Regulation of eukaryotic gene expression

- The levels of eukaryotic gene regulation include the following:

1-Alteration of gene content.

2- Transcriptional regulation.

3- Post-transcriptional regulation.

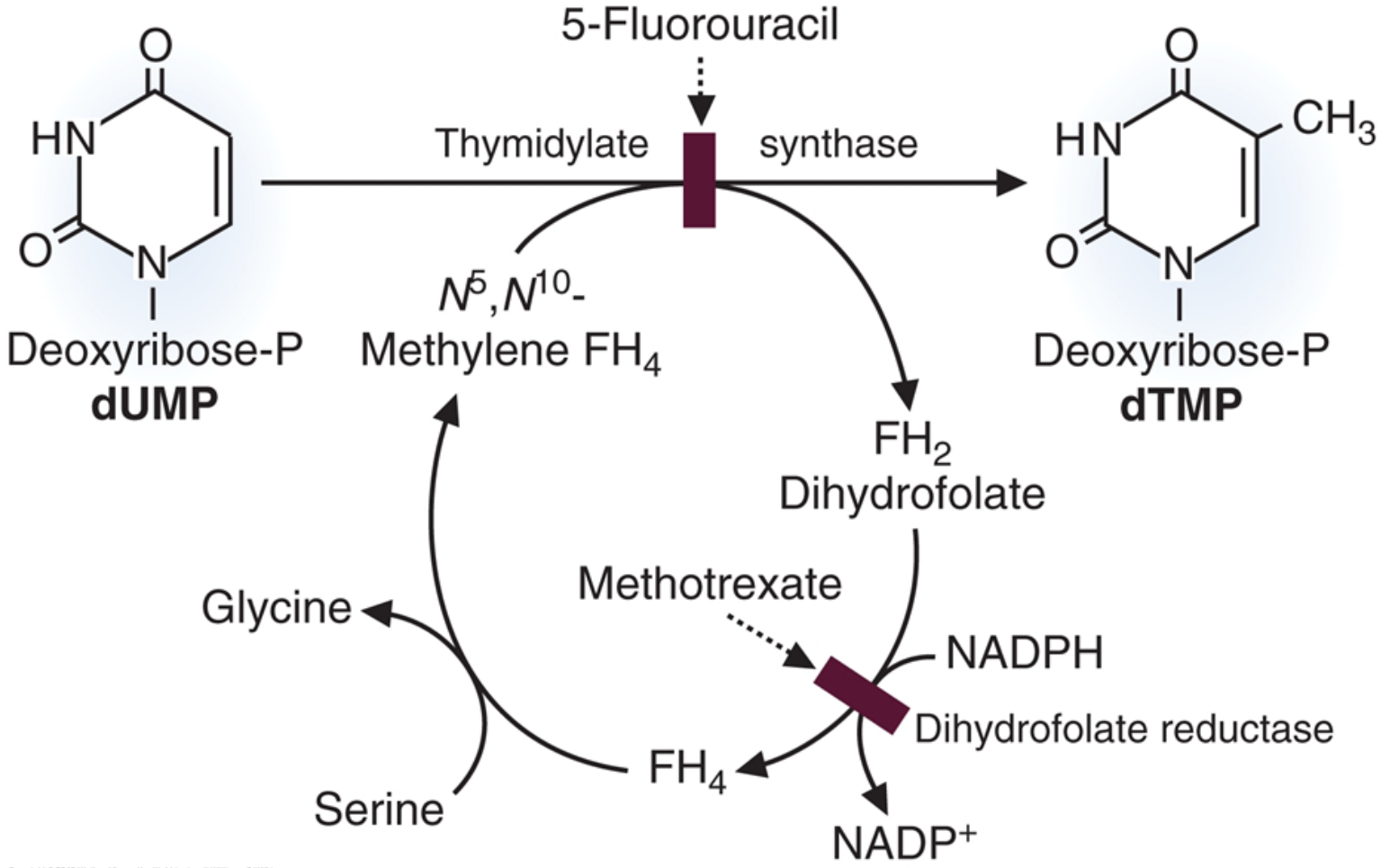
1-Alteration of gene content (regulation through modification to DNA)

- The eukaryotic genome may be changed by the following mechanisms:

Gene amplification:

- It is the increase of a gene product by increasing the number of genes coding for that product e.g. histone & rRNA genes.
- More than 20 genes are known to be amplifiable e.g. dihydrofolate reductase genes.

