Cell Commitment

- The generation of specialized cell types is called differentiation.
- But differentiation is only the last, overt stage of a series of events that commit a particular blastomere to become a particular cell type.
- These overt changes in cellular biochemistry and function are preceded by a process resulting in the commitment of the cell to a certain fate.

Cell Commitment

- The process of commitment can be divided into two stages.

- The first stage is a labile phase called **specification**. The fate of a cell or a tissue is said to be specified when it is capable of differentiating autonomously (i.e., by itself when placed into a petri dish or test tube) into an environment that is neutral with respect to the developmental pathway. At the stage of specification, cell commitment is still capable of being reversed.

- The second stage of commitment is **determination**. A cell or tissue is said to be determined when it is capable of differentiating autonomously even when placed into another region of the embryo--a decidedly non-neutral environment. If a cell or tissue type is able to differentiate according to its specified fate even under these circumstances/ it is assumed that commitment is irreversible.

Autonomous Specification

- The first mode of commitment is autonomous specification. Here, the blastomere inherits a set of transcription factors from the egg cytoplasm, and these transcription factors regulate gene expression, directing the cell into a particular path of development.
- In other words, the egg cytoplasm is not homogeneous, but rather contains different morphogenetic determinants (transcription factors or their mRNAs), which will influence the cell's development.
- In this type of specification, the cell "knows“ what it is to become very early and without interacting with other cells.

