CANCER STEM CELL BIOLOGY and CANCER VACCINES
(7)

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Epigenetic Regulation of Cancer Stem Cells

What is Epigenetic?

- Epigenetics refers to a number of mechanisms that control the reversible regulation of gene expression by changing the chromosome without altering the DNA sequence.

- Epigenetic changes in the overall structure of chromatin occur through at least three interrelated mechanisms:

i. posttranslational modifications of histones,

ii. ATP-dependent chromatin remodelling,

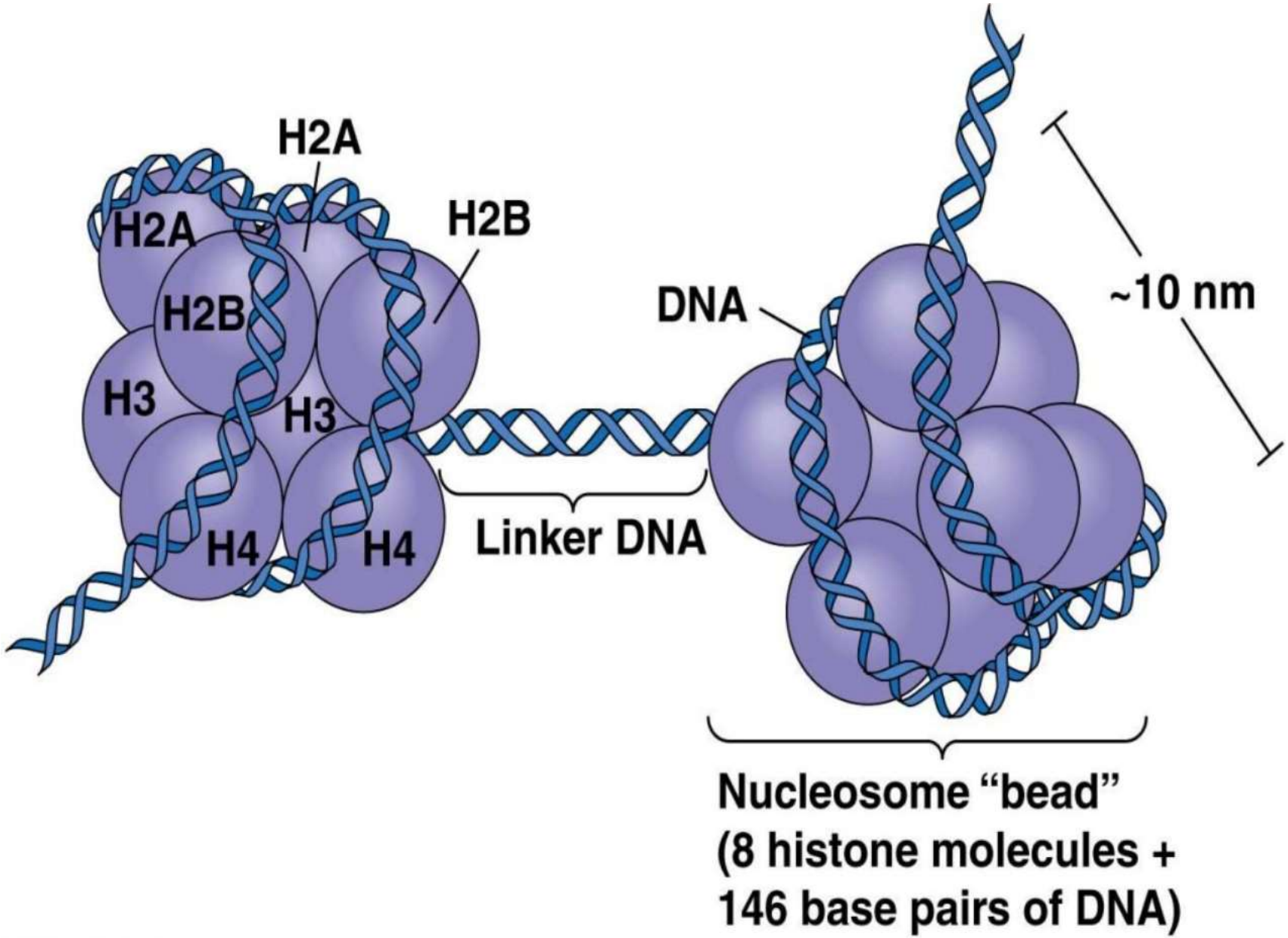
iii. the incorporation (or replacement) of specialised histone variants into chromatin,

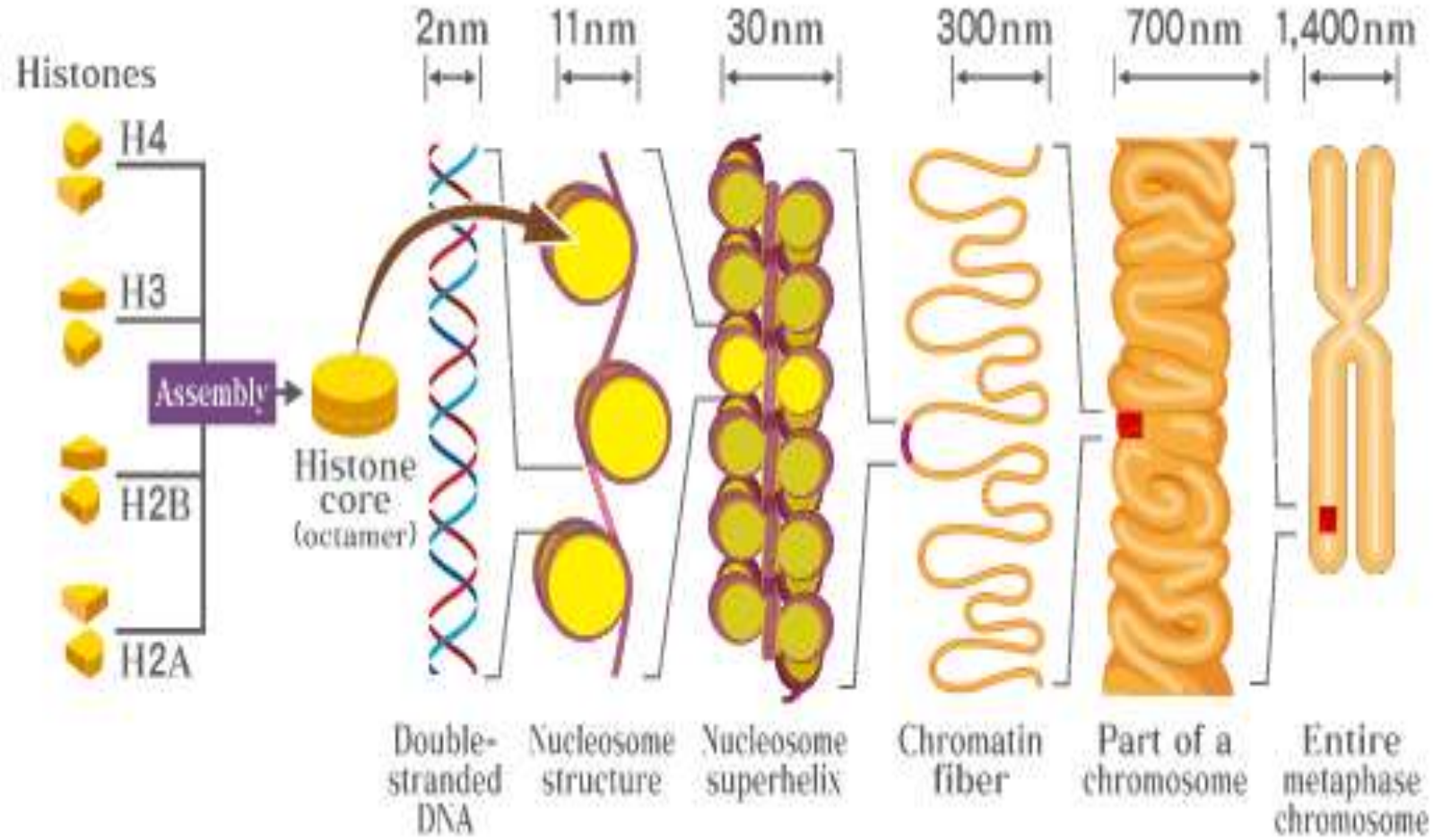
iv. DNA methylation and demethylation of CpG nucleotides and

