

EPIGENETIC CONTROL OF GENE EXPRESSION

- epigenetic alterations

- heritable through cell division

- behave similarly to mutations in terms of stability (in some instances)

- reversible → can be (potentially) manipulated therapeutically

- sensitive to the environment (e.g. diet)

1.1 INTRODUCTION TO THE CONCEPTS OF EPIGENETIC CONTROL

- all cells in an organism share the same genetic code (DNA), but there are many types of cells expressing different genes

- only a few genes out of total ~25,000 (human) are expressed by each cell

- mechanisms [activity of transcription factors specific for each cell lineage]

[epigenetic marks applied to the DNA]

- epigenetic marks [demarcate start/end of genes]

[provide structure to the chromosome]

[alter how a gene is being read] [expressed (active)]

[not expressed (silent, inactive)]

[+ more subtle changes]

DNA → RNA → Protein

	dopamine	haemoglobin	moglobin
neuron	+	-	-
red blood cell	-	+	-
muscle cell	-	-	+

1.2 MITOTIC HERITABILITY OF EPIGENETIC MARKS

- epigenetic marks are [important during development of an organism (from zygote to sperm/egg)] removed between generations (sperm + egg → zygote step)

Q: Is it really true that no epigenetic marks are passed down to next generation?

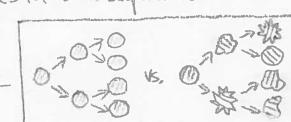
- aberrant epigenetic control would have deleterious effects in all stages of organism life-cycle

- Epigenetics: study of MITOTICALLY HERITABLE changes in gene expression that occur WITHOUT changes in DNA sequence

- mitotic heritability of epigenetic state: helps to maintain cell identity

[same sets of genes are expressed in daughter cells]

[tissue homogeneity (lack of mitotic heritability would result in tissue heterogeneity)]



1.3 CHROMATIN AND THE NUCLEOSOME

- DNA + histones = chromatin (~2m DNA packed into nucleus w/ diameter ~10 μm)

- tightness of chromatin compaction negatively correlates w/ transcriptional activity (more compressed → less accessible)

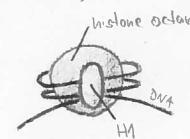
- nucleosome: the smallest unit of chromatin

[histone octamer (2xH2A, 2xH2B, 2xH3, 2xH4)]

[146bp DNA wrapped 1 3/4 times around histone octamer]

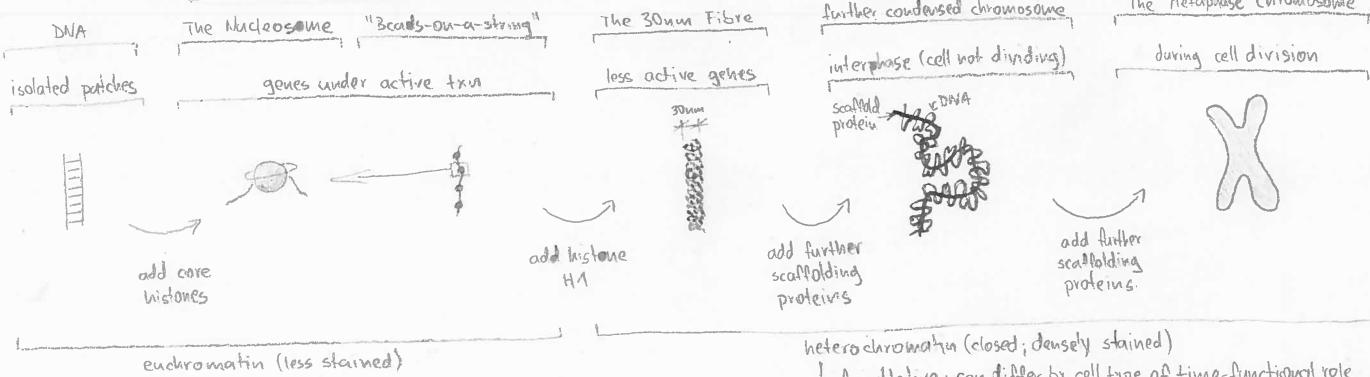
[histone H1 locking DNA in place]

- N-terminal histone tails protrude from the octamer



for txn, replic, repair
histones: positively charged (lysine & arginine-rich)
- DNA: negatively charged

1.4 CHROMATIN COMPACTION - HETEROCHROMATIN VS. EUCHROMATIN



heterochromatin (closed; densely stained)

[facultative: can differ by cell type or time-functional role (e.g. tissue specific, X chromosome silencing)]

[constitutive: same in all cell types - performs a structural role (e.g. centromeres, telomeres, portions of sex chromosomes - mainly Y)]

- functions [gene silencing]

[structural integrity of the genome]

- different epigenetic marks are associated w/ euchromatin and heterochromatin

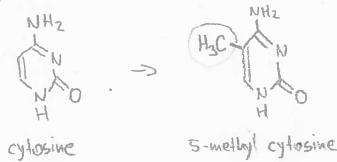
1.5. DNA METHYLATION AT CpG ISLANDS

- specific epigenetic modifications

\downarrow DNA methylation

- almost exclusively occurs at CpG dinucleotides in mammals

- symmetrical \rightarrow can be maintained through cell division



Cytosine-Phosphate-Guanine

- de novo: DNMT3a, DNMT3b
- maintained by: DNMT1

- laid down by de novo methyltransferases DNMT3a and DNMT3b in mammals

see pg. 1.3

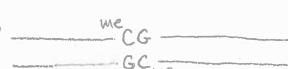


symmetrical methylation at CpG dinucleotide



hemi-methylated DNA

\downarrow methylation by DNMT1 see also pg. 2.1, ch. 2.2: HP1 & H3K9me



symmetrical methylation restored (maintained) by DNMT1

- CpGs clustered into CpG islands \leftarrow often at gene promoters

usually protected from methylation - i.e. NOT methylated

if methylated (rarely), then almost always associated w/ gene silencing

mostly studied for X inactivation randomly chooses one of two X chromosomes
occurs at gastrulation in the embryo
mitotically inherited by all daughter cells
inactive X chromosome shows DNA methylation of CpG islands

- silencing mechanism

\leftarrow 1^o mechanism - ^{me}CpG bound by methylated CpG binding proteins (e.g. MeCP1, MeCP2)

\leftarrow have DNA binding domain and tru repression domain
recruit other factors that condense chromatin

\leftarrow 2^o mechanism - methylation can prohibit tru factor binding and alter gene expression

- for rare tru factors & promoters with few CpGs



1.6. DNA METHYLATION AT INTERGENIC REGIONS AND REPETITIVE ELEMENTS

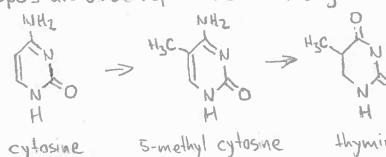
- occurrence of DNA methylation

- CpG islands: usually UNmethylated

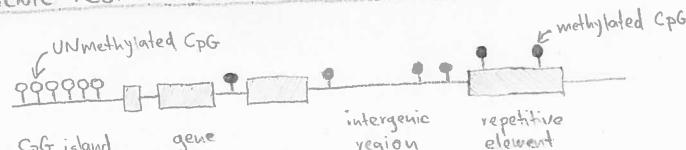
- intergenic regions: usually methylated

- repetitive elements: usually methylated

- CpGs are underrepresented in the genome: cytosine is prone to deamination to thymine



\leftarrow DNA methylation is mutagenic



- function of DNA methylation

- at intergenic regions: maintain genomic integrity

- Dnmt1-null cells display genomic instability

DNMT knockouts die in utero

- silence cryptic tru start sites or cryptic splice sites



tru start site (occurring either by chance or due to presence of a gene) needs to be silenced - otherwise, there will be collisions b/w RNA Pol II transcribing A and C

\uparrow
may result in truncated (i.e. non-functional) protein product

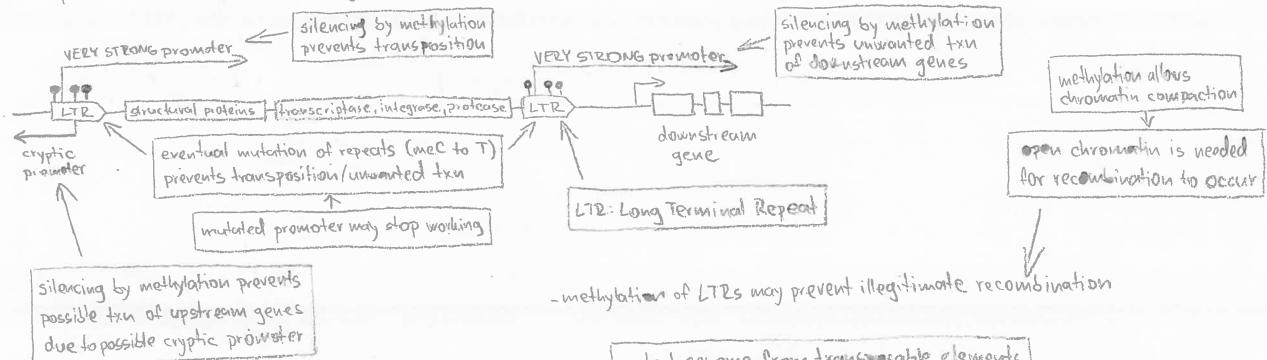
hypomethylation (see ch. 7.4, pg. 7.2)

\leftarrow illegitimate recombination b/w repeats

activation of repeats & transposition

activation of cryptic promoters & disruption of neighbouring genes (cf. A^{N} , $\text{A}^{\text{in}} \text{in } \text{A}^{\text{N}}$ alleles) see ch. 6.3, 6.5 pg. 62, 63

at repetitive elements; maintain genomic integrity



- genome defense model (Prof. Timothy Bestor)

- DNA methylation is mutagenic \Rightarrow there must be also a benefit for cells to retain it

- DNA methylation in cancer: mainly demethylation in intergenic regions, more rare methylation of CpG islands

- lack of methylation historically the earliest epigenetic aberration found

[found in all cancers studied, more consistently than genetic mutations]

- DNA demethylation

[passive: dilution of DNA methylation with every cell division due to lack of DNMT1

- requires DNA replicu

active

- originally, methylation was thought to be irremovable, ~ year 2000: shown demethylation can occur w/o replicu

- shown to occur in early development, primordial germ cells development, later specific stages of differentiation

- not simple removal of $-\text{CH}_3$; C-C bond is very hard to break

- enzymatic removal via intermediates, using multiple different systems

- TET proteins (main players), AID

[only expressed at very restricted times in development (primordial germ cells, embryonic stem cells)]

2.1 INTRODUCTION TO HISTONE TAIL MODIFICATIONS

- specific epigenetic modifications

↳ post-translational histone modifications

- methylation, ubiquitination, phosphorylation, sumoylation, acetylation of residues in N-terminal tails of histones (+ many other modifications)
- predominantly H3 and H4, less so H2A and H2B
- histone code: Thomas Jenuwein, C. David Allis - Translating the Histone Code (10.8.2001, Science, vol. 293, issue 5532)

Q: What about C-terminal tails of H2A and H2B?

MODIFICATION	ABBREV.	TARGET RESIDUES	FUNCTION	BINDING DOMAINS
methylation (mono, di, tri)	me	lysine (K), arginine (R)	txn, repair (H)	Chromo, MBT, PHD, Tudor
acetylation	ac	lysine (K)	txn, repair, replication, condensn	Bromo
ubiquitination	ub	lysine (K)	txn	
sumoylation	su	lysine (K)	txn	
ADP-ribosylation	N/A	glutamate (E)	txn	
phosphorylation	ph	serine (S), threonine (T)	txn, repair, condensn	A4-B-3-3
citrullination	cit	deimination of arginine (R)	txn	

↳ results in citrulline

2.2 HISTONE ACETYLATION AND HISTONE METHYLATION

- histone acetylation: universally associated w/ gene activity (i.e. active gene that is being transcribed)

- mechanism: reduces positive charge by neutralizing positive lysines (K) → decreases attraction b/w histones (+) and DNA (-)

↳ acts as docking site for other proteins

↳ e.g. bromodomain proteins that open the chromatin (chromatin remodelers)

↳ recruit proteins that act on chromatin

chromodomain: CHromatin Organization Modifier

sometimes referred to as KAT (K as in lysine)

the name is derived from the relationship of this domain w/ Brahma (the drosophila gene, which study led to discovery of bromodomain); the name is UNRELATED to the chemical element bromine.

- enzymes: HAT (Histone Acetyltransferase) acetylates histones (HAT: 18 different genes in mouse)

↳ HDAC (Histone DeAcetylase) DEacetylates histones (HDAC: 18 different genes in mouse)

- arguably, not an epigenetic modification (rather chromatin modification)

↳ rapid acetylation/deacetylation dynamics (circadian rhythm)

↳ lack of mechanism for mitotic heritability



- histone methylation (mono, di, tri)

- does NOT change histone charge (unlike acetylation)

- can be associated w/ both activity and inactivity of a gene - depending on the context

- examples: H3K4me: active locus, around promoter

↳ H3K9me: inactive locus, spread over the gene - usually associated w/ constitutive heterochromatin (centromere, telomeres, ...)

