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TOPIC: EPIGENETICS

COURSE : M.Sc. ZOOLOGY

ELECTIVE PAPER : CELL & MOLECULAR BIOLOGY

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EPIGENETICS

- Monozygotic twins have identical genotypes but are not always phenotypically identical.

- Epigenetics, a branch of modern genetics, describes a situation in which two cells have different phenotypes, although their DNA sequence are identical at the locus responsible for the phenotype, i.e., it explains how a change in phenotype may occur without a change in genotype.

- Epigenetics literally means "on the top of" or "in addition to" genetics, actually refers to changes in the pattern of gene expression/activity resulting from external changes in chromatin that do not involve alterations in the DNA sequence.

- Conrad Hal Waddington (1942) : Credited with coining the term "epigenetics" (derived from the Greek word "epigenesis") to describe how environmental influences on developmental events can affect the phenotype of the adult. Using *Drosophila melanogaster*, Waddington showed that environmental alterations during development induced alternative phenotypes in organisms with identical genotypes through phenomenon "genetic assimilation."

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Epigenetics

A TIMELINE

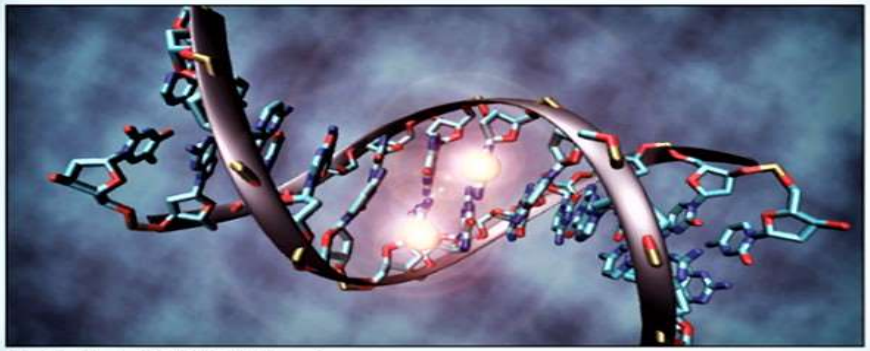

Godfrey Argent Studio

C.H. Waddington

1942 C.H. Waddington coins the term **epigenetics**.

1975 Riggs and Holliday propose that methyl modifications of DNA could influence gene expression.

1984 Solter demonstrates that differences in DNA methylation from parental nuclei impact allele-specific expression and imprinting.



Methylated DNA Molecule

Christoph Bock-Max Planck Institute — http://en.wikipedia.org/wiki/File:DNA_methylation.jpg

1987 Chromatin immunoprecipitation (ChIP) method developed.

1992 Frommer et al. describe bisulfite modification and sequencing-based method to identify individual 5-methylcytosine residues in DNA.

2000 Allis and Strahl propose histone code hypothesis.

2003 ENCODE consortium launched.

2005 Involvement of RNA in epigenetic regulation of expression is proposed.

2008 NIH launches Epigenomics Roadmap Program.


fasboff/iStock

Genetically identical mice that have different coat colors due to epigenetic effects.

2009 First descriptions of 5-hydroxymethyl cytosine as a "6th base" released.

2012 Initial results of ENCODE project published.

2013 Tom Cech proposes that promiscuous binding of PRC2 allows it to scan RNA for genes that have escaped repression leading to maintenance of the repressed state.

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- **Waddington's work, the methylation model of Holliday and Pugh, and the discovery that expression of genes from both the maternal and paternal genomes is required for normal development, all helped set the stage for the birth of epigenetics and epigenomics as fields of scientific research.**

- **A good example of epigenesis is provided by bone marrow stem cells which differentiate into several types of blood cells. A hematopoietic stem cell divides into two daughter cells, one of which continues to have the properties of a hematopoietic stem cell, including the potential to differentiate into all the different types of blood cells. But the other daughter cell becomes either a lymphoid progenitor cell or a myeloid progenitor cell. Lymphoid progenitor cells generate daughter cells that differentiate into lymphocytes, which perform many of the functions involved in immune responses to pathogens. Myeloid progenitor cells divide into daughter cells that are committed to differentiating into red blood cells, different kinds of phagocytic white blood cells, or the cells that generate platelets involved in blood clotting. Lymphoid and myeloid progenitor cells both have the same DNA sequence as the zygote (generated by fertilization of an egg cell by a sperm cell) from which they developed, but they have restricted developmental potential because of epigenetic differences between them.**

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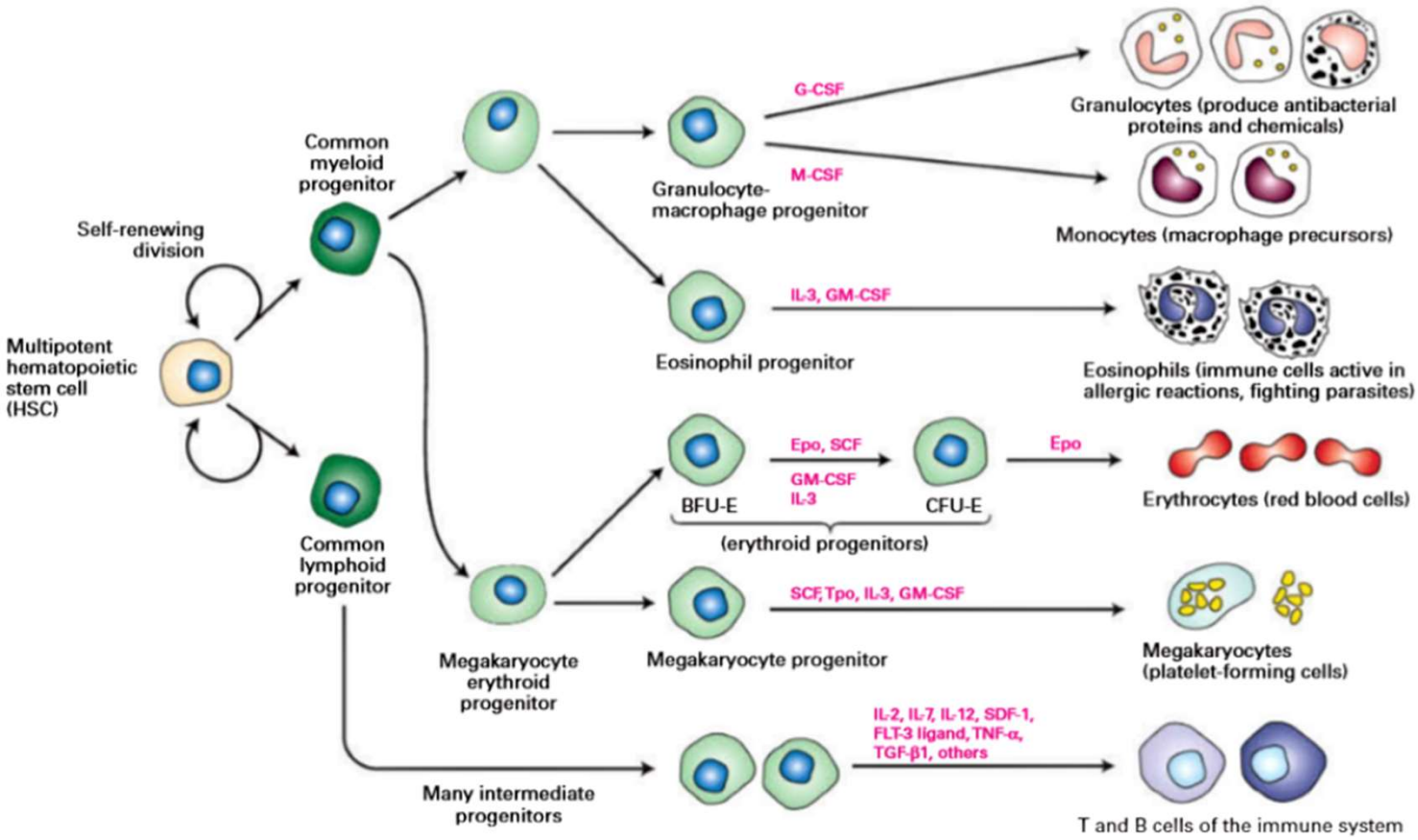