v. noncoding RNA

What is DNA Packaging?

- Nucleosomes are the basic unit of chromatin that constitute the bulk of compacted chromosomes.
- A mononucleosome consists of genomic DNA wrapped around histone octamer scaffolds. The protruded NH2-tails of histones in the nucleosomes can be modified in a number of ways, for example, lysine and arginine methylation, lysine acetylation, serine, threonine and tyrosine phosphorylation, and lysine ubiquitination and sumoylation .
- These modifications can change the charge of chromatin leading to a more condensed or open state, which subsequently dictates the accessibility of regulatory proteins including transcriptional regulators.
- In transcriptionally non-permissive chromatin, regulatory repressor proteins recognize modified histones tails for recruitment, leading to chromatin condensation and obstruction of transcriptional activator binding.
- Mammalian genomes are compacted into highly condensed chromatin via histones and other scaffold proteins to maintain a compartmentalized three-dimensional conformation. This topological organization is thought to control gene expression directly.





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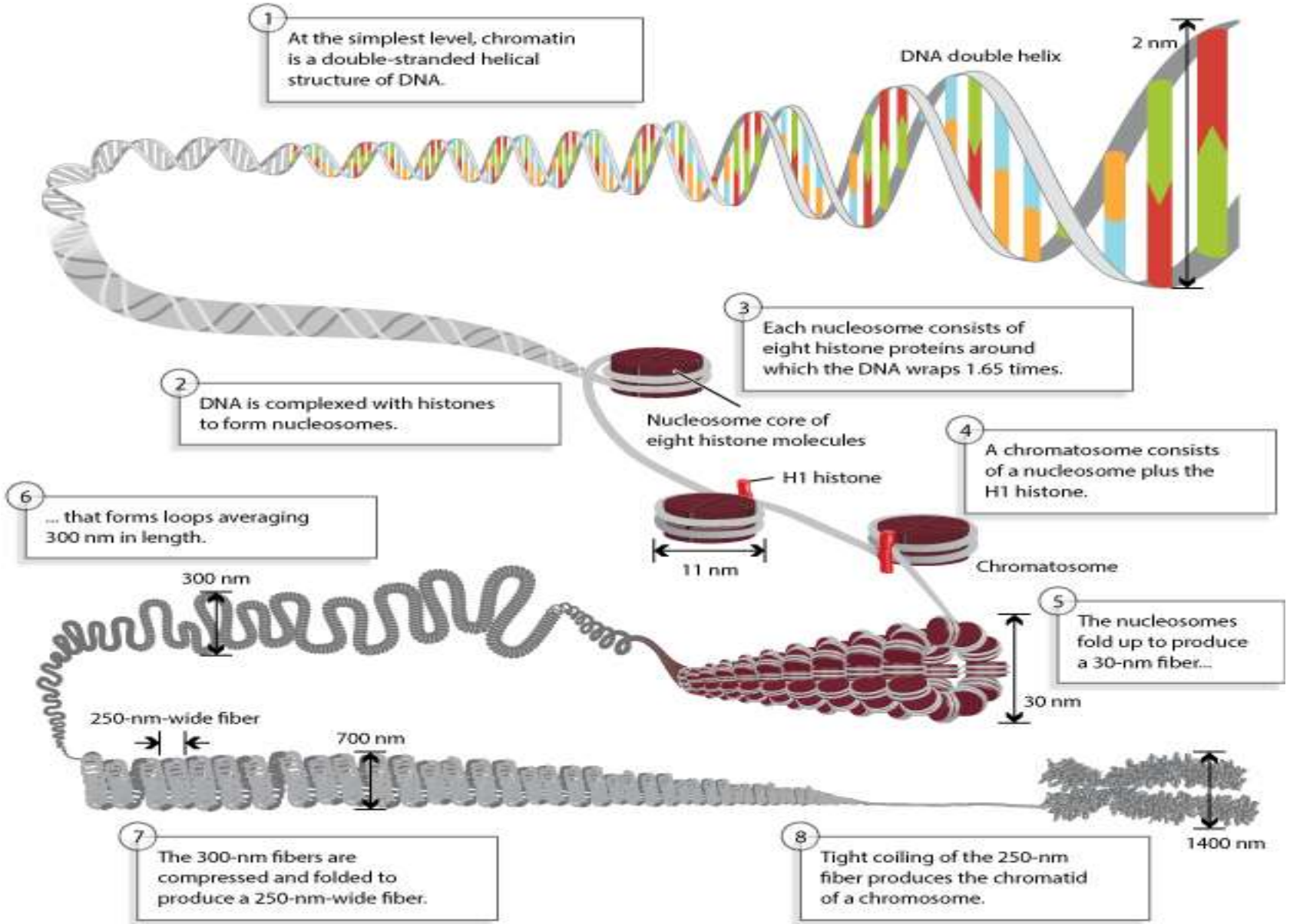
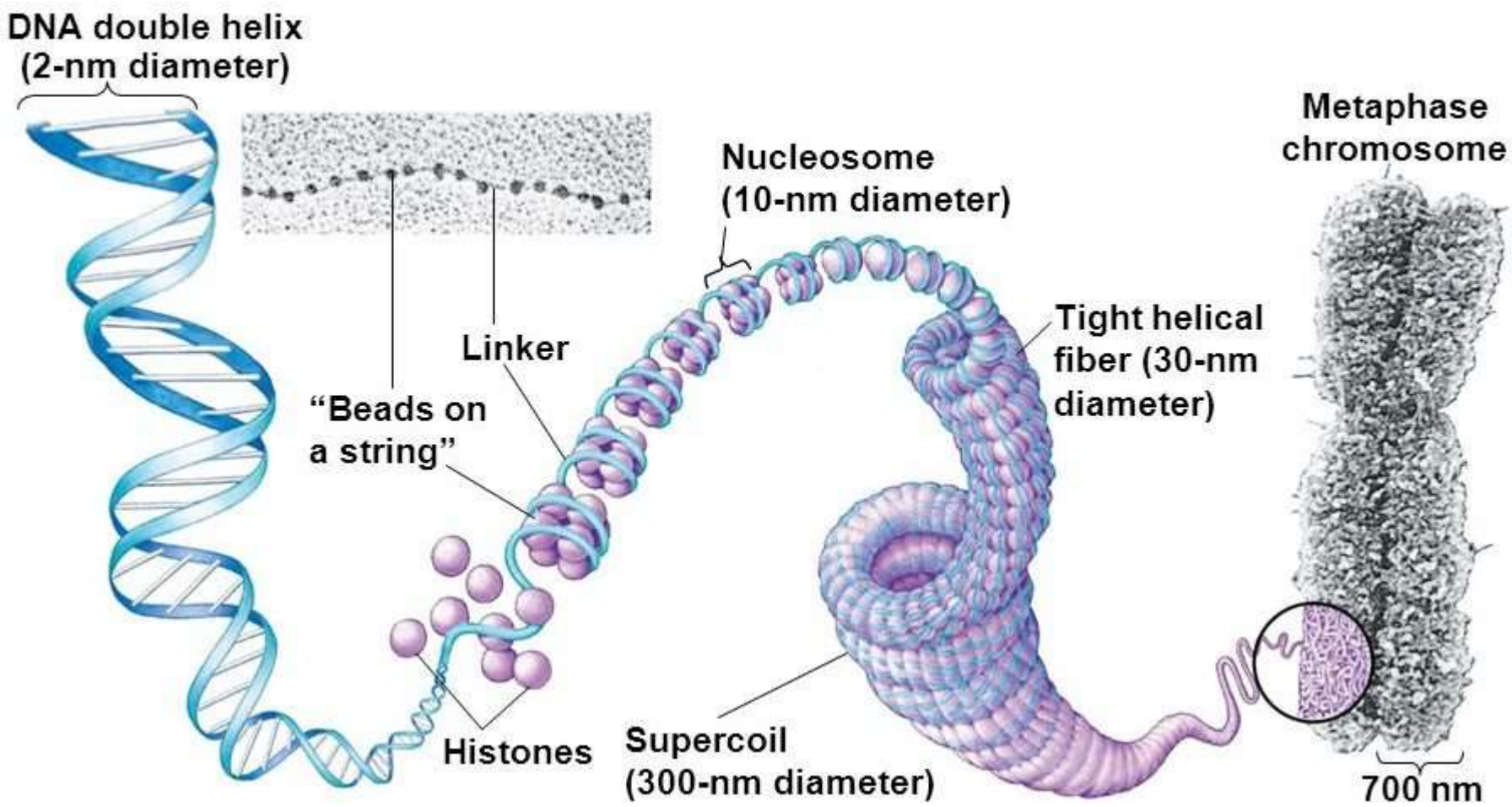


Annunziato, 2008



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DNA Methylation and Demethylation in CSC Plasticity

- DNA methylation is the most commonly studied epigenetic mark in the mammalian genome.
- It is primarily thought to suppress the binding of transcription factors to gene promoters, thus controlling gene expression. Recently, repressor proteins that can read and bind methylated DNA have been shown to be a major mechanism of transcriptional repression.
- Gene methylation has vital importance during mammalian development.
- DNA methylation also contributes to other developmental events like X chromosome inactivation and genome stability. DNA methylation patterns are faithfully inherited during both mitosis and meiosis.
- Recent studies have demonstrated that while some CpG regions are stably methylated, a small number of dynamic methylated regions could play a major role in controlling the transcription network of cells.
