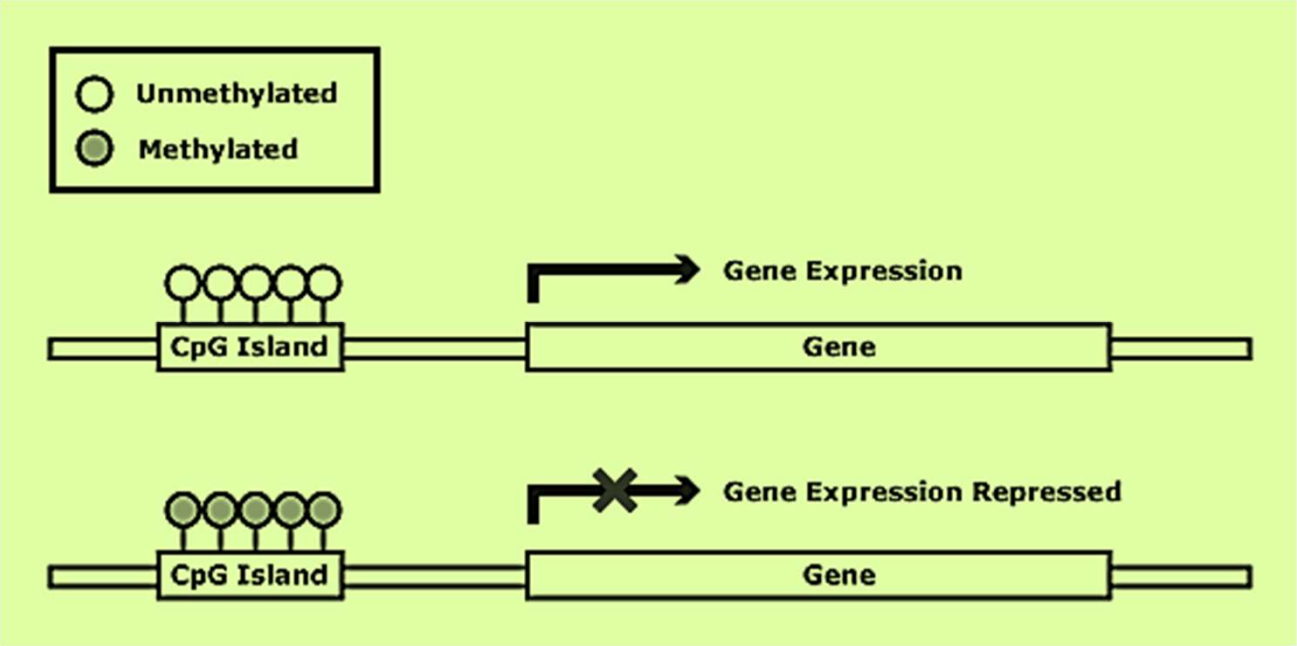
Subhadipa 2021

DNA Methylation

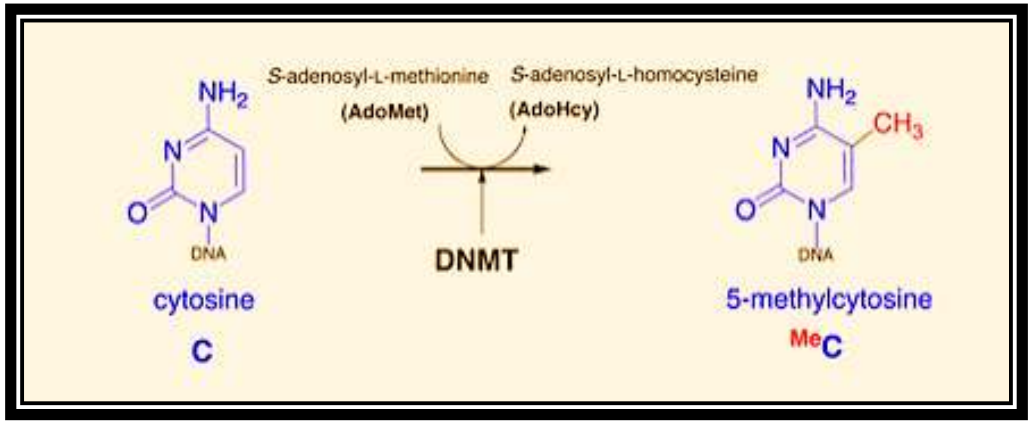
In eukaryotes, Methylation of DNA (not to be confused with histone methylation) is a common epigenetic signaling tool that cells use to lock genes in the "off" position.

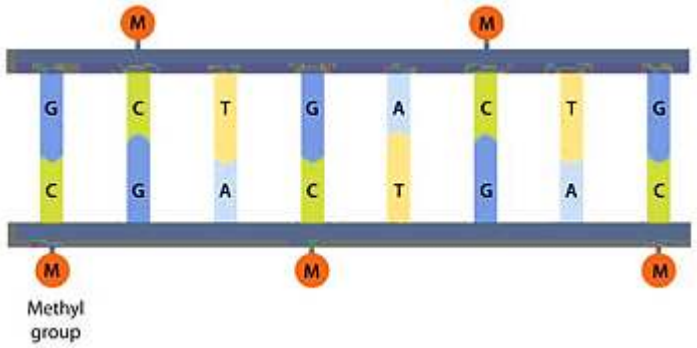


'Epigenetics' is defined as the inheritable changes by modulating the frequency, rate, or extent of gene expression in a mitotically or meiotically heritable way that does not entail a change in the DNA sequence.

What is DNA Methylation?