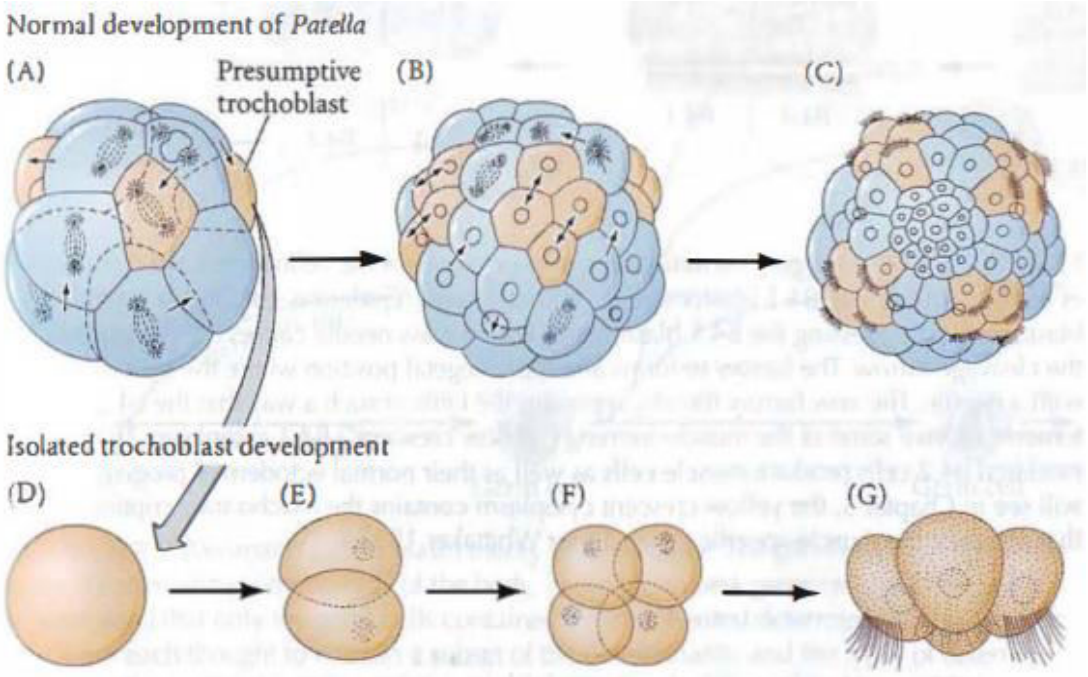
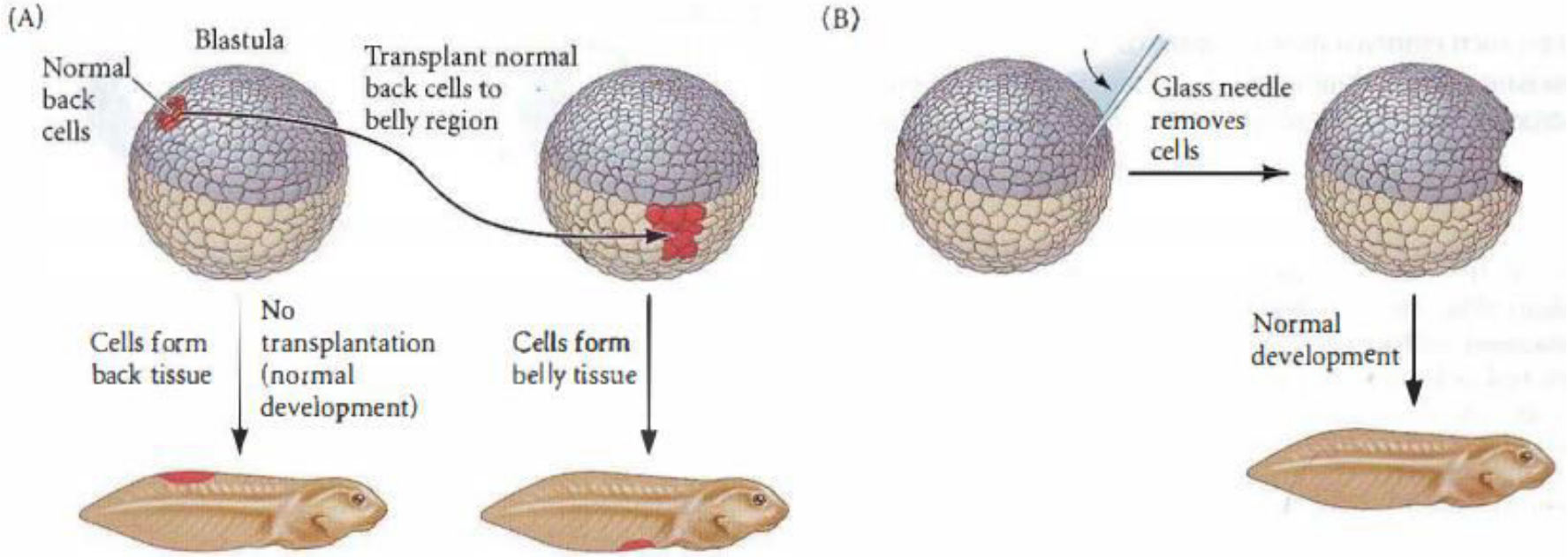


FIG. **Autonomous (mosaic) Specification** (A-C) Differentiation of trochoblast (ciliated) cells of the mollusk Patella. (A) 16-cell stage seen from theside; the presumptive trochoblast cells are shaded. (B) 48-cell stage. (C) Ciliated larval stage, seen from the animal pole. (D-G) Differentiation of a Patella trochoblast cell isolated from the 16-cell stage and cultured in vitro. (E,F) Results of the first and second divisions in culture. (G) Ciliated product, (F). Even in isolated culture, these cells divide and become ciliated at the correct time.

Conditional Specification

Conditional specification is the ability of cells to achieve their respective fates by interactions with other cells (Figure 11.4). Here, what a cell becomes is in large measure specified by paracrine factors secreted by its neighbors



Conditional specification. (A) What a cell becomes depends on its position in the embryo. Its fate is determined by interactions with neighboring cells. (6) If cells are removed from the embryo, the remaining cells can regulate and compensate for the missing part.

Syncytial Specification

- It uses elements of both.
- In early embryos of insects, nuclei divide within the egg; but the cell does not divide.
- Many nuclei are formed within one common cytoplasm.
- A cytoplasm that contains many nuclei is called a syncytium, and the specification of presumptive cells within such a common cytoplasm is called syncytial specification.
- As in the other eggs, the insect egg cytoplasm is not uniform. Nuclei in the anterior part of the cell will be exposed to cytoplasmic transcription factors that are not present in the posterior part of the cell, and vice versa.
- The interactions of nuclei and transcription factors, which eventually result in cell specification, take place in a common cytoplasm.