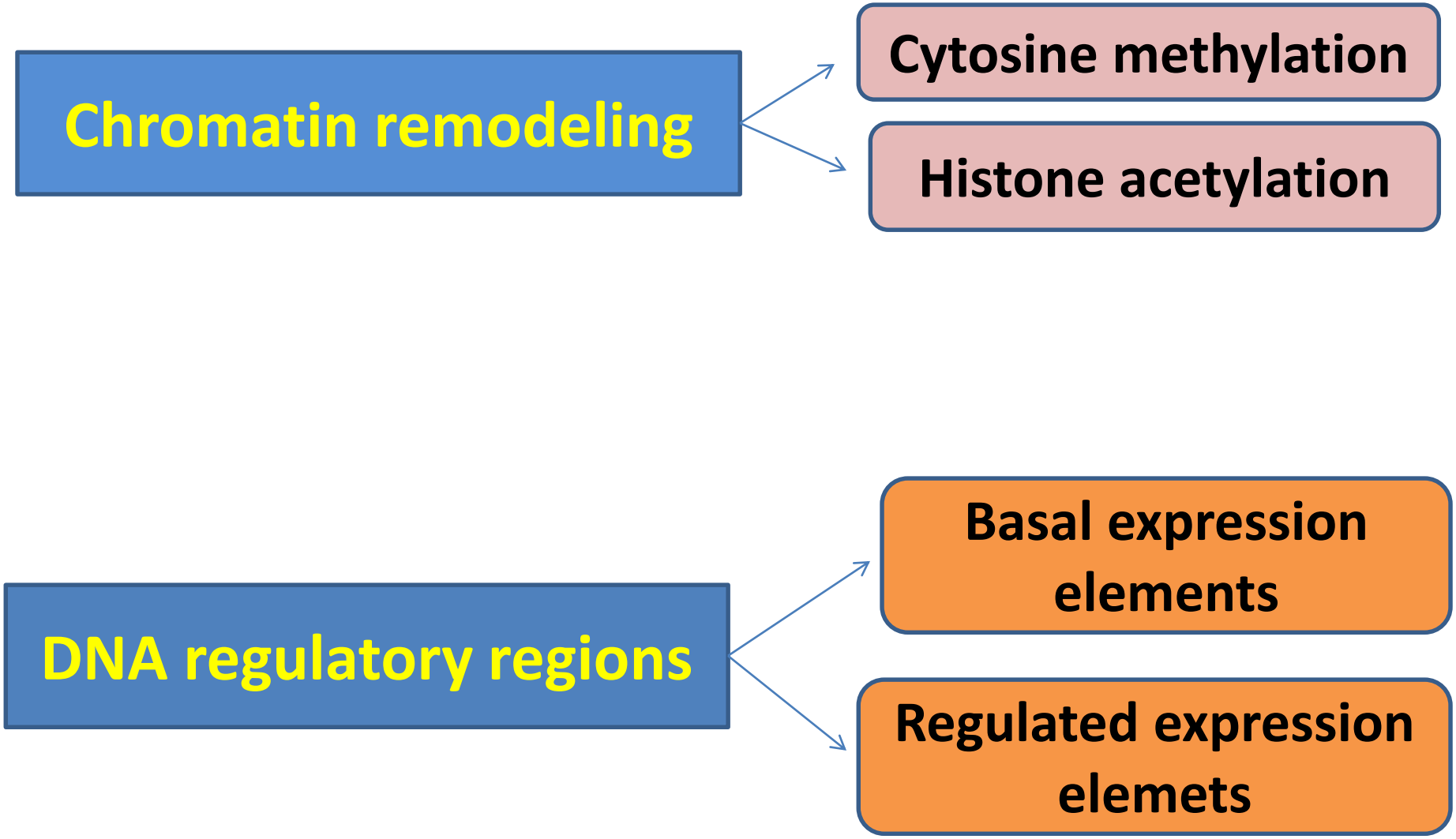
- Dihydrofolate (FH2 is a derivative of folic acid) is reduced to tetrahydrofolate (FH4) by dihydrofolate reductase.
- Methylene-FH4 is required for conversion of dUMP to dTMP which is utilized for DNA synthesis.
- It has been demonstrated in patients receiving methotrexate (an inhibitor of FH2-reductase) as a treatment for cancer that malignant cells can develop **drug resistance by increasing the number of genes for dihydrofolate reductase.**
- ***For cancer, methotrexate competitively inhibits dihydrofolate reductase (DHFR) (methotrexate is structurally similar to folate). The affinity of methotrexate for DHFR is about 1000-fold that of folate.***