- Failure to maintain correct methylation patterns leads to aberrant DNA methylation, often observed in human diseases including neurodevelopmental defects, neurodegenerative, neurological and autoimmune diseases, and cancers.

DNA Methylation and Demethylation in CSC Plasticity

- DNA methylation occurs mainly to CpG dinucleotides which cluster in CpG islands; areas of high CpG density usually found at promoters.
- To methylate cytosines, DNA methyltransferases (DNMTs) catalyse the transfer of a methyl group from cofactor S-adenosylmethionine to the carbon of the cytosine ring to generate 5-methylcytosine (5mC).
- This functions to inhibit gene expression either by recruitment of methyl-CpG-binding domain proteins which in turn recruit histone-modifying and chromatin remodelling complexes, or by preventing the recruitment of DNA binding proteins, that is, transcription factors.
- To reactivate expression after silencing, 5-mCs can be oxidised to 5-hydroxymethylcytosine (5hmC) and back to the unmodified state by TET proteins and base excision repair (BER), to ultimately restore the unmethylated cytosine.

DNA Methylation and Demethylation in CSC Plasticity

- Methylation patterns are altered in cancer; localised hypermethylation occurs in CpG islands of the promoters of tumour suppressor genes, silencing their expression.

- Global hypomethylation in intergenic regions causes oncogene activation and ultimately results in genomic instability.

DNA Methylation and Demethylation in CSC Plasticity

- In general, CpG methylation is required for differentiation whereas demethylation is essential for induction of the pluripotent state.

- However non-CpG methylation, although rarer, is associated with pluripotency and is lost upon development in all tissues except the brain.

- There is evidence to suggest that loss of methylation is also required for the generation of CSCs and tumour initiation. CSC formation from cancer cells was found to depend on loss of methylation of the Nanog promoter via DNMT1 inhibition.