TABLE II.2 Modes of cell type specification and their characteristics

I. Autonomous specification

Predominates in most invertebrates.

Specification by differential acquisition of certain cytoplasmic molecules present in the egg.

Invariant cleavages produce the same lineages in each embryo of the species. Blastomere fates are generally invariant.

Cell type specification precedes any large-scale embryonic cell migration.

Produces "mosaic" development: cells cannot change fate if a blastomere is lost.

II. Conditional specification

Predominates in vertebrates and a few invertebrates.

Specification by interactions between cells. Relative positions are important.

Variable cleavages produce no invariant fate assignments to cells.

Massive cell rearrangements and migrations precede or accompany specification.

Capacity for "regulative" development; allows cells to acquire different functions.

III. Syncytial specification

Predominates in most insect classes.

Specification of body regions by interactions between cytoplasmic regions prior to cellularization of the blastoderm.

Variable cleavage produces no rigid cell fates for particular nuclei.

After cellularization, both autonomous and conditional specification are seen.

Source: After Davidson 1991.

Developmental Genetics

Genomic Equivalence

- The chromosome in each cell of an organisms body are the mitotic descendants of the chromosome present at the time of fertilization.
- Therefore each somatic cell nucleus has the same chromosome and therefore the same set of genes.
- This fundamental concept is called genomic equivalence.

Differential Gene Expression

- Every cell nucleus contains the complete genome established in the fertilized egg. Therefore the DNAs of all differentiated cells are identical.
- The unused genes in differentiated ceUs are neither destroyed nor mutated, but retain the potential for being expressed.
- Only a small percentage of the genome is expressed in each cell, and a portion of the RNA synthesized in each cell is specific for that cell type.

Regulation of gene expression

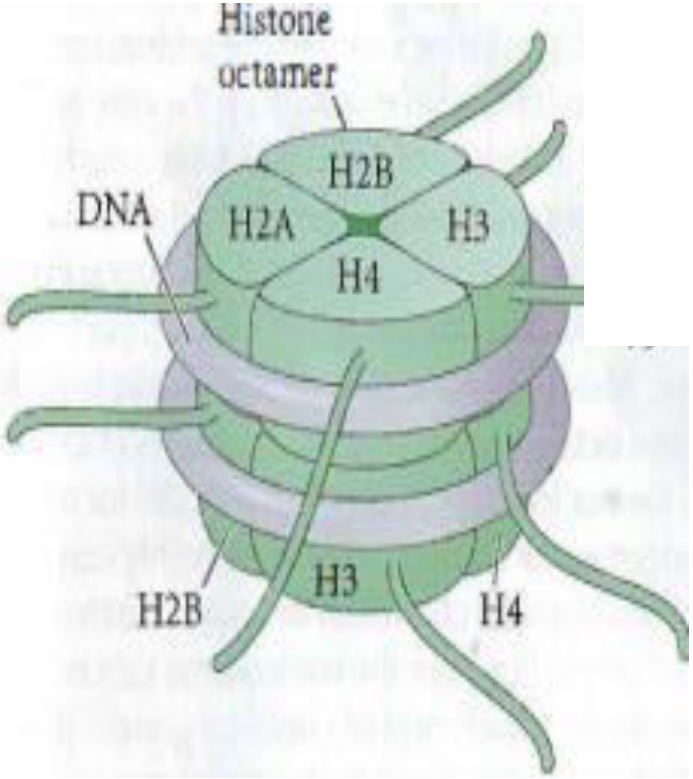
Gene expression can be regulated at several levels such that different cell types synthesize different sets of proteins:

- **Differential gene transcription** regulates which of the nuclear genes are transcribed into nuclear RNA.
- **Selective nuclear RNA processing** regulates whiclh of the transcribed RNAs (or which parts of such a nuclear RNA) are able to enter into the cytoplasm and become messenger RNAs.
- **Selective messenger RNA translation** regulates which of the mRNAs in the cytoplasm are translated into proteins.
- **Differential protein modification** regulates which proteins are allowed to remain and/or function in the cell.