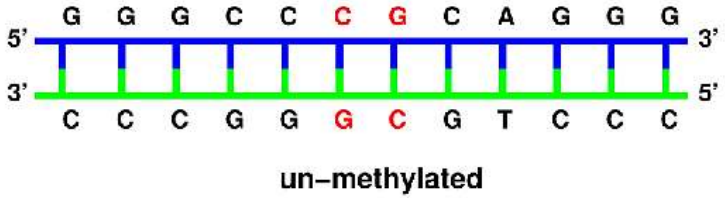
- DNA methylation is a **heritable epigenetic mark involving the covalent transfer of a methyl group to the C-5 position of the cytosine** ring of DNA.
- The **cytosine bases of eukaryotic DNA are converted to 5-methylcytosine** by **DNA methyltransferase (DNMT) enzymes.**
- The **altered cytosine residues are usually immediately adjacent to a guanine nucleotide**, **resulting in two methylated cytosine residues sitting diagonally to each other on opposing DNA strands.**



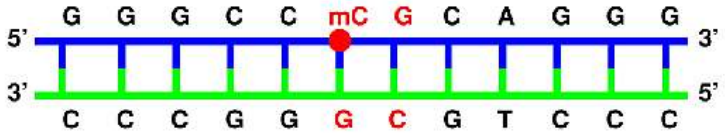


Unmethylated, hemimethylated and fully methylated DNA

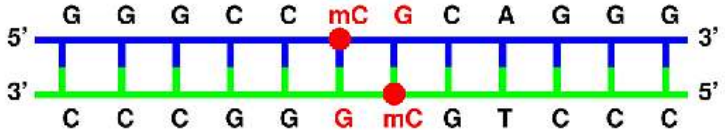
Subhadipa 2021