↳ H3K27me: inactive locus, spread over the gene - usually associated w/ facultative heterochromatin (cell-type specific)

- enzymes: HMT (Histone MethylTransferase) methylates histones (a.k.a. KMT - Lysine Methyl Transferase)

↳ HDM (Histone DeMethylase) DEMethylates histones (a.k.a. KDM - Lysine DeMethylase)

- mechanism: unmodified histone tails act as docking sites for other chromatin proteins

↳ e.g. H3K4me → CHD1 (ATP-dependent chromatin remodeler)

↳ H3K9me → HP1 (Heterochromatin Protein 1: essential heterochromatin protein, can recruit DNMT1)

↳ H3K27me → CBX2 (part of PRC1) that lays down H2AK119ub epigenetic mark

DNA Methyltransferase 1
see pg. 1.2, ch 1.5

Polycomb Repressive Complex 1

- general rule: modified histone tails are "read" by other chromatin proteins; they act as docking sites for other epigenetic factors

- interacting proteins can have ability to alter chromatin packaging (chromatin remodelers)

↳ recruit other proteins that can bring about further epigenetic modifications

example: H3K9me3 → HP1 → DNMT1 → CpG → ^{me}CpG

↳ note that all modifications in this example are on H3K9 (i.e. the same residue)

↳ HMT → spread H3K9me3

↳ see also MITx 7.28.2x: pg. 10.3

2.3 CHROMATINE REMODELLING

- specific epigenetic modifications

Chromatin remodelling: ATP-dependent chromatin remodelling complexes shift nucleosomes

- changes in chromatin compaction

- more dense/sparsely packed (sliding nucleosomes along DNA)
- nucleosome eviction (disassembly): e.g. around TATA box sites
- histone variant deposition

nucleosome turnover: role in stability of epigenetic marks

- disrupt DNA-histone interactions → allow movement of nucleosomes

- chromatin remodelling complexes

- types SWI-SNF (SWItch/Sucrose Non Fermentable) [Bromodomain → ac
ATPase]

[ISWI (Imitation SWI) [SANT domain → ? (we do not know)
ATPase]

CHD (Chromodomain and Helicase-like Domain) [Chromodomain → me
ATPase (Helicase)]

me: context dependent
(active/inactive chromatin)

- role in development and disease

- almost all are essential for viability (knockout studies in mice)

[cell differentiation during development (need rapid changes in gene expression)]

- histone modifiers & chromatin remodelers

- chromatin remodelling complexes recognize histone modifications

- some complexes have ability to both act as chromatin remodeler

[modify histones]

eg: NURD complex [HDAC protein (deacetylate histones)
Chd3/Chd4 (nucleosome remodelling proteins)]

- order & hierarchy (causality): histone modification and nucleosome repositioning: which comes first? or are they simultaneous?

likely varies by circumstance

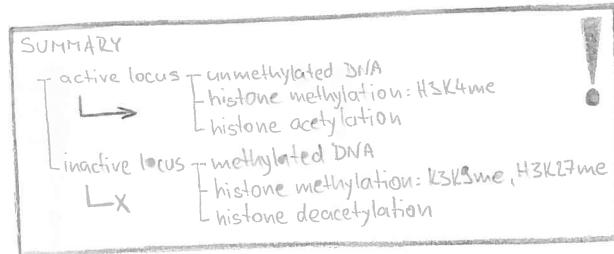
- open questions

- mitotic heritability

- interplay b/w epigenetic marks, mechanisms of their action

- factors that: make/remove/recognize epigenetic marks

- how are p.icular factors being recruited to specific sites?



2.4 HISTONE VARIANTS

- specific epigenetic modifications

Histone variant: histones w/ varying stabilities or specialist domains that alter the function of the nucleosome

- known variants of: H2A, H3, H1

- variants → increased/decreased stability

[different amino acids: e.g. Serine (can be phosphorylated) not found in canonical histones]

[others we do not understand]

- deposition of histone variants: ATP-dependent chromatin remodellers

[replication dependent]

[replication independent (see e.g. H2A.X below)]

- variants examples

H3: Centromeric histone variants: e.g. CNEP-A (different names in different species)

- centromere specific (centromere: the place where chromosome attaches to the mitotic spindle)

- defines position of centromere by altering DNA packaging structure

H2A.X

- universal histone variant, highly conserved

- C-terminal motif differs from canonical H2A: H2A.X has Serine 133 (can be phosphorylated)

- H2A.X-ph (phosphorylated H2A.X) aka. γ-H2A.X localised in double strand breaks and involved in DNA repair

- DSB repair Serine 133 phosphorylated by kinases at DSB

[γ-H2A.X → DNA repair proteins]

[epigenetic factors that alter chromatin state at DSB (DNA needs to be accessible to repair proteins)]

[phosphatase cleaves phosphate group off Ser133 after the repair is complete]

MacroH2A: inactive X chromosome

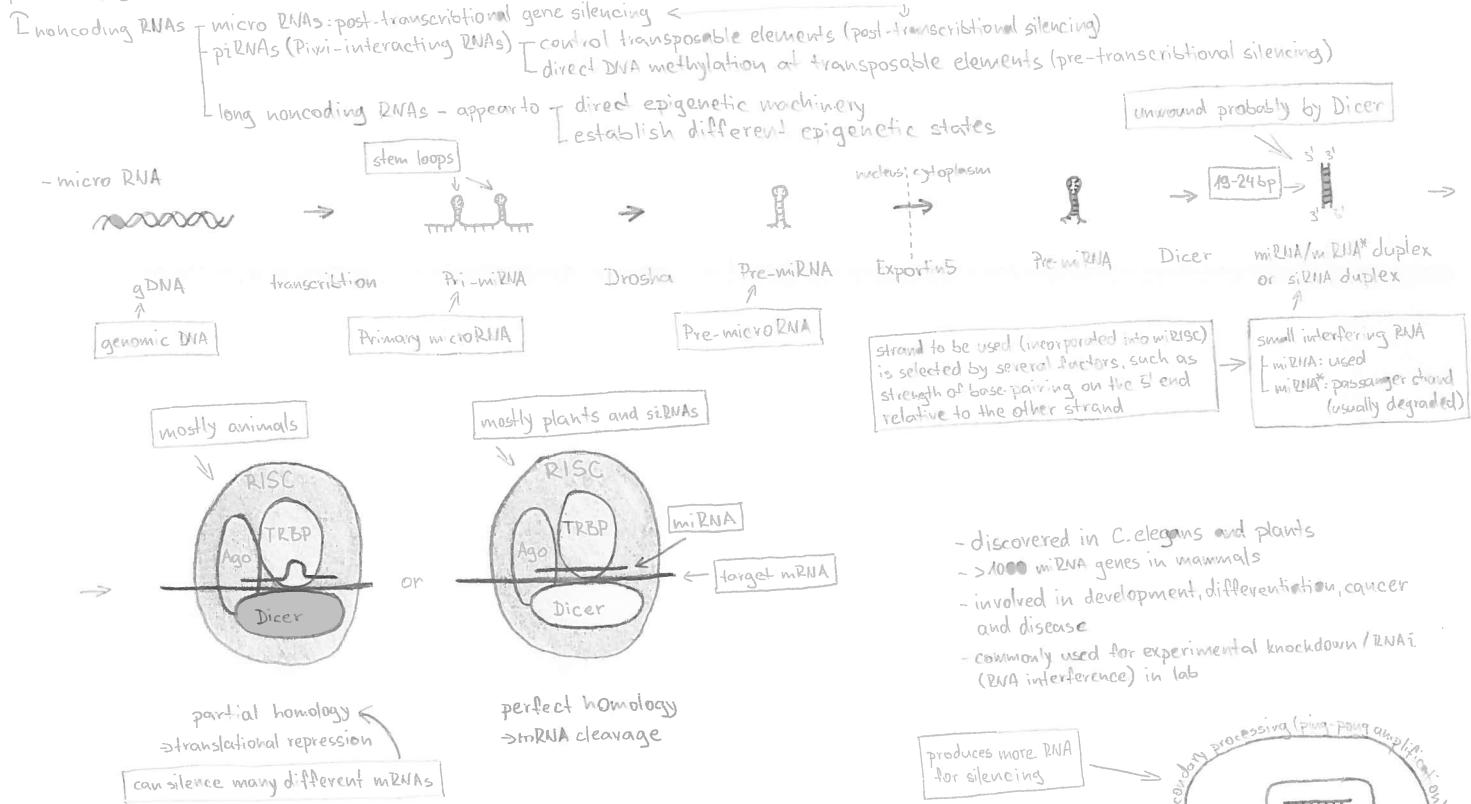
- found only in vertebrates

- contains large 200 amino acid C-terminal "macro" domain (mechanism of action of this "macro" domain is still unknown)

- enriched on the inactive X chromosome (facultative heterochromatin)

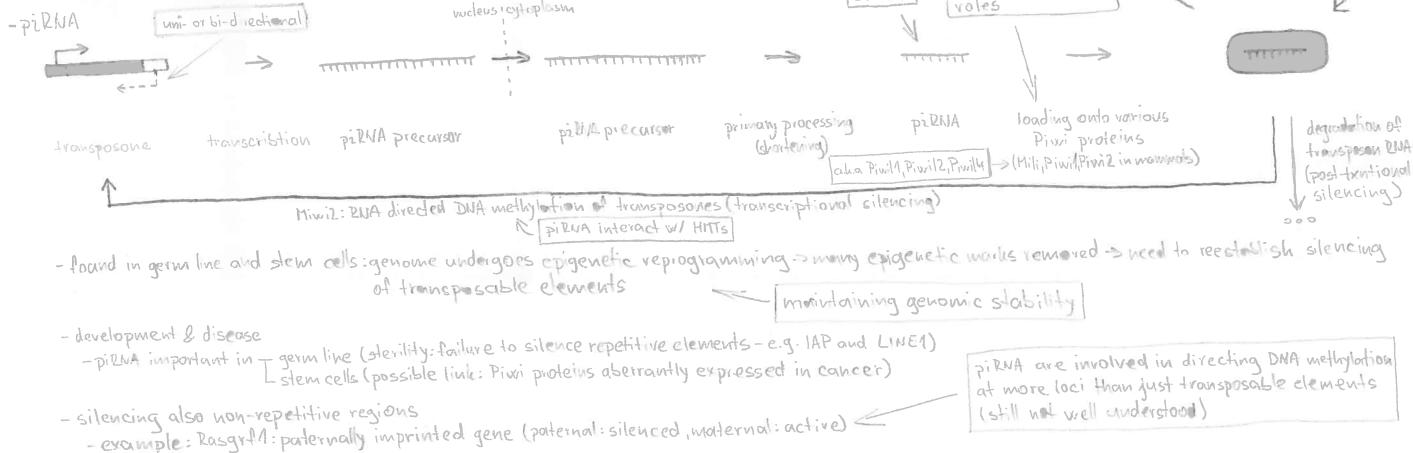
2.5. NONCODING RNAs - microRNAs

- specific epigenetic modifications



- discovered in *C. elegans* and plants
- >1000 miRNA genes in mammals
- involved in development, differentiation, cancer and disease
- commonly used for experimental knockdown / RNAi (RNA interference) in lab

2.6. NONCODING RNAs - piRNAs



2.7. NONCODING RNAs - LONG NON-CODING RNAs INTRODUCTION

length > 200 nt: most are several kilobases or less

- long non-coding RNAs (lncRNA)
- > 200 nt long, usually spliced, capped (5' cap), polyadenylated (3' polyA tail) ← much like mRNA: see Mix 7.28.2x pg. 9.1
- predominantly constrained to nucleus
- expressed in developmentally controlled manner
- number of unique long non-coding RNAs: ~ 10,000 - 200,000 in mammalian genome
- many of long non-coding RNAs found (expressed) only in very few copies
- able to regulate epigenetic processes
- examples: X inactivation: Xist, Tsix
 - genomic imprinting: Airn, Kcnq1OT1, H19
 - Hox genes (control the body plan of an embryo along the cranio-caudal axis) silencing: HOTAIR, HOTTIP
 - DNA damage response: lncRNA-p21
- function: direct epigenetic complexes to specific sites in DNA
 - most do not have DNA binding capability
 - bind DNA via trn factors → specificity does NOT allow for unique targeting
 - bind existing epigenetic marks → often insufficient specificity (-ii-)
 - in cis: on chromosome from which they are transcribed
 - in trans: somewhere else than ↑
- specificity: can act in allele-specific way (tethered to the locus in cis during trn) } specificity not always required (depending on their function)
 - strong site specificity (long sequence)

- possible mechanisms
 - guides (e.g. cis: Xist, Longlott, Airn; trans: HOTAIR)
 - scaffolds (e.g. trans: HOTAIR, NEAT1)
 - decoys (e.g. Tsix, MALAT1)
 - signal (e.g. Xite, Airn): txrn process itself influencing epigenetic state, lncRNA may be only a (lost degraded) byproduct
 - enhancers (e.g. cis: eRNAs)
 - reservoirs (e.g. H19-reservoir for microRNAs: produces microRNAs after being cut)