OVERVIEW OF FATE OF LYMPHOID AND MYELOID PROGENITOR CELLS

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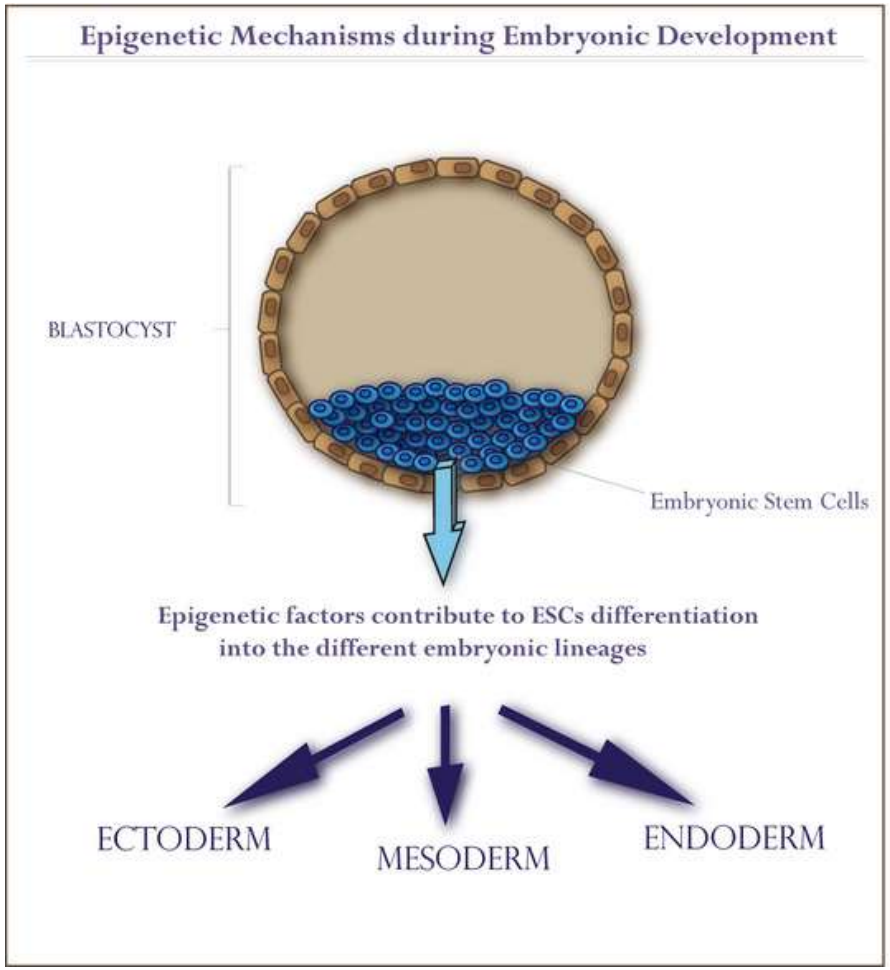

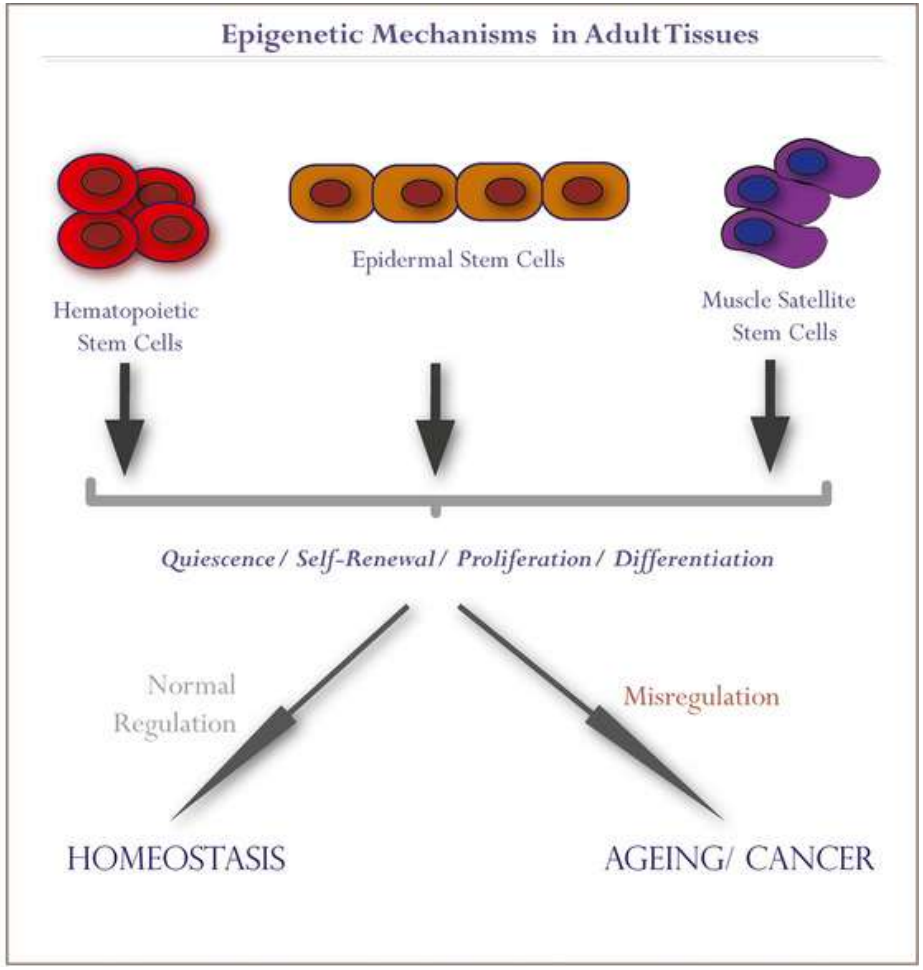

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MOLECULAR BASIS OF EPIGENETICS