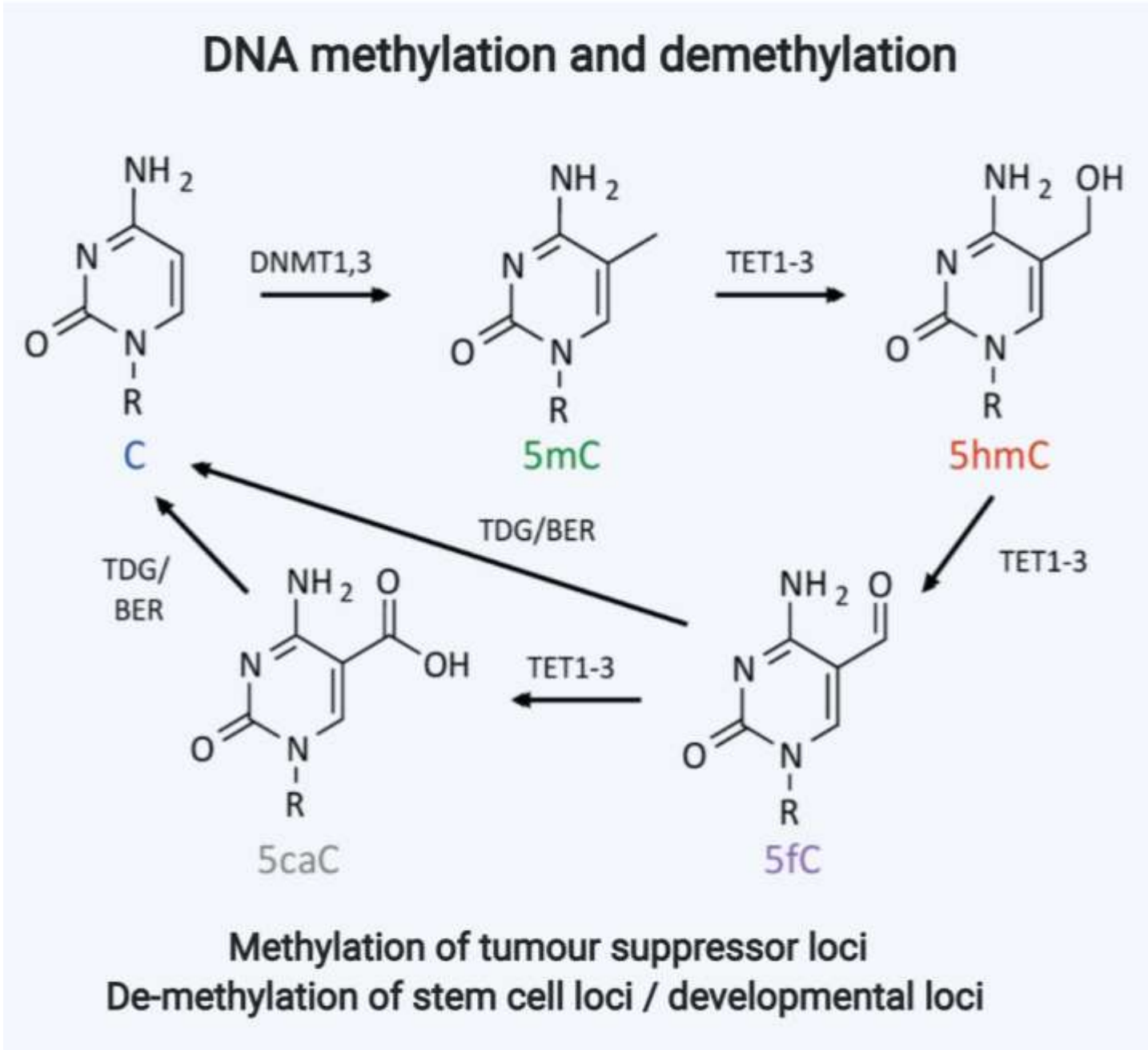
- Loss-of-function mutations in DMNT3A also led to the expansion of preleukaemic SCs, indicating a role for dysregulated DNA methylation in inducing tumour-initiating cells.

DNA Methylation and Demethylation in CSC Plasticity

- Demethylation is highly important in pluripotency, both in ESCs and induced pluripotent stem cells (iPSCs).

- Reprogramming of somatic cells to a stem-like state can be achieved by expression of the Yamanaka factors (Oct3/4, Sox2, Klf4 and c-Myc), and is accompanied by epigenetic changes including demethylation.

- Reprogramming is dependent on TET1, which affects Nanog levels and can even act as a substitute for Oct4. However, knockout of TET1 in ESCs did not perturb the pluripotent state.



French et al., 2020

DNA Methylation and Demethylation in CSC Plasticity

- However, opposing roles for DMNT1 have been demonstrated as it can both promote or inhibit CSC formation.

- DNMT1 was required for the initiation of colon cancer. DNMT was also important for CSC function in established leukaemia, breast and lung tumours whereas inhibition of DNMT1 promoted CSCs and EMT in prostate cancer.

- These discrepancies could be due to tumour-specific effects or local hypermethylation of tumour suppressor genes which may promote the generation of CSCs in some cases.