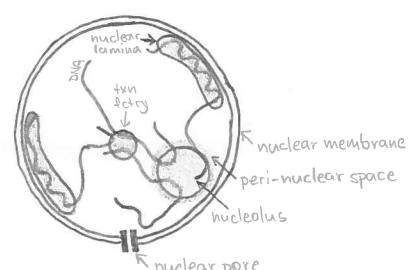
ooo

2.8 LONG NONCODING RNAs - XIST AND HOTAIR

- Xist (X Inactivation Specific Transcript): ~10kb, spliced, polyadenylated, constrained to the nucleus, non-coding (no protein product)
- epigenetic dosage compensation mechanism (X chromosome inactivation)
 - requires allele specificity
 - Xist is critical determinant for X inactivation (the first measurable step)
 - Xist expressed only from one of the two X chromosomes: determines the chromosome that will be inactivated
 - Xist RNA coats the inactive X in cis
 - mechanism
 - Xist contains many repeats in the transcript
 - RepA (RepeatA) binds the histone methyltransferase complex PRC2 (Polycomb Repressive Complex 2), which lays down H3K27me
- HOTAIR
 - acts in trans (more controversial)
 - expressed from HOXC locus (chr12)
 - represses the HOXD locus (chr2): HOXD cluster is very similar to HOXC cluster \Rightarrow contains the sequence homologous w/r (part of) HOTAIR
 - acts in other regions (w/o sequence specificity) - in cancer
 - HOTAIR $\xrightarrow{\text{PRC2}} \text{add H3K27me}$
 - $\xrightarrow{\text{LSD1}} \text{remove H3K4me}$

2.9. 3D ORGANIZATION OF THE NUCLEUS

- nuclear space
 - chromosomes (~1/2 of the space)
 - chromosome territories: areas, where each chromosome resides
 - nuclear sub-compartment
 - nuclear lamina: attached to inner side of nuclear membrane
 - attached DNA regions: lowly expressed
 - ~70% - 90% same b/w cell types
 - txrn factories
 - appears static & anchored, DNA passes through RNA Pol II
 - high concentration of RNA Pol II (~1000-fold higher than elsewhere) & high concentration of txrn factors (and varied by txrn factory)
 - coregulated genes localised to the same txrn factory (\Rightarrow efficient)
 - open question: are txrn factories stable or do they rapidly self-associate?
 - nuclear pore
 - euchromatin, actively transcribed
 - reason unknown (fast export to cytoplasm for translation?)
 - nucleolus ("txrn factory for ribosomal DNA")
 - aggregation of ribosomal DNA (rDNA) repeats from different chromosomes
 - rRNA transcription by RNA Pol I, processing and ribosome subunit assembly
 - peri-nuclear space (localized around nucleolus)
 - RNA Pol III
 - transcription of tRNA
 - polycorn body
 - paraspeckles
 - splicing body



3.1. HISTORY AND BACKGROUND OF X CHROMOSOME INACTIVATION

- X chromosome inactivation - good model for study of epigenetics

- X inactivation history

1949, Barr and Bertram: identified structure at nuclear periphery, present only in female cells → Barr body

1953, Ohno: identified Barr body as densely packaged X chromosome

1961, Lyon: Lyon hypothesis (now law) - dosage compensation by inactivation of one X chromosome per female diploid cell

X chromosome to be inactivated chosen by random chance: mice, calico cats,...

- X inactivation in karyotypically abnormal cells

XX, 44 autosomes: normal diploid female → 1 inactive X

XY, 44 autosomes: normal diploid male → 0 inactive X

XXX, 44 autosomes: Trisomy X patients → 2 inactive Xs

XXY, 44 autosomes: Klinefelter patients → 1 inactive X ⇒ X inactivation does not depend on sex (presence of Y chromosome)

XXXX, 88 autosomes: Tetraploid female cells → 2 inactive Xs ⇒ only 2 X chromosomes inactivated (correct to achieve relative dosage compensation)

Xφ, 44 autosomes: Turner syndrome patients → 0 inactive X ⇒ X inactivation depends on number of X chromosomes

1966 Olympics used presence of Barr body to check sex of "female" athletes

Fun fact: Susanna Ohno, DNA music

3.2 TIMING OF RANDOM AND IMPRINTED X CHROMOSOME INACTIVATION

- forms of X inactivation in mammals

- random

- in embryo proper around gastrulation (epiblast)

- inactive X chosen randomly, then propagated through mitosis

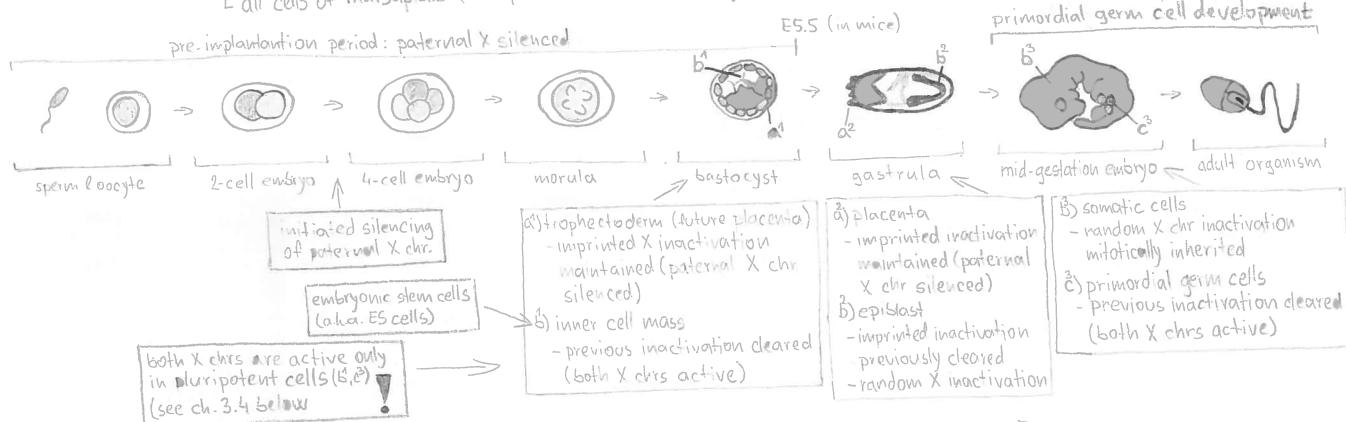
see picture below (gastrula)

- imprinted

- paternal X chromosome selectively silenced

- occurs in pre-implantation development (before an embryo has made placenta) and extra-embryonic tissues (placenta) ← (at least in mice)

[all cells of marsupials (non-placental mammals, indigenous to Australia & South America)]



3.3 STAGES OF X INACTIVATION - COUNTING AND CONTROL OF XIST EXPRESSION

- stages: counting: ratio of X:A (X chrs: autosomal chrs) - determine if X inactivation required

choice: choose X chr to be inactivated

initiation: Xist expression from XIC (X inactivation centre)

spreading: spread inactivation in cis along the X chr

establishment: turn Xist signal into transcriptional silence

maintenance: maintain inactivation through the life of the cell and its progeny

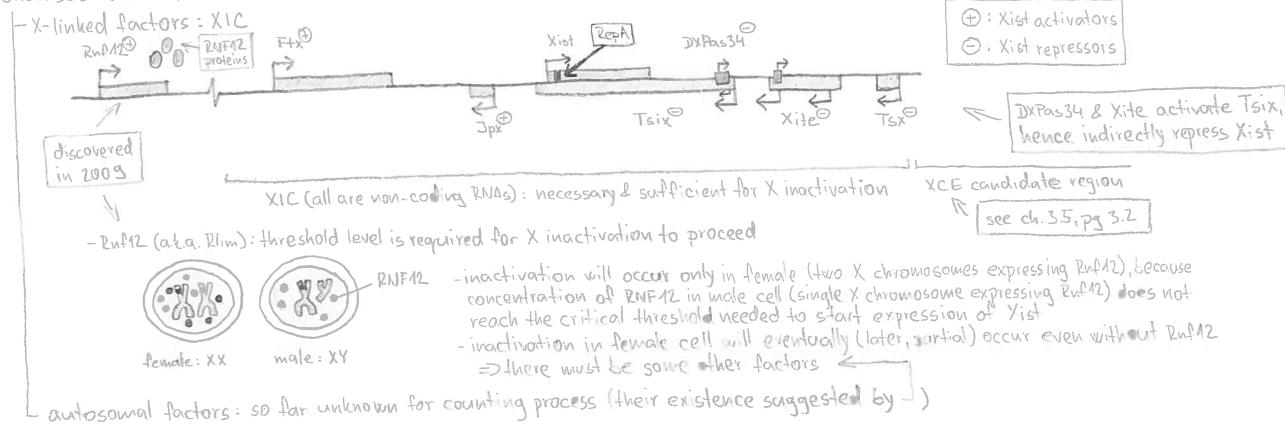
Xist: X-Inactive Specific Transcripts

- X inactivation is accomplished via a progressive accumulation of epigenetic marks on the inactive X chromosome

- X chromosome counting

- very little known, controversial theories

- consensus: X-linked and autosomal-linked factors to determine X:A ratio



autosomal factors: so far unknown for counting process (their existence suggested by)

3.4. CONTROL OF XIST EXPRESSION BY PLURIPOTENCY FACTORS

- pluripotency factors: help maintain the pluripotent state of ES cells and other pluripotent cells

- e.g.: Oct4, Sox2, Nanog, Lhx1 ← txn factors that activate the expression of the network of genes in the pluripotency circuitry

- mechanism directly repress expression of Xist (bind in its first intron)

activate Tsix by directly activating Dpxas34 and Xite

directly repress Rnf12

- upon differentiation, pluripotency factors are reduced, relieving the silencing of Xist

3.5. STAGES OF X INACTIVATION - CHOICE OF WHICH X TO INACTIVATE

- very little is known
- transient pairing of X chromosomes: may allow exchange of information to allow the choice (exchange of bound factors)
 - via XIC (X Inactivation Centre) and surrounding regions
 - followed by Xist transcript stabilisation on a single X chr
 - pairing is critical for counting and choice: if X made to pair w/ autosome instead (by adding many XIC copies to autosome), Xist upregulation fails
- skewed choice & inactivation
 - 1972, Bruce Cataniah
 - genetic differences in XCE (X-chromosome Controlling Element - region near to XIC) result in skewed X inactivation
 - studied in mice ↑, but occurs also in humans ↓
 - Rett syndrome
 - X-linked neurodevelopmental disease
 - caused by mutations in methyl binding domain protein MeCP2
 - lethal in males before birth
 - heterozygous females survive
 - Rett syndrome phenotype variable on specific mutations
 - imprinted X chr inactivation (e.g. placenta)
 - Tsix plays a role in mutations in Tsix
 - applies universally to all X-linked phenotypes (calico cats, diseases,...)
 - skewing usually 50:50
can be up to 5:95/95:5
 - X:X^{mut} e.g. 80:20 → less severe disease
20:80 → more severe disease
 - disease severity depends also on where the clonal patches are in the affected organs (e.g. brain)

3.6. STAGES OF X INACTIVATION - INITIATION AND SPREADING OF SILENCING

- Xist required by initiation: the chromosome must be coated by Xist
 - initiation initially Xist dependent and reversible
 - becomes fired and more irreversible over developmental time
 - plays only minor role in maintenance: turning off Xist expression will not immediately reactivate the chromosome
 - only limited number of cell types capable of initiating X inactivation ⇒ other factors are involved (e.g. SATB1, SATB2: may enable nuclear reorganization and silencing)
- mechanism
 - silent nuclear compartment occurs before gene silencing (devoid of RNA Pol II)
 - chromosome being silenced drawn into the silent nuclear compartment
 - Xist coats the chromosome, SATB1 & SATB2 bind the chromosome
 - repetitive elements present on the chromosome are silenced first
 - genes are silenced by pulling them into silent nuclear compartment
 - some genes escape silencing (reside outside of silent nuclear compartment): number differs b/w species
 - pseudo-autosomal regions: shared w/ Y chr, allow X-Y or X-X pairing
 - non-pseudo-autosomal escapee genes: double dose required in female organism?

3.7. STAGES OF X INACTIVATION - ESTABLISHMENT OF SILENCING

- fuzzy distinction b/w spreading and establishment of silencing
- Xist
 - PRC2 → add H3K27me
 - PRC1 → add H2AK119ub
- epigenetic marks
 - repressive (added): H2AK119ub, H3K27me3, H3K9me2
 - active (removed): H3ac, H4ac, H3K4me

see ch. 15, pg. 12

3.8. STAGES OF X INACTIVATION - MAINTENANCE OF SILENCING (DNMT1)

- imprinted (e.g. placenta) and random (e.g. somatic cells) X inactivation have variations in requirements for maintenance
- progressive layering of redundant mitotically heritable epigenetic marks to lock in epigenetic silencing
- factors: e.g. Dnmt1, Smchd1 (if removed, previously inactivated X chr. will reactivate)
- Dnmt1: maintenance methyltransferase (recognizes hemimethylated DNA - produced by DNA replication during cell division)
 - DNA methylation one of the final steps of X chr inactivation
 - locks in the silent state

- experiment

embryonic day	tissue	X ^{lacZ} X _m , Dnmt1 ^{-/-}	X ^{lacZ} X _m , Dnmt1 ^{+/+}
8.5	embryo	50% blue cells (random XCI)	50% blue cells (random XCI)
	placenta	0% blue cells (imprinted XCI, X _p silent)	0% blue cells (imprinted XCI, X _p silent)
9.5	embryo	100% blue cells (failed random XCI)	50% blue cells (random XCI)
	placenta	0% blue cells (imprinted XCI, X _p silent)	0% blue cells (imprinted XCI, X _p silent)

- embryonic day 5.5: initiation of random XCI (in mice)