Several systems and pathways that result in the establishment, maintenance, and inheritance of the epigenetic state are recognized. These pathways are organized into three categories :-

First Category - Epigenators

- ✓ **Epigenetors are the 'environmental signals' that are received by the cell and that stimulate a response via an intracellular pathway.**

Second Category -Epigenetic Initiators

- ✓ **Epigenetic initiators are the 'responses' to epigenator signals that produce epigenetic changes.**
- ✓ **Components of initiators include protein–protein signal transduction pathways, DNA binding proteins, and noncoding RNAs. The actions of initiators define the location at which epigenetic changes in chromatin will take place**

Third Category -Epigenetic Maintainers

- ✓ **Epigenetic maintainers are 'molecular elements' that maintains the epigenetic modifications that have occurred.**
- ✓ **Components of maintainers include DNA methylation and histone modifications that conserve and sustain the epigenetic changes in the present and future generations.**
- ✓ **They are not sequence-specific, they operate anywhere in the genome, and they depend on initiators to specify the loci at which chromatin modifications will take place.**
- ✓ **Epigenetic maintainers ensure that epigenetic modifications are transmitted by mitosis to daughter cells, or by meiosis to gametes and to subsequent generations.**

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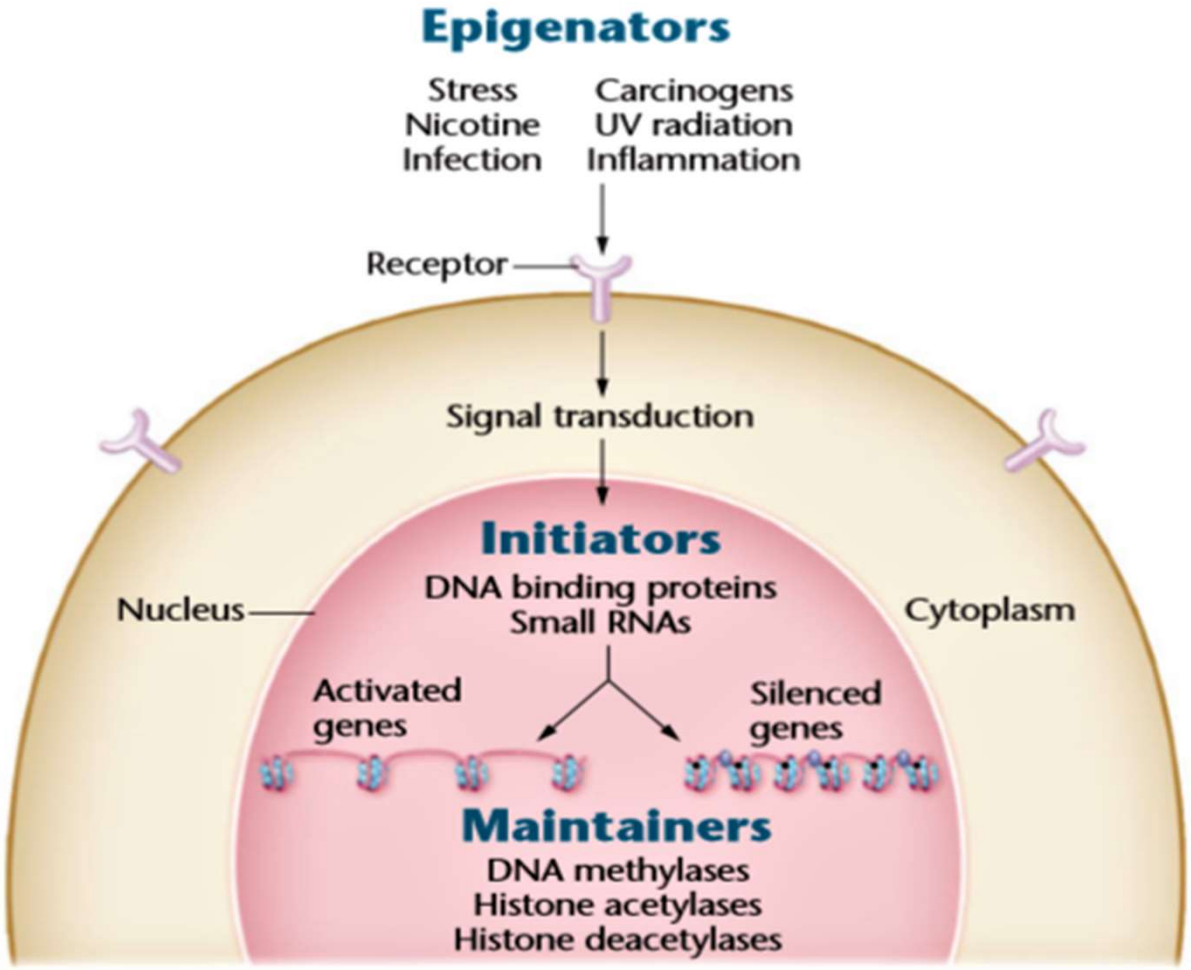
OVERVIEW OF AN EPIGENETIC PATHWAY ORGANISED INTO CATEGORIES