Gene diminution:

- It is a rare form of regulation by removing a gene or genes from the genome e.g. complete loss of all genes in red blood cells during development.
- *A gene whose expression is only needed at a particular developmental point or in a particular tissue may be shut off by gene diminution. As reticulocytes mature into red blood cells all of their genes are lost as the nucleus is degraded.*

2- Transcriptional regulation.



Cytosine methylation

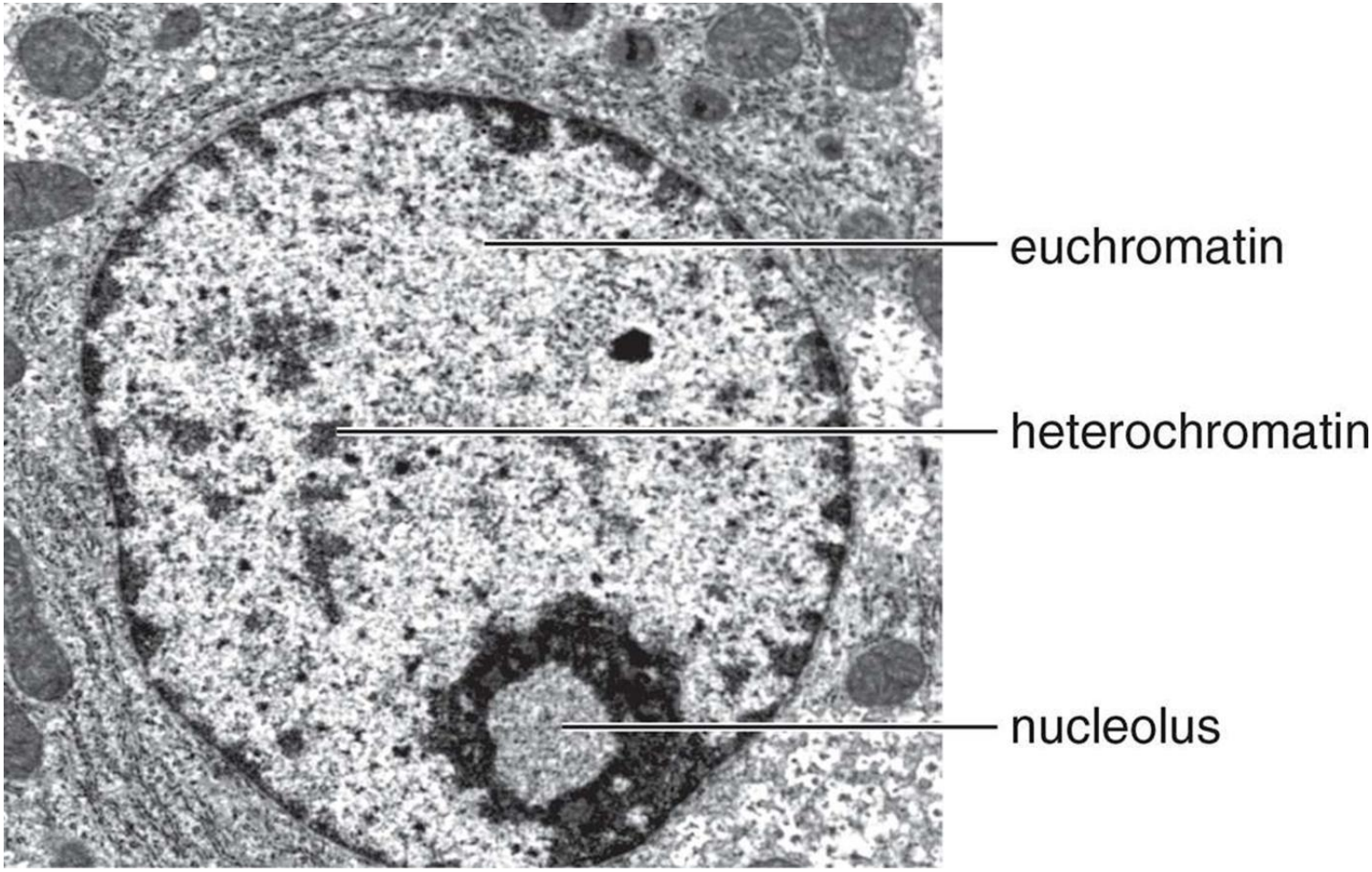
- **Many mammalian genes have CG- rich regions upstream of the coding region, that provide multiple sites for methylation.**

