Multiple Choice Questions

1. c) They grow in multilayered clumps known as foci.
2. c) Dnmt3a and Dnmt3b.
3. a) The XIST gene produces XIST RNA that binds to the chromosome and turns off its genes.
4. a) Methyl marks.

Subjective Questions (Attempt one question between Q1 and Q2; and one question from Q3 and Q4). Each question = 10 marks

1.(a) Explain Waddington's concept of the "epigenetic landscape," describing how the "ball" and the "valleys" represent a cell's developmental potential and fate. *(5 marks)*
(b) Discuss the molecular mechanisms, such as DNA methylation and histone modification, that influence a cell's movement in this landscape, and provide a real-world example (e.g., phenotypic variation in monozygotic twins). *(5 marks)*

Ans: (a) Waddington's Epigenetic Landscape and Its Molecular Basis

Waddington's "epigenetic landscape" is a metaphorical model that describes the process of cell differentiation. The "ball" represents a cell with its full developmental potential, like a totipotent zygote. The landscape's surface, with its hills and valleys, represents the different possible developmental paths and cell fates. As the cell progresses through development, it rolls down the landscape. Once the cell enters a "valley," its path becomes increasingly restricted, representing its commitment to a specific cell lineage or fate, which is a process known as canalization.

(b) Molecular Mechanisms and a Real-World Example

The movement of the cell "ball" in the epigenetic landscape is governed by molecular mechanisms, primarily DNA methylation and histone modification. DNA methylation involves adding methyl marks to cytosine bases, which typically represses gene expression. Histone modification, such as acetylation, can either open up the chromatin structure for gene transcription (making a path easier to follow) or compact it to silence genes (forming a "hill" that the cell cannot easily traverse). The combination of these marks constitutes the "epigenetic code" that directs which genes are active or inactive, thereby guiding the cell's trajectory.

This non-sequence-dependent inheritance can explain phenotypic differences in genetically identical individuals, such as monozygotic twins. For example, identical twin mice can exhibit different coat colors even though they have the same DNA sequence. This is because differences in environmental factors, such as diet or exposure to toxins, can influence DNA methylation and histone modification patterns, altering gene expression and leading to different phenotypes.

2.(a) Explain the primary purpose of X-inactivation in mammalian females and describe the molecular mechanism by which it occurs, starting from the expression of the XIST gene to the formation of a Barr body. *(5 marks)* (b) Discuss how the presence of Barr bodies supports the role of cytosine methylation in regulating gene expression and maintaining chromosomal stability. *(5 marks)*

Ans: (a) Purpose and Molecular Mechanism

The primary purpose of X-inactivation, also known as Lyonization, is to ensure dosage compensation of X-linked genes between males (XY) and females (XX). Without this process, females would have twice the dosage of X-linked gene products compared to males, which could be lethal or lead to severe developmental abnormalities.

The molecular mechanism begins with the XIST gene on one of the X chromosomes turning on and producing XIST RNA. This non-coding RNA then coats the chromosome from which it was transcribed. This binding of XIST RNA to the chromosome triggers a cascade of events that leads to its silencing. The entire chromosome becomes highly condensed, forming a Barr body in the nucleus.

(b) Support for Epigenetic Regulation

The existence of Barr bodies strongly supports the role of cytosine methylation in regulating gene expression and maintaining chromosomal stability. The silencing of the entire X chromosome is a clear example of how a widespread change in DNA packaging, driven by epigenetic marks like methylation, can lead to a stable and heritable change in gene expression without altering the underlying DNA sequence. The compacted, silenced state of the Barr body is a form of heterochromatin, which is heavily associated with dense DNA methylation. This demonstrates how methylation can serve as a powerful and stable mechanism for long-term gene silencing and the maintenance of chromosomal integrity.