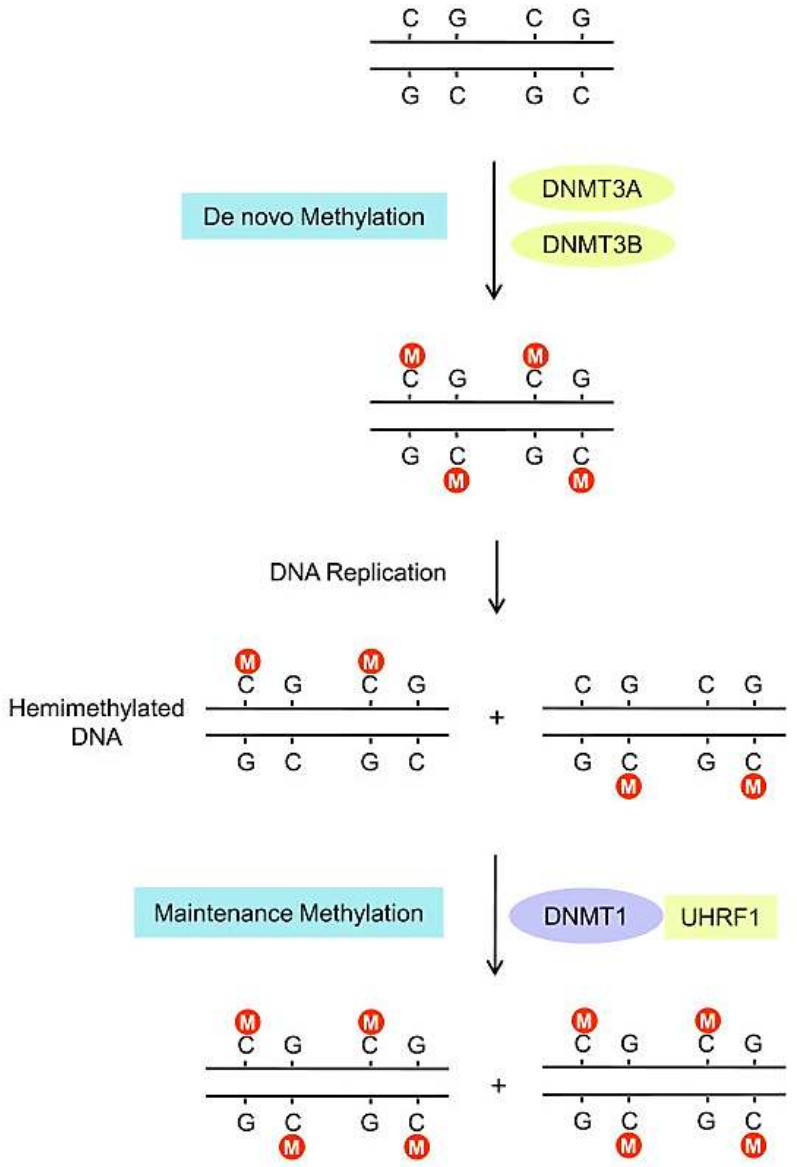
un–methylated



hemi–methylated



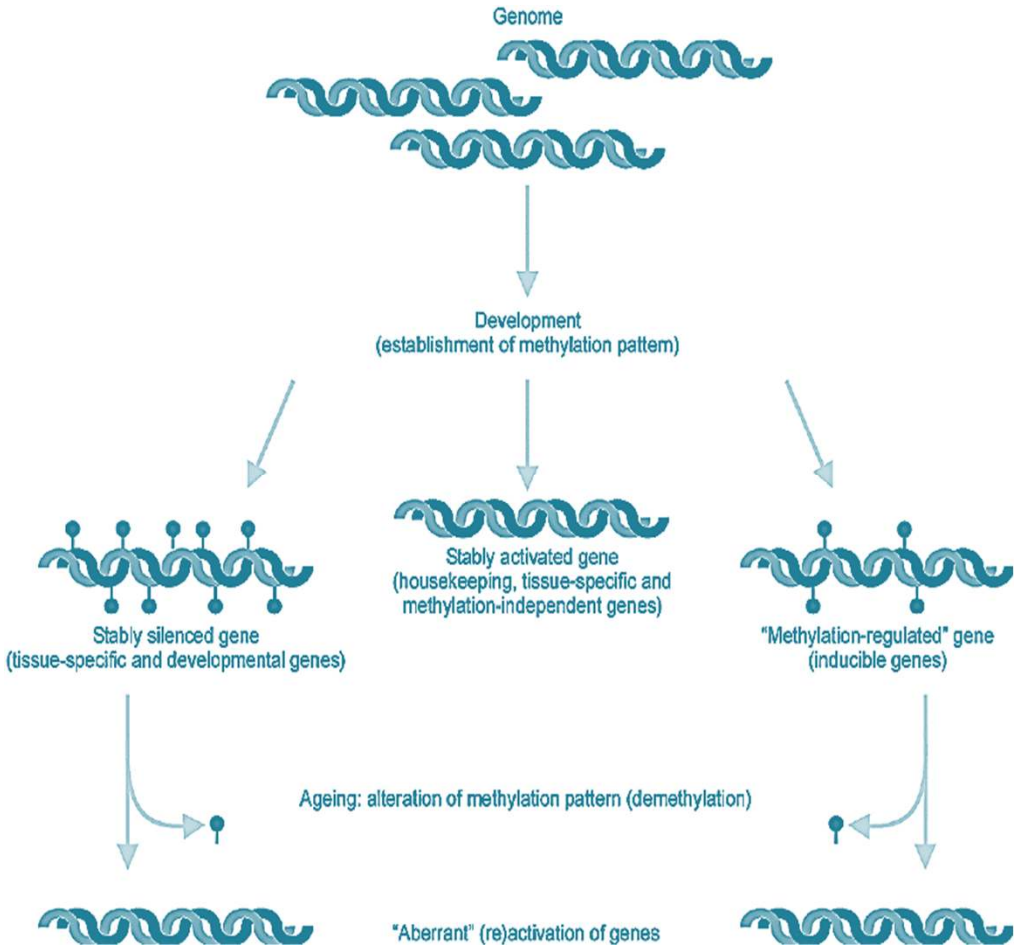
fully–methylated



Functions of DNA Methylation

Subhadipa 2021

Most DNA methylation is essential for normal development, and it plays a very important role in a number of key processes like:



1. Genomic **imprinting.**

2. **X chromosome inactivation, and tumorigenesis.**

3. Essential for **silencing retroviral elements.**

4. **Regulating tissue-specific gene expression.**

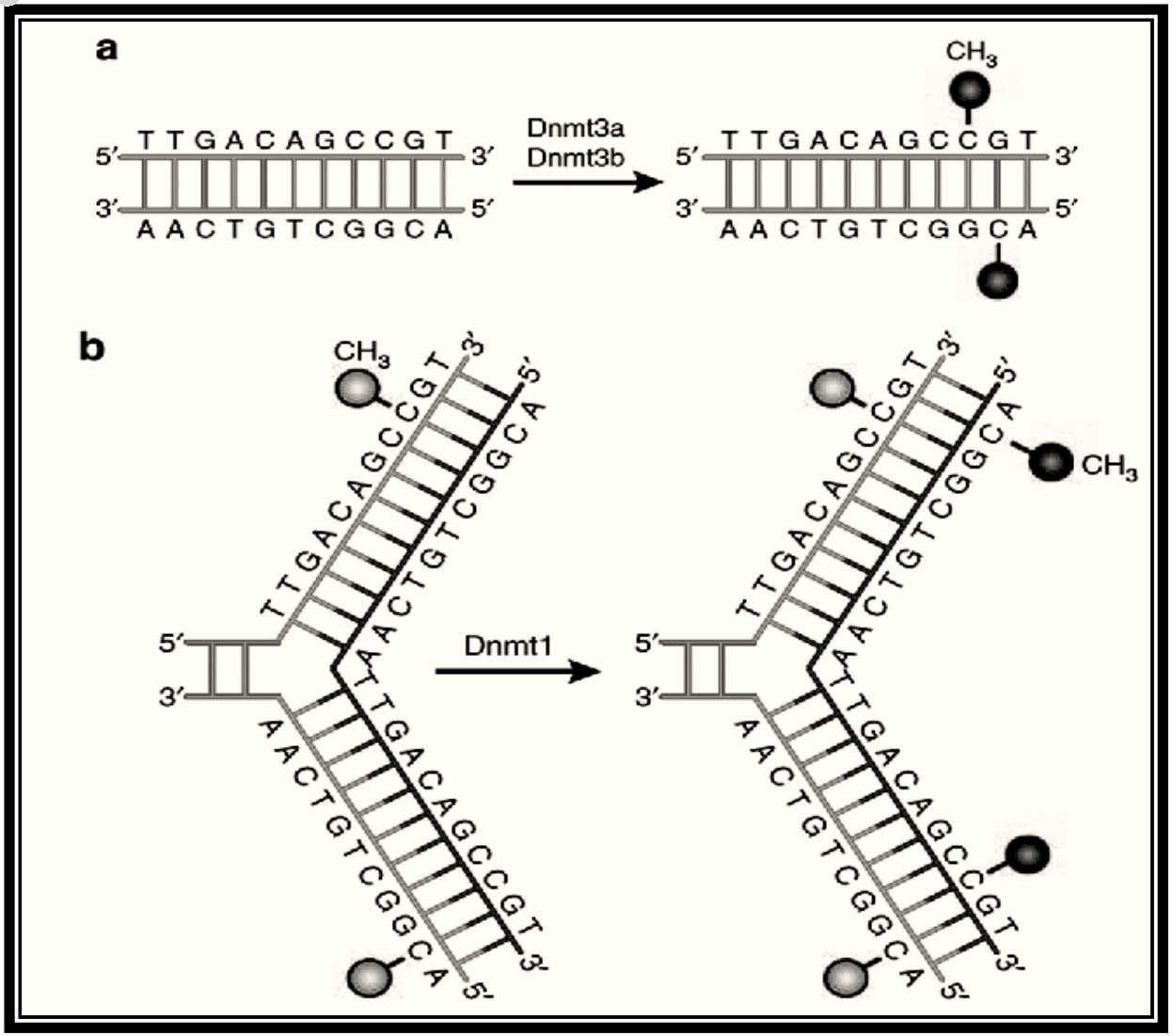
5. DNA methylation in different genomic regions may exert different influences on gene activities based on the underlying genetic sequence.