- random XCI established

- maintenance of random XCI failed due to lack of Dnmt1

- maintenance of imprinted XCI does not rely on DNA methylation

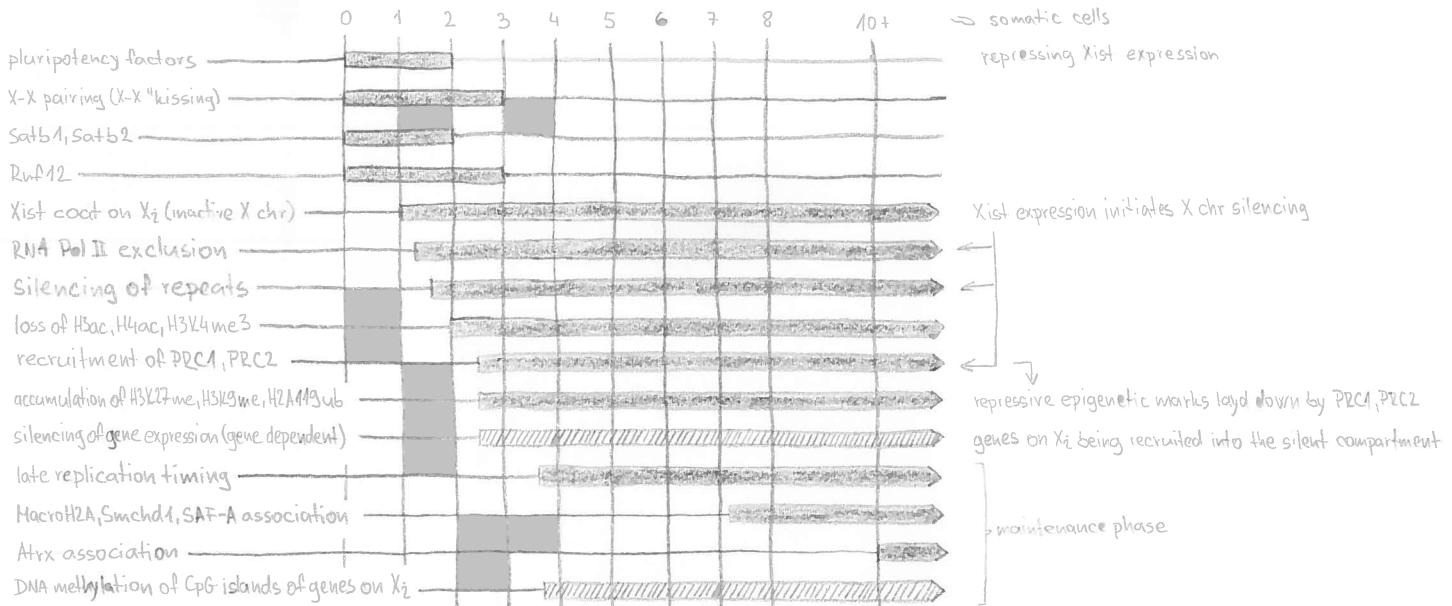
3.9. STAGES OF X INACTIVATION - MAINTENANCE OF SILENCING (Smchd1)

- Smchd1 bound to inactive X chr at E13.5 (embryonic day 13.5), i.e. well into maintenance phase ← compare w/ E8, E9.5 for Dnmt1
 - Smchd1^{-/-}: females die at ~E11 (~4-5 days after initiation of X inactivation at E5.5)
 - both random and imprinted X inactivation are abnormal
 - Xist expression & PRC2 recruitment is normal
 - very little DNA methylation on "inactive" X chr
 - failure of trn silencing on "inactive" X chr
 - experiment
 - mouse embryos → Smchd1^{-/-} (lacking Smchd1, homozygote)
 - Smchd1^{+/+} (wild type control)
 - | | |
|----------------|---------------------------------|
| X _m | X _p , X _m |
|----------------|---------------------------------|
 - lacking Xist on maternal X chr, paternal chromosome Xist intact → "random" X inactivation always inactivates paternal X chr
 - paternal -X-linked GFP (green fluorescent protein)
 - results
 - Smchd1^{-/-}: Both embryo and placenta green
 - Smchd1^{+/+}: Both embryo and placenta normal (not green)

⇒ Smchd1 affects silencing in both embryo (random) and placenta (imprinted)
 - not really a formal proof of ↑, because there is no evidence that X chr has been ever inactivated in the first place (there may be nothing to maintain by Smchd1) - in order to provide that evidence, ↑ experiment is needed
 - experiment
 - mouse embryonic fibroblasts (MEFs) from E13.5 X_m^{-/-} X_p^{+/+} Smchd1^{+/+} embryos (X_p silenced, maintenance phase of XCI)
 - microRNAs against Dnmt1, Smchd1, MacroH2A (see ch 24, pg. 2.2)
 - 5-azacytidine: Dnmt1 inhibitor
 - results:

5-azacytidine	+	+	+	+
Dnmt1 microRNA	-	+	-	-
Smchd1 microRNA	-	-	+	-
MacroH2A microRNA	-	-	-	+
green (not all, only a few)	-	+	+	+
- aberrant inactivation will result green:
- embryo: X_m^{-/-}
- placenta: imprinted X inactivation targets X_p
- no evidence of reactivation ↗ provided here
- Maintenance of XCI is very stable: there are still only a small proportion of cells w/ reactivated X chr
- there are several redundant layers of epigenetic marks ensuring heritable epigenetic silencing

3.10. X CHROMOSOME INACTIVATION SUMMARY



- replication timing
 - temporal segregation of replication for euchromatin and heterochromatin (euchromatin replicated early, heterochromatin late in S phase)
 - likely relates to ability to transfer epigenetic marks onto newly formed chromatin
- screening for new epigenetic modifiers involved in XCI
 - fluorescently tag expression from each of X chrs (one red, one green)
 - use microRNAs against various targets to reduce their expression
 - study green/red output in absence of ↑
- XCI (X Chromosome Inactivation) summary
 - initiated by Xist long non-coding RNA expression
 - coats and silences in cis (the chromosome it has been synthesized from)
 - X chrs pairing: influences choice of X chr to be silenced
 - Xist creates silent nuclear compartment via nuclear reorganization
 - Xist recruits various epigenetic modifiers, histone variants and chromatin proteins
 - results progressive accumulation of repressive epigenetic marks on X_i (inactive X chromosome)
 - locks in the inactive state

Smchd1:
Structural Maintenance
Of Chromosomes
flexible Hinge Domain
containing 1

3.11. DOSAGE COMPENSATION IN FLIES AND WORMS, COMPARED w/ MAMMALS

- sex chromosomes in different species

- mammals male: $X \uparrow Y$ (upregulated X)

 [female: $X \uparrow X \downarrow$ (one X upregulated, one X downregulated)]

- worms male: X

 [hermaphrodite: $X \downarrow X \downarrow$ (both downregulated by 50%)]

flies male: $X \uparrow Y$ (upregulated X)

 [female: XX]

upregulation of active X in mammals:

- still very controversial

- theory is that upregulation is needed to match expression of autosomes, which come in pairs

why both up and down?

- we do not know

- worms (downregulation of both Xs by 1/2)

- involves SMC proteins (condensin or condensin-like proteins normally involved in chromatin condensation during mitosis)

- Smchd1 (see ch. 3.9, pg. 33) involved in mammalian dosage compensation is SMC protein

- not core SMC protein, but has hinge domain

- flies (male X upregulation)

- involves long noncoding RNAs (roX1, roX2)

 [histone acetyltransferases]

 [RNA/DNA helicase]

 [creation of ACTIVE nuclear compartment (enriched in transcription factories)]

see ch 29, pg 26

↑
contrast w/ SILENT nuclear compartment in mammalian XCI

- comparison w/ mammals

 upregulation (controversial): active X chr in humans associated w/ long noncoding RNA (XACT)

3.12. LESSONS FROM THE FLY

- position effect variegation (PEV)

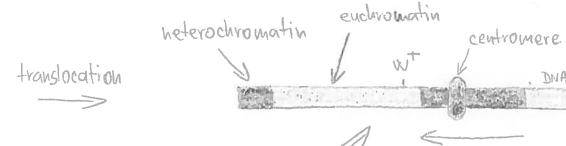
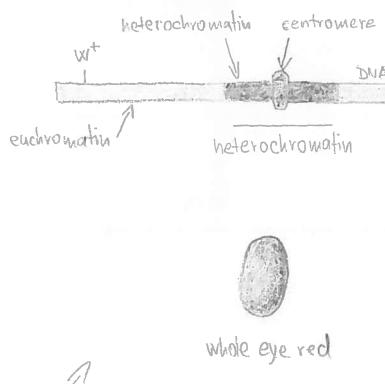
 - position of a gene relative to heterochromatin alters expression of the gene

 - variegated expression: mosaic expression of a gene (within the same tissue, sometimes on and sometimes off)

- fly: eye color phenotype

 - white gene (wt) codes for red pigment ← "white", because mutations in it may fail to produce the red pigment, turning eyes white (historical name)

in some cells heterochromatin manages to silence the gene, in others it doesn't



heterochromatin manages to silence the white gene in some cells, but not quite in all of them, producing red patches

epigenetic effect

- heterochromatin spreading

 - common feature of heterochromatin in all organisms

 - relevant to all heterochromatin (not just pericentromeric heterochromatin)

 - occurs in
 [centromeres]
 [telomeres]
 [repetitive elements]
 ...

 - limited by boundary elements

- mutagenesis screen: looking for mutations that alter eye color phenotype (likely to be important for epigenetic control)

 - Suvar (suppressors of variegation) - eyes more red: H3K9 methyltransferases, HDACs, chromobox protein H1 (binds to H3K9)

 - Evers (enhancers of variegation) - eyes less red:

 - much of what we know about epigenetic control in mammals comes from fly

- experiments in mice

 - GFP (green fluorescent protein) transgene directed to expressed in red blood cells

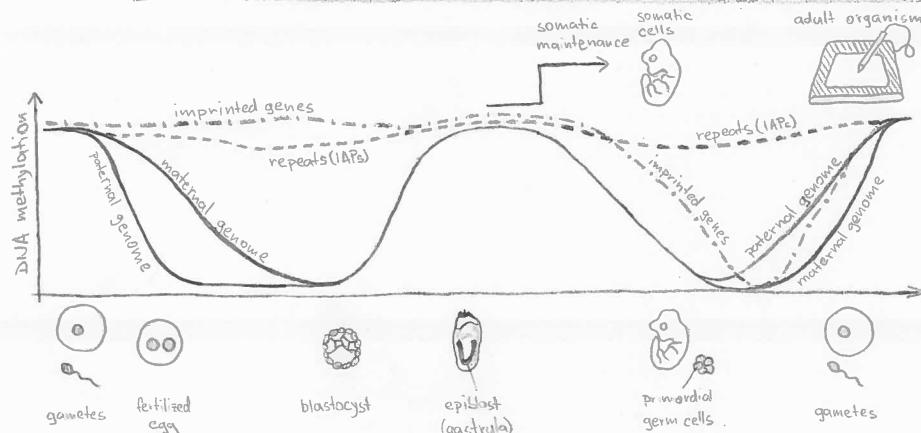
 [known] fly homologs

 [novel proteins]

 - unique to higher organisms

 - involved in higher-organism-specific processes (XCI)

4.1 INTRODUCTION TO EPIGENETIC REPROGRAMMING OF THE MATERNAL AND PATERNAL GENOMES



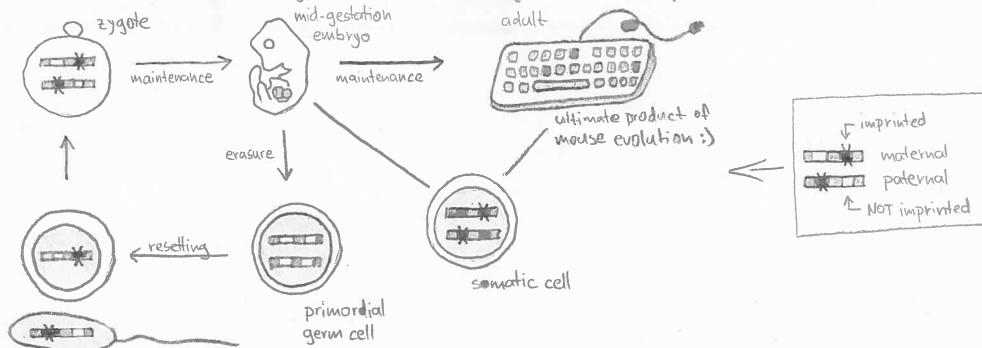
- DNA methylation
 - pre-implantation period
 - paternal genome: rapid and active demethylation (TET proteins)
 - indirect via hydroxylation → C-C bond is very stable and hard to break
 - maternal genome: passive demethylation (DNMT1 excluded from nucleus → can not maintain DNA methylation over cell division)
 - primordial germ cell development: differential resetting in spermatogenesis and oogenesis

4.2. EPIGENETIC REPROGRAMMING OF THE MATERIAL AND PATERNAL GENES

- repeats (see the graph in previous chapter: -----)
 - kept methylated in general
 - brief windows in time ↑ slight demethylation
 - ↑ upregulation of piRNAs (involved in targeted remethylation of repeats - see ch. 2.6, pg. 2.3)
- imprinted genes (see the graph in previous chapter ---)
 - genomic (a.k.a. parental, gametic) imprinting: monoallelic expression (only one of the two alleles is expressed)
 - ↑ based on parent-of-origin of the allele
 - known to be critical for embryo viability (must have one set of chromosomes from father and one from mother)
 - expression of imprinted genes controlled by ICRs (Imprint Control Regions), a.k.a. DMRs (Differentially Methylated Regions), DMDs (Differentially Methylated Domains)
 - imprint associated w/ DNA methylation at the ICR ↑
 - consequences (gene expression vs. silencing) of imprinting (methylation of ICR) differ by locus
 - depends on mechanism
 - ↑ long noncoding RNAs
 - ↑ enhancer/insulator blocking (see MIT + 7.28.2x, pg. 7.1)
 - ICR methylation established in primordial germ cells ← parent-of-origin specific marks
 - ↑ maintained in embryo

contrasts DNA methylation of imprinted genes vs. rest of paternal/maternal genome
(see graph at the top of this page)

