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.

