DNA methylation: important in development, imprinting, stem cell regulation, XCI
Addition of a methyl group to C5 position of pyrimidine ring of cytosines
Usually forms in the context of a CpG dinucleotide
60-80% of individual CpGs are methylated
CpG islands are clusters in gene regulatory regions that tend to be unmethylated
DNA methylation is particularly concentrated on repetitive elements

Aberrant DNA methylation observed in cancer for more than 2 decades
Promoter hypermethylation leads to silencing of tumour suppressor genes
Global hypomethylation observed, thought this was associated with genomic instability
CpG island "shores" show great variation in methylation across different types of cancer

DNMT3L acts as an accessory protein to DNMT3A during embryonic development, imprinting
DNMT3A and 3B carry out de novo DNA methylation
DNMT3A: 130 kDa protein, encoded by 23 exons on human chromosome 2p23
98% homology between human and mice, highly conserved across mammals
3 major domains: PWWP domain, ADD domain, PHD domain
ADD and PWWP interact with proteins involved in transcriptional repression
N terminus thought to be involved in DNA binding
ADD inhibits the catalytic domain by forming an auto-inhibitory loop that is released by interaction with H3K4me0, that links DNMT3A and H3 chromatin

Methylation activity of DNMT3A
Methylates DNA at unique sites and at repetitive elements
In mouse ESCs localizes to discrete nuclear foci and joins DNMT3B at pericentromic heterochromatin
Genomic studies show DNMT3A methylates specific regions of the genome, overlaps with those methylated by 3B
Methylates free or linker DNA with higher efficiency than nucleosome-bound DNA
Interacts with H3K4me0, mark of inactive gene transcription
Interacts with histone modifications involved in gene repression
EZH2 interacts with DNMT3A, but is insufficient for de novo DNA methylation
Poor understanding of how DNMT3A is recruited more broadly
How is DNMT3A recruited to chromatin?
How does it target specific sites?
How is the protein regulated through interactions with other protein partners?
In hematopoietic stem cells, DNMT3A regulates methylation at DNA methylation "canyons"
DNMT3A prefers sequences rich in C, T, and A around CpG target sites
Relative importance of DNA methylation sites is unclear
Also active in CpA methylation, which occurs at extremely low levels in most tissues

Role of DNMT3A in development

Deletion of both Dnmt3a and Dnmt3b genes in ESCs leads to persistent self-renewal and inefficient differentiation
Dnmt3a also involved in somatic stem cel differentiation
When deleted in ESCs, self-renewal favoured over differentiation
Caused HSCs with deleted gene to accumulate in the bone marrow
Could still differentiate into blood cell types, but the efficiency was reduced relative to wild-type cells
Dnmt3a null HSCs show net loss of DNA methylation, especially at edges of hypomethylated canyon regions
These regions are enriched for genes associated with self-renewal and cancer: Homeobox A9, Meis homeobox 1, MDS1 and EV11 complex locus
In Dnmt3a null mice, genes associated with HSC self-renewal fail to be appropriately repressed in differentiation, and are increased in expression
Suggests that absence of DNMT3A abrogates ability to switch from self-renewal to a differentiation program
Beta catenin may contribute to the phenotype
Ctnnb1 promoter (encodes beta catenin) became hypomethylated and gene and target gene expression increased with Dnmt3a and 3b null HSCs
Knockdown of Ctnnb1 partially rescued the differentiation block
Questions that remain to be answered:
Mechanisms dictating the activity of DNMT3A at specific genomic loci
Link between changes in DNA methylation at canyons and other regions and the phenotype unclear
Possible role for DNMT3A in maintaining "stemness" beyond differentiation
DNMT3A or 3B may be involved in differentiation of other somatic stem cell types
DNMT3A implicated in neural stem cell differentiation

DNMT3A mutation in blood malignancies
First mutations in DNMT3A associated with cancer identified in 2010
3 groups reported mutations in AML, with frequency of up to 22%
Mutational hotspot at arginine 882 highlighted, other mutations also seen
Found mutations in DNMT3A in most types of blood malignancies at varying frequency
Mutations distributed through all functional domains
Most of the specific mutations have not been functionally characterized
The relevance of certain mutations for the cancer phenotype has not been explained

DNMT3A mutations typically found at higher variant allele frequencies than others in hematological malignancies
This suggests they were one of the first to arise
HSCs in mice have self-renewal advantage, so this could be a possible mechanism leading to their expansion over time
DNMT3A could serve as a precancerous lesion
DNMT3A mutations found in AML patients' ESCs in the absence of other leukemia associated mutations