Differential Gene transcription

NUCLEOSOME

- Eukaryotic genes are contained within a complex of DNA and protein called chromatin.
- The protein component constitutes about half the weight of chromatin and is composed largely of histones.
- The nucleosome is the basic unit of chromatin structure (Figure). It is composed of an octamer of histone proteins (two molecules each of his tones H2A, H2B, H3, and H4) wrapped with two loops containing approximately 140 base pairs of DNA.
- Histone HI is bound to the 60 or so base pairs of "linker" DNA between the nucleosomes



Histone Regulation

- The histones are critical because they are responsible for maintaining the repression of gene expression.
- This repression can be locally strengthened (so that it becomes very difficult to transcribe those genes in the nucleosomes) or relieved (so that transcribing them becomes relatively easy)
- Repression and activation are controlled to a large extent by modifying the tails of histones H3 and H4 with two small organic groups: methyl (CH_3) and acetyl ($COCH_3$) residues.

Histone Regulation

Histone Acetylation

- The addition of negatively charged acetyl groups to histones-neutralizes the basic charge of lysine and loosens the histones. This activates transcription.
- Enzymes known as histone acetyltransferases place acetyl groups on histones (especially on lysines in H3 and H4), destabilizing the nucleosomes so that they come apart easily.
- The enzymes that remove acetyl groups-hjstone deacetyJases--stabilize the nucleosomes and prevent transcription.

Histone Regulation

Histone Methylation

- The addition of methyl groups to histones by histone methyltransferases, can either activate or further repress transcription, depending on the amino acid being methylated and the presence of other methyl or acetyl groups in the vicinity.

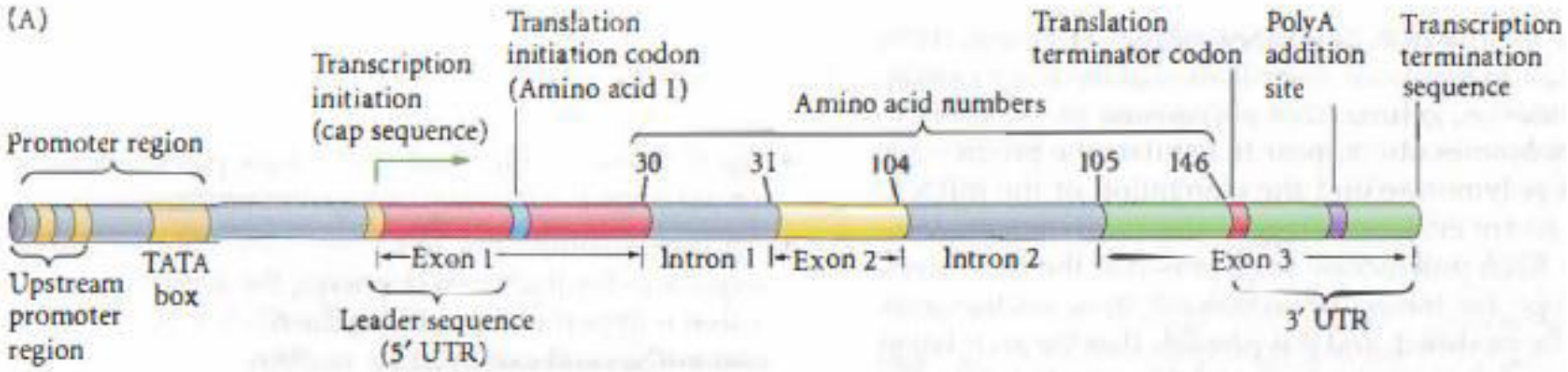
In addition to regulating the initiation of the transcriptional complex (i.e., getting RNA polymerase on the promoter), nucleosomes also appear to regulate the progression of RNA polymerase and the elongation of the mRNA.