- maternal and paternal imprinting erasure in primordial germ cells development

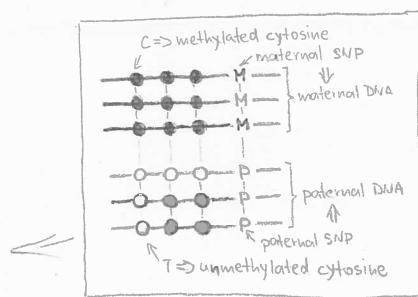
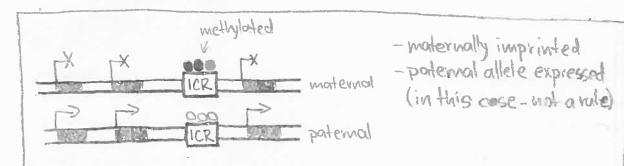
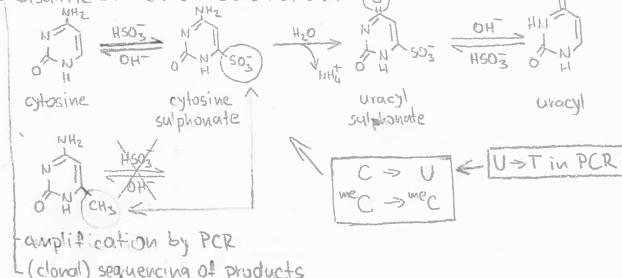


4.3. LOCATION OF IMPRINTED GENES IN THE GENOME AND BISULFITE SEQUENCING

- genomic imprinting
 - ~150 genes imprinted in humans
 - imprinted genes exist in clusters (rather than in isolation)
 - each cluster has its own ICR (Imprint Control Region)
 - tissue specific (unlike X inactivation) - despite presence of ICR methylation
 - imprinted gene expression common in placenta
 - ↑ brain

- bisulfite sequencing: method for measurement of DNA methylation

- mechanism: bisulfite chemical conversion of DNA

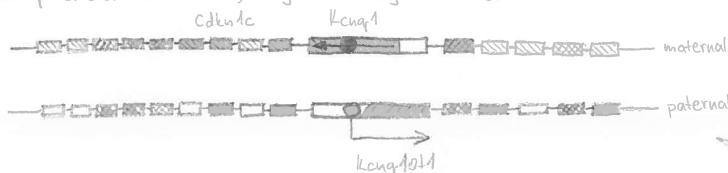


4.4. Kcnq1 AND H19/IGF2 MECHANISMS OF ACTION AND BECKWITH WIEDEMANN SYNDROME

- mechanism of action of ICRs: varies w/ each cluster ↙ ICR methylation does not necessarily imply cluster silencing !

- examples
 - Kcnq1 cluster, human chr 11: long noncoding RNA
 - H19/Igf2 cluster, human chr 11: enhancer blocking
 - Snrpn cluster, human chr 7: long noncoding RNA

- Kcnq1 cluster: controlled by long noncoding RNA in cis



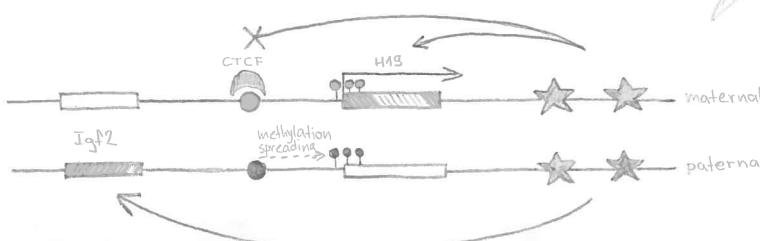
maternal allele

- methylated ICR suppresses expression of Kcnq1ot1 lncRNA
- imprinted genes maternally expressed
- paternal allele
- no methylation ICR → expression of Kcnq1ot1 lncRNA
- Kcnq1ot1 → G3a → add H3K9me
- PRC2 → add H3K27me

works similar to Xist (silences in cis)

- Cdkn1c: cycle independent kinase inhibitor
cell cycle regulator, involved in growth restriction (tumor suppressor)

- H19/Igf2 cluster: enhancer blocking



- H19 has nothing to do w/ imprinting of this cluster ← unlike Kcnq1ot1, Xist
reservoir of microRNA

- maternal allele
 - unmethylated ICR → CTCF (insulator protein)
 - prevents preferred DNA looping, where enhancers promote expression of Igf2 (growth promoting, oncogene)
 - forces less preferred DNA looping, where enhancers promote expression of H19 lncRNA
 - prevents spreading of DNA methylation to H19 promoter region
- paternal allele
 - methylated ICR → CTCF → opposite effect

- Beckwith-Wiedemann syndrome (opposite of Silver-Russell syndrome)

- imprinted disorder, results from various abnormalities in linked Kcnq1 and H19/Igf2 clusters at 11q15.5 (1Mb)

loss of Cdkn1c (tumor suppressor, growth suppressing)
↑ upregulation of Igf2 (oncogene, growth promoting)

- caused by maternal allele behaving like paternal allele

- mutation/deletion to cause loss of imprinting
- UPD (Uniparental Disomy): two copies of one parental chromosome (here paternal UPD)
- epigenetic disruption for loss of imprinting (rare)

- symptoms

- fetal and post-natal overgrowth
- macroglossia ("large tongue")
- predisposition to embryonic/childhood tumors, but NOT adult tumors (e.g. Wilms tumor - kidney)
- many imprinted genes are involved in growth (loss of imprinting is common feature of cancer)

Angelman syndrome

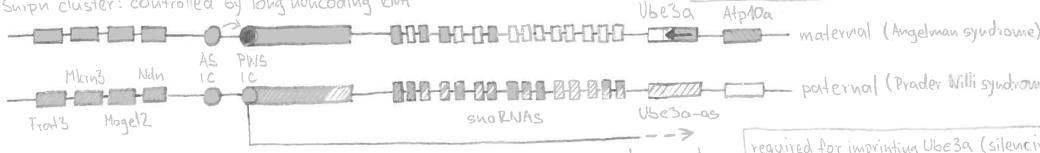
- brain growth retardation (microcephaly)
- severe mental retardation
- characteristic puppet-like jerky arm movement
- seizures
- happy dispositions, inappropriate bouts of laughter

Prader-Willi syndrome

- low muscle tone, failure to thrive as infants
- small stature
- hypogonadism
- OCD behaviour (overeating → obesity later in childhood)

4.5 SURPN ICR MECHANISM, PRADER WILLI AND ANGELMAN SYNDROMES

- Surpn cluster: controlled by long noncoding RNA



- split imprint centre

- AS-IC (Angelman Syndrome Imprint Centre)
- unmethylated on both chrs
- required to set up methylation of PWS IC during oogenesis

PWS-IC (Prader Willi imprint centre)

- Angelman syndrome: failure to express Ube3a
- deletion/inappropriate silencing of MATERNAL allele
- deletion of AS-IC → PWS-IC left unmethylated → Ube3a silenced
- paternal uniparental disomy
- specific mutation in UBE3A
- failure to methylate PWS-IC

maternally transmitted

paternally transmitted

- Prader-Willi syndrome

- deletion/inappropriate silencing of PATERNAL allele
- no expression of paternally expressed genes (Fnt3, Mkrn3, Magel2, Ndu)

- SNORDAs: role in determining brain phenotype

- deletion of PWS-IC leads to maternal-like allele (Surpn/Surpn start site removed)

4.6. SUMMARY OF EPIGENETIC REPROGRAMMING AND IMPRINTING

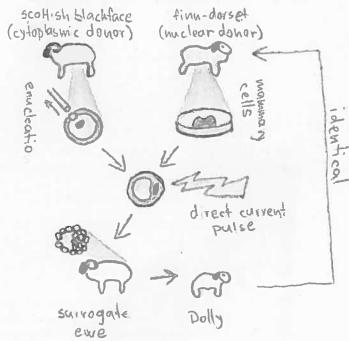
- epigenetic reprogramming
 └ germ cells
 └ early development (pre-implantation)
 + differentiation

5.1. DISRUPTED EPIGENETIC REPROGRAMMING IN ASSISTED REPRODUCTIVE TECHNOLOGIES (ART)

- sensitive periods of exposure [early embryonic development | germ cell development] during epigenetic reprogramming
 - requirement for mitotic heritability of epigenetic changes induced by environment
 - transgenerational epigenetic inheritance through gametes: **CONTROVERSIAL!**
 - [many mouse studies, few human]
 - [diet, maternal care, chemical exposure]
 - [disruption by scientific/medical intervention (assisted reproductive technologies, cloning, somatic cell reprogramming)]
 - open questions
 - [what proportion of genome is sensitive to environment?]
 - [what proportion of humans are sensitive to environmental disruption?]
 - [what proportion of changes are meiotically or mitotically heritable?]
 - disrupted epigenetic reprogramming in assisted reproductive technologies
 - [IVF (In Vitro Fertilization) | induce production of oocytes and harvest them]
 - dish oocytes w/ sperm
 - culture until blastocyst stage
 - implant back into mother
 - [ICSI (Intracytoplasmic Sperm Injection) | oocyte held by holding needle]
 - sperm injected directly into the egg
 - culture on dish until blastocyst stage
 - implant back into mother
- possible reasons
- [oocytes harvested at time of epigenetic reprogramming]
 - [embryos cultured in vitro during epigenetic reprogramming]
 - [underlying problem associated w/ need of ART]
- higher incidence of Beckwith-Wiedemann and Angelman syndromes
- maternally transmitted imprinting disorders
 - especially in ICSI (but also in IVF)
 - still very rare: e.g. 3-5 fold increase from 1/300.000
 - also broader epigenetic abnormalities, not necessarily disease-specific
- epigenetic abnormalities present also in animal ART, even without underlying fertility problem
⇒ there likely is a procedural issue in ART

5.2. DISRUPTED EPIGENETIC REPROGRAMMING IN SOMATIC CELL REPROGRAMMING AND CLONING

- somatic cell nuclear transfer for cloning

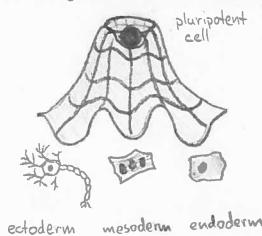


- extremely low efficiency: few % of live births
- large offspring syndrome (placental and foetal overgrowth)
 - imprinting defect due to disrupted epigenetic reprogramming?
 - imprinted genes contribute to growth (especially placenta)
 - pre-mordial germ cell development skipped [ICR not reset]
 - germ cell genome packaging not achieved
- ICR methylation eroded during early development, lack of maternal effect proteins binding ICRs?
- "successful" clones: genome-wide tm differences indicative of epigenetic abnormalities
- compounds altering epigenetic state (e.g. inhibit epigenetic machinery) can increase efficiency of cloning

patient specific, addresses ethical issues (iPS do not come from, nor can produce embryo)
can not make placenta

- somatic cell reprogramming

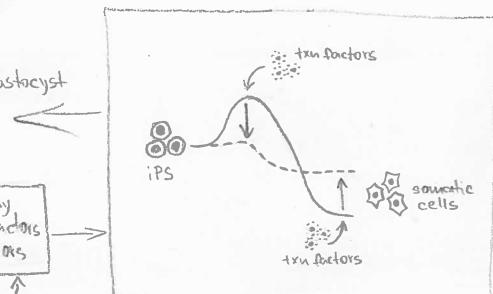
- somatic cell → iPS (induced pluripotent stem cell) → differentiate to produce particular tissue/organ
- epigenetic landscape (Conrad Waddington, 1942)



- to reprogram a somatic cell, need to "climb up the hill": somatic cell → blastocyst
 - remove lineage-specific epigenetic marks
 - restore pluripotent epigenetic marks
 - restore pluripotent chromatin state (much more open)
 - retain imprints at ICR
 - remove X inactivation (females)
 - extremely difficult (efficiency: few %)
 - some lineages more difficult than others

can be improved by adding epigenetic factors (on top of trn factors)

$<1\% \rightarrow \sim 5\%$



5.3. INTRODUCTION TO TRANSGENERATIONAL EPIGENETIC INHERITANCE, EFFECTS OF ENVIRONMENT AND SENSITIVE PERIODS IN EPIGENETIC CONTROL

- non-mammalian examples
 - sex determination in turtles: temperature dependent
 - flowering of some flowers requires vernalisation (cold during winter)
 - diet dependent bee development: royal jelly causes difference b/w workers and queens

- environmental effects on epigenetic control in mammals

- sensitive periods: pre-implantation and early post-implantation period, pre-embryonic germ cell development
- brief time-windows for individual organs, later in embryo development (during development of particular organ)

- transgenerational epigenetic inheritance

- inheritance of -phenotypes
gene expression patterns

- through germ cells !!!

- not explained by genetic differences!!!

- possible proposed mechanisms for passing epigenetic marks

- incomplete epigenetic reprogramming?