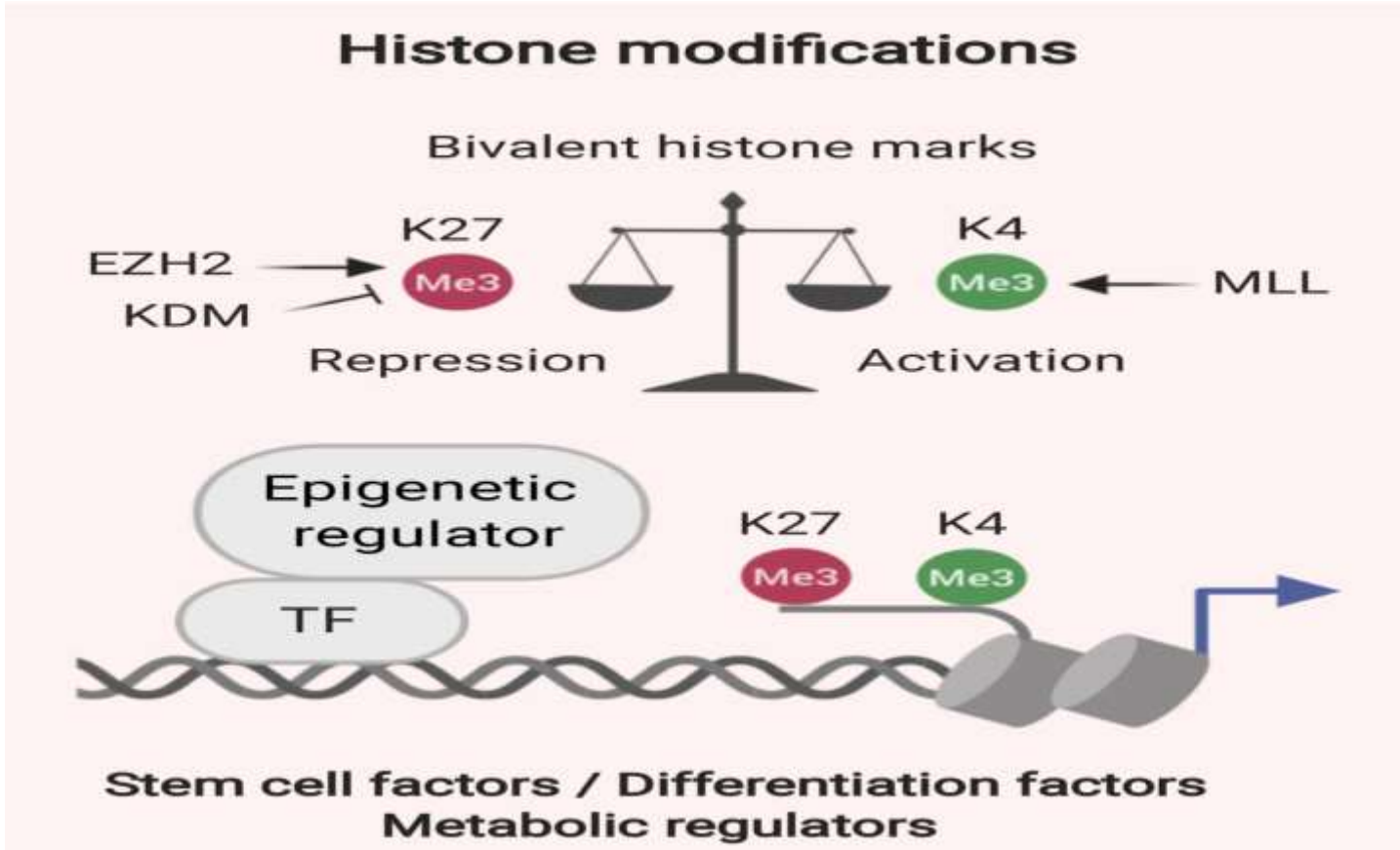
Histone Modifications in CSC Plasticity

- The amino acid residues located on the N- and C-terminal tails of histones can be modified to influence gene expression, including by acetylation, methylation and ubiquitylation.

- When occurring on promoters or enhancer regions, these modifications confer chromatin states that affect gene expression by altering the ability of protein complexes to bind.

- Chromatin formation that permits protein binding and gene expression is known as **euchromatin**, and repressive is known as **heterochromatin**. These states are mediated by the Trithorax group (TrxG) proteins and Polycomb group (PcG) proteins, respectively.

- Modifications are named by the histone type (eg, H3) followed by the amino acid and modification, for example, K4 me3 (trimethylation on lysine 4). The most studied modifications are those that occur to histone 3 found primarily at active enhancers (H3K9ac, H3K27ac), promoters (H3K4me3) and within the bodies of actively transcribed genes (H3K36me3).



French et al., 2020

Histone Modifications in CSC Plasticity

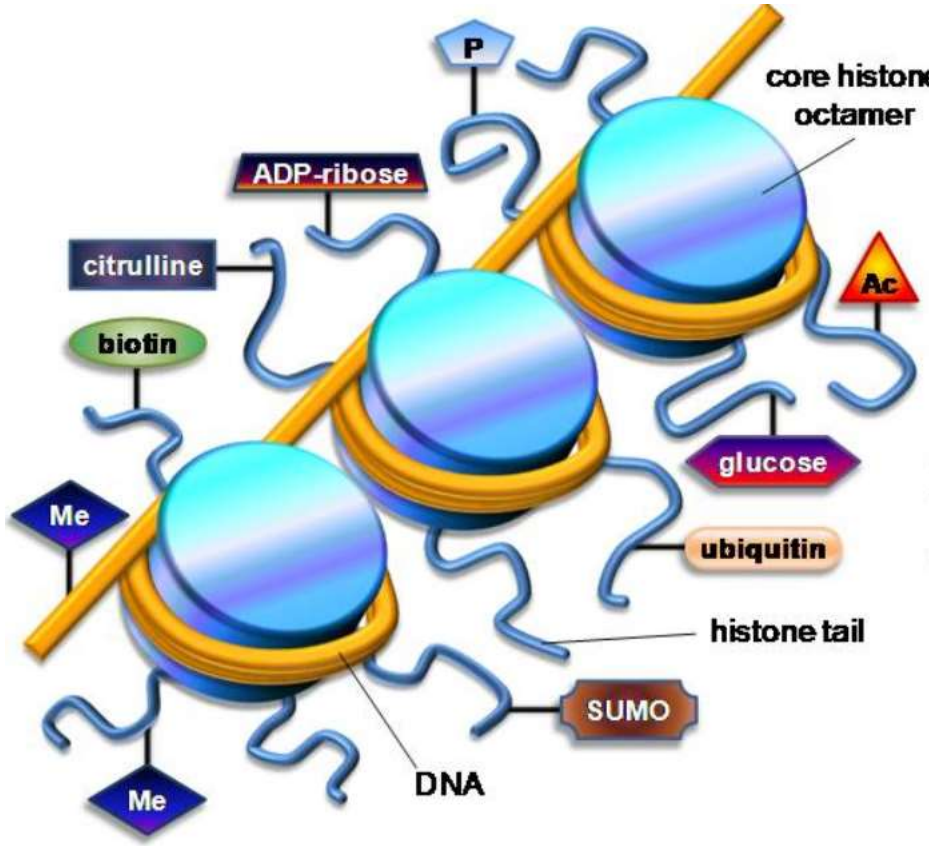
- In addition to histone modifiers, dysregulation of some proteins that regulate chromatin structure, such as cohesins, are involved in promotion of stem cells and the generation of CSCs in leukaemias.

- Furthermore, mutations that disrupt the function of chromatin-remodelling complexes, and are found at high frequency in cancers, can cause aberrant activation of stem cell related pathways.

- Nearly all the genes involved in ESC identity, including KLF4, Sox2, Oct4 and Nanog, are regulated by super-enhancers; large genomic regions with very high levels of transcription factors that are highly important in controlling pluripotency and differentiation.