Histone Regulation

Epigenetic Memory: Keeping the right genes on or off

- The modifications of histones can also signal the recruitment of the proteins that can retain the memory of transcriptional state from generation to generation through mitosis.
- These are the proteins of the Trithorax and Polycomb families.
- **Polycomb proteins** bind to condensed nueleosomes, keep the genes in an inactive state.
- The Polycomb proteins fall into two categories that act sequentially in repression.
- The first set has histone methyltransferase activities that methylate lysines H3K27 and H3K9 to repress gene activity.
- A second set of Polycomb factors, which bind to the methylated tails of histone 3 and keep the methylation active and also methylate adjacent nudeosomes, thereby forming tightly packed repressive complexes.
- **Trithorax proteins** keep these genes active when bound to the nucleosomes of active genes.
- The Trithorax proteins help retain the memory of activation; they act to counter the effect of the Polycomb proteins.
- It can modify the nucleosomes or alter their positions on the chromatin, allowing transcription factors to bind to the DNA previously covered by polycomb proteins.

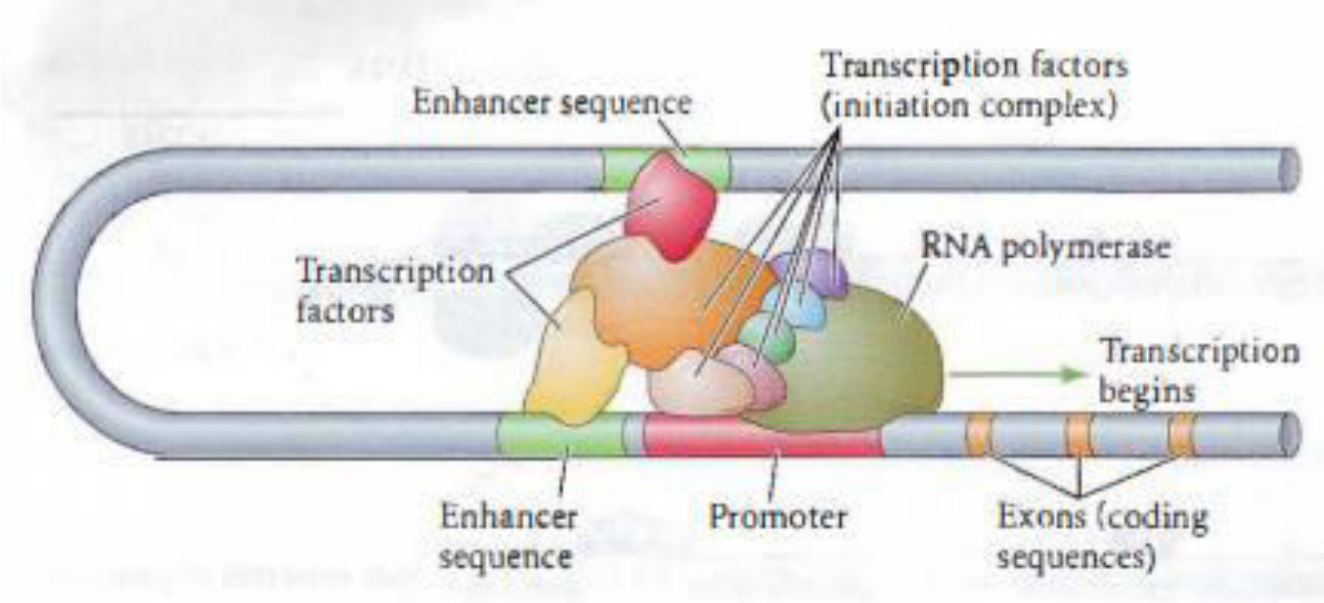
Anatomy of gene



Promoters

- Necessary for controlling where and when a particular gene is transcribed.
- RNA polymerase binds to the given region initiate transcription.
- located immediately upstream from the site where the RNA polymerase initiates transcription.
- Most of these promoters contain the sequence TATA, to which RNA polymerase will be bind. This site, known as the TATA box, is usually about 30 base pairs upstream from the site where the first base is transcribed.