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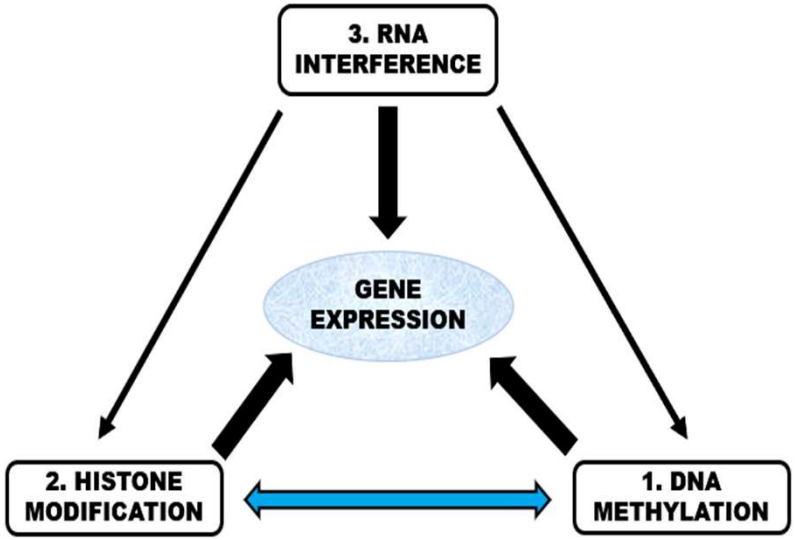
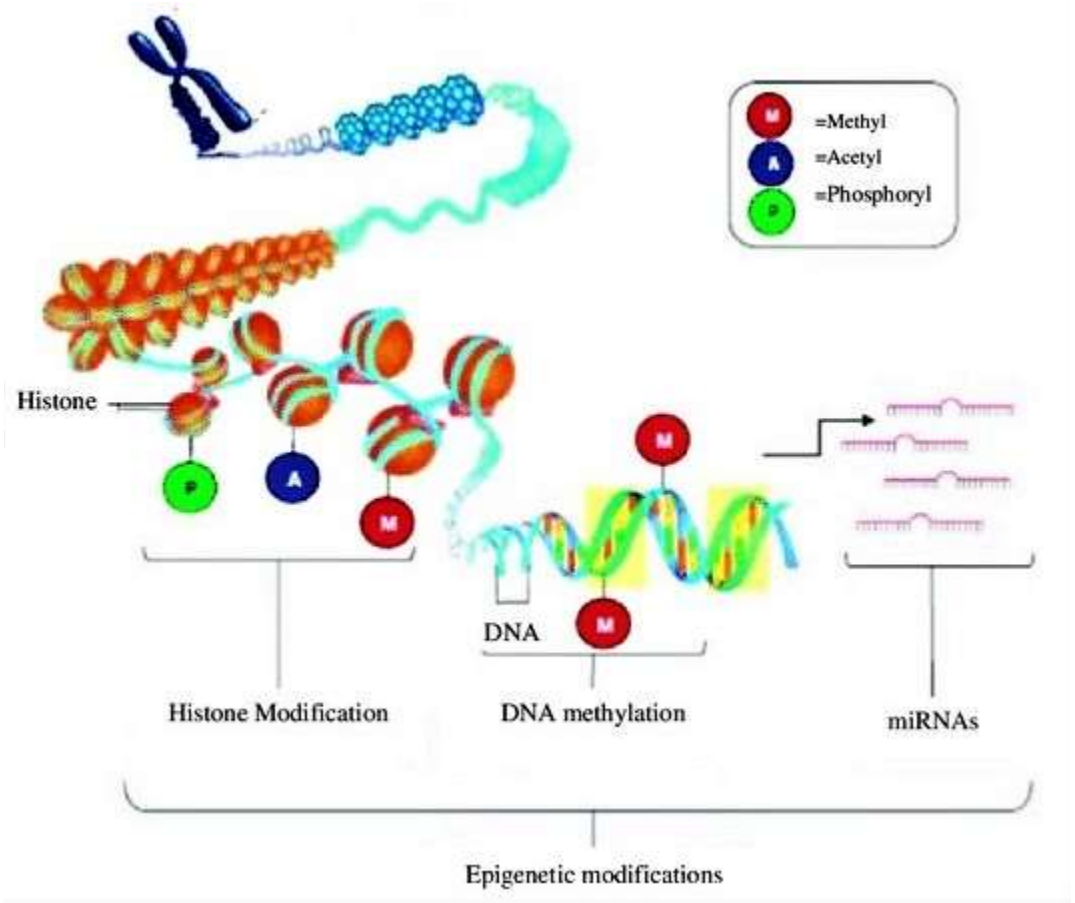
EPIGENETICS MECHANISMS





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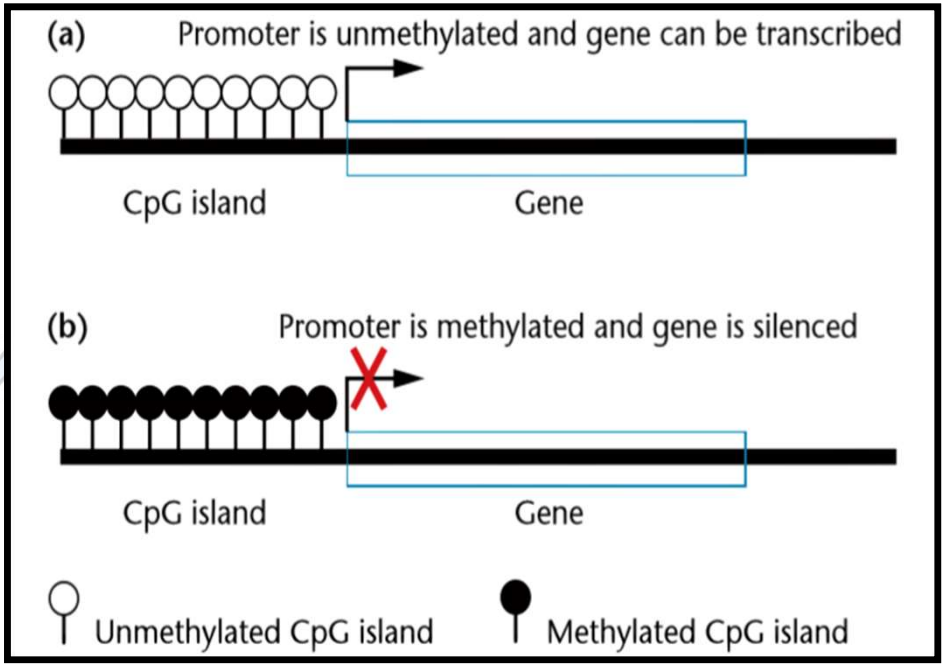
I. METHYLATION

- Mechanism of reversible modification of DNA by the addition or removal of methyl group.

- In mammals, methylation of DNA takes place after replication and involves the addition of a methyl group (–CH3) to cytosine, a reaction catalyzed by methyltransferase enzymes. DNA methylation also occurs during the differentiation of adult cells. In both instances, methylation takes place almost exclusively on cytosine bases adjacent to a guanine, a combination called a CpG dinucleotide. Many of these dinucleotides are clustered in regions, called CpG islands, located in and near promoter sequences adjacent to genes.

LOCATION OF CpG ISLANDS UPSTREAM OF PROMOTER REGIONS



- Methylation of CpG sequences in CpG island promoters in mammals generates binding sites for a family of methyl binding proteins (MBDs) that associate with histone deacetylases, inducing hypoacetylation of the promoter regions and transcriptional repression.