Histone Modifications in CSC Plasticity

- Chromatin at promoters and enhancers can be classed as active, repressed or poised.
- Poised regions are bivalent in terms of histone modifications as they contain both H3K27me (repressive) and H3K4me (activating) histone marks. In ESCs, the majority of bivalency occurs in the promoters of transcription factors and half of bivalent domains have binding sites for pluripotency transcription factors.
- In ESCs, bivalency tends to be lost upon differentiation with some enhancers becoming active (loss of H3K27me3, gain of H3K27ac) and some become repressed (H3K27me3 enrichment), this occurring in a cell type-specific manner.
- Bivalent marks at pluripotency loci have also been identified in solid cancers. In theory, poised chromatin can allow cellular identity to switch in any direction, that is, not only differentiation but also de-differentiation, and may therefore be important in the generation of CSCs.
- Bivalency also allows for the highly dynamic state so characteristic of pluripotency, and may be the single most important epigenetic process for conferring CSC plasticity.



Association of DNA with histones showing some common posttranslational modifications that influence this interaction

http://www.wiringthebrain.com/2013/01/the-trouble-with-epigenetics-part-1.html

Nucleosome Positioning in CSC Plasticity

- The basic unit of chromatin is the nucleosome, which is formed of 147 bp of DNA wrapped around eight histones, two each of histones H2A, H2B, H3 and H4.
- Gene expression is regulated at the level of chromatin structure in an ATP-dependant process by chromatin modifiers which act to remove or slide assembled nucleosomes along with the DNA, and can also exchange histone H2A-H2B dimers with dimers of histone variants.
- The presence of nucleosomes naturally represses gene expression by preventing the access of transcription factors.
- The absence or loss of nucleosomes at a transcription start site (nucleosome-free region [NFR]) allows for assembly of the transcription machinery and rapid activation of gene expression.

Nucleosome Positioning in CSC Plasticity

- The position of nucleosomes must therefore be precisely regulated at promoters, enhancers and repressors.
- This is achieved by four known families of ATP-dependent chromatin remodelling complexes;

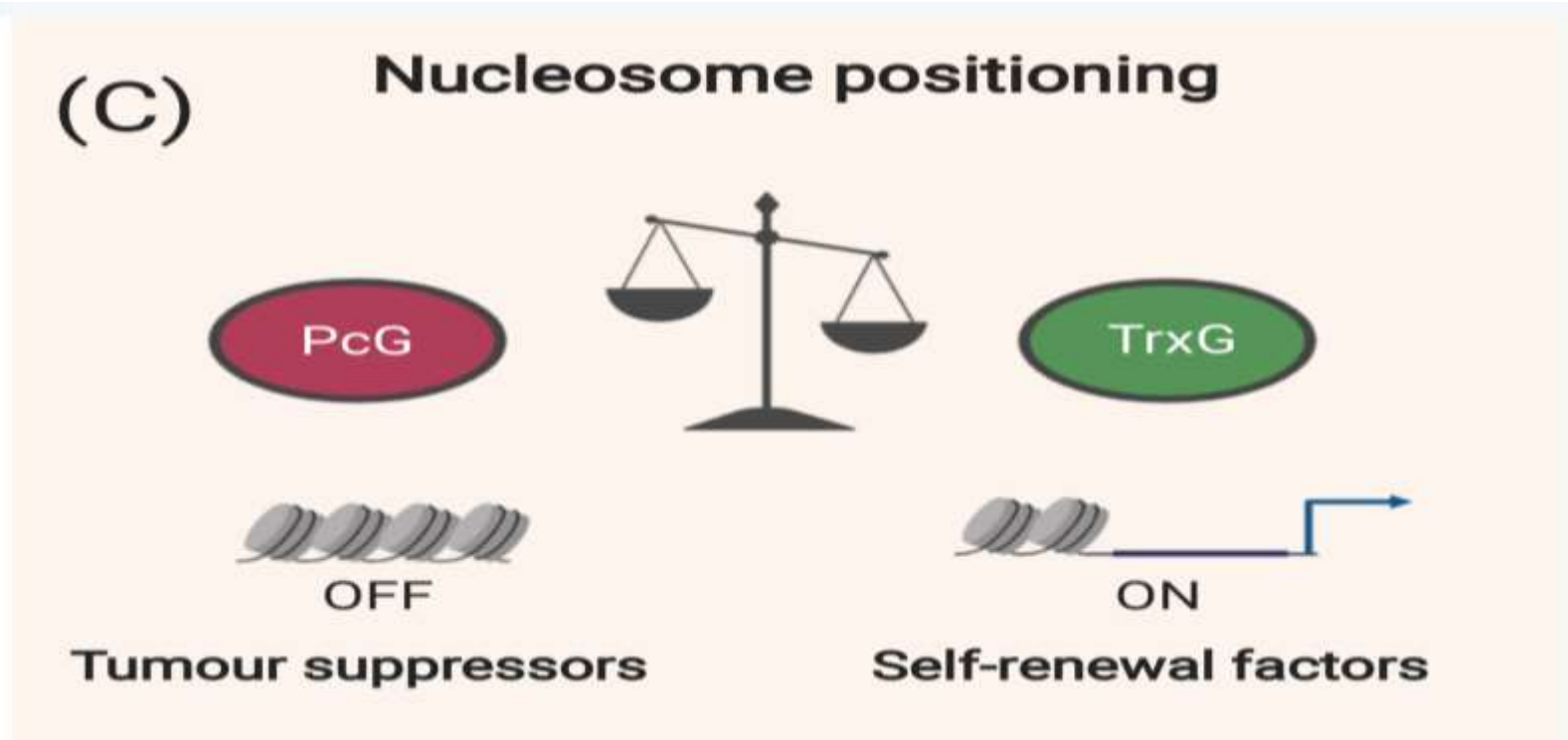
i. switch/sucrose nonfermenting (SWI/ SNF),

ii. imitation switch (ISWI),

iii. inositol requiring 80 (INO80) and

iv. NuRD/Mi-2/CHD helicase binding domain.

- Chromatin remodelling complexes use ATP hydrolysis to catalyse the assembly, sliding and ejecting of nucleosomes. DNA-sequence specificity is achieved by interaction with transcription factors.
- Remodelling complexes cause DNA to loop or twist to disrupt connection with histones and thus forcing translocation across a nucleosome.
- Chromatin modifiers can also interact with methylated DNA and covalent histone modifications to affect global gene expression patterns and chromatin architecture.



French et al., 2020

Nucleosome Positioning in CSC Plasticity

- Nucleosome positioning is also important in the reprogramming of somatic cells to pluripotency.