Anatomy of gene



Enhancers

- It is a DNA sequence that controls the efficiency and rate of transcription from a specific promoter.
- Enhancers bind specific transcription factors, proteins that activate the gene by (1) recruiting enzymes (such as histone acetyltransferases) that break up the nucleosomes in the area or (2) stabilizing the transcription initiation complex.
- Enhancers can activate only cis-linked promoters (i.e., promoters on the same chromosome); therefore they are sometimes caned cis regulatory elements.

Anatomy of gene

Transcription Factors

- Transcription factors bind to the enhancer DNA with one part of the protein and use other sites on the protein to interact with one another to reruit histone modifying enzymes.
- Transcription factors have three major domains.
- The first is a DNA-binding domain that recognizes a particular DNA sequence in the enhancer.
- The second is a trans-activating domain that activates or suppresses the transcription of the gene whose promoter or enhancer it has bound.
- The protein-protein interaction domain that allows the transcription factor's activity to be modulated by TAFs (Transcription Associated factors) or other transcription factors.

Anatomy of gene

Pioneer Transcription Factors

- Certain transcription factors that penetrate repressed chromatin and bind to their enhancer DNA sequences called "pioneer" transcription factors
- These are critical in establishing certain cell lineages.
- **FoxA proteins**: bound to the DNA during mitosis, providing a mechanism to re-establish normal transcription in presumptive liver cells.
- **Pax 7 proteins:** activates muscle-specific gene transcription in a population of muscle stem cells by binding to its DNA recognition sequence.
- **Pbx proteins:** helps MyoD transcription factors (critical for muscle development initiation in embryo) which bind to DNA element adjacent to DNA sequence recognized byb MyoD.

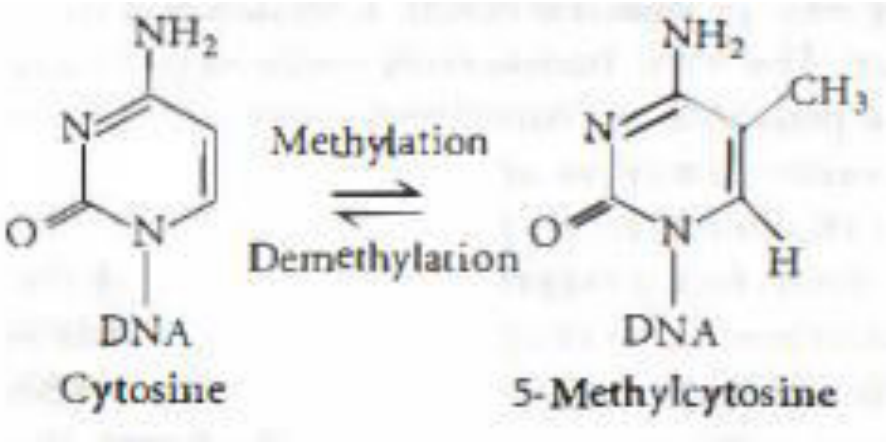
Anatomy of gene

Silencers

- DNA regulatory elements that actively repress the transcription of a particular gene. They are called "negative enhancers."
- **Neural restrictive silencer element (NRSE**) is a DNA sequence in mouse that prevents a promoter activation in any tissue except neuron.
- Its expression is limited to the nervous system: those encoding synapsin I, sodium channel type II, brain-derived neurotrophic factor, Ng-CAM, and Ll.
- The protein that binds to the NRSE is a zinc finger transcription factor called neural restrictive silencer factor (NRSF) expressed in every cell except a mature neuron.

DNA methylation and the control of transcription

- The promoters of inactive genes become methylated at certain cytosine residues, and the resulting methylcytosine stabilizes nucleosomes and prevents transcription factors from binding.