Human DNMT3A null HSCs appear to have an advantage relative to wild-type HSCs in xenograft models
Multiple studies show that cancer-associated mutations were enriched in 5-10% of 70 year olds that have almost all peripheral blood cells arising from a single HSC
DNMT3A was by far the most common mutation observed in these individuals
Somatic mutations were collectively associated with increased risk of leukemia and all-cause mortality
Mutations are insufficient for the development of leukemia, but can predispose the development
Need to understand the mechanisms that endow mutant HSCs with their competitive advantage
Domains that are enriched for mutations and the frequency of heterozygosity vs. homozygosity varies between different blood malignancies
Myeloid lineage: DNMT3A most common in adults with AML, 20-25% frequency of mutation in de novo disease
Around 60% of the mutations found at the residue R882 in methyltransferase domain
R882 position most frequently mutated in MDS, CMML, MPN as well, although not as frequently as in AML
Mutations are typically heterozygous, biallelic involvement confined to non-R882 mutants
T lymphoid malignancies have more diversity in DNMT3A mutations
PTCL – DNMT3A mutations clustered in methyltransferase domain, <20% affect the R882 position
T-ALL has a similar proportion of R882 mutations
Frequency of biallelic involvement very high (up to 62%)
R882 mutation leads to a hypomorphic protein, acts as a dominant negative inhibiting the methyltransferase activity of the remaining DNMT3A protein
Approximately 20% of wild type function remains
Heterozygous mutation at other sites may only lower activity to 50%, be insufficient to drive malignancy
Selection for a second mutation (loss of heterozygosity) more common in non-R882 mutants
Some DNMT3A activity may be required for the myeloid lineage choice and cancer development by action at specific gene targets
Myeloid malignancies may tolerate or require more complete functional loss

R882 mutation in AML correlates with global hypermethylation, particularly at CpG islands, shores, promoters
Hypomethylation of genes implicated in AML but role in disease development not understood
Murine leukemias generated from Dnmt3a-/- HSCs loss of methylation at intergenic regions associated with AML
Hypermethylation was observed in T-ALL
Poor correlation of genetic changes with gene expression poses a challenge to the field

DNMT3A-related disease features
Possible association between DNMT3A mutational status and clinical features e.g. blood cell counts, percentage of blasts in bone marrow, blood at time of diagnosis
Increased incidence of DNMT3A mutations in myeloid and lymphoid cancers with advanced age reported

DNA methylation pathways and crosstalk with histone methylation

H3K4 and H3K9 among most highly conserved epigenetic marks, correlate with gene activation and gene silencing, respectively
DNA methylation also correlated with gene silencing
Established by specialized de novo DNA methyltransferase enzymes, present in 3 different DNA sequence contexts:
CG, CHG (H is A, T, or C) which are symmetrical sequences
CGG, an asymmetrical sequence
DNA methylation perpetuated through mitotic and meiotic divisions by maintenance DNA methyltransferases
Extensive links, crosstalk between histone modifications and DNA methylation
Readers of histone methylation include PHDs, chromodomains, bromo adjacent homology domains, readers of DNA methylation including SRA, CXXC domain, MBD

DNA and histone methylation in mammals
Occurs primarily at CG residues, with non-CG methylation only seen in the stemm cells of the body in actively transcribed genes
60-80% of CG residues genome wide are methylated
In CpG islands and active regulatory regions only 10% of CG are methylated
Methylation of repetitive DNA important in maintaining DNA integrity
DNA and H3K9 methylation strongly associated in mammals
H3K9 methylation catalyzed by a member of the SET-containing SUV39 protein family
Suv39H1 and Suv39H2 null mice ESCs had reduced DNA methylation in major satellites but not minor satellites or C-type retroviruses
Knockout of G9a in mouse ESCs led to DNA hypomethylation at specific loci throughout the genome
No effect seen in Dnmt3a null ESCs
H3K9 methylation found to be dependent on DNA methylation in human cancer cells
May show that mouse ESCs are undifferentiated, use different mechanisms to maintain H3K9 methylation
2 waves of global demethylation occur in mammals
One in early embryogenesis, other during PGC specificiation
Takes place around the time of implantation, occurs via DNMT3A and 3B
PGC de novo methylation establishes imprints and requires catalytically inactive homologue DNMT3L

Active promoters have CpG islands protected from methylation
Recruitment of H3K4 methyltransferases and binding of transcription factors assists with this
DNMT3A contains an ADD domain that recognizes unmodified H3, inhibited by H3K4 methylation
Evidence for inhibitory effect of H3K4 observed at imprinted genes, which do not become methylated in cells that have reduced H3K4 demethylase citation 119
G9A and GLP recruit DNMT3A directly
G9a and GLP methylate DNA at some promoters during differentiation
Acts on 126 different genes
DNA methylation lost in cells with mutations in G9a or Glp

Potential experiment:

DNMT3A mutations associated with blood cancer
H3K4 methylation inhibits DNMT3A activity
Could increased H3K4 methylation also lead to blood cancers?
Could decreased methylation of H3K4 reduce the incidence of blood malignancy?

Overexpress a H3K4 methyltransferase in normal hematopoietic stem cells?