- As nucleosome repositioning is required for the generation of the pluripotent state it may be important for the formation of CSCs.

- This may occur via aberrant functions of ATP-dependent chromatin remodelers, which has also been linked to cancer, especially in terms of the SWI/SNF complex, which is frequently found mutated.

Histone Variants in CSC Plasticity

- ATP-dependent chromatin remodelling complexes can also regulate transcription via incorporation of histone variants into nucleosomes.
- There are eight histone variants of H2A, and six of H3, which are deposited in specific locations along the genome. Histone variants can influence gene expression by directly altering the structure and stability of nucleosomes, or by recruiting readers of histone modifications to induce local chromatin changes.
- For example, nucleosomes that contain the histone variant H2A.Bbd, bind less DNA and are not as stable, resulting in less compact chromatin. Less stable nucleosomes including those that contain H2A.Bbd, H2A.Z or H3.3 are localised at active promoters, enhancers and insulators, and may serve to prevent the formation of stable nucleosomes around these regulatory regions and facilitate transcription.
- In contrast, nucleosomes containing the histone variant macroH2A are relatively more stable and inhibit transcription. High mobility and exchange of histone variants is a key feature of SCs and this dynamism is thought to contribute to SC plasticity.

Non-coding RNAs in CSC Plasticity

- Noncoding RNAs (ncRNA) are transcribed from regions of the genome that do not encode for proteins.
- The resulting RNA transcripts function to regulate the expression of protein-coding genes and are therefore essential for control of cellular function and identity.

- ncRNAs can be divided into two major groups based on their size:

i. small ncRNAs being 200 nucleotides or less, and

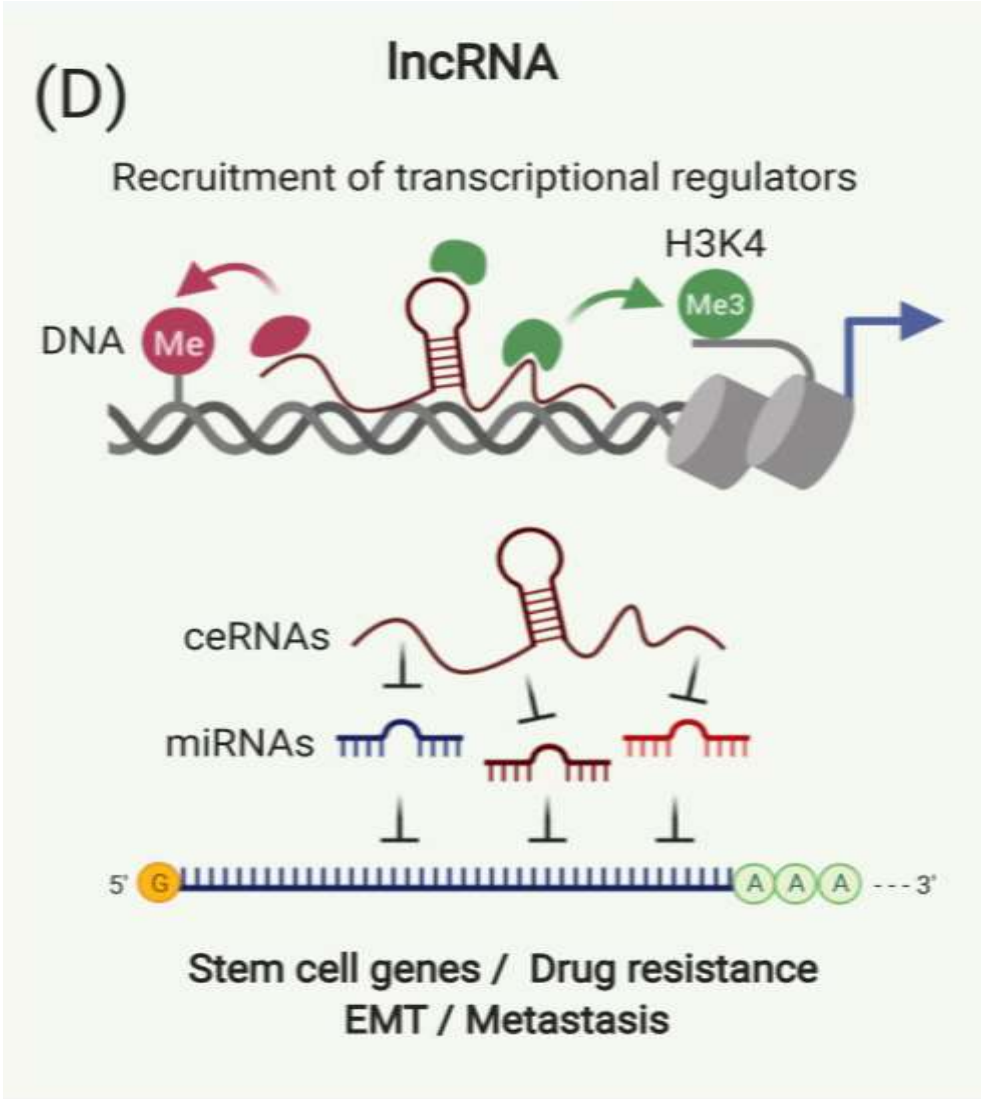
ii. long ncRNAs (lncRNAs) 200 nucleotides or more.

- Small ncRNAs can be further subcategorized based on length, function and subcellular localization and include microRNAs (miRNAs) and short interfering RNAs (siRNAs) amongst others.

Non-coding RNAs in CSC Plasticity

Long ncRNAs:

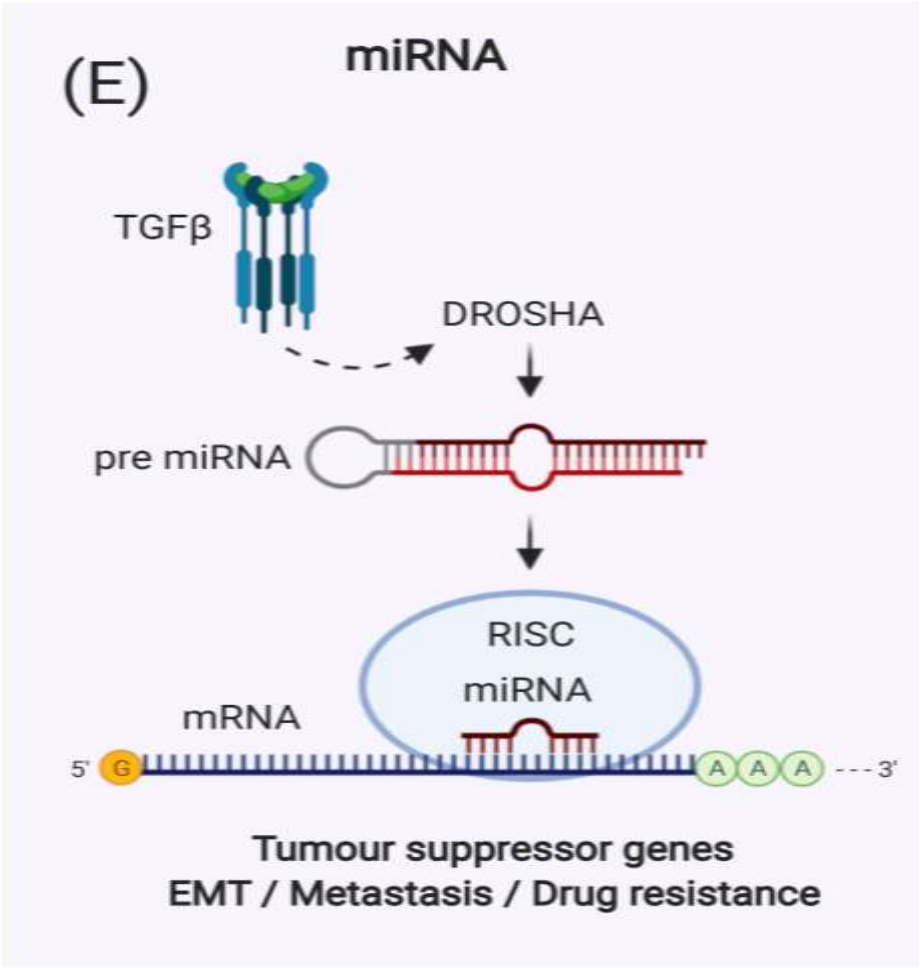
- LncRNAs can physically associate with DNA or proteins to either promote or repress gene expression.
- To promote transcription, lncRNAs can function as guides or scaffolds for the assembly of protein complexes at specific loci.
- To repress transcription lncRNAs can function as decoys, binding and preventing functions of RNA or protein targets.
- Many lncRNAs are known to be involved in the regulation of pluripotency and cell fate transitions and are important in many types of adult stem cells.
- Furthermore, the expression of many lncRNAs is altered during the early stages of reprogramming to iPSCs. Some lncRNAs associated with pluripotency have also been found to be upregulated in CSCs.
- Importantly, many lncRNAs have been implicated in the generation of CSCs via roles in cellular transformation and EMT.



French et al., 2020

Non-coding RNAs in CSC Plasticity

Micro RNAs: Modify gene expression by directly affecting transcription.



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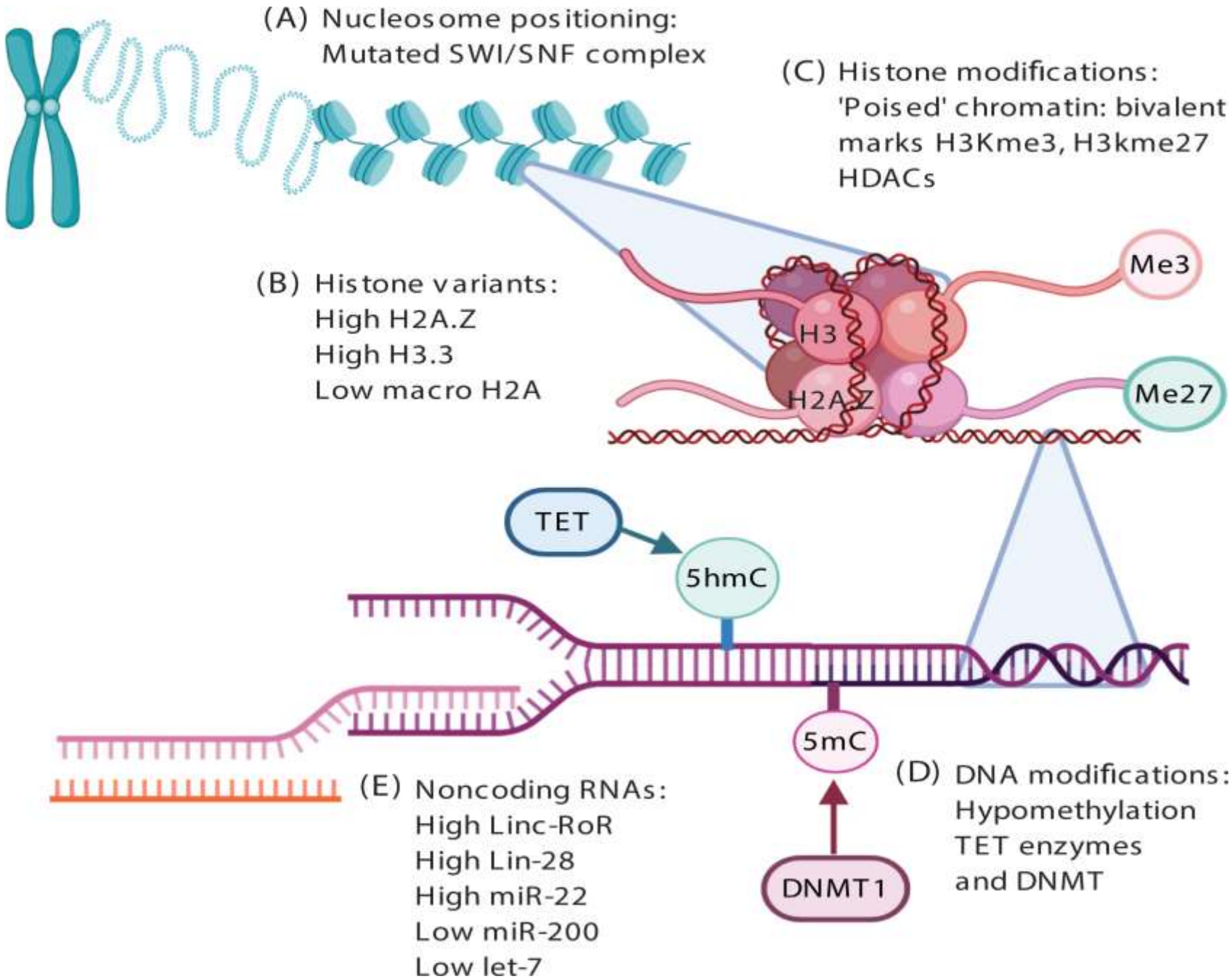

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