- **The methyl group is added by DNA methylase on both strands of DNA in 5`-CG-3` dinucleotides.**

- **Heavy methylation is associated with genes for which the rate of transcription is low.**

- **Transcribtionally inactive chromatin is densly packed (**a highly coiled and compact structure) **during interphase as observed by electron microscopic studies and is referred to as heterochromatin; transcriptionally active chromatin stains less densely and is referred to as euchromatin.**

- **methylation converts the active euchromatin into inactive heterochromatin and it may result in transcriptional silencing. Reactivation occurs by demethylation.**


euchromatin
heterochromatin
nucleolus

- **Heavy methylation is one of epigenetic mechanisms that marks a gene for silencing.**

- **The Greek prefix *epi-* (ἐπι- "over, outside of, around") in *epigenetics* implies features that are "on top of" or "in addition to" the traditional genetic basis for inheritance. *Therefore epigenetic refers to heritable changes in gene expression that are not due to changes in the DNA sequence itself.***

Histone acetylation

- **Acetylation at the N-terminal (lysine) reduces the histone positive charges & decreases the binding affinity of histones for the negative charged DNA, which allows the access of the different transcription factors to act.**

- **Deacetylation reverses the process.**

DNA regulatory regions

- **Each gene can be divided into coding & regulatory regions, as defined by the transcription start site.**

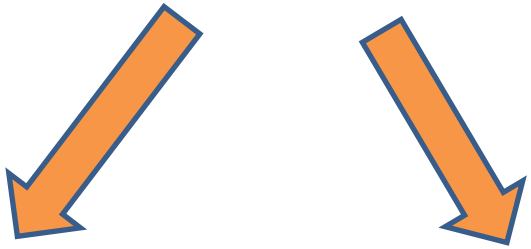
- **In case of class II gene (transcribed by polymerase II), the coding region contains the DNA sequence that is transcribed into mRNA, which is translated into protein.**

The regulatory region consists of two classes of elements as follows:

A- Basal expression elements

B- Regulated expression elements (cis-acting elements)

- **Basal expression elements**: it contains



proximal element or TATA box that directs the RNA polymerase II to the correct start site (+1)

The upstream element e.g. CAAT box or GC box that specify the frequency of initiation

- **Regulated expression elements (cis-acting elements): they are specific DNA sequences that are present on the same gene, so termed cis-elements, and are responsible for regulation of expression & include the following elements:**

Enhancers	Silencers	Other regulatory elements
they interact with gene regulatory proteins or trans-factors (so termed because they are produced by other genes) and increase the rate of expression (they facilitate initiation of transcription)	they interact with gene regulatory proteins or trans-factors and decrease the rate of expression (they inhibit initiation of transcription)	They mediate response to various signals including chemicals, metals and hormones. In the latter case, it is termed the hormone response elements (HRE)

3-Post-transcriptional regulation

- **Alternative splicing**: for example, in the thyroid gland, the calcitonin gene produces a transcript that codes for the hormone calcitonin , the same gene is expressed in neurons and produces a transcript that codes for calcitonin-related peptide which is involved in taste.

❖ **Regulation of RNA stability**:

- RNAs have different half-life time e.g. the longer the poly A tail, the longer the half-life time of mRNA.

- Certain proteins interact with mRNA, forming ribonucleoproteins. Some of these proteins protect mRNA from digestion by Rnase enzyme, enhancing translation.

❖ mRNA editing:

- The only example known in humans involves the editing of **apolipoprotein B mRNA.**
- Apo B-48 is synthesized by the intestine, and Apo B-100 is synthesized by the liver.
- The apolipoprotein B mRNA synthesized by the intestine is primarily the same as that synthesized by the liver. **However, intestinal cells convert a site-specific cytosine of mRNA to uracil.** This results in the formation of a stop codon near the middle of the mRNA that terminates the synthesis of the growing polypeptide at 48% that of apo B-100.
- The differences in the translated proteins **is not due to alternative splicing but is due to the tissue specific RNA editing event.**