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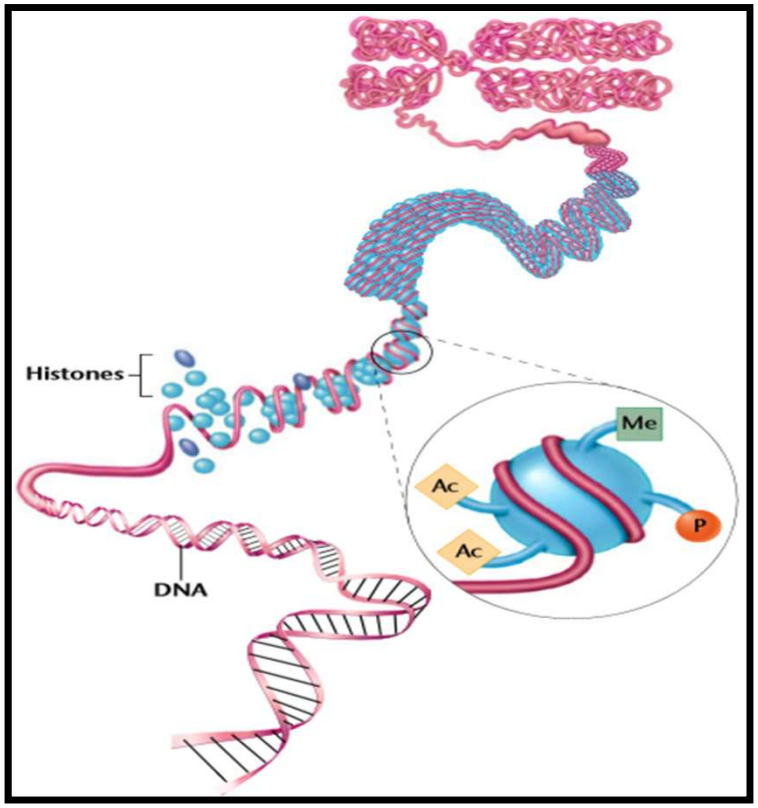
II. HISTONE MODIFICATION

- Mechanism of modification of histones by the addition or removal of chemical groups.

- Chromatin is composed of DNA wound around an octamer core of histone proteins to form nucleosomes. Amino acids in the N-terminal region of these histones can be covalently modified in several ways, including acetylation, methylation, and phosphorylation. These modifications occur at conserved amino acid sequences in the N-terminal histone tails which protrude from the nucleosome. Chemical modification of histones alters the structure of chromatin, making genes accessible or inaccessible for transcription.



Clusters of histones, nucleosomes are the focus of epigenetic modifications. (Ac = acetyl groups, Me = methyl groups, P = phosphate groups)

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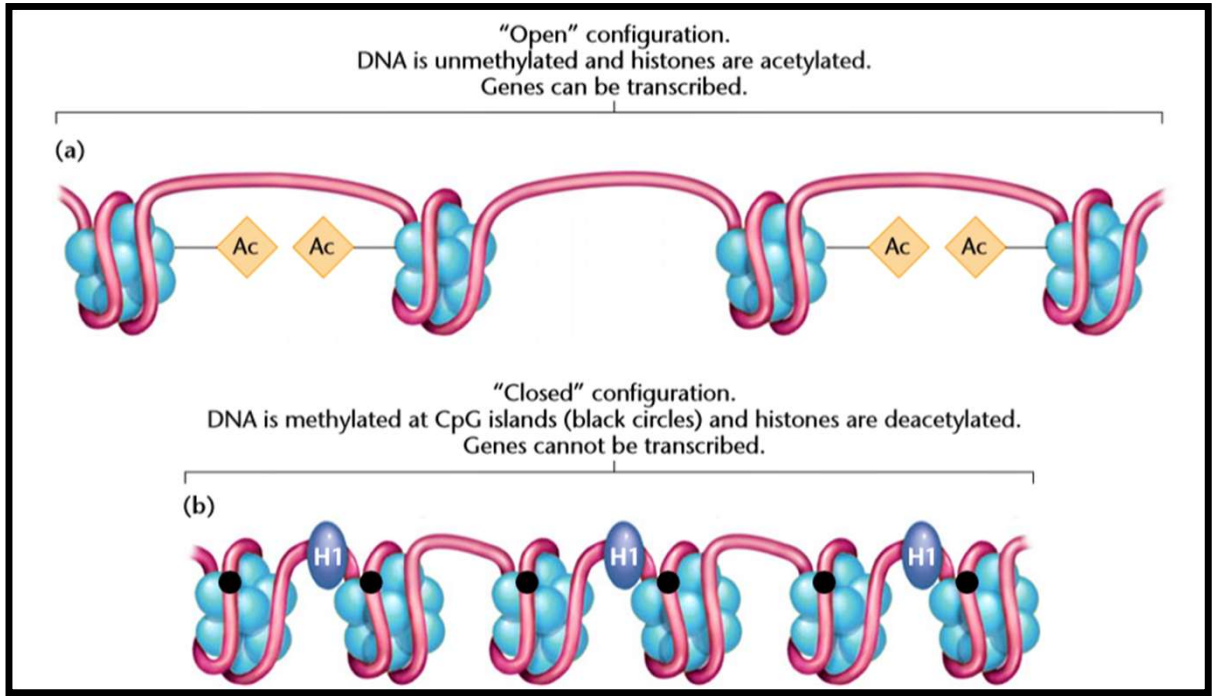
- **Normally, when histones are modified by acetylation, a reaction catalyzed by the enzyme histone acetyltransferase (HAT), chromatin structure becomes "open," making genes on these modified nucleosomes available for transcription (ST Figure a.). This modification is reversible, and acetyl groups can be removed by another enzyme, histone deacetylase (HDAC), changing the chromatin to a "closed" configuration, and silencing genes by making them unavailable for transcription (ST Figure b.). However, the program of histone modification depends on other events, including the presence or absence of methyl groups on histones.**



EPIGENETIC MODIFICATIONS/ALTERATIONS TO THE GENOME.