DNMT family: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3-like (DNMT3L)

- DNA methylation is accomplished by three enzymes: **DNMT1, DNMT3a and DNMT3b.**
- These enzymes can be further classified as **de novo methyltransferases (DNMT3a and DNMT3b containing PWWP domain),** enzymes **that are able to methylate previously unmethylated CpG sequences,** or **maintenance methyltransferases (DNMT1), which copy pre-existing methylation marks onto new DNA strands during replication.**
- **DNMT1 is the most abundant DNA methyltransferase in mammalian cells** and **predominatly methylates hemimethylated CpG dinucleotides as a maintenance methyltransferase.**
- DNMT3a methylated CpG dinucleotides at a slower rate than DNMT1, but a greater rate than DNMT3b.
- In addition to DNMT1, DNMT3a and DNMT3b there are **two non-canonical family members, DNMT2 and DNMT3L.**
- DNMT2 is not a DNA methyltransferase, it methylates cytosine 38 in the anticodon loop of tRNA16 but does not methylate DNA.
- DNMT3L is closely related to DNMT3a and DNMT3b structurally, but is catalytically inactive as a DNA methyltransferase. DNMT3L is known to associate with both DNMT3a and DNMT3b and may be responsible for the recruitment of histone deacetylases to direct repression onto newly established imprints.

