overexpression of satellite and LINE-1 transcripts in cancer may reflect global alterations in heterochromatin silencing.

Aberrant Satellite Methylation in human Cancer

• **Whole-genome DNAm** (methylome) **analysis** (not just CpG islands) by MeDIP-seq **enables unbiased analysis of cancer methylomes**

• Malignant peripheral nerve sheath tumors (MPNSTs), benign neurofibromas, and normal Schwann cells examined

tumeur périphérique de la gaine nerveuse

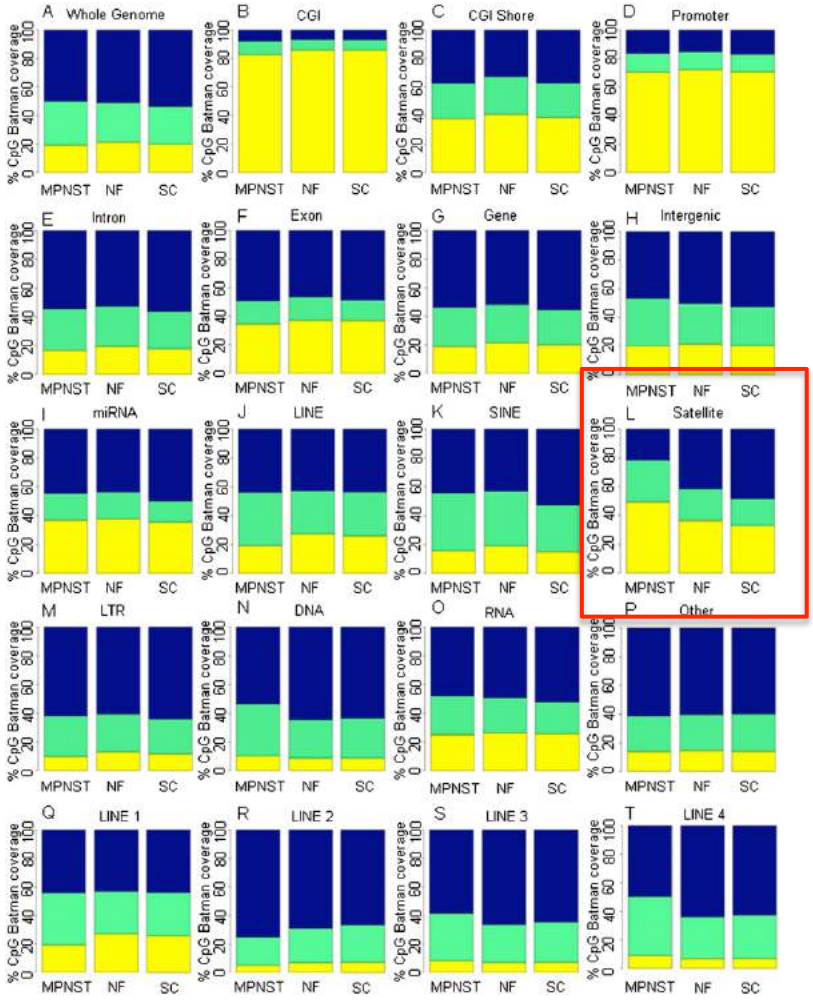
• No significant global hypomethylation was observed in MPNSTs

• However, a highly significant ($P < 10^{-100}$) directional difference in DNAm was found in satellite repeats, suggesting these repeats to be the main target for hypomethylation in MPNSTs.

• However, genomic instability does ***NOT*** appear to be a common feature in MPNSTs (Serra et al. 1997; Kobayashi et al. 2006),

⇒ Absence of DNA methylation alone is not a trigger for chromosome instability in this case

⇒ Different cell types may have different requirements for satellite DNA methylation



Feber et al, Genome Res.(2011)

Other Repeat Elements in the Genome

Transposable elements represent about 45% of the human genome.

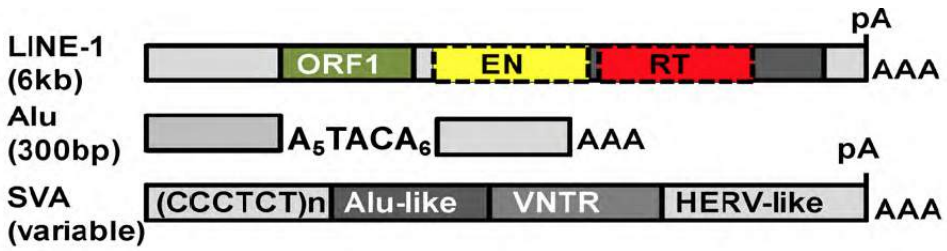
Long interspersed nuclear elements (LINEs), comprising 20% of the human genome are a type of non-LTR retrotransposon.

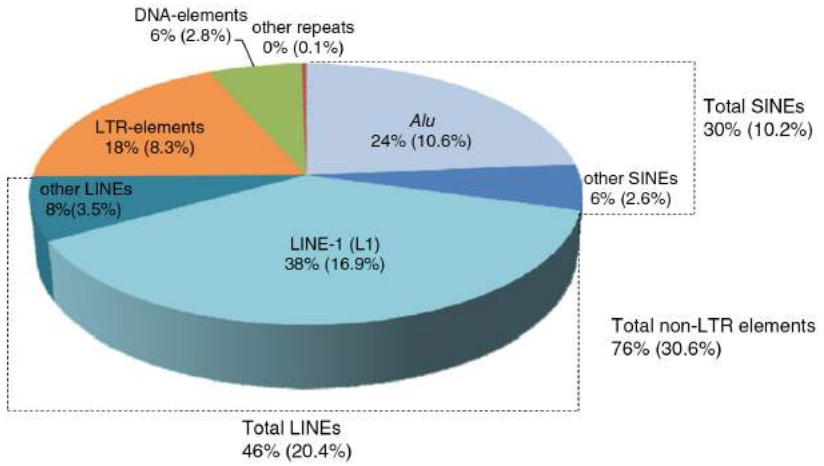
Non-autonomous retrotransposons are a third class of retrotransposons, of which the short interspersed nuclear elements (SINEs- Alu's – SVA's) comprise ~13% of the human genome (Lander et al.,2001).

The human genome contains millions of copies of retrotransposons; however, only the LINE-1 (L1) family, remains the primary source of retrotransposition.

L1 retrotransposon activity has persisted over time within the human genome and its derepression is associated with genomic instability and tumor development (Gasior et al., 2006; Lee et al., 2012).

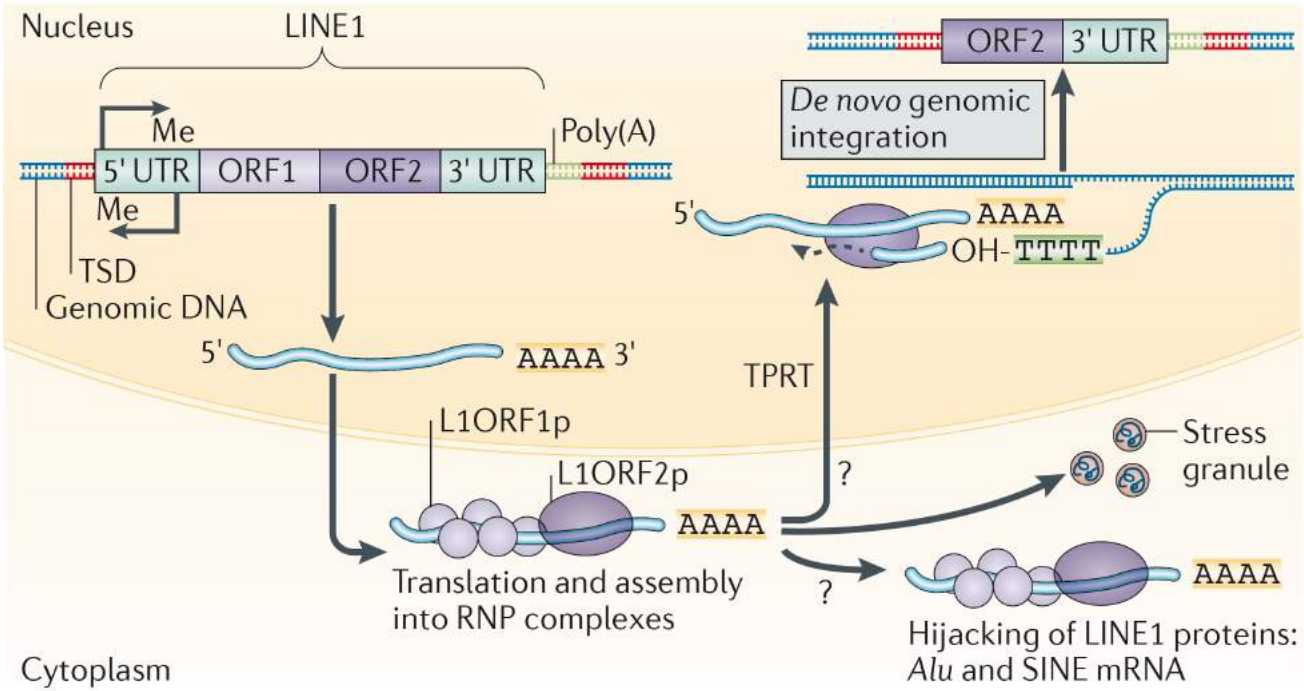
Over 100,000 L1 sequences exist in the human genome; however, most are rendered inactive by point mutations, rearrangements, or truncations (Brouha et al., 2003).





E. Heard, 2016

LINE-1 Elements



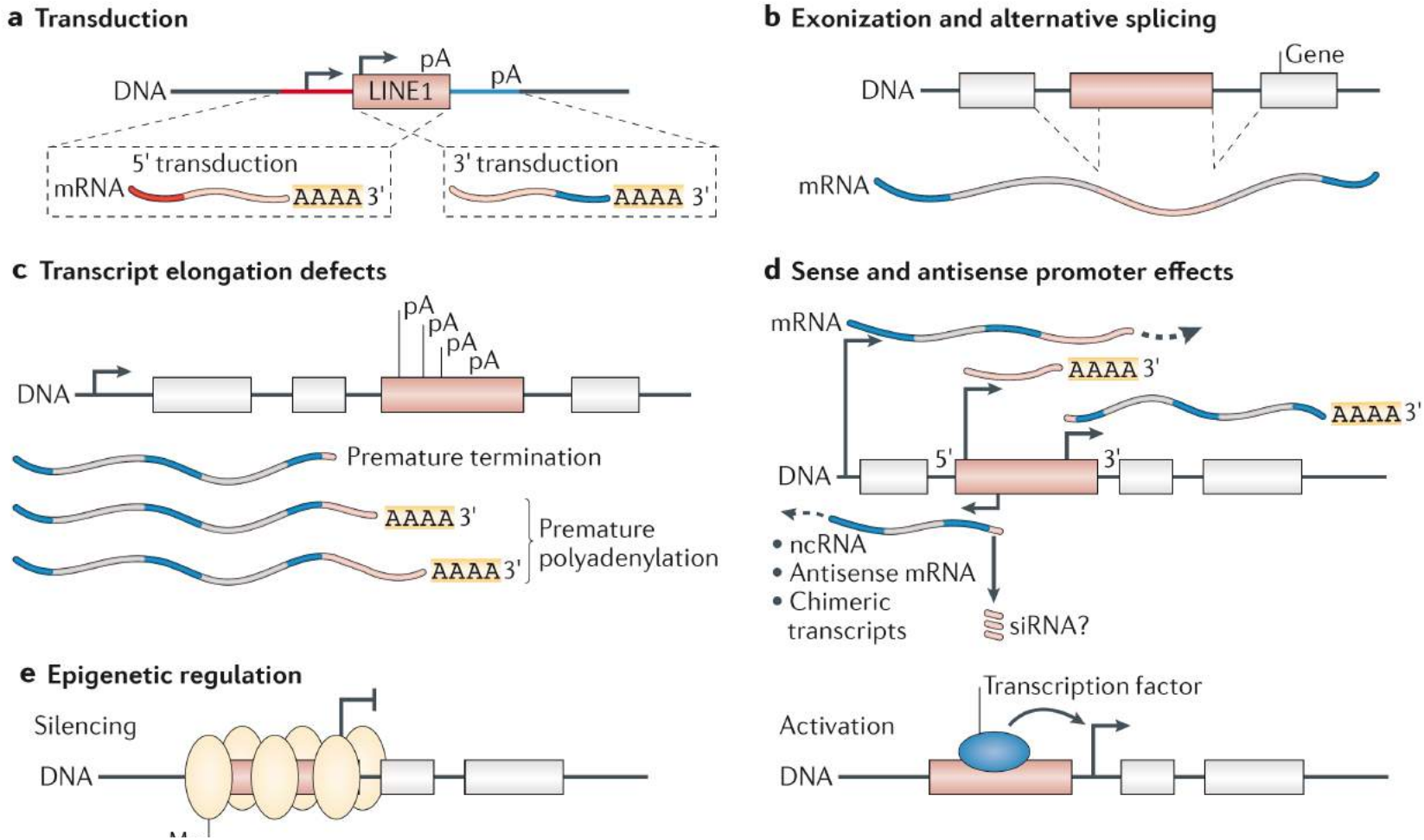
A few (<10) "Hot" L1s responsible for the bulk of L1 retrotransposition within the human genome (Brouha et al., 2003). Recently, several newly inserted "hot" L1s found – and are extremely polymorphic and specific to a few individuals, (Beck et al., 2010; Huang et al., 2010; Iskow et al., 2010).

LINEs are potentially mutagenic ; new insertions can influence gene expression;

May participate in creating somatic variation during life span

(Mobile DNA elements in the generation of diversity and complexity in the brain, Erwin, Marchetto & Gage, 2014, Nature Reviews Neuroscience 15, 497–506)

LINE-1 element impact on genome functions



Epigenetic control of LINEs and other transposons is key to survival of the host

Both in the germ line, during development and in the adult

Epigenetic mechanisms have even been proposed to have evolved for this purpose!

Such control may also be *exploited* to generate **differential expression states** of host genes

As B. McClintock originally proposed: transposable elements may act as “controlling elements”

Epigenetic Control of Repeats

DNA methylation represents a key control mechanisms for the repression of repetitive elements (Liang et al, 2002; Kato et al, 2007)

Several repressive histone modifications, including H3K9me3, H3K27me3, and H4K20me3, are also enriched at interspersed repeats (Martens et al 2005; Mikkelsen et al, 2007; Leeb et al, 2010) and Suv39/H3K9me3 has been shown to repress LINEs in the mouse.

Aberrant repetitive DNA methylation eg hypo-methylation of L1, Alu, LTR, and satellite repeats, is significantly associated with tumor progression in multiple cancers such as gastrointestinal stromal tumors, myeloma, and lung cancer (Rauch et al, 2008; Bollatio et al, 2009; Igarashi et al 2010)

Hypomethylation of L1 DNA has been observed in various cancers and is associated with an increase in transcriptional activation and expression of L1 (Alves et al., 1996; Asch et al., 1996; Kitkumthorn et al., 2012; Murata et al., 2013; Criscione et al., 2014; Park et al., 2014).

L1 hypomethylation can occur early in tumorigenesis and is associated with bladder (Patchsung et al., 2012; Salas et al., 2014), gastric (Shigaki et al., 2013; Baba et al., 2014a), colon (Ogino et al., 2008; Antelo et al., 2012; Murata et al., 2013), lung (Saito et al., 2010), and breast cancers (Park et al., 2014).

LINE or SINE/Alu related Cancers

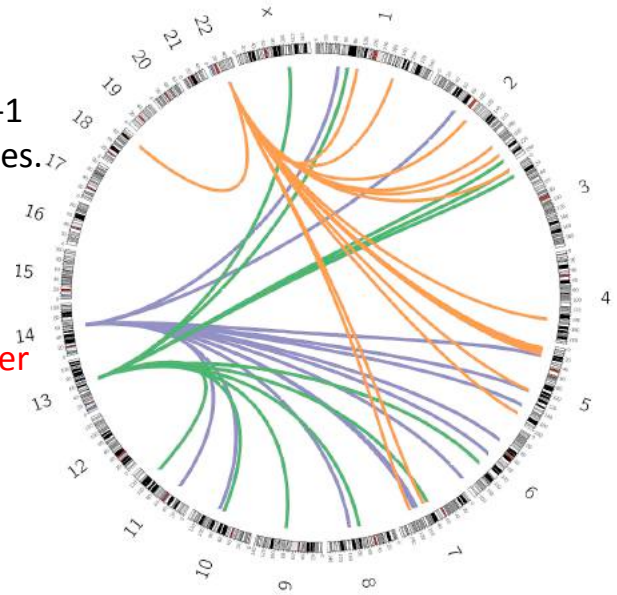
Examples of TE insertion and TE-mediated chromosomal rearrangements associated with cancer.

Locus and/or gene	Associated cancer	TE	Distribution
Insertion			
APC, adenomatous polyposis coli gene	Desmoids tumors	*Alu*	Germline
APC	Colon cancer	L1	Germline
APC		L1	Somatic
BRCA1, breast cancer 1 gene	Breast/ovarian cancer	*Alu*	Germline
BRCA2, breast cancer 2 gene	Breast/ovarian cancer	*Alu*	Germline
MYC, c-myc proto-oncogene	Breast carcinoma	L1	Somatic
NF1, neurofibromatosis 1 gene	Neurofibroma	*Alu*	Germline
Chromosomal deletions			
VHL, von Hippel Lindau gene	von Hippel Lindau disease	*Alu*	Germline
BRCA1	Breast/ovarian cancers	*Alu*	Germline
BRCA2	Breast/ovarian cancers	*Alu*	Germline
CDH1, cadherin 1 gene	Hereditary diffuse gastric cancer	*Alu*	Germline
CAD, caspase activated DNase gene	Hepatoma	*Alu*	Somatic
Chromosomal duplication			
MLL1, myeloid/lymphoid mixed lineage leukemia gene	Acute myeloid leukemia	*Alu*	Somatic
MYB, myb transcription factor gene	T-acute lymphoblastic lymphoma	*Alu*	Somatic
BRCA1	Breast/ovarian cancers	*Alu*	Germline
Chromosomal translocation			
EWSR1-ETV, t(5q23q31)(18q12)	Ewing sarcoma	*Alu*	Somatic
BCR-ABL, t(9;22)(q34;q11)	Chronic myeloid leukemia	*Alu*	Somatic

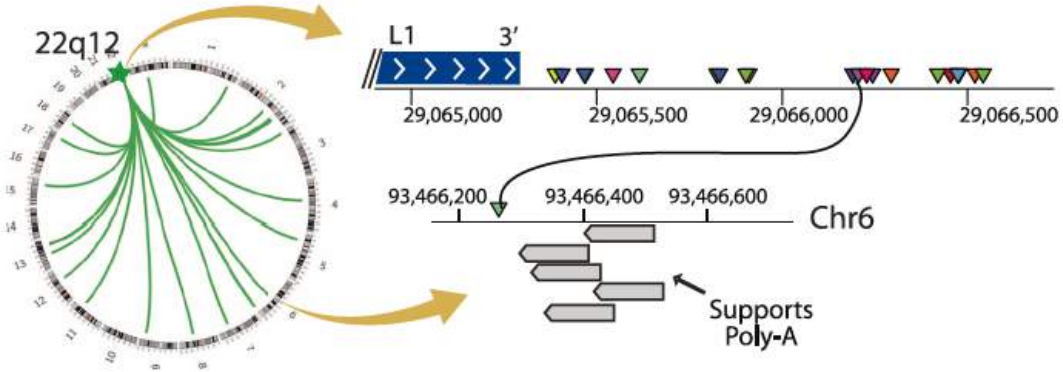
Mobile DNA elements can restructure cancer genomes

• Examined **290 tumour samples** from **12 different cancer types**, found 3,000 sites where LINE-1 elements were mobilised solely in the tumour.
• In 24% of sites, small pieces of non-repetitive DNA were transduced by LINE-1 into another position in the genome, mobilising exons and even complete genes.
• Particularly common in lung and colorectal cancers.
• **LINE1 activity correlates with hypomethylation, which can be caused by environmental factors.**

Majority of LINE-1 events are ***passenger*** mutations rather than drivers in cancer evolution –need to look at thousands of cancer genomes to detect true driver events (integrated with other mutational processes and transcriptional data)



In a lung cancer genome, **three LINE-1** copies located at human chromosomes 22, 14, and 13 spread non-repetitive DNA by transduction.



Sanger Center Press office 2014

Mobile DNA in cancer. Extensive transduction of nonrepetitive DNA mediated by L1 retrotransposition in cancer genomes. Tubio JM, Li Y, Ju YS, Martincorena I, Cooke SL et al. Science, 2014;345;6196;1251343

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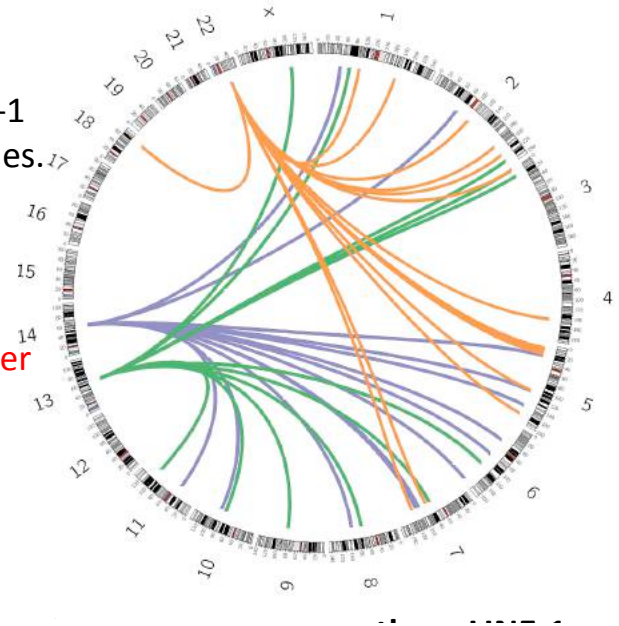

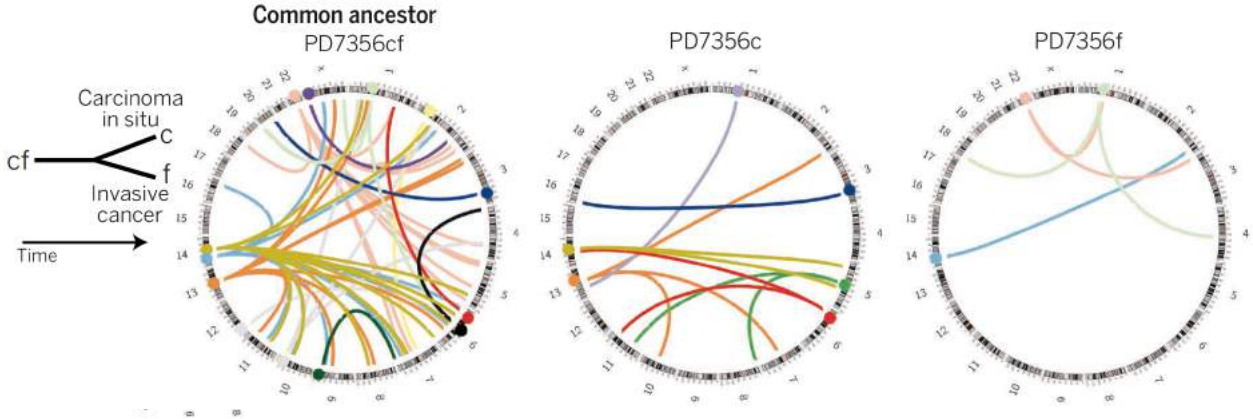

Lung tumor progression: hundreds of 3' transductions arose from a small number of active L1 source elements (colored circles on outer rim of circle). As the tumor evolved from the preinvasive common ancestor to invasive cancer, individual elements exhibited variable activity over time.

Sanger Center Press office 2014

Mobile DNA in cancer. Extensive transduction of nonrepetitive DNA mediated by L1 retrotransposition in cancer genomes. Tubio JM, Li Y, Ju YS, Martincorena I, Cooke SL et al. Science, 2014;345;6196;1251343

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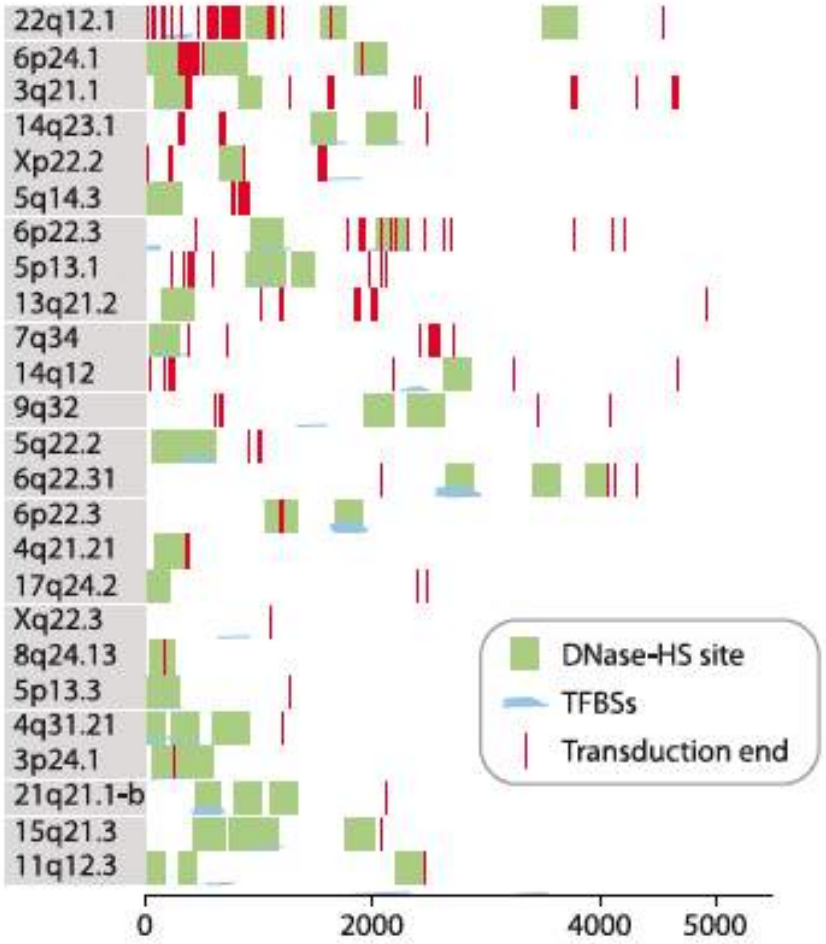
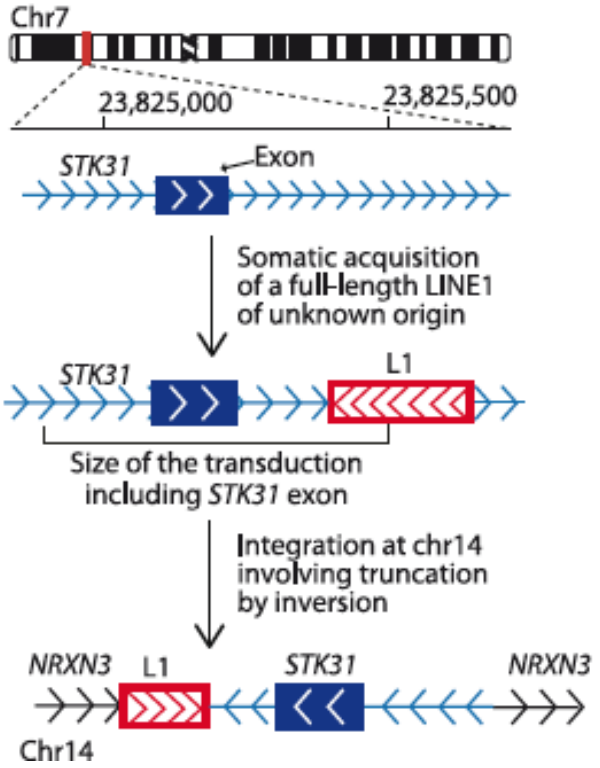


Somatic transductions frequently mobilize DNA sequences with regulatory potential.
Gray rectangles represent the 3' end of the L1 source elements.
The x axis = distance downstream of each source element.
Green rectangles represent DNAse-I–hypersensitive sites,
Horizontal blue lines represent transcription factor binding sites.
Red lines represents end points of a somatic transduction event.



STK31 exon shuffling mediated by a somatic L1 element:

An intact L1 element inserts somatically immediately downstream of an exon of *STK31*.

A further partnered transduction event occurs in which the exon of *STK31* and a portion of the somatic L1 element retrotranspose to an intron of *NRXN3*

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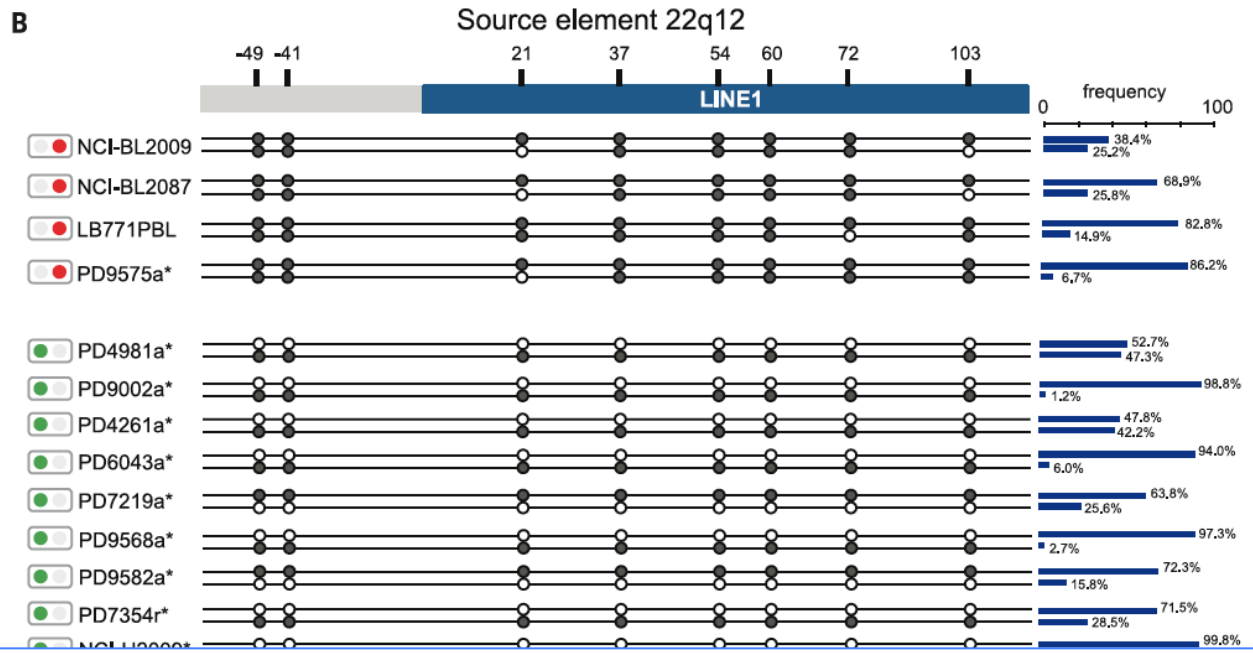
Mobile LINE-1's are always Hypomethylated

Mobile LINE-1s show 5' end DNA hypomethylation in cancer:



Nevertheless, the control systems that are lost and enable L1 activity to be triggered may be multiple and not just at the level of DNA methylation

Mobile DNA in cancer. Extensive transduction of nonrepetitive DNA mediated by L1 retrotransposition in cancer genomes. Tubio JM, Li Y, Ju YS, Martincorena I, Cooke SL et al. Science, 2014;345;6196;1251343

Pathological impact of LINE-1 Mobility

Widespread somatic L1 retrotransposition occurs early during gastrointestinal cancer evolution

Extensive somatic insertional mutagenesis occurs early during the development of GI tumors, probably before dysplastic growth.

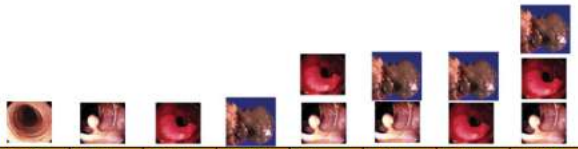
- L1-targeted resequencing (L1-seq) on different stages of 4 colorectal cancers arising from colonic polyps, seven pancreatic carcinomas, as well as seven gastric cancers.
- Found somatic L1 insertions not only in all cancer types and metastases but also in colonic adenomas, well-known cancer precursors.
- Some insertions were also present in **low quantities in normal GI tissues,** occasionally caught in the act of being clonally fixed in the adjacent tumors.

Early insertion (mutation) timing? Normal-appearing cells may harbor tumor-initiating genetic lesions.

- Insertions in adenomas and cancers numbered in the hundreds, and many were present in multiple tumor sections, implying clonal distribution.
- Many insertions occurred in known or candidate cancer driver genes eg within 1.9 kb of two exons of the CYLD gene (a known tumor suppressor)
- No enrichment of cancer driver genes targeted by somatic L1 insertions was observed when compared to germline insertions

Speculate: Early LINE activation – due to loss of epigenetic control (triggered by stress? eg chronic inflammation) may lead to new tumor initiating mutations

PCR-verified and Sanger-sequenced somatic L1 insertions

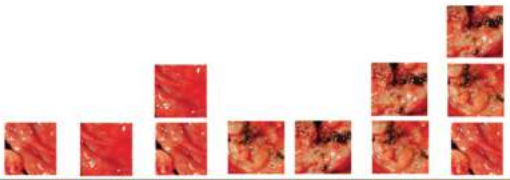


Colorectal cancer cases

Patient ID	N-only	P-only	C-only	M-only	P+C	P+M	C+M	P+C+M	Total:
1BV	0	0	13	no M	0	no M	no M	no M	13
2BV	1	10	1	2	0	0	4	0	18
3BV	0	0	18	no M	0	no M	no M	no M	18
4BV	0	1	0	0	0	0	7	0	8
Total:	1	11	32	2	0	0	11	0	57
Projected Additional	11	181	168	53	0	0	78	0	491

Pancreatic cancer cases

Patient ID	N-only	C1-only	C2-only	C1+C2	M-only	C+M	Total:
A33	0	0	0	0	2	1	3
A43	0	2	0	0	2	2	6
A55	0	0	0	0	2	5	7
A57	0	PanIN: 0	0	0	3	2	5
A82	0	0	0	0	0	0	0
A83	0	0	0	1	0	2	3
A146	0	0	0	0	no M	no M	0
Total:	0	2	0	1	9	12	24
L1-seq validated by TIP-seq	0	0	0	0	0	10	10
Projected Additional	0	55	0	2	183	16	256



Gastric cancer cases

Patient ID	N1-only	N2-only	N1+N2	C1-only	C2-only	C1+C2	N+C	Total:
2028	0	0	0	0	0	0	0	0
2034	0	0	0	0	0	5	0	5
2043	0	0	0	2	3	8	1	14
2044	0	0	0	0	0	1	0	1
2049	0	0	0	0	0	0	0	0
2670	0	0	0	0	0	0	1	1
2812	0	0	0	0	0	2	0	2
Total:	0	0	0	2	3	16	2	23
Projected Additional	0	0	0	105	79	20	0	204

Ewing et al (2015) Genome Res 25:1536–1545

LINEs as Drivers in Cancer?

Possible oncogenic role of L1 insertions given that mutated genes are candidate drivers of tumorigenesis

L1 can also mobilize other nonautonomous retrotransposons such as Alu and SVA, potentially leading to additional genomic lesions that could function in tumorigenesis.

Expression of L1 ORF1p is a hallmark of many human cancers, with almost half (47%) of the human neoplasms examined being immunoreactive for L1 (Rodic et al., 2014).

L1 DNA hypomethylation is common during tumorigenesis => L1s can be reactivated and participate in cancer initiation and progression.

Several correlations between tissue L1 hypomethylation and increased cancer risk or poor prognosis. (Ashktorab et al, 2014; Gualtieri 2013,40–42)

L1 methylation status as a cancer prognostic marker in peripheral blood of patients ? (Controversial…)

Target-site analysis: somatic L1 insertions are biased away from transcriptional active regions and toward regions such as intergenic or heterochromatic regions, cancer-specific hypomethylation regions, or genes frequently mutated in cancer

LINEs as Drivers in Cancer?

Possible oncogenic role of L1 insertions given that mutated genes are candidate drivers of tumorigenesis

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LINE1 ORF1p and ORF2p levels are upregulated in breast cancers compared to normal tissues.

Cytoplasmic levels of ORF1p and ORF2p are elevated in DCIS breast cancers compared to highly invasive cancers.

Conversely, nuclear levels of ORF1p and ORF2p were found to be higher in invasive breast cancers and correlated with increased lymph node metastasis and poor patient survival (Harris et al., 2010; Chen et al., 2012).

Furthermore, inhibition of the L1-encoded reverse transcriptase in breast cancer cells was demonstrated to **reduce the rate of proliferation and promote cellular differentiation** (Patnala et al., 2014).

Can Epimutated Genes drive Cancer?

Cancer-associated pathway	Gene
Cell cycle	Rb, $p16^{INK4a}$, $p15^{INK4b}$, 14-3-3, cyclin D2, cyclin E, $p14^{ARF}$
Signal transduction	ErbB2, *RASSF1, LKB1/STK11, APC*
Apoptosis	Death-associated protein kinase gene (*DAPK*), caspase-8 gene
DNA repair	O^6-methylguanine-DNA methyltransferase gene (*MGMT*), *MLH1, BRCA1, FNACF*
Carcinogen metabolism	Glutathione S-transferase P1 gene (*GSTP1*)
Hormonal response	Oestrogen receptor gene, progesterone receptor gene, retinoic acid receptor b2 gene (RAR-b2)
Senescence	*TERT, TERC*
Invasion/metastasis	Tissue inhibitor of metalloproteinase 3 gene (*TIMP-3*), E-cadherin gene, von Hippel-Lindau gene (*VHL*)
Transcription	Runx3, Twist, ER α, ER β, PR, RAR, vitamin D receptor
Drug responsiveness	Glutathione S-transferase, thymidylate synthase

Cause or Consequence?

Acquired epimutations - proposed as a "second hit" in tumors associated with familial cancer syndromes cause by heterozygous germline mutations; or aberrantly activated oncogenes

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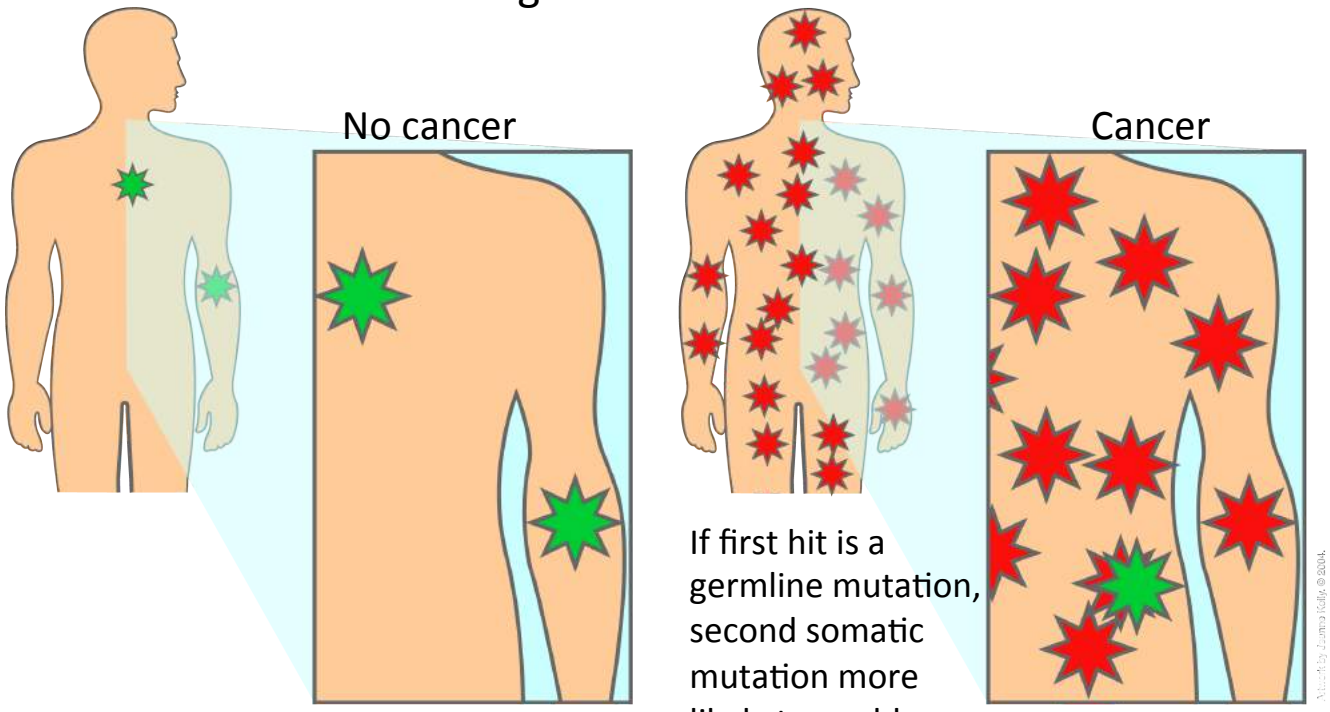
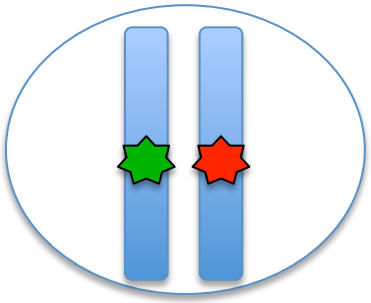
Can Epimutated Genes drive Cancer?

Epimutations involved in Familial Cancer?

An epiallele or silenced allele of a gene can be equated to the 'first hit' or 'second' hit as proposed by Knudson in his two-step model for carcinogenesis.





Germline mutation
Somatic mutation

or epimutation

irreversible

reversible?

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Can Epimutated Genes drive Cancer?

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Cause or Consequence?

Acquired epimutations - proposed as a "second hit" in tumors associated with familial cancer syndromes cause by heterozygous germline mutations; or aberrantly activated oncogenes

Constitutional epimutations – already present (and widespread) in somatic cells, prior to disease onset

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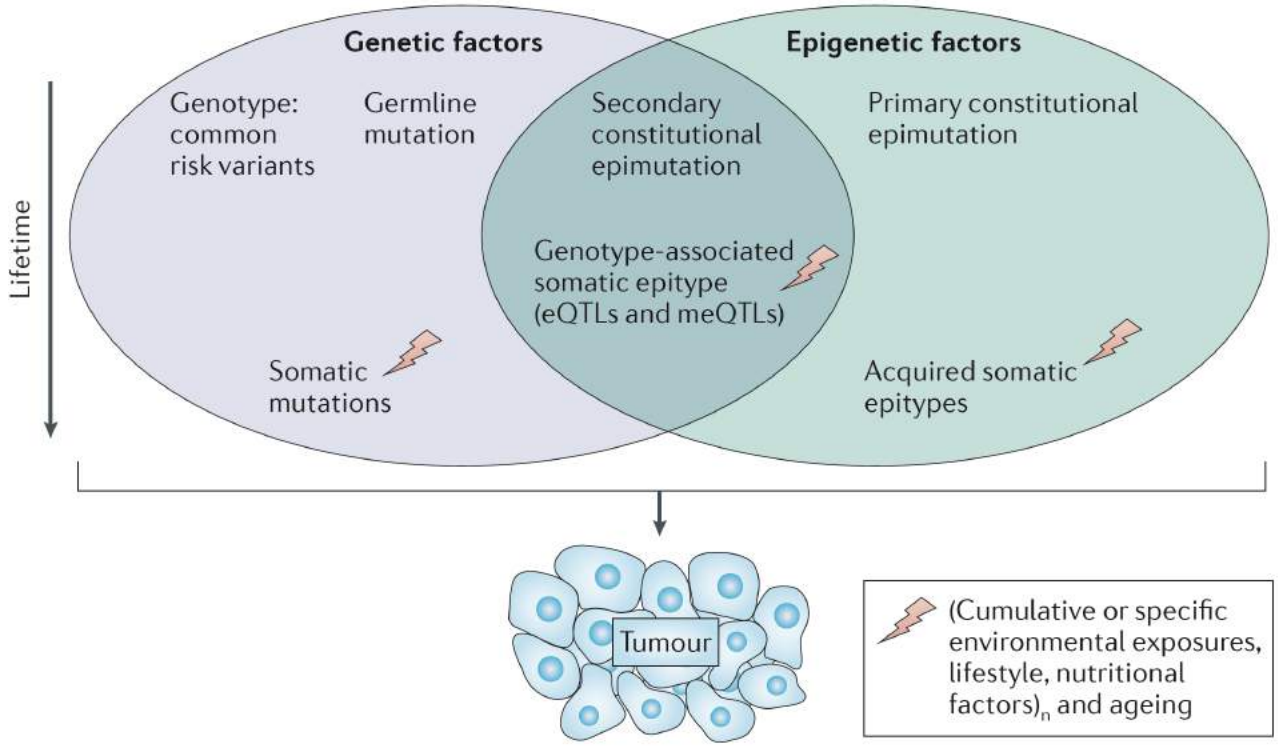
Constitutional Epimutations

• ***Constitutional epimutation***: an aberration in gene expression due to an altered epigenotype that is widely distributed in normal tissues (albeit frequently mosaic)
• Provides ***alternative mechanism*** to genetic mutation for cancer predisposition.
• In cancer-affected families, can sometimes see inter-generational inheritance of constitutional epimutation… ***primary*** (ie non-DNA sequence based) or ***secondary*** (ie do to DNA seq variant)?



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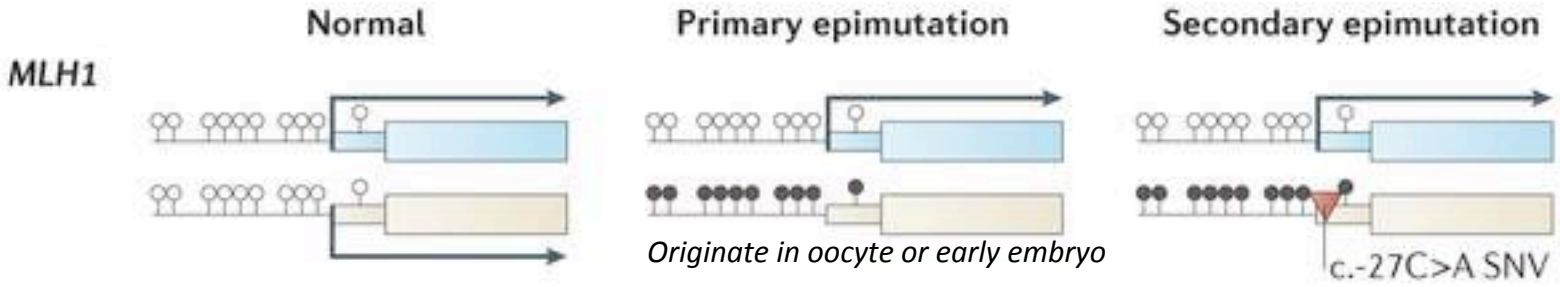
Epimutations: primary versus secondary

Primary: epigenetic change induced in parental germ line or early embryo?
Secondary: consequence of DNA sequence polymorphism/mutation?



MutL homologue 1 (*MLH1*) mutations cause Lynch syndrome: young onset of colorectal, endometrial and other cancers that typically demonstrate *microsatellite* instability (MSI) (and high mutations rates) owing to a deficiency in DNA mismatch repair.

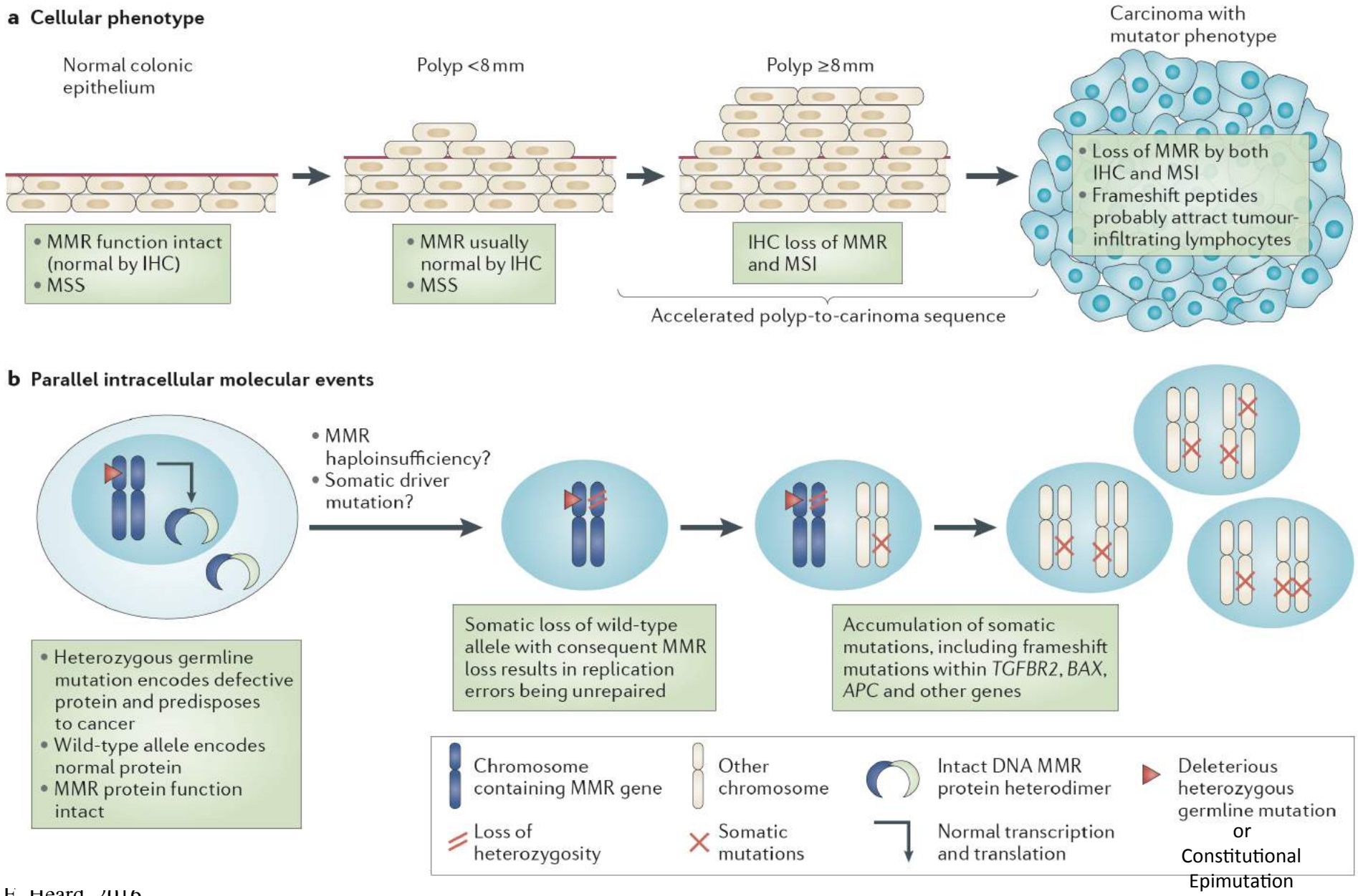
Lynch syndrome can be **hereditary** - caused by heterozygous *germline mutations* within one of four key DNA mismatch repair genes, most frequently within *MLH1* or *MSH2*

Or by constitutional *epimutation* of MLH1 in **sporadic** forms of Lynch syndrome (1-10% cases) (Hitchins and Lunch, 2014)

Cancer Risks in Individuals with Lynch Syndrome Age ≤70 Years Compared to the General Population

Cancer Type	General Population Risk	Lynch Syndrome (*MLH1* and *MSH2* heterozygotes)	
		Risk	Mean Age of Onset
Colon	5.5%	52%-82%	44-61 years
Endometrium	2.7%	25%-60%	48-62 years
Stomach	<1%	6%-13%	56 years
Ovary	1.6%	4%-12%	42.5 years
Hepatobiliary tract	<1%	1.4%-4%%	Not reported
Urinary tract	<1%	1%-4%	~55 years
Small bowel	<1%	3%-6%	49 years
Brain/central nervous system	<1%	1%-3%	~50 years
Sebaceous neoplasms	<1%	1%-9%	Not reported

Lynch Syndrome: Mismatch Repeair Deficiency



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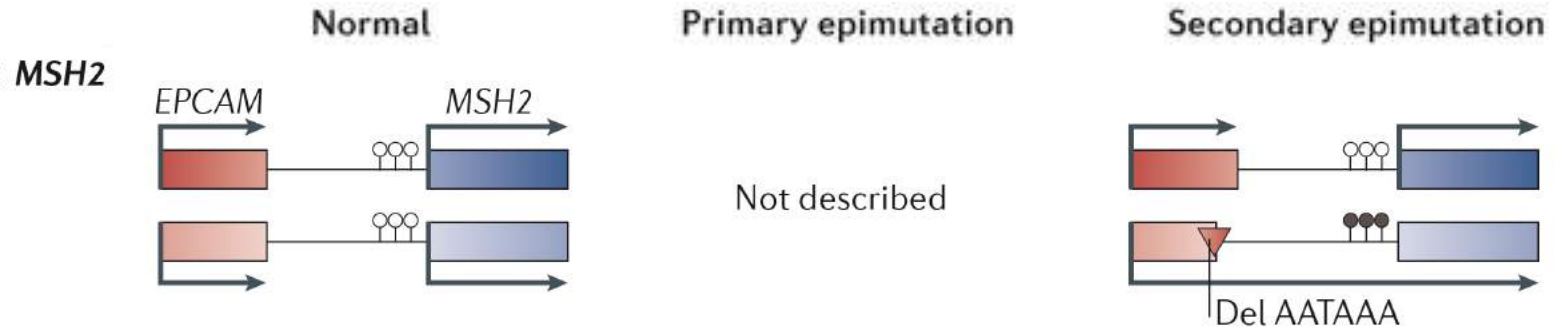


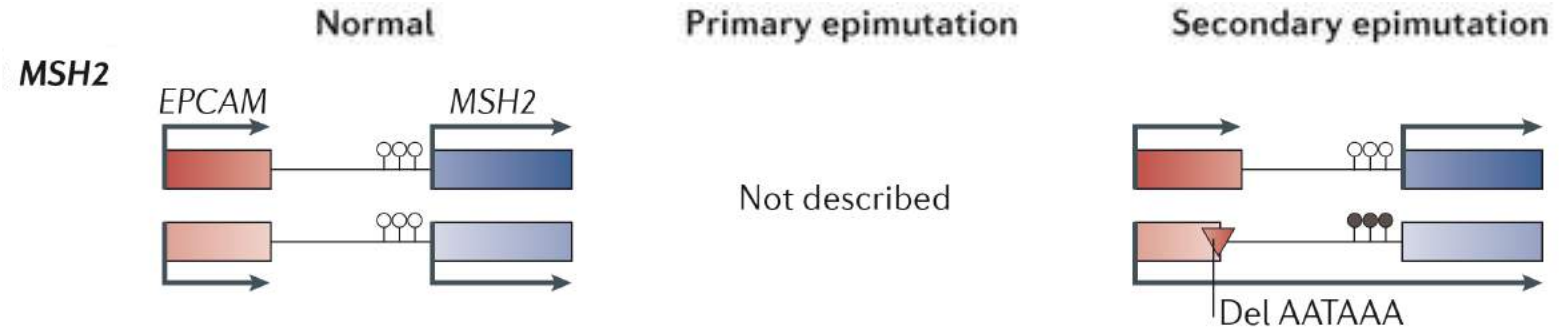
Primary Epimutations of other cancer-related genes?
E-cadherin (CHD1)? (just one case…)
BRCA1 or *RAD51* – mosaic constitutional methylation (0.01-20%) – but no evidence that this is cancer causing.

A DNA sequence change (mutation) affects a transcription factor binding site
⇒ no/low transcription
⇒ CGI/promoter DNA methylation arises

Apparent (Secondary) Epimutations: due to cis-acting genetic lesion…
• *MLH1* c.-27C>A single nucleotide variant (SNV) in four distinct families from a common European ancestral haplotype: mistaken for Primary (trans generational) epimutation
• *MSH2* secondary epimutation due to EPCAM deletion
• *DAPK1* 2ary epimutation linked to a SNV within a regulatory element 64 kb upstream of *DAPK1* allowed erroneous binding of the homeobox B7 (HOXB7) transcriptional repressor

Epimutations: primary versus secondary

Primary: epigenetic change induced in parental germ line or early embryo?
Secondary: consequence of DNA sequence polymorphism/mutation?



A DNA sequence change (mutation) affects a transcription factor binding site
ion
NA methylation

Primary Epimutations of other cancer-related genes?

E-cadherin (CH
BRCA1 or *RAD5*
(0.01-20%) – b
causing.

Apparent (Sec
• *MLH1* c.-27C
European ance
• *MSH2* second
• *DAPK1* 2ary e
allowed errone

DAPK1

Cancer causality?

Methylation testing of specific candidate genes in mutation-negative patients with a clinical suspicion of a high-penetrance cancer syndrome: primary /secondary epimutations for *MLH1*, *H19* and a few others, but not for *APC*, or *CDKN2A.*

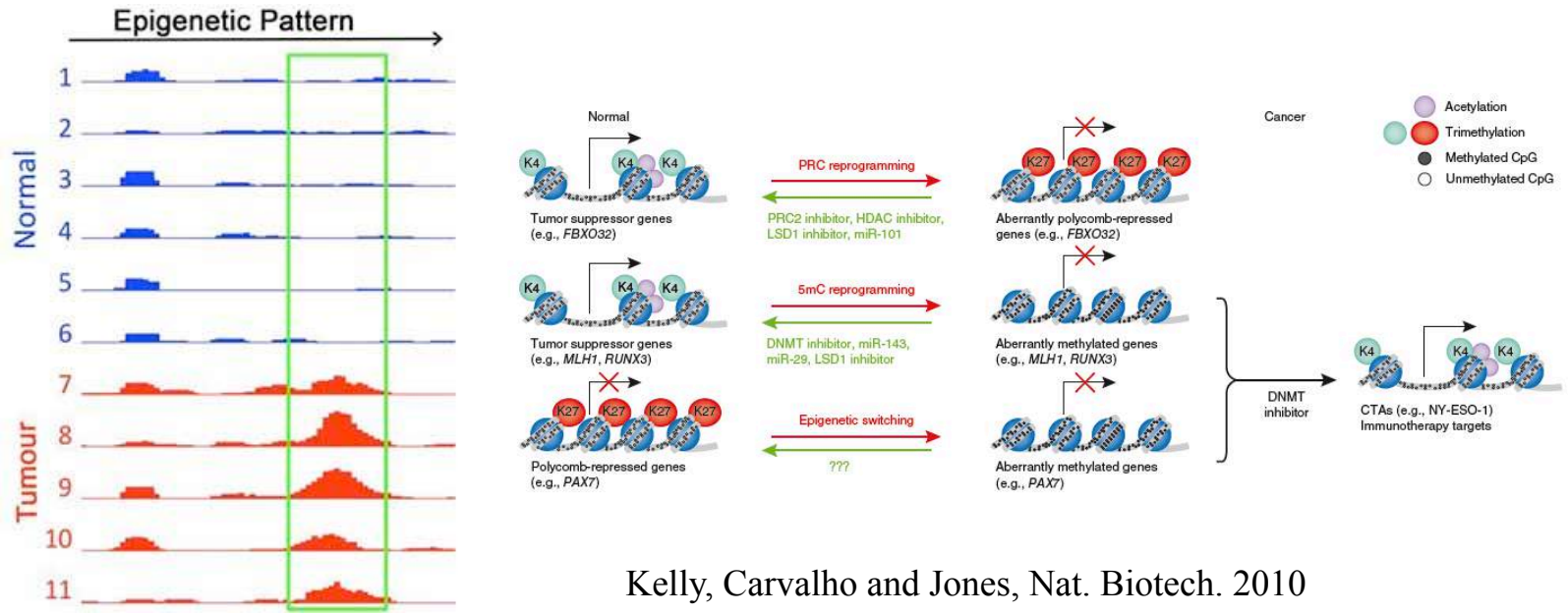
⇒ **No evidence of a generalized role for this mechanism in other cancer-causing genes?**

However, many epimutations may be missed in sporadic cases of cancer as patients are <u>not</u> usually tested for epimutations…
Also epigenetic marks other than DNA methylation may be involved!

Primary Epimutations versus Sequence Variants?