- A globin gene that is activated in a red blood cell precursor has the same nucleotides as the inactive β-globin gene in a fibroblast or retinal cell of the same animal. In vertebrates after DNA replication, the "fifth base" of cytosine is enzymatically methylated to form 5-methylcytosine. In mammal about 5% of the cytosines are converted and it occur only when the cytosine residue is followed by a guanosine. This is the major mechanism of transcriptional regulation which control the level of transcription.

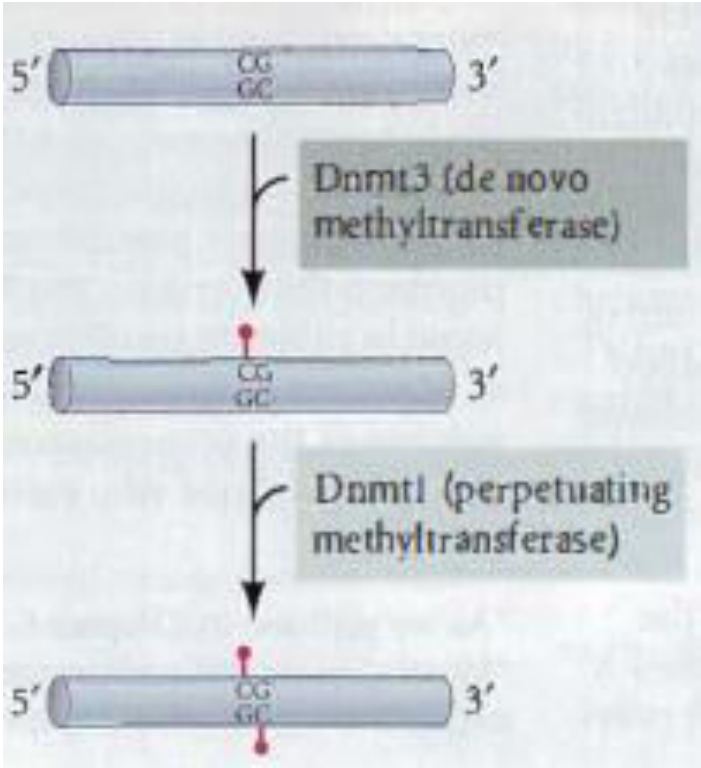
DNA methylation and the control of transcription

Mechanism

- DNA methylation appears to act in two ways to repress gene expression.
- First, it can block the binding of transcription factors to enhancers. Several transcription factors can bind to a particular sequence of unrnethylated DNA, but they cannot bind to that DNA if one of its cytosines is methylated.
- Second, a methylated cytosine can recruit the binding of proteins that facilitate the methylation or deacetylation of histones, thereby stabilizing the nucleosomes forming tight complexes with the DNA and don't allow other transcription factors and RNA polymerases to find the genes.

Inheritance and stabilization of DNA methylation patterns

- Methylated cytosines in DNA can bind particular proteins such as MeCP2.
- MeCP2 recruit enzyme DNA methyltransferase-3 (Dnmt3) to the chromatin which methylates previously unmethylated cytosines on the DNA resulting in repression of relatively large region of DNA.
- The newly established methylation pattern is then transmitted to the next generation by DNA methyl transferase-I (Dnmt1).
- This enzyme recognizes methyl cytosines on one strand of DNA and places methyl groups on the newly synthesized strand opposite it. This is why it is necessary for the C to be next to a G in the sequence. Thus, in each cell division, the pattern of DNA methylation can be maintained. It can be stably inherited by all the progeny of that cell.



Differential RNA Processing

- To become an active protein, the RNA must be

(1) processed into a messenger RNA by the removal of introns,

(2) translocated from the nucleus to the cytoplasm,

(3) translated by the protein synthesizing apparatus.

(4) posttranslational modification of protein to become active.