Subhadipa 2021

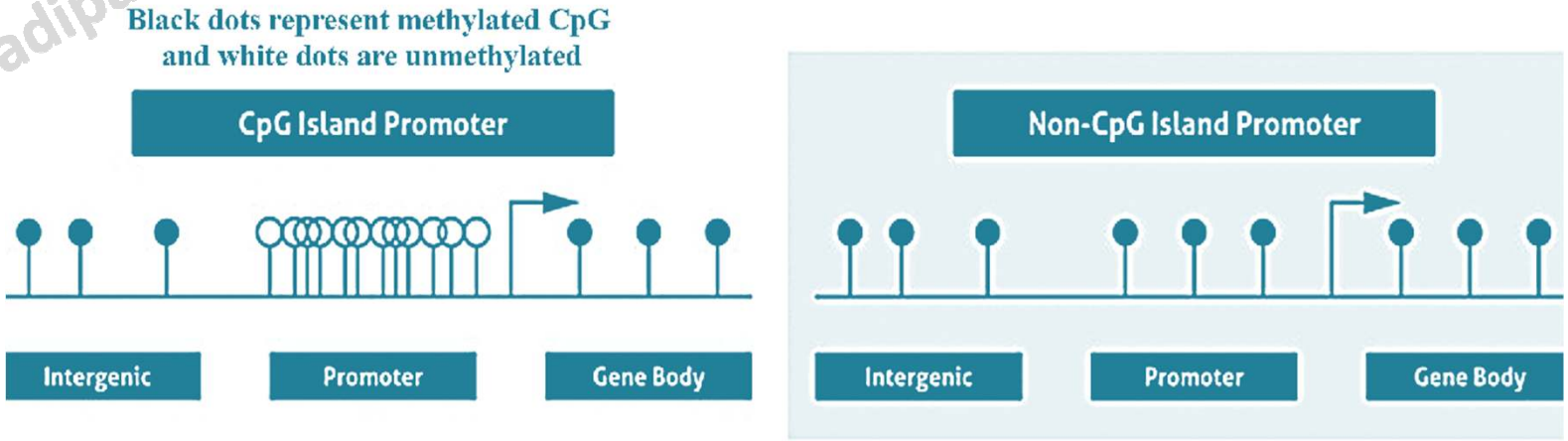
Functions of Methyltransferase



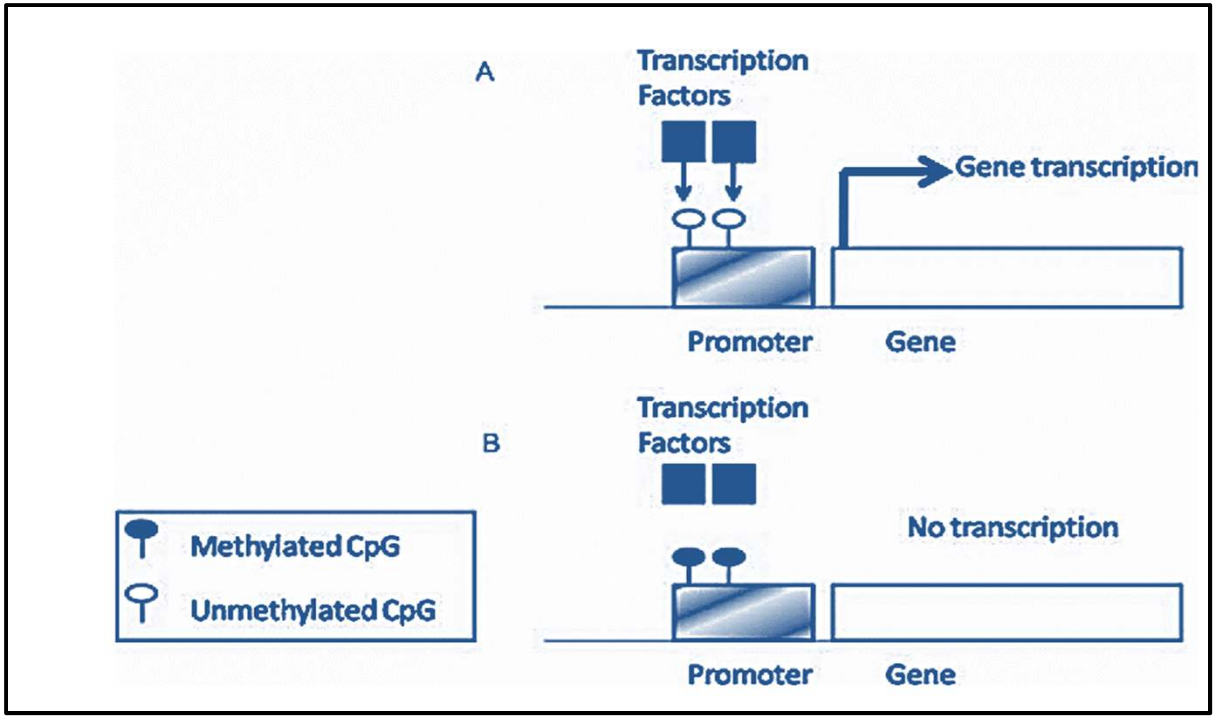
De Novo

Maintenance

Location of DNA methylation (CpG sites Vs. CpG Island)

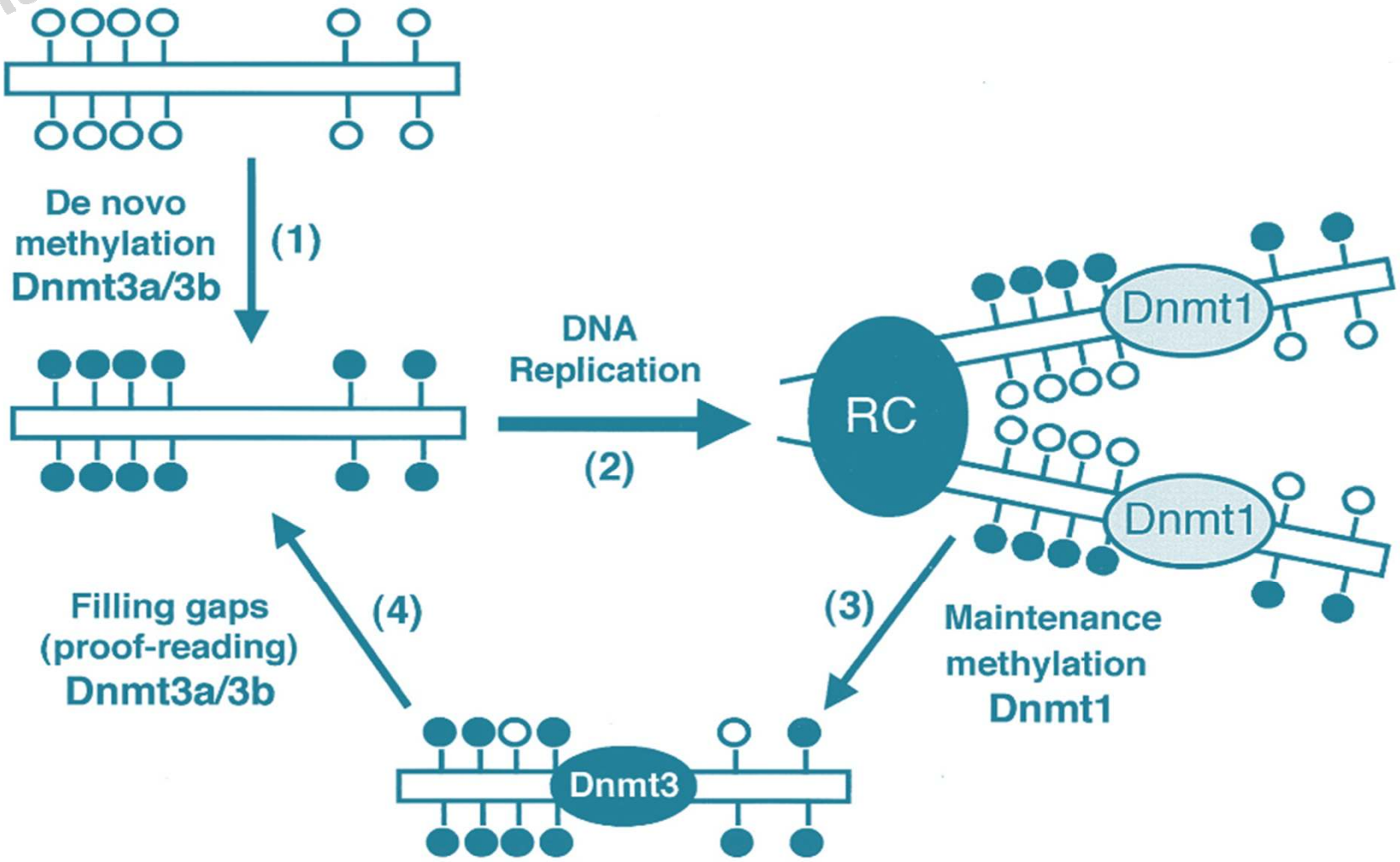


56 million CG sites, about 60–80% of which are methylated.