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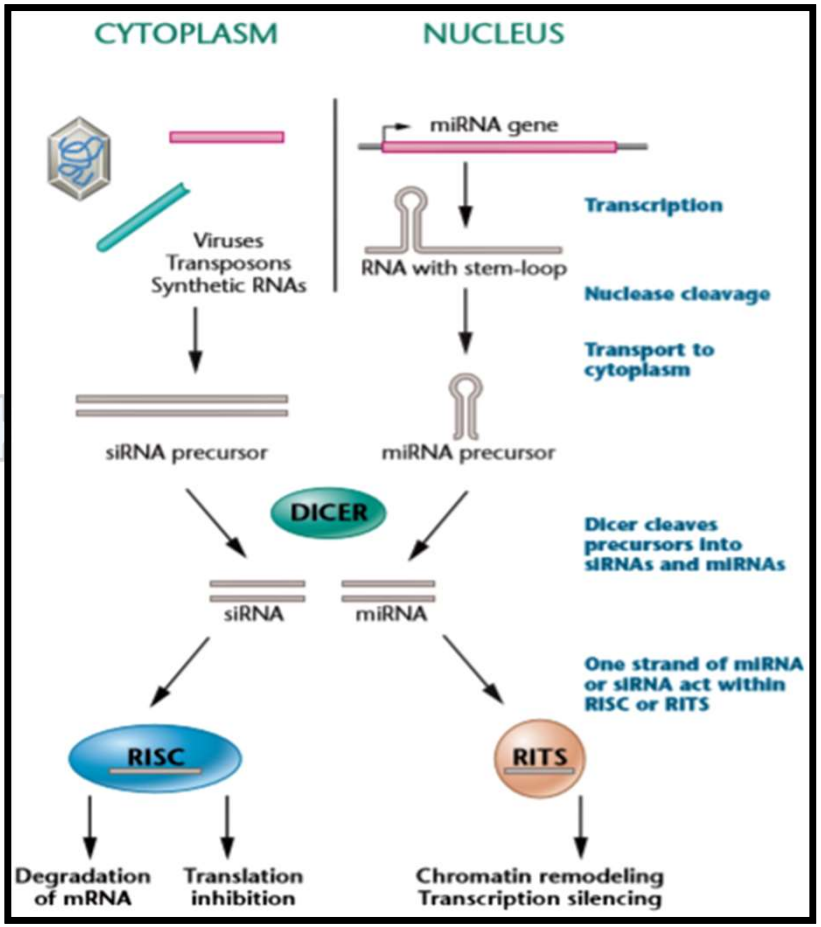
III. RNA INTERFERENCE

- In addition to DNA methylation and histone modification, small, noncoding RNA molecules also participate in epigenetic regulation of gene expression.

- After transcription, these small interfering RNA (siRNA) molecules associate with protein complexes to form RNA-Induced Silencing Complexes (RISCs). RISCs bind to mRNA molecules that carry sequences complementary to siRNA in the RISC. If the siRNA is not perfectly complementary to the mRNA, the binding interferes with translation, resulting in downregulation of gene expression. If, however, the siRNA in the RISC is perfectly complementary to sequences in the mRNA, the mRNA is cleaved and destroyed, effectively silencing the gene.

OUTLINE OF RNA-INDUCED SILENCING COMPLEXES



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- **Recently, it has been discovered that siRNAs can silence genes by directly interfering with transcription initiation. This does not involve any changes in existing epigenetic promoter modifications, nor does it require new modifications. Instead, siRNAs complementary to promoter regions bind to a promoter. Binding blocks the assembly of the preinitiation complex by preventing binding of transcription factor TFIIB and RNA polymerase.**

- **Short RNA molecules can also associate with protein complexes to form RNA-Induced Transcriptional Silencing (RITS) complexes. RITS complexes initiate formation of facultative heterochromatin that silences genes located within these newly created heterochromatic regions. Unlike the heterochromatin at telomeres and centromeres, which is constitutive, the heterochromatic state in facultative heterochromatin is reversible and can be converted to euchromatin, with genes in this region once again accessible for transcription.**

In sum epigenetic modifications alter chromatin structure by several mechanisms including DNA methylation, histone acetylation, and RNA interference, without changing the sequence of DNA. These epigenetic changes create an epigenome that in turn, can regulate normal development or generate responses to environmental signals

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FUNCTIONAL CONSEQUENCES OF EPIGENETICS

A. EPIGENETICS AND IMPRINTING

- Genomic imprinting is an epigenetic phenomenon that causes genes to be expressed in a parent-of-origin-specific manner, i.e., the expression of imprinted genes is determined by parent that contribute them.

- Mutations in imprinted genes can arise by changes in the DNA sequence or by epigenetic changes, called epimutations, both of which are heritable changes in the activity of a gene.

- The pattern of imprinting in mammals is reprogrammed every generation. For example, females receive a maternal and a paternal set of chromosomes. In somatic cells and in germ cells, the maternal chromosome set has female imprints, and the paternal set contains male imprints. When gamete formation begins in female germ cells, both chromosome sets have their imprints erased and are each reprogrammed by changing the pattern of methylation to carry a female imprint pattern that is transmitted to the next generation through the egg . Similarly, in male germ cells, the paternal and maternal chromosome sets have their imprints erased and are reprogrammed by methylation to become a male imprinted set.

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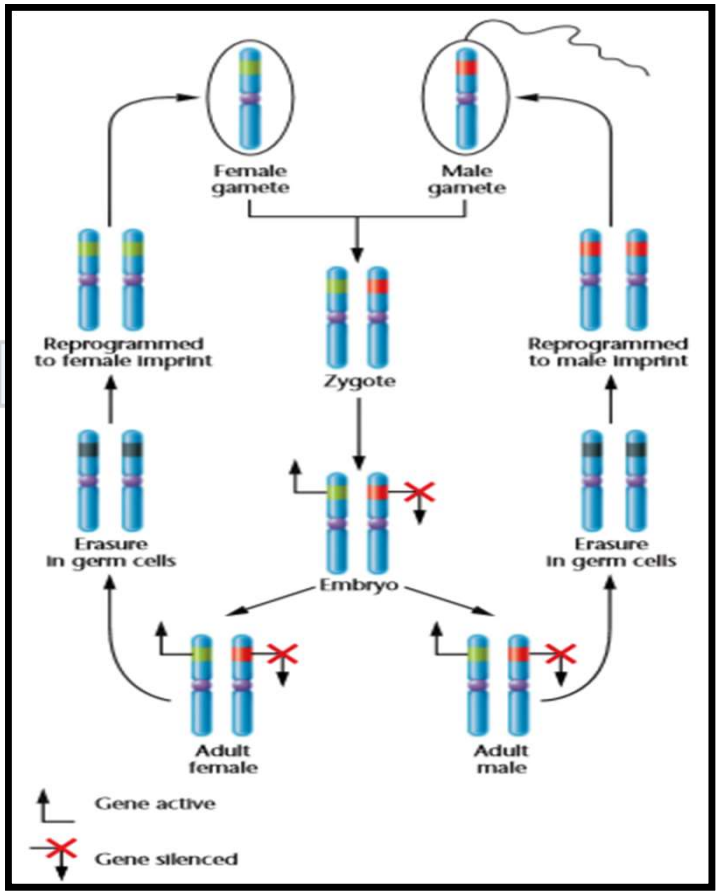
- Reprogramming occurs at two stages: in the parental germ cells and in the developing embryo just before implantation.

- In the first stage, erasure by demethylation and reprogramming by remethylation lay down a parent-specific imprinting pattern in germ cells of the parent.