3. (a) Differentiate between somatic and germ-line mutations in terms of their location and inheritance potential. Explain the different types of point mutations (silent, missense, nonsense) with their effects on proteins. *(5 marks)*
(b) Describe the mechanism of a transition mutation caused by wobble base pairing, explain why mismatch repair corrects this error, and outline how the Ames test is used to identify chemical mutagens. *(5 marks)*

Ans:

Feature	Somatics mutation	Germ-line mutation
Location	Occurs in non reproductive somatic cells	Occurs in reproductive cells that give rise to gametes (sperm or eggs)
Inheritance	Non inherited by offspring	Inherited by offspring if passed through gametes
Transmission	Passed to the daughter cells through mitosis, producing a clone of mutant cells	Passed to approximately half of the offspring in the next generation
Distribution in Offspring	Restricted to affected tissues of the individual	Present in all cells of the offspring if inherited
Impact	Affects only the individual; may cause diseases like cancer	Affects both the individual and potentially future generations

Point mutations, which are single base-pair changes, have different effects on the resulting protein:

- Silent mutation: Changes a sense codon to a synonymous codon, resulting in no change to the amino acid sequence of the protein.
- Missense mutation: Changes a sense codon into a different sense codon, leading to the incorporation of a different amino acid.
- Nonsense mutation: Changes a sense codon into a nonsense (stop) codon, causing premature termination of translation.

(b) Wobble Base Pairing and Mutagen Identification

A transition mutation (a purine-to-purine or pyrimidine-to-pyrimidine change) can be caused by wobble base pairing. During DNA replication, a base can exist in a rare tautomeric form that allows it to pair with a non-complementary base. For example, a guanine base can pair with a thymine (G-T wobble pair) instead of a cytosine. This creates a mismatched base pair, which becomes a permanent mutation in the next round of replication. The mismatch repair system is designed to correct these errors by identifying the mismatch and excising the incorrect base from the newly synthesized strand, which is temporarily unmethylated.

The Ames test is a method used to identify chemical mutagens. It uses bacterial strains that have been mutated to be unable to produce a specific nutrient, such as histidine (his−). The bacteria are exposed to a test chemical and then plated on a medium lacking the nutrient. If the chemical is a mutagen, it will cause a reverse mutation (his−→his+), allowing the bacteria to

grow and form colonies. The number of colonies is proportional to the mutagenic potential of the chemical.

4. (a) Explain how DNA methylation patterns change as a cell develops from a totipotent zygote to a differentiated unipotent cell. Discuss the significance of these changes in regulating developmental potential, with reference to pluripotency and multipotency. *(5 marks)*

(b) Explain why reprogramming a differentiated cell back to a pluripotent state is challenging, focusing on the role of established DNA methylation patterns. *(5 marks)*

Ans: (a) DNA Methylation and Developmental Potential

As a cell develops from a totipotent zygote to a differentiated unipotent cell, its DNA methylation patterns undergo a dramatic change. In early embryogenesis, global DNA methylation is largely erased, or reprogrammed. This is followed by the re-establishment of new, cell-specific methylation patterns that "restrict the developmental potential" of progenitor cells and channel them toward specific lineages.

A totipotent cell, like a zygote, can give rise to all cell types of an organism and extraembryonic tissues. A pluripotent cell, such as an embryonic stem cell, can differentiate into any cell type within the three germ layers but not extraembryonic tissues. A multipotent cell, like an adult stem cell, can differentiate into a limited number of cell types, usually within a single lineage. This progression is meticulously supervised by epigenetic regulatory machinery, and the gain of DNA methylation on specific genes is a key mechanism for restricting a cell's developmental potential and committing it to a specific lineage.

(b) Reprogramming a Differentiated Cell

Reprogramming a differentiated cell back to a pluripotent state is challenging because the cell's identity is maintained by stable, long-lasting DNA methylation patterns and other epigenetic marks. Differentiated cells have a specific "methyl signature" that keeps lineage-specific genes active while permanently silencing genes related to pluripotency. This process is unidirectional and makes the cell's fate difficult to reverse. To reprogram a differentiated cell, these established methylation patterns must be erased and remodeled. This requires the induction of genes that are normally silenced in a differentiated state, which is a major hurdle in the reprogramming process.