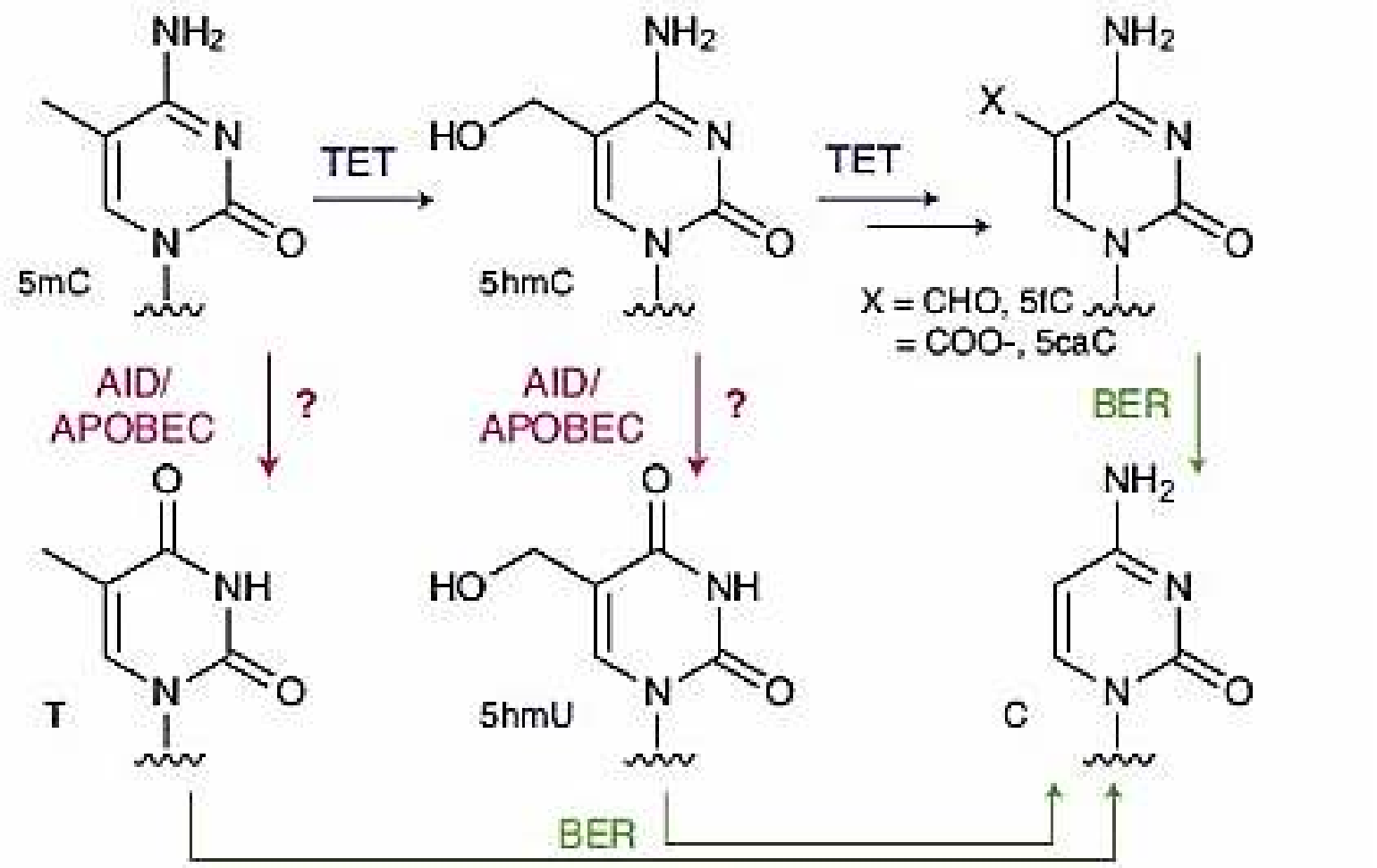


Subhadipa 2021

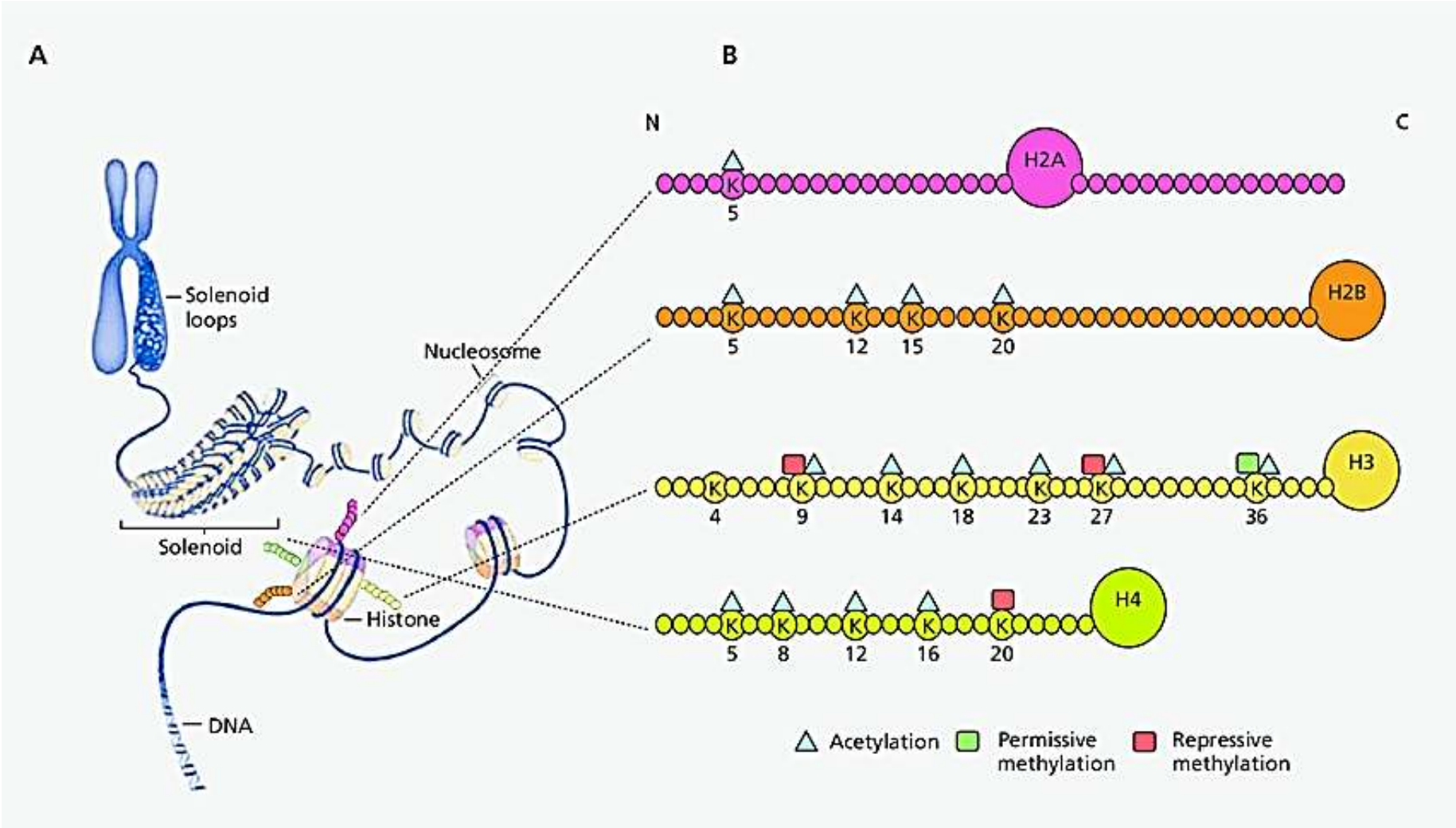
Model for the distinct roles of DNMT1 and DNMT3a/3b in de novo and maintenance methylation