- In the second stage, large-scale demethylation occurs in the embryo sometime before the 16-cell stage of development. After implantation, differential genomic remethylation recalibrates which maternal alleles and which paternal alleles will be inactivated. It is important to remember that once imprinted, alleles remain inactive in all cells, while genes silenced by epigenetic methylation can be reactivated by external signals during or after differentiation.

OUTLINE OF IMPRINTING PATTERNS REPROGRAMMED EACH GENERATION



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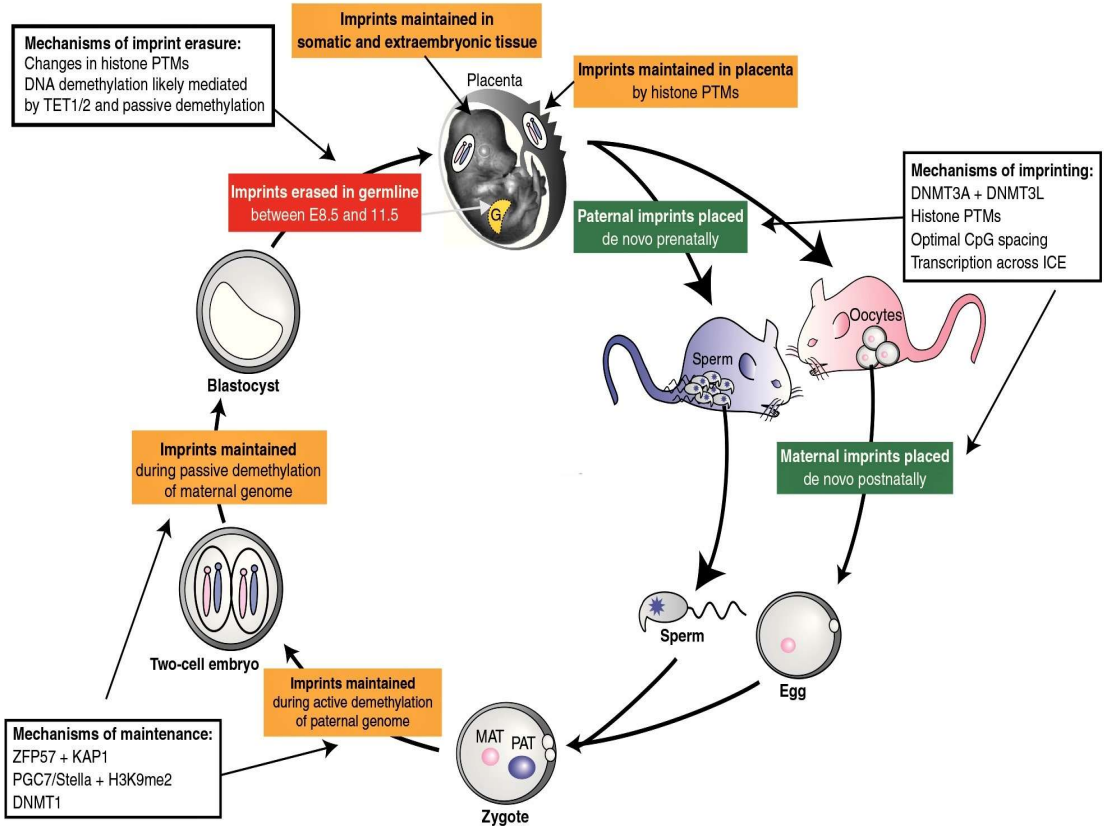
- **Another example of imprinting involves inactivation of one of the X-chromosomes in mammalian females. In mice, prior to development of embryo, imprinting occurs in tissues such that the X-chromosome of paternal origin is inactivated in all cells, while the genes on the maternal X-chromosome remain active. As soon as the embryonic development is initiated, the imprint is "released" and random inactivation of either paternal or maternal X- chromosome occurs.**



GENOMIC IMPRINTING DURING MOUSE LIFE CYCLE

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- **Most human disorders associated with imprinting have their origins during fetal growth and development. Imprinting defects cause Prader–Willi syndrome, Angelman syndrome, Beckwith–Wiedemann syndrome, and several other diseases . However, given the number of candidate genes and the possibility that additional imprinted genes remain to be discovered, the overall number of imprinting-related genetic disorders may be much higher.**

Disorder	Locus
Albright hereditary osteodystrophy	20q13
Angelman syndrome	15q11-q15
Beckwith–Wiedemann syndrome	11p15
Prader–Willi syndrome	15q11-q15
Silver–Russell syndrome	Chromosome 7
Uniparental disomy 14	Chromosome 14

SOME IMPRINTING DISORDERS IN HUMANS

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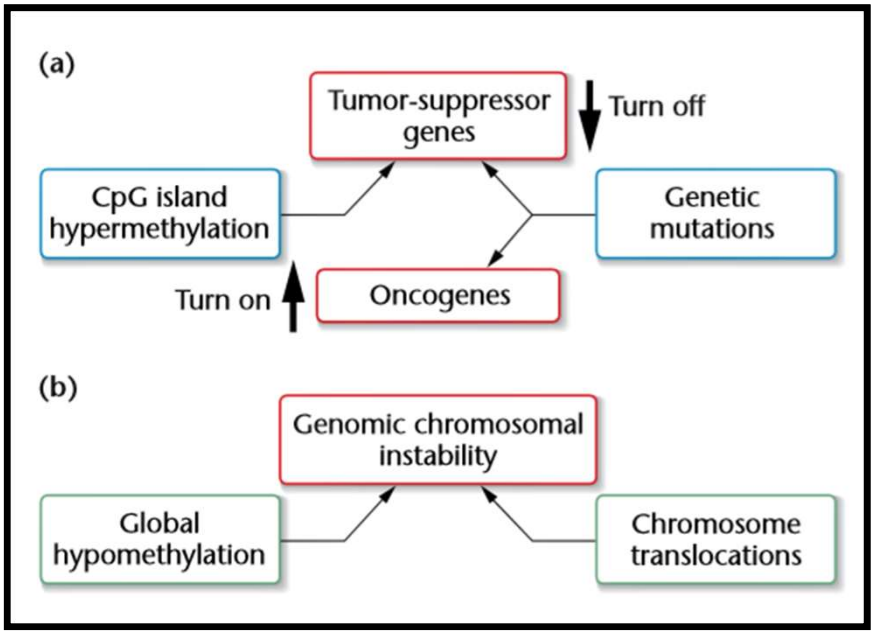
B. EPIGENETICS AND CANCER

- The relationship between epigenetics and cancer was first noted in the 1980s by Feinberg and Vogelstein who observed that colon cancer cells had much lower levels of methylation than normal cells derived from the same tissue.

- Subsequent research by many investigators showed that global hypomethylation is a property of all cancers examined to date. In the ensuing years, it has become clear that the epigenetic states of normal cells are greatly altered in cancer cells and that other epigenetic changes, including selective hypermethylation and gene silencing, are also present in cancer cells. Cancer is now being viewed as a disease that involves both epigenetic and genetic changes that lead to alterations in gene expression.