- messenger molecule transmitted in gametes causing

- altered re-establishment of epigenetic marks?

distinction from effects NOT transmitted via gametes

- e.g. altered placenta

- mothering style

- newborn nutrition via milk

5.4. DUTCH FAMINE, HUMAN EPIDEMIOLOGICAL STUDIES AND THE DEVELOPMENTAL ORIGINS OF ADULT HEALTH AND DISEASE

- Dutch Famine / Dutch Hunger Winter (1944-1945)

- birth cohort study
- exposure to famine during peri-conceptional period (before and shortly after conception)
- increased mental and metabolic disorders
 - altered glucose tolerance \rightarrow diabetes
 - obesity
 - cardio-vascular disease
- subtle changes in DNA methylation of a small number of genes
 - { IGF2
GNAS
MEG ICR } imprinted genes
- no such problems in children of SAME PARENTS conceived outside of famine periods
- famine later in gestation
- however, oligos w/ sensitive periods (gametogenesis, pre-implantation period) !
- original data: suggested transgenerational effect
- subsequent studies (better controlled): suggests NO transgenerational effect !!!
- Barker hypothesis (CONTROVERSIAL!!!)
 - used to explain extensive and permanent effects of in-utero/neonatal environment on adult health/disease
 - e.g. under-nutrition: correlates w/ obesity, type II diabetes, CVD in adults
 - "thrifty phenotype": remembers scarce food supply & tries to store energy for the case of repeating such low-food period
 - proposed to have epigenetic basis

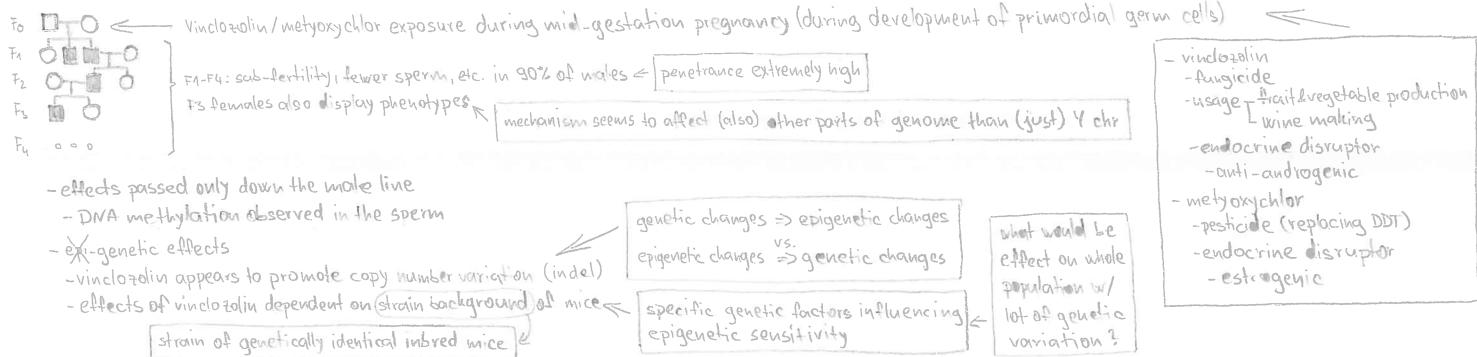
5.5 HUMAN EPIDEMIOLOGICAL STUDIES ON THE ÖVERKALIX COHORT, GRANDPARENTAL EFFECTS AND POSSIBILITY OF TRANSGENERATIONAL EPIGENETIC INHERITANCE IN HUMANS

- Överkalix: isolated region/town (?) in northern Sweden
- frequently subjected to famine
- good historical records (of harvest and food prices \Rightarrow periods of famine)
- studies: INCREASED grandparental food \rightarrow decreased grandchild longevity
 - sensitive period: "slow growth period" (9-12 yo in grandfathers, 8-10 yo in grandmothers)
 - grandparental effects
 - sex specific: paternal grandfather \rightarrow grandson, PATERNAL grandmother \rightarrow grand daughter
 - complicated (confounded by sex specific effects)
 - not seen in all cohorts
 - no current replica data (ongoing e.g. in Britain) or molecular data
 - may explain grandMATERIAL effect but not grandpaternal
 - grandpaternal: - Y chr? - genetic?
- grandmothers: also fetal/infant life
- CONTROVERSIAL
 - not enough data
 - consider exposure of germ cells: pregnant mother \rightarrow fetus \rightarrow germ cells developing in fetus
- studies: prepubertal smoking
 - slow-growth period
 - increased BMI in sons
 - discussion: germ cell epigenetic marks (Y chr!) potentially compromised by exposure
 - smoking has mutagenic effect \leftarrow complicates things !!!
- 3 generations influenced in the same time !
- Summary
 - transgenerational epigenetic inheritance possible (but not proven)
 - diff. cult to study
 - important: may have very large impact

6.1. MOUSE AND RAT STUDIES ON PATERNAL EFFECTS OF CHEMICAL EXPOSURE.

EFFECTS OF MATERNAL BEHAVIOUR ON EPIGENETIC MAKEUP

- paternal effects due to environment



- effects passed only down the male line

- DNA methylation observed in the sperm

- ex-*genetic* effects

- vinclozolin appears to promote copy number variation (indel)

- effects of vinclozolin dependent on strain background of mice

strain of genetically identical inbred mice

genetic changes → epigenetic changes
vs.
epigenetic changes → genetic changes

specific genetic factors influencing epigenetic sensitivity

what would be effect on whole population w/
lot of genetic variation?

- maternal behaviour

✓ life-long alterations in the nature of stress response

mother licks pups → less "stressed" adults : decreased epigenetic silencing at glucocorticoid receptor (hippocampus)

mother does NOT lick pups → more "stressed" adults : increased

- cross-fostering (change pups and mothers) confirms there is no genetic influence

- overall considerations

- what proportion of genome is sensitive?

- what proportion of population is genetically susceptible

- what is the role of ex-*genetic* alterations? (e.g. exposure to mutagenic substances)

- what proportion of changes can be transgenerationally heritable?

6.2. TRANSGENERATIONAL EPIGENETIC INHERITANCE VIA THE GAMETES

plants: *Linaria vulgaris* heritable epimutation

- two flower types: originally thought to be two different species

wild-type: bilateral symmetry

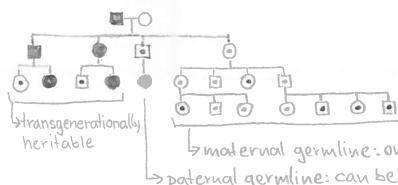
- no ex-*genetic* difference

peloric mutant: radial symmetry

- epigenetic cause (1993): heritable DNA methylation at Lcyc

epigenetic reprogramming in plants is different than the one described for mammals

mammals: first observed for transgenes (more often for those derived from "foreign" DNA, bacterial sequences)



F	M
□	wild-type
●	active transgene
○	silent transgene

Q: Does the behaviour depend on exact position of imprinted transgene?
- e.g. close to a maternally imprinted region → methylated → silenced

contrast w/ imprinted genes: reset in each generation (not transgenerational)

- experiments need controls to establish epigenetic heritability via gametes

- rule out other possible mechanisms for epigenetic modifications during embryogenesis

- when is phenotype established?

- effect of uterine environment

- cross-fostering if phenotype is established late

best addressed w/ mouse/rat studies
(usually impossible in humans)

- importance of discrimination ↑

- consequences for interpretation of genetic information and heredity
analysis of mechanism

6.3. THE AGOUTI VIABLE YELLOW ALLELE IN MICE

- agouti: type of fur coloration in which each hair displays alternating bands of dark and light pigmentation

- agouti viable yellow allele (A^y)

- caused by IAP (Intracisternal A-Particle) insertion

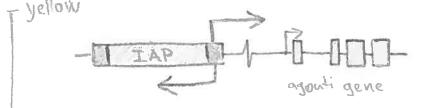
- sensitive to epigenetic state

yellow

IAP elements: family of retrovirus-like elements

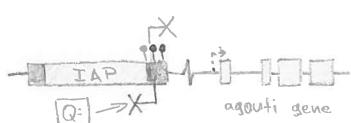
- code for virus-like particles (IAPs) found regularly in rodent-early embryos

- transcribed under certain circumstances (DNA hypomethylation)



- constitutive agouti yellow coat
obesity
type II diabetes

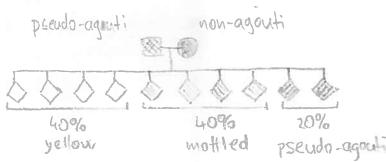
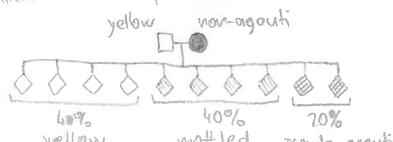
- pseudoagouti



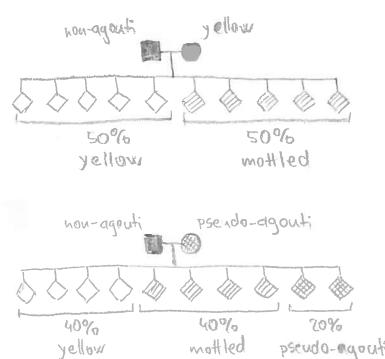
mottled (patches of yellow/dark)

- mosaic expression results in variegated, mottled coat colour
- relates to expression state during gastrulation (cf. calico cats)

A^y allele inheritance patterns



paternal inheritance



maternal inheritance

- some memory of phenotype & epigenotype
- only from mother, not from father

- possible explanations
 - intruterine environment
 - different behaviour of mother (mothering style)
- ruled out by IVF (transferring zygote to non-agouti mother: still produces 50% yellow and 50% mottled pups)
- differences in oogenesis
- ruled out by (not in scope of this course)
- inheritance through gametes

6.4. ENVIRONMENTAL EFFECTS ON THE AGOUTI VIABLE YELLOW ALLELE

- altered maternal diet: during sensitive period (E0.5-E8.5), preconception (alteration to oogenesis?)
control (no diet alteration)

- 40%/40%/20% (yellow/mottled/pseudo-agouti)

- methyl donors (folate, B12, choline, betaine/?ethanol?) / genistein (soy protein)

- 0%/30%/70% (yellow/mottled/pseudo-agouti) \Rightarrow shift towards MORE methylated DNA

- folate, B12, choline, betaine and ethanol (?) act as methyl donors

- increase SAM (S-Adenosyl Methionine) - donor of methyl group in DNA methylation

- genistein is a phyto-oestrogen (plant-derived xenoestrogen)

- alters DNA methylation in all germ layers

- early embryonic effect

- effects independent of SAM \Rightarrow does not act via alteration of methyl donor availability (!)

BPA (Bisphenol-A): anti-androgenic (endocrine disruptor)

- 60%/30%/10% (yellow/mottled/pseudo-agouti) \Rightarrow shift towards LESS methylated DNA

- BPA found in many polycarbonate plastics

thermo-paper

- effects on human health controversial

- effects can be counteracted with methyl donors or genistein (!)

- diet during pregnancy can alter epigenetic makeup and adult health

- due to mitotic heritability of epigenetic state

- mechanisms: availability of methyl donors (SAM)

alteration of endocrine factors

other unknown mechanisms (e.g. genistein)

- effect of folic acid on humans

- prevents spina bifida (proven)

- other possible effects?

6.5. THE AXIN FUSED ALLELE IN MICE AND METASTABLE EPIALLELES

- axin fused allele ($Axin^{fu}$)

- caused by IAP insertion (see ch.6.3, pg. 6.2)

- sensitive to epigenetic state

- kinky tail (penetrant)



- two kinds of Axin
 - wild-type
 - truncated transcript (exon 7 onwards)

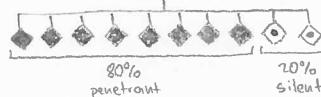
straight tail (silent)



- only wild-type Axin

- $Axin^{fu}$ allele inheritance patterns

penetrant unaffected

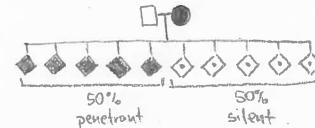


penetrant unaffected

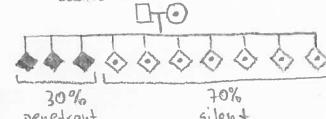


paternal inheritance

unaffected penetrant



unaffected silent



maternal inheritance

- paternal transgenerational inheritance observed
 - \Rightarrow intrauterine environment
 - \Rightarrow mothering style
 - \Rightarrow oogenesis

} do NOT play a role

- humans

- monozygotic twins

- very few DNA methylation differences

- observed differences subtle

- potential metastable epialleles (very controversial)

- searching for hypervariable b/w individuals

} similar b/w tissues (of the same individual) characteristics observed in mice

- difficult to prove transgenerational epigenetic inheritance in humans \leftarrow requires environment controls that are (ethically) impossible in humans

- example: caudal duplication anomaly

- monozygotic twins \Rightarrow genetically identical

- DNA methylation difference (Axin gene)

- only one of the twins affected

6.6 POTENTIAL MECHANISMS OF TRANSGENERATIONAL EPIGENETIC INHERITANCE:

INCOMPLETE EPIGENETIC REPROGRAMMING

- epigenetic reprogramming

↳ germ cells: both sperm and oocytes escape demethylation

early development: both paternal and maternal genomes demethylated as usual

DNA methylation is UNLIKELY to be the inherited epigenetic mark

- histone mark?