Erasing DNA Methylation



Histone tail methylation: Separate Event



Epigenetic crosstalk

Subhadipa 2021

- Both DNMT1 and DNMT3a are known to bind to the histone methyltransferase UV39H1 that restricts gene expression by methylation on H3K9.

- DNMT1 and DNMT3b can both bind to histone deacetylases that remove acetylation from histones to make DNA pack more tightly and restrict access for transcription.

- DNMTs cooperate with histone-modifying enzymes involved in adding and/or stripping histone markers in order to impose a repressive state on a gene region.

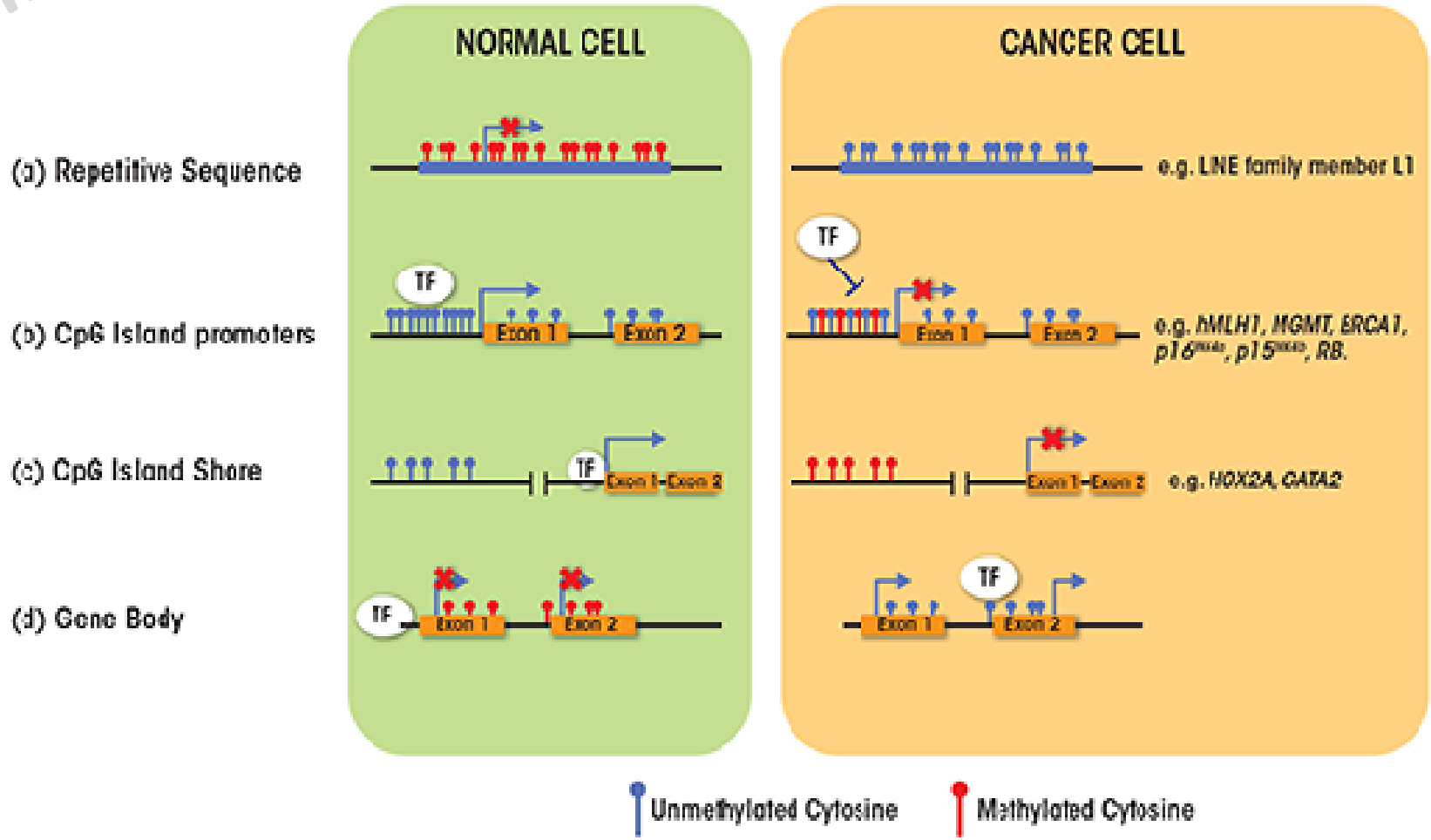
- The direct binding of DNMT3a to the H3 histone tail, sometimes facilitated by H3K36 trimethylation, a repressive histone mark, also stimulates its methyltransferase activity.