CANCER: EPIGENETIC AND MUTATIONAL CHANGES TO THE GENOME.

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- The widespread hypomethylation is a hallmark of cancer cells, hypermethylation at CpG islands and inactivation of certain genes, including tumor-suppressor genes , are also found in many cancers, often in a tumor-specific pattern.

- In addition to altered patterns of methylation, many cancer cells also have disrupted histone modification profiles. In some cases, mutations in the genes encoding members of the histone-modifying proteins histone acetyltransferase (HAT) and histone deacetylase (HDAC) are linked to the development of cancer. For example, those affected with Rubenstein–Taybi syndrome inherit a germ-line mutation that produces a dysfunctional HAT and have a greater than 300 fold increased risk of cancer. In other cases, HDAC complexes are selectively recruited to tumor-suppressor genes by mutated, oncogenic DNA binding proteins. Action of the HDAC complexes at these genes converts the chromatin to a closed configuration and inhibits transcription, causing the cell to lose control of the cell cycle.

Some Cancer-Related Genes Inactivated by Hypermethylation in Human Cancers

Gene	Locus	Function	Related Cancers
BRCA1	17q21	DNA repair	Breast, ovarian
APC	5q21	Nucleo-cytoplasmic signaling	Colorectal, duodenal
MLH1	3p21	DNA repair	Colon, stomach
RB1	13q14	Cell-cycle control point	Retinoblasto-ma, osteosar-coma
AR	Xq11-12	Nuclear receptor for androgen; transcriptional activator	Prostate
ESR1	6q25	Nuclear receptor for estrogen; transcriptional activator	Breast, colorectal

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- The mechanisms that cause epigenetic changes in cancer cells are not known, partly because they take place very early in the conversion of a normal cell to a cancerous one, and partly because by the time the cancer is detected, alterations in the methylation pattern have already occurred.

- However, three lines of evidence support the fact that such changes occur very early in the transformation process has led to the proposal that initiating epigenetic changes leading to cancer may occur in stem cells residing in normal tissue.
  - First, epigenetic mechanisms can replace mutations as a way of silencing individual tumor-suppressor genes or activating oncogenes.
  - Second, global hypomethylation may cause genomic instability and the large-scale chromosomal changes that are a characteristic feature of cancer.
  - Third, epigenetic modifications that silence multiple genes, they are more effective than serial mutations of single genes in transforming normal cells into malignant cells.

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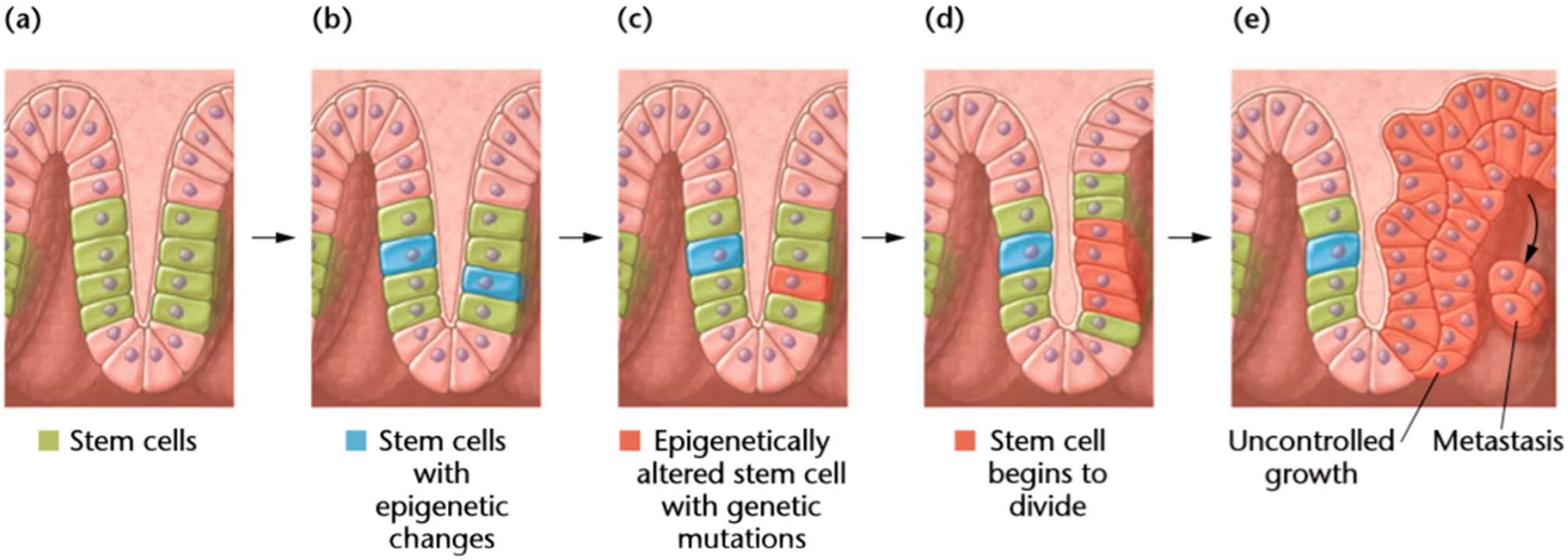
A MODEL OF CANCER BASED ON EPIGENETIC CHANGES IN COLON STEM CELLS



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- In addition to changing ideas about the origins of cancer, the fact that epigenetic changes are potentially reversible makes the development of therapeutic drugs for cancer treatment a possible new approach to chemotherapy.

- The focus of epigenetic therapy is the reactivation of genes that have been silenced by methylation or histone modification, essentially reprogramming the pattern of gene expression in cancer cells. Several drugs for altering epigenetic genome modifications are in clinical trials, and one (decitabine, marketed as Vidaza) has been approved by the U.S. Food and Drug Administration for treatment of acute myeloid leukemia and myelodysplastic syndrome, a precursor to leukemia. This drug is an analog of cytidine and is incorporated into DNA during replication during the S phase of the cell cycle. Methylation enzymes (methyltransferases) bind irreversibly to decitabine, preventing methylation of DNA at many other sites, effectively reducing the amount of methylation in cancer cells.

- Other drugs that inhibit histone deacetylases (HDAC) are also being investigated for use in epigenetic therapy. Experiments with cancer cell lines indicate that inhibiting HDAC activity results in the reexpression of tumor-suppressor genes. One HDAC inhibitor now in clinical trials may be approved for the treatment of some forms of lymphoma. Further research into the mechanisms and locations of epigenetic genome modification in cancer cells will allow the design of more potent specific drugs to target epigenetic events as a form of cancer therapy

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THANK YOU