- There are two major ways in which differential RNA processing can regulate development

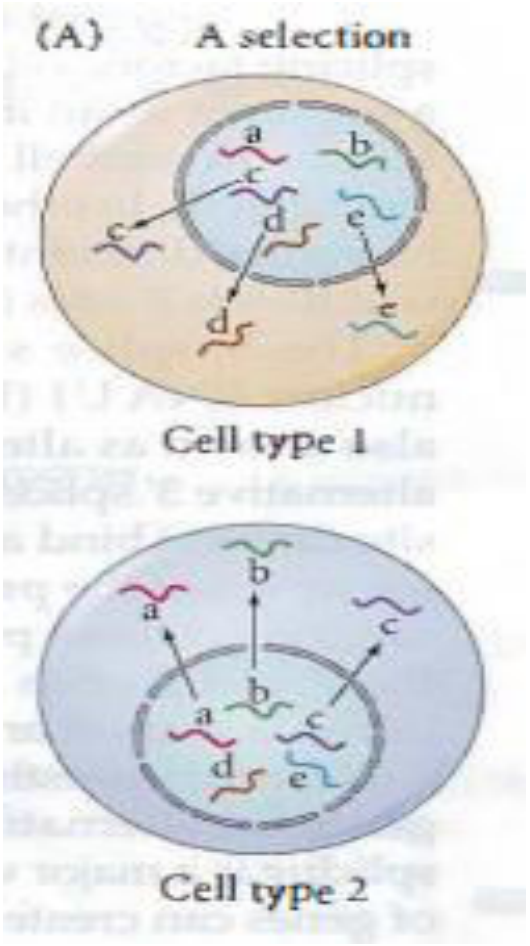
(A) Nuclear RNA Selection

(B) Differential RNA Splicing

Differential RNA Processing

(A) Nuclear RNA Selection-

- Selecting which nuclear transcripts are processed into cytoplasmic messages.
- Different cells select different nuclear transcripts to be processed and sent to the cytoplasm as messenger RNA.
- Thus, the same pool of nuclear transcripts can give rise to different populations of cytoplasmic mRNAs in different cell types.

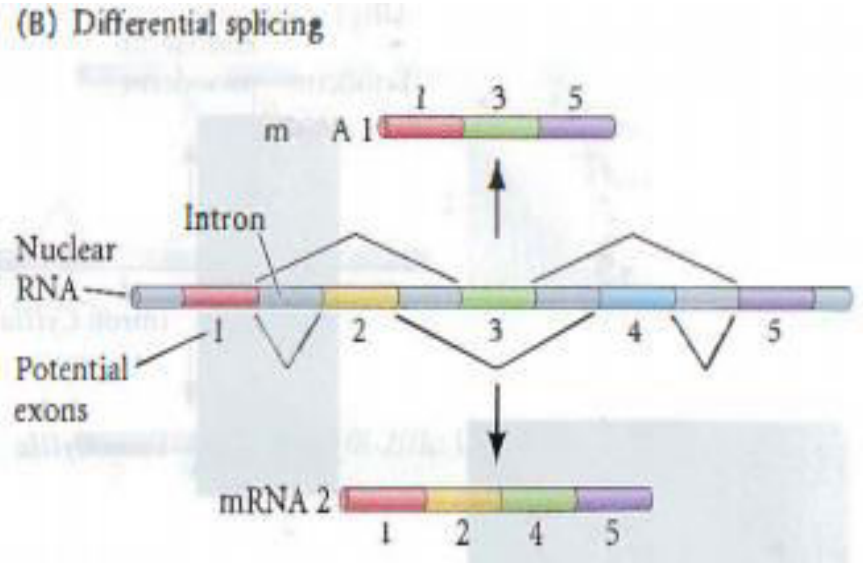


Same nuclear RNA transcripts are made in tvvo cell types, but the set that becomes cytoplasmic messenger RNA is different.

Differential RNA Processing

(B) Differential RNA Splicing-

- Splicing of mRNA precursors into messages that specify different proteins by using different combinations of potential exons.
- If an mRNA precursor had five potential exons, one cell type might use exons 1, 2, 4, and 5; a different type might use exons 1,2, and 3; and yet another cell type might use all five.
- Thus a single gene can produce an entire family of proteins.



Same nuclear RNA is spliced into different mRNAs by selectively using different exons.