- The presence of the active histone modification H3K4 trimethylation (H3K4me3) impairs the binding of DNMT3a, DNMT3b, and DNMT3L to H3 histone tails and prevents methylation. CpG islands contain particularly high levels of H3K4me3.

- histone tails in this region often contain H3K4me3 that inhibits DNMT binding to unmethylated CpG sites and maintains a permissive environment for transcription.

Methylation and Cancer

Subhadipa 2021



Methylation and Genomic imprinting

- gDMR or ICR (imprinting control region), CpG-rich and differential DNA methylation

- PEGs and MEGs.

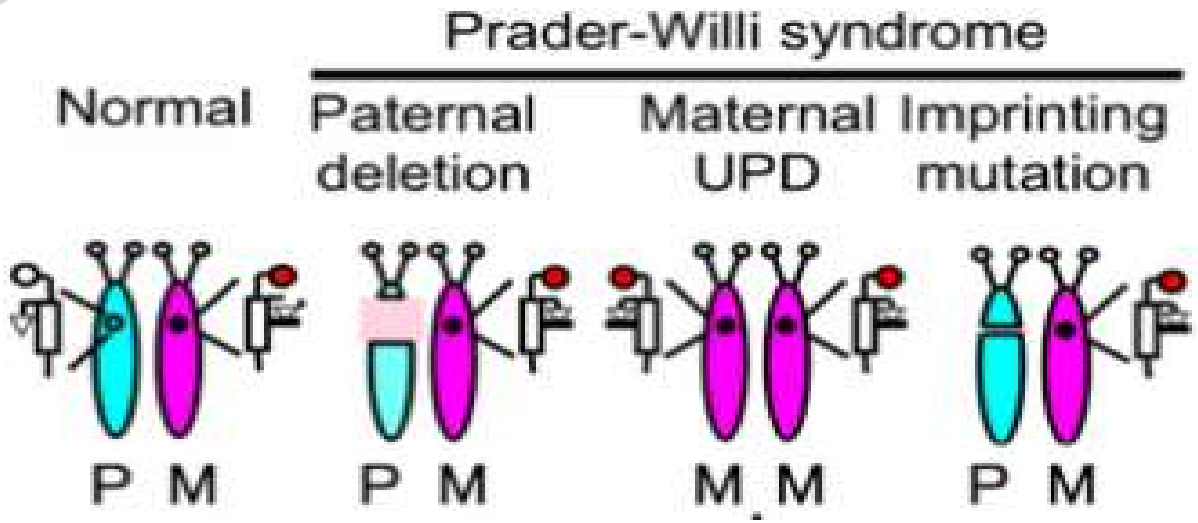
- The maternal allele of the promoter region of the small nuclear ribonucleoprotein polypeptide N (SNRPN) gene is suppressed; by contrast, the paternal allele of region is not methylated and expressed.

Prader-Willi syndrome

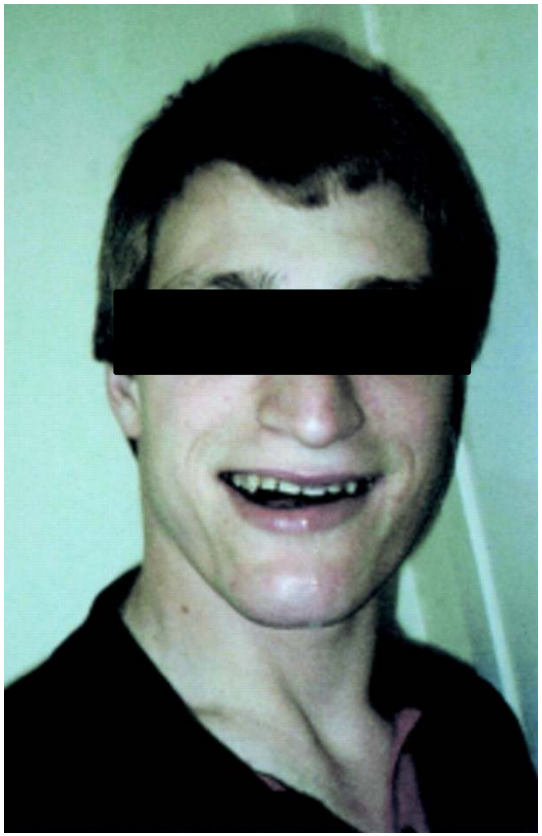
Subhadipa 2021

Indications for Prader-Willi testing:
Neonatal hypotonia & feeding difficulties
Persistent hypotonia
History of poor suck
Developmental delay
History of above, plus excessive eating

- PWS have a 15q11.2–q13 deletion on the paternally inherited chromosome 15
- 25% have maternal uniparental disomy (UPD)
- 5% have an imprinting center sequence variant



Angelman syndrome



Indications for Angelman testing:
Developmental delay, intellectual disability
Seizures
Expressive speech impairment
Ataxia
Inappropriate happy demeanor, hand flapping

- **15q11–q13 deletion on the maternal allele.**
- **Paternal Uni Parental Disomy 15.**
- **An imprinting mutation on the maternal chromosome 15.**