- not feasible to measure single alleles in small cell samples

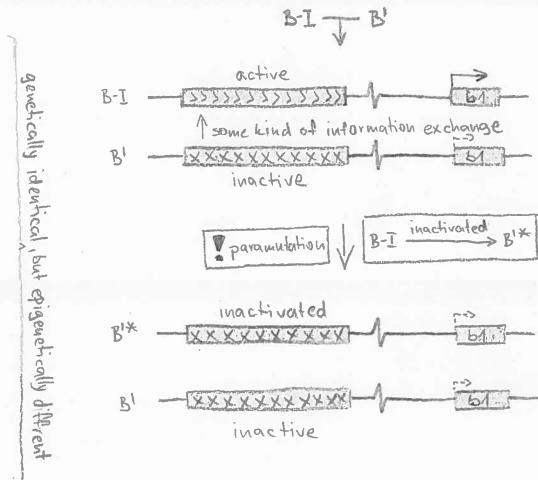
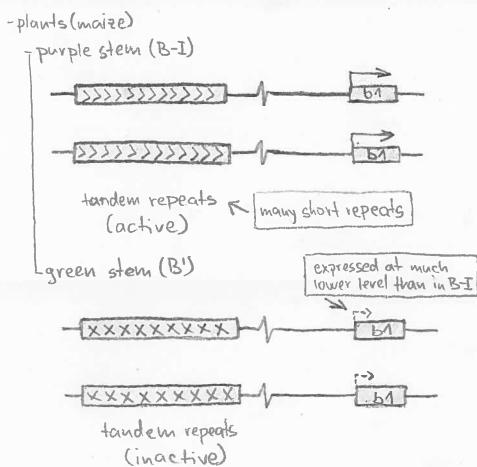
- histone to protamine exchange in spermatogenesis \leftarrow it has been thought that there are no histones in sperm (replaced by protamines)

- RNA carrying the mark and causing re-establishment of DNA methylation?

✓ Axin^{fu} inherited also paternally

recent work shows that many histones remain

6.7. PARAMUTATION IN PLANTS AND PARAMUTATION-LIKE EFFECTS IN MICE



- mice: Kit^{tm1Afl}

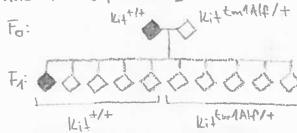
- caused by transgene insertion
- decrease in Kit mRNA (messenger RNA) → less Kit protein
- missing pigmentation (melanocytes) at belly, tail, paws, nose, ...

Kit protein

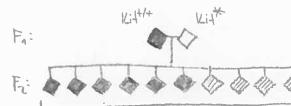
- responsible for melanocyte (pigment-producing cell) migration during development
- melanocytes start at spinal cord, then "wrap around" body of the animal

distant parts of the body (melanocyte migration-wise)

- inheritance patterns



✗-genetically identical to $Kit^{+/+}$ (wild-type)
- paramutation-like effect
- one allele alters the other one in trans
- DNA sequence NOT altered



◆ black coat (wild-type)
◇ white spotted
◆ partial white spotted

transgenerational epigenetic inheritance

- RNA mediator of paramutation-like effect?
- expression in ALL white spotted offspring
- somatic cells: less full-length Kit mRNA
- testis/mature sperm: HIGHER txn rates at Kit locus

experiment

- fertilized egg of black wt parents
- RNA extract from testis/somatic tissues of white spotted mice
- microinject the RNA extract to nucleus of the egg

results

- produces white spotted phenotype
- rarely gives the same outcome w/ RNA extract from black wt mice
- same outcome w/ miRNA targeting Kit (such miRNA NOT found in sperm/testis)
- control miRNA NOT targeting Kit does NOT produce white spotted phenotype

6.8. CONSTITUTIONAL EPIMUTATION IN HUMANS - NOT TRANSGENERATIONAL INHERITANCE

- Lynch syndrome: hereditary nonpolyposis colorectal cancer (HNPCC)

- mutations in mismatch repair genes (e.g. MLH3) → microsatellite instability (cause of HNPCC)

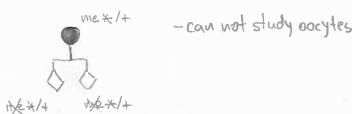
- some cases: NO mutations in mismatch repair genes

MLH3 CpG island DNA hypermethylation of one allele

- in ALL tissues examined
- MLH3 epimutations can run in families

Lynch syndrome occurs

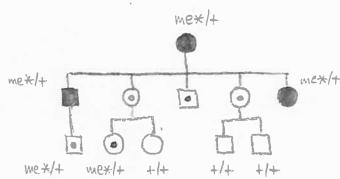
- inheritance patterns



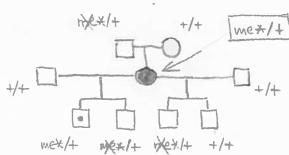
- sperm of the father
- 1/1 methylation of allele that carried methylation in the soma
- escaped demethylation during spermatogenesis?
- potentially consistent w/ transgenerational inheritance via sperm

fourth one has not inherited the allele (+/+) ↓

- transmission of epimutation to one of three sons
- incomplete penetrance
- affected son did not develop Lynch syndrome (yet?)
- sperm of affected son
- completely unmethylated
- better controls than w/ sperm of the father above
- e.g. purify only mature sperm (that can sire)



- 100% penetrant transmission of methylated allele, when that allele has been passed on
- found to be due to SNP in 5'UTR, which causes decreased expression of MLH3 and is associated w/ DNA methylation



controversial: potential evidence may be only due to measurement error

! no evidence found for transgenerational inheritance of epigenetic state via gametes in humans

7.1. OVERVIEW OF CANCER EPIGENETICS

- aberrations in epigenetic control seen in cancer
 - DNA methylation
 - histone modifications & histone variants
 - nuclear architecture
 - noncoding RNA

- clinical implications of aberrant epigenetic control
 - diagnosis: epigenetic alterations as biomarkers
 - prognosis: specific alterations associated w/ outcome
 - therapy: epigenetic modifier inhibitors

- hallmarks of cancer

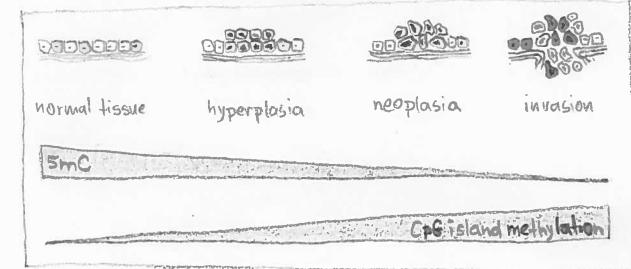
- evading growth suppressors
- sustaining proliferative signalling
- deregulating cellular energetics
- resisting cell death
- avoiding immune destruction
- inducing angiogenesis (formation of new blood vessels)
- activating invasion and metastasis
- tumor-promoting inflammation
- enabling replicative immortality
- genome instability and mutation

emerging hallmarks

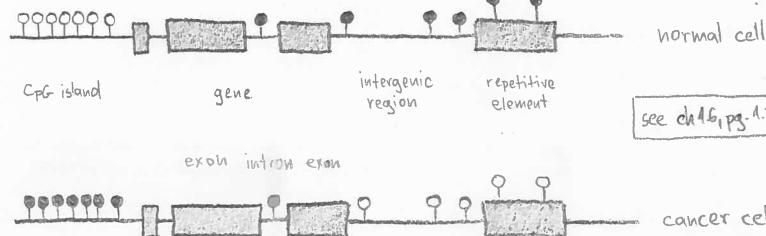
enabling characteristics

- cancer oversimplified

- activation of oncogenes
- inactivation of tumor suppressors
- mechanism [genetic] \rightarrow direction of causality?
- epigenetic \leftrightarrow between the two



7.2 HYPERMETHYLATION OF CPG ISLANDS IN CANCER



- CGI (CpG Island) hypermethylation \leftarrow often MORE frequent than ~~ex~~-mutations
- alternative to genetic mutation \rightarrow silences tumor suppressor genes in cancer
- occurs frequently in tumors: different CGIs in different tumor types
 - mitotically heritable (preserved in daughter cells)
 - selected for (cells w/ downregulated tumor suppressor or upregulated oncogene have "selective advantage")
 - can be one of the hits in the Knudson hypothesis (cancer as a result of multiple hits to DNA)
 - progresses w/ age of the tumor
 - is cancer "just" a result of aging?
 - age of an organism in general
- biomarker
 - distinguish b/w normal and cancer cell
 - identify specific feature of the cancer
 - DNA hypermethylation biomarkers preferred (hyper- vs. hypo- methylation \leftarrow easier to detect hypermethylation at specific loci)

DNA hypermethylation (locus specific)

- CpG islands & CpG island shores of tumor suppressor genes
- ICRs (see ch 4.2, 4.3, pg. 4.1): loss of imprinting

DNA hypomethylation

- repetitive regions
- CpG poor promoters
- ICRs: loss of imprinting

ch. 7.4, pg. 7.2



epimutations (unlike ~~ex~~-mutations) are reversible: therapeutic target

examples

- Rb: retinoblastoma
- MLH1: colorectal cancer (see ch 6.8, pg. 6.4)
- ERCC1: breast cancer
- MGMT: gliomas and colorectal tumors

7.3. HYPERMETHYLATION OF SETS OF CPG ISLANDS IN CANCER

- CIMP (CpG Island Methylator Phenotype): CpG islands of a set of genes methylated
- described for: colorectal cancer, gliomas (start in glial cells & non-neuronal cells nervous system), neuroblastoma...
- different cancer type \leftrightarrow different set of CpG islands
- CpG island shores (regions of ~2kb on both sides of CpG islands)
 - same behaviour as CpG islands themselves
 - normal cell: unmethylated
 - cancer cell: hypermethylated
 - better predictor than CpG islands themselves (?)
- loss of imprinting
 - common early event
 - both hyper-/hypo- methylation can cause loss of imprinting (?)
- example: Wilms' tumor (kidney tumor in children)
 - H19/Igf2 cluster (see ch 4.4., pg. 4.2)
 - hypermethylation of ICR \rightarrow Igf2 overexpression (maternal allele)

- diagnosis

- e.g. GSTP1 hyper-me in prostate cancer \Rightarrow malignancy
- prognosis
 - e.g. miR-34b/c hyper-me \Rightarrow metastasis
- treatment
 - e.g. MGMT hyper-me (glioma) \Rightarrow temozolamide chemotherapy (alkylating agent vs. inactivation of MGMT that repairs alkyl-guanine lesions)



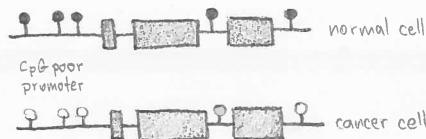
- diagnosis

- tumor identification
 - type
 - subtype \leftarrow more accurate than gene expression
 - primary tumor (when unknown)
- prognosis
 - tumor load/burden
 - hyper-me increases w/ tumorigenicity
 - different cancer (sub)types \Rightarrow different prognosis
- treatment
 - monitoring tumor decline/recurrence
 - cell-free DNA found in blood can be easily accessed & tested

7.4. HYPMETHYLATION GENOME-WIDE IN CANCER

see also ch.7.2, pg. 7.1

- historically, earliest epigenetic abnormality found
- occurs in all tumor types (to some degree)
- progresses w/ tumorigenicity (similar to CPG hypermethylation - see ch.7.3, pg.7.1)
- consequences: location dependent
 - repeats: genomic instability (see ch.1.6, pg. 1.2!)
 - CPG poor promoters
 - can result activation of genes



See also ch. 6.3, 6.5, pg. 6.2, 6.3

examples

- R-RAS gene activation → gastric cancer
- miR21 (miRNA targeting PTEN tumor suppressor gene) → glioma

evidence

- mouse models: Dnmt1 deletion → hypomethylation → increased genomic instability → embryo dies mid-gestation or results cancer when selectively turned off in adult individual
- human disease: DNMT3B mutation → ICF syndrome (immodeficiency and cranio-facial defects syndrome) characterised by genomic instability

human cancers

- role of DNA methylation: context dependent

- e.g. deletion of Dnmt1 can
 - suppress tumorigenesis: e.g. in tumor suppressor hypermethylation
 - enhance tumorigenesis: e.g. in chromosomal instability (e.g. hypomethylation of repetitive elements)
- stage-specific: early vs. late in tumorigenesis

7.5. ALTERED HISTONE MODIFICATIONS IN CANCER

- altered histone modifications/variants → can increase genome instability

- heterochromatin vs. euchromatin

- different histone variants

repair mechanisms: heterochromatin

- unwind
- inhibit tyro during repair
- repair (enzymes access DNA)
- re-compact

increased mutation rate in heterochromatin

less efficient than euchromatin

high/low grade: more/less aggressive

repeats (likely genome-wide)

normal

- more compacted DNA → silenced

cancer

- decreased [H4K16ac (active mark)]
- [H4K20me (inactive mark)]

- less compacted DNA → active

CpG islands

normal

- increased [H3K4me]
- [H3ac, H4ac]

- less compacted → active

cancer (hypermethylated CpG islands - see ch.7.2, 7.3, pg. 7.1)

increased [H3K23me]

decreased [H3K4me]

[H3ac, H4ac]

- more compacted DNA → silenced

other tumor-type specific alterations

- can be prognostic

- mutations in histones and histone variants

- e.g. childhood high grade glioma (not adult, not low grade)

H3.1 (canonical H3)

- [H3.3]

L27 → M

- can not be acetylated/methylated

- may mimic methylation?

H3.1 G34 → V

- no direct marks on G34, but

- appears to alter ability to methylate K36 (active mark) two residues downstream

- environmental contribution

- carcinogens

mutagenic

non-mutagenic: how do they act?