DNA sequence based predisposition to somatic epimutation, rather than primary epimutation, may be a prevalent phenomenon – involving short/long range regulatory elements

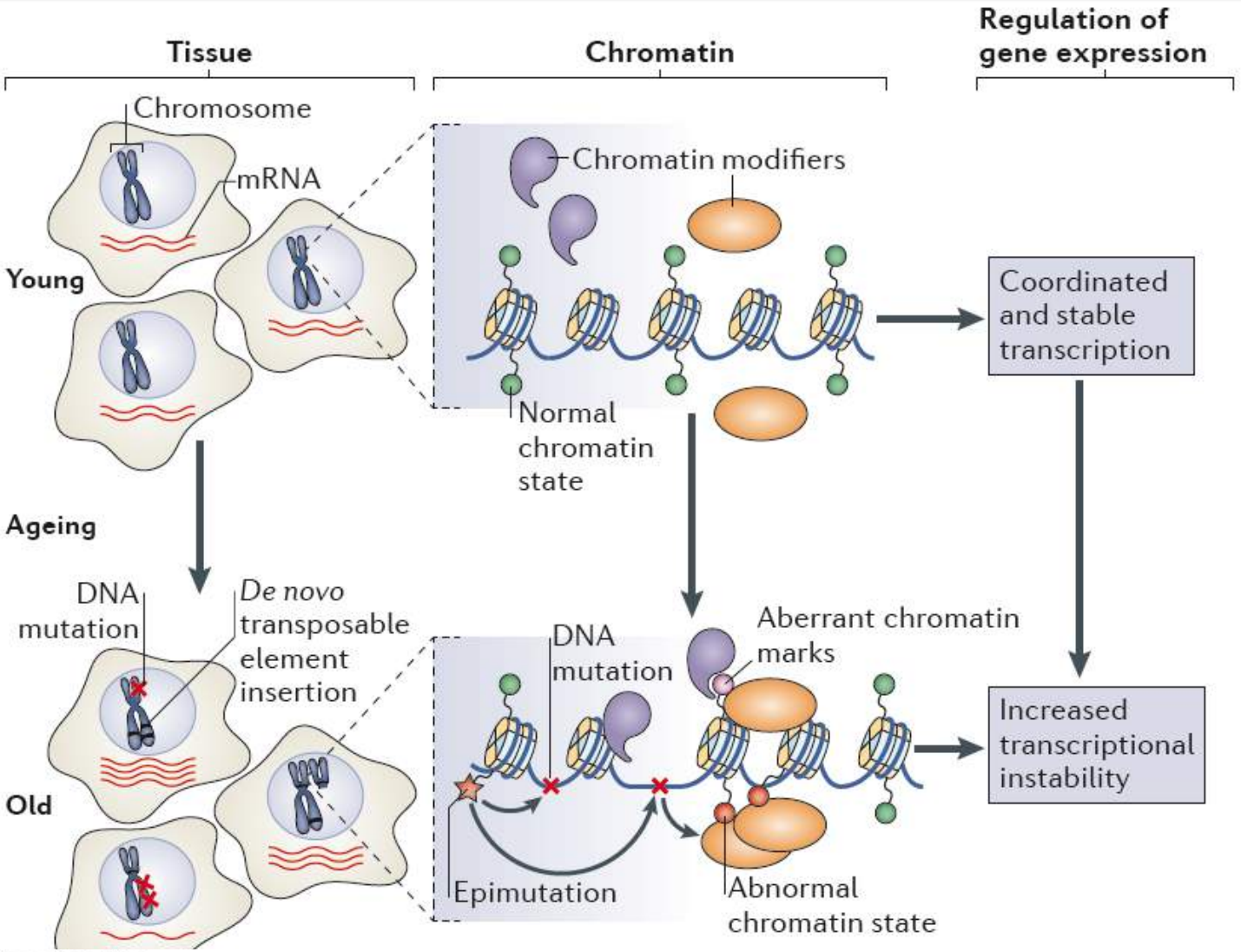
> This has important implications for disease – epimutations can be useful biomarkers, and they can be reversed: regulatory element variants can be 'shifted' to activate/inactivate a gene, using *epidrugs* that change epigenetic status



Kelly, Carvalho and Jones, Nat. Biotech. 2010

SNP-directed epimutations may also be influenced by diet, toxins, stress etc
> Can now explore extent and stability of epimutations with epigenomic mapping

How do Epigenetic Changes Arise: Ageing



Epigenetic regulation of ageing: linking environmental inputs to genomic stability

Bérénice A. Benayoun[1,2*], Elizabeth A. Pollina[1,3*] and Anne Brunet[1–3]

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How do Epigenetic Changes Arise: Ageing

Environmental inputs	Effects on chromatin	Effects on healthspan and lifespan
Diet (dietary restriction)	• Modulation of chromatin modifiers • Heterochromatin maintenance • rDNA chromatin structure • Inhibition of recombination • Nucleosome positioning	Increased
Circadian cycle (regular)	Circadian epigenome	Increased
Circadian cycle (perturbed)	Modulation of chromatin modifiers	Decreased
Exercise	• Modulation of chromatin modifiers • Chromatin modifications	Increased
Pheromones	Signalling through chromatin modifiers	Increased
Systemic factors (sex steroid hormones)	• Chromatin structure • Chromatin modifications	Increased

Mechanistic link ?

Epigenetic regulation of ageing: linking environmental inputs to genomic stability

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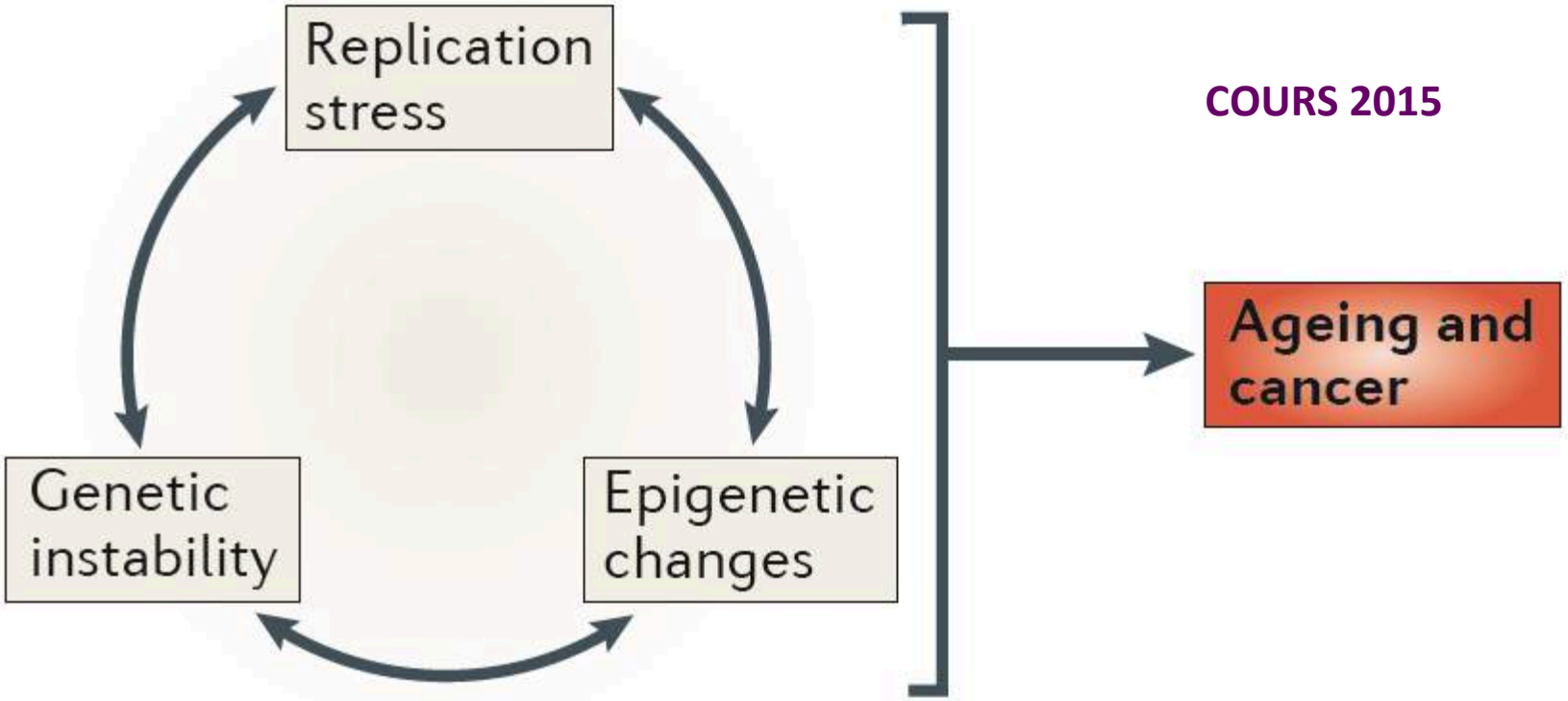
Replication stress: loss of chromatin memory

Replication stress can lead to both DNA mutations
and epigenetic changes (chromatin memory loss) that can impact on:
gene expression, repeat element activity, centromere function…
leading to further genetic and epigenetic aberrations

Oncogenic activity can trigger replication stress, including unscheduled initiation, fork stalling and collapse. This can result in epigenetic aberrations in cancer…



COURS 2015

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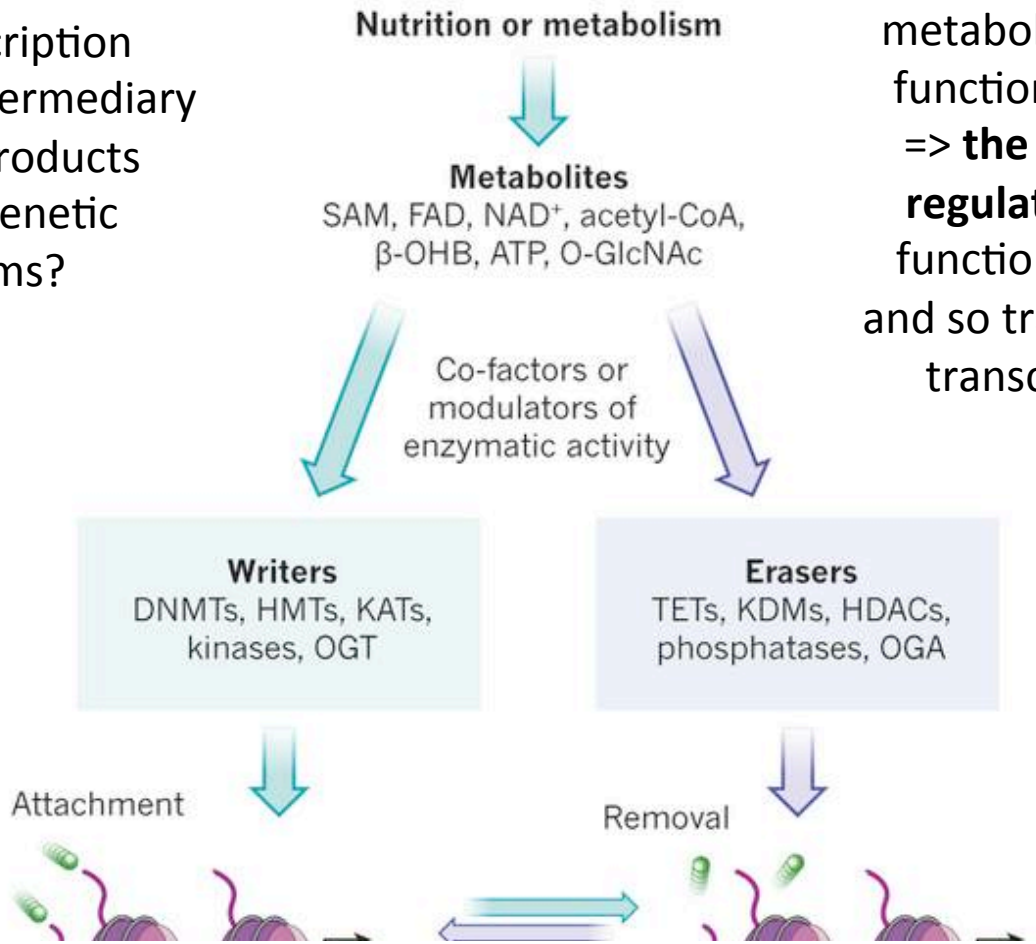
How do Epigenetic Changes Arise: Metabolic Stress

Metabolic stress and chromatin changes

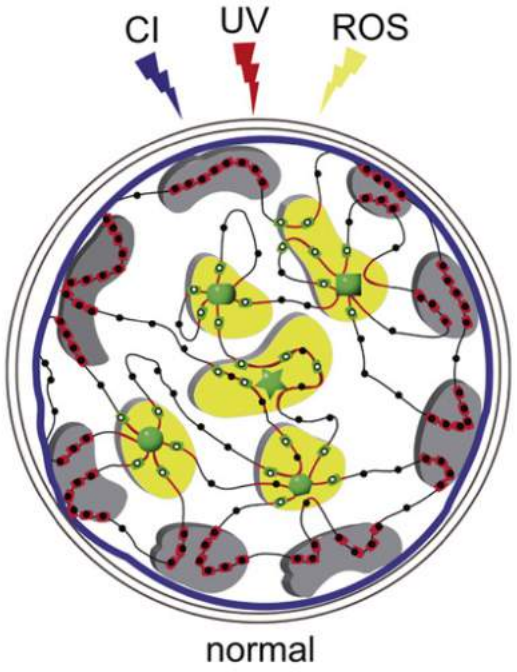
Is gene transcription influenced by intermediary metabolism products through epigenetic mechanisms?



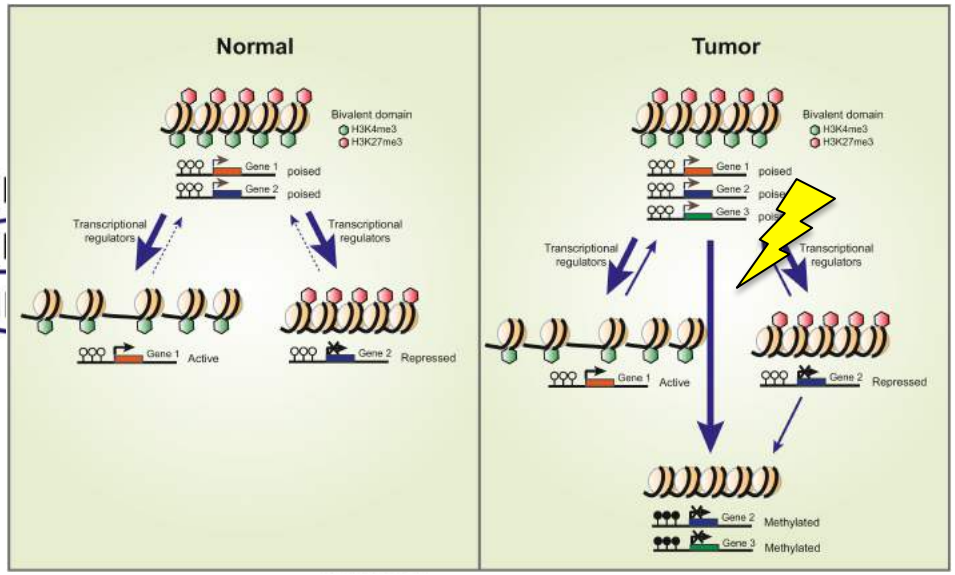
Cellular concentrations of metabolites can fluctuate as a function of a cell's metabolic => **the activity of chromatin regulators** may change as a function of **metabolic status** and so transduce a homeostatic transcriptional response?

Kaelin, W. G. & McKnight, S. L. Influence of metabolism on epigenetics and disease. *Cell* **153,** 56–69 (2013).
Lu, C. & Thompson, C. B. Metabolic regulation of epigenetics. *Cell Metab*. **16,** 9–17 (2012).
Wellen & Thompson. A two-way street: reciprocal regulation of metabolism and signalling. *Nature Rev. Mol. Cell Biol*. **13,** 270–276 (2012).
Katada, S., Imhof, A. & Sassone-Corsi, P. Connecting threads: epigenetics and metabolism. *Cell* **148,** 24–28 (2012).
Teperino, R. et al Histone methyl transferases and demethylases; can they link metabolism and transcription? *Cell Metab*. **12,** 321–327 (2010)

How do Epigenetic Changes Arise: Oxidatve Stress



Work of S. Baylin and colleagues: Several hundred bivalently marked genes (developmental regulators) switch to (>stable?) DNA methylation during tumor progression – perhaps due to stress-induced redistribution of Polycomb proteins? Thus some cells become too stably "locked" in to a primitive (stem cell like?) state…

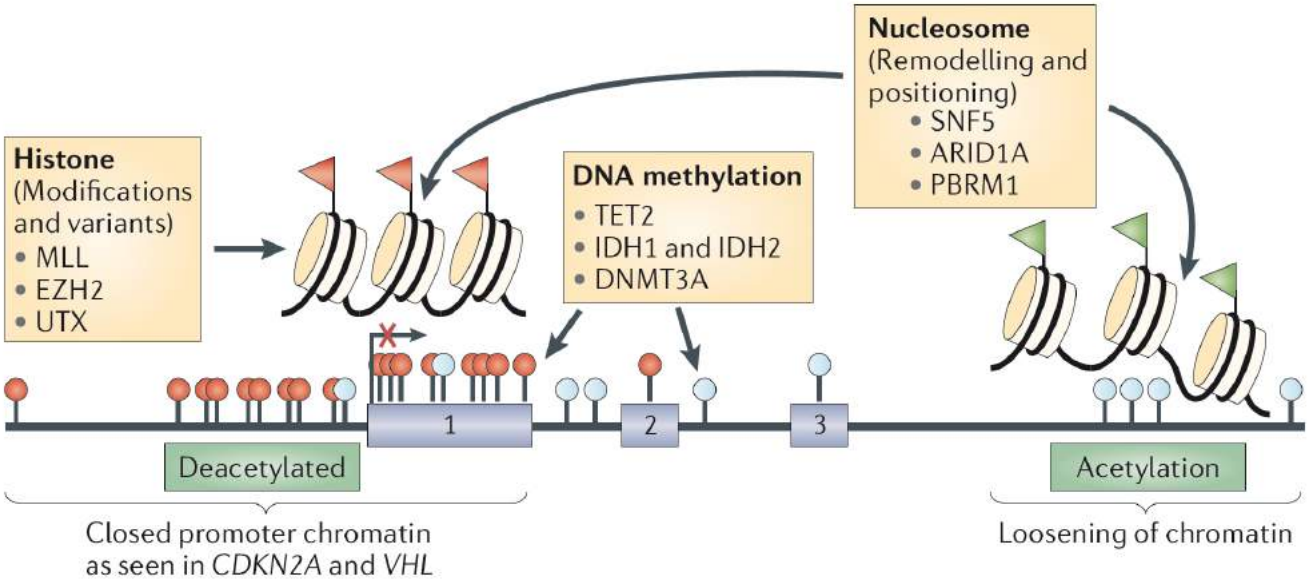


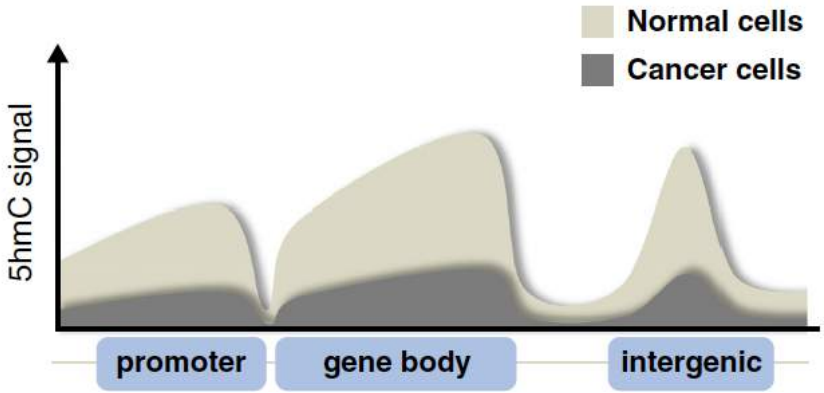
Oxidative damage induces formation and relocalization of a silencing complex that may explain cancer-specific aberrant DNA methylation and transcriptional silencing

A potential role for increased levels of cellular ROS that accompany cancer risk states such as inflammation, in the formation of cancer-specific aberrant patterns of DNA methylation and transcriptional silencing?

When cells are exposed to chronic oxidative damage that is present during all phases of tumorigenesis, see induced shifts in chromosome localization -> may be associated with losses of DNA methylation observed in cancer cells. (O'Hagan et al, 2012, Cancer Cell)

How do Epigenetic Changes Arise: Mutations in Chromatin modifers





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