- some heavy metals: alter activity of histone modifier enzymes

- environment alters epigenotype → promotion of tumorigenesis?

- not sensitive period (e.g. adult organism): cancer can result from single cell w/ uncorrected error

7.6. LONG RANGE EPIGENETIC ALTERATIONS IN CANCER AND ALTERATIONS IN NUCLEAR ARCHITECTURE

- long-range epigenetic alterations: large regions (Mbs in length)

- LRES (Long Range Epigenetic Silencing): appears to be common

gain of repressive marks (in whole region)

normal: hypomethylated DNA

[hyperacetylated H3, H4]

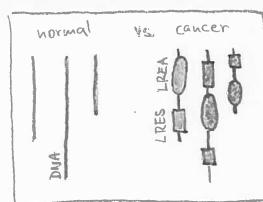
cancer: hypermethylated DNA

[hypoacetylated H3, H4]

H3K3me, H3K27me

vs ↑*

silenced



reasons why large regions are altered in sympathy

- nuclear

- nuclear architecture?

- consolidation of repressive marks (in whole region)

normal: hypomethylated DNA

[hypoacetylated H3, H4]

H3K3me

[hyper(?)-methylated DNA]

[hypoacetylated H3, H4]

H3K3me, H3K27me

silenced

stronger/weaker silencing

- exchange of repressive marks (in whole region)

normal: hypomethylated DNA

[hypoacetylated H3, H4]

H3K3me, H3K27me

silenced

silenced

different kind of silencing

silenced

silenced

! methylation: better biomarker than expression (more sensitive: subtle changes)

- nuclear chromatin organisation in cancer

- used for past 140 years (diagnosing cancer tissue)

- still today (by pathologists)

- nuclear size

[shape]

- ploidy (# of copies of genome)

- chromatin organisation

- fundamental differences b/w cancer vs normal tissue

- specific differences: NOT well defined

LREA (Long Range Epigenetic Activation): recently found

- related to LOCks (large Organised Chromatin lysine (K) altered regions)

7.7 ALTERED EXPRESSION OF PIRNAs AND LONG NONCODING RNAs IN CANCER

- noncoding RNAs

- miRNA (see ch. 2.5, pg. 2.3)

- globally misexpressed in cancer

- do NOT alter epigenetic state itself ← post-translational silencing

- piRNA (see ch. 2.6, pg. 2.3)

- piRNA expression deregulated in cancer

DEregulated, NOT DOWNregulated

hypomethylation of repeats?

stem cell behaviour of cancer?

- piRNA expressed in stem cells - they have important role in stem cells
germline

pg. 2.4: HOTAIR

long noncoding RNA (see ch. 2.7, pg. 2.3, 2.4)

- globally dysregulated

- unclear role/molecular mechanism

- can have both poor prognosis [favourable prognosis] depending on cancer type, lncRNA type, ...

- example: HOTAIR

- acts in trans [expressed from HOXC cluster] (some) sequence specificity
[acts at HOXD cluster]

see ch. 2.8, pg. 2.4

- breast cancer

- overexpressed

- associated w/ metastasis

- in vitro [retargets PRC2
, LSD1 (no data)] gene expression like embryonic fibroblasts

retargetting suggests targets other than HOXD

breast cancer: HOTAIR
targets metastasis
suppressor genes

- other cancers

- esophageal cancer: HOTAIR - poor prognostic indicator
- colon cancer, liver cancer: HOTAIR upregulated [also associated w/ metastasis]

- diagnostics

- detection of aberrant lncRNA expression

- e.g. PCA3 in prostate cancer

- urine test: NON-invasive

- therapeutics

- lncRNA knock down in vivo (under research)

7.8 MUTATIONS IN EPIGENETIC MODIFIERS IN CANCER

- mechanisms of occurrence of epigenetic marks

[stochastic alteration + subsequent selective pressure (mitotically heritable & promoted cell growth)?

influence of cellular stress?

- may alter epigenetic state

EPIGENETICS

- CGI hypermethylation
- genome-wide hypomethylation
- LREs & LREA, LOCS
- altered histone marks
- altered lncRNAs, piRNAs
- altered nuclear structure

GENETICS

- point mutations
- translocations
- amplification
- deletions
- copy number variations

genomic instability,
aberrant DNA repair
in heterochromatin

genetic mutations in epigenetic regulators (possible in cancer)

- TET: DNA demethylation through hydroxylation

- chromatin remodelers: opening/closing chromatin

- DNMT: DNA methylation

- MBD: Methyl CpG Binding Domains

- HDAC: histone deacetylation

- HAT: histone acetylation

- HDM: histone demethylation

- HMT: histone methylation

- chromatin readers: reading histone marks

Enzyme	Target	Disruption	Function	Cancer
DOT1L	H3K79	translocation	loss	AML
EZH2	H3K27	amplification	gain	breast, prostate
EZH2	H3K27	mutation	gain	lymphoma
EZH2	H3K27	mutation	loss	MDS
MLL1	H3K4	translocation	loss	AML, ALL
MLL3	H3K4	deletion	loss	leukemia
RIZ1	H3K9	CpG hypermethylation	loss	breast, liver
UTX	H3K27	mutation	loss	multiple types
LSD1	H3K4, H3K9	amplification	gain	prostate, bladder, lung, colon

role of epigenetic modifier is CONTEXT DEPENDENT

- can be both [oncogenic tumor suppressive] in different contexts

- different target genes in different tissue type

e.g. EZH2 (enzymatic component of PRC2)

- inhibiting EZH2 [Beneficial: some solid tumors (breast, prostate)]

[bad: e.g. MDS (Myelodysplastic Syndrome)]

blood disorder

7.9. DRUGS THAT TARGET THE EPIGENETIC MACHINERY AS CHEMOTHERAPEUTICS

- clinical implications of aberrant epigenetic control

| diagnostics: epigenetic alterations as biomarkers

| prognostics: specific alterations associated w/ outcome

| therapy: inhibitors of epigenetic machinery

- small molecule inhibitors | small molecules

| very specific binding to epigenetic modifiers
| inhibition of bound epigenetic modifier

- epigenetic machinery as therapeutic target

- epigenetic alterations are reversible → suitable for pharmaceutical interventions

- side effects | systemic use: drug acting in all tissues (not just targeted cells)

| patient survival as primary aim | disregarding long-term side effects in elderly patients/patients w/ poor prognosis

| aiming at prolonging lifespan & quality of life

| current treatments (e.g. chemotherapy) already do have lots of side-effects

- targets: enzymatic epigenetic regulators: enzymes that

- DNMT (DNA +me): DNMT inhibitors

- nucleoside analogues

- irreversibly bind DNMTs → prevent DNMTs from further methylating nascent DNA strands after replication

DNA demethylation | toxic, nonspecific



| lay epigenetic marks
| remove epigenetic marks
| chromatin remodellers

| most readily targeted
| b) small molecule inhibitors

inhibitor(s)
DNMTi:
DNA replication dependent

see EZH2: ch.7.8, pg.7.3

↑ first used in 1970s in much higher doses: lots of (unpleasant) side effects

recently used in far lower doses: good anti-neoplastic effect

- mechanism of action still unclear, long-term effects unknown

- effective in haematological malignancies

| patients respond extremely well (life expectancy)
| side effects NOT too severe (quality of life)

| dependent on hypermethylation of CpG islands of tumor suppressor gene

- drugs | Decitabine (FDA approved)
| Vidaza (FDA approved)

| more in clinical trials
- also for solid tumors

rapidly dividing cancer cells are hit the most, but normal cells need to divide as well, so they will probably be hit also

version of effectiveness
of DNMTi in haematological
malignancies?

- HDAC (Histone -ac): HDAC inhibitors

- 4 different classes (targetting different types of HDACs)

- precise mechanism of action unknown

- pleiotropic effect: targetting Histone DACs

nuclear

other proteins DACs (e.g. tru factors)

Cytoplasmic

not selective

- effective in lymphoid malignancies (cutaneous T-cell lymphoma, others?)

| extremely effective (life expectancy)

| well tolerated (quality of life)

- HAT (Histone +ac): HAT inhibitors

- drugs | CBP/EP300; (preclinical tests)

| more in development

HDM (Histone -me): HDM inhibitors

- drugs | LSD1; (2 compounds in preclinical tests)

| more in development

- HMT (Histone +me): HMT inhibitors

- drugs | EP25676-DOTALi (clinical trial)

| GSK126-E2H2i (preclinical)

| more in development (targets: GSa, CARMA, etc.)

TET (DNA -me)

| no publicly known drug development

Chromatin Remodellers (non-enzymatic epigenetic regulators)

- recent advance

- targetting protein-protein interaction domains

- BET family proteins (contain bromodomains: bind to acetylated histone tails)

- e.g. BRD 2,3,4

- BET: | specifically alter expression of small number of genes - including MYC

| alter tru elongation: main mode of action?

- drugs | Vorinostat (FDA approved)

| Zomidepsin (FDA approved)

| Panobinostat (ph III) | FDA approved: 23.2.2015

| Valproic acid (ph III)

| Belinostat (ph II pivotal) | FDA approved: 3.7.2014

| more early trials and preclinical

- targetting epigenetic machinery - very active area of research

- early weariness (side effects, long-term effects) no longer seen

- drugs appear surprisingly effective

- future | specific drugs

| combined treatment w/ standard chemotherapeutics

| treatment of disorders other than cancer

- caution needs to be applied (treatment of cancer or otherwise)

- younger patients (e.g. effects on germ cells)

potent oncogene

7.10. EXTENSION LECTURE: AGING - PART 1

- hallmarks of aging
- causes of damage: always deleterious
 - epigenetic alterations
 - genomic instability
 - telomere attrition
 - loss of proteostasis: ability to make & degrade proteins at correct rates
- responses to damage: beneficial when low, damaging when high
 - deregulated nutrient sensing
 - cell senescence: cells do not divide, but do not die (protection mechanism against cancer?)
 - mitochondrial dysfunction
- causes of phenotype
 - stem cell exhaustion (e.g. slower healing rate)
 - altered intercellular communication

- epigenetic changes in ageing

- hypervariable [b/w tissues
[b/w individuals] → epigenetic drift
- lifetime environmental effects?

aging as introduction of many random errors, randomly distributed across genome, cells, tissues, ... ← as opposed to specific concerted effects

epigenetic differences in twins: the older the twins, the greater the differences
→ genome-wide studies

epigenetic drift? environment? ↑

7.11. EXTENSION LECTURE: AGING - PART 2

- DNA methylation
 - young [unmethylated CpG islands
[methylated intergenic regions]
 - old [hypermethylated CpG islands
[hypomethylated intergenic regions]
- strikingly similar to DNA methylation changes in cancer (see ch. 7.2, pg. 7.1)

- affected genes: enriched in genes silenced by PRC2-mediated H3K27me3 in embryonic & adult stem cells

- bivalent genes (in stem cells)
 - H3K27me3 (suppressing)
 - H3K4me3 (activating)

differentiation

→ -H3K27me3: rapidly activated ↑

→ -H3K4me3: (rapidly) repressed ✗

both, in the same time

↑

not expressed "poised"

easy to react, fast to differentiate?

! there is no DNA methylation there
(yet? : to be added to repressed genes later?)

aging/cancer: these genes are silenced by DNA methylation

not well understood,
needs more research

- usually genes involved in developmental regulation (e.g. lineage specific txn factors in ES cells / adult stem cells)

- H3K16ac: decreased (other studies say increased) in aging cells

- involved in [higher order chromatin organization
DNA damage response]

worms, flies, yeast: Ser2 - overexpression prolongs lifespan

mice: Ser1 - overexpression does NOT prolong lifespan

improves some aspects of aging

- H3K9ac: depleted in aging, variably changed in cancer

- Deacetylation mediated by Sirt6 - important in DNA repair → mutations in Sirt6 can cause defective DNA repair → faster aging
- Overexpression: prolongs mammalian lifespan
- Deletion: accelerates aging

- H4K20me3: decreased: aged human fibroblasts (most common cell in mammalian connective tissue, critical in wound healing)

increased: aged rat kidney/liver samples; Hutchinson-Gilford Progeria (human) samples

enriched: centromeres, telomeres, ICR & repeats (role in transcriptional repression)

see ch. 2.9, pg. 2.4

- Hutchinson-Gilford Progeria Syndrome: premature aging

- (de novo) mutations prevent normal processing of LaminA - protein required for nuclear architecture (nuclear laminae)

→ nuclear laminae w/ abnormal structure → abnormal function (disorganized heterochromatin, disrupted DNA repair, increased genomic instability)

- it is unknown if HGPS patients experience "normal" (just accelerated) aging, or aging via completely different mechanisms

- cancer vs. aging

- incomplete data
- high degree of correlation, but there are differences

- fewer changes in expression of epigenetic modifiers identified in aging compared w/ mutations and expression differences in cancer

- mechanisms of epigenetic changes in aging

- stochastic events (drift)?
- decrease in DNMT1 (maintenance DNA methylation)
- DNMT3A (de novo DNA methylation)

DNMT1 mislocalised in such cells

- increase in - DNMT3B (de novo DNA methylation) in cell senescence (see ch. 7.10)

- treatment for aging?

- not necessarily prolonging lifespan, but rather extending productive period within the lifespan

anti-cancer drugs - use against aging as well?

activation of Sirt6 (see H3K9ac above), or other Sirts?

combination of epigenetic drugs w/ other anti-aging treatments

caloric restriction

anti-inflammatory drugs (e.g. aspirin)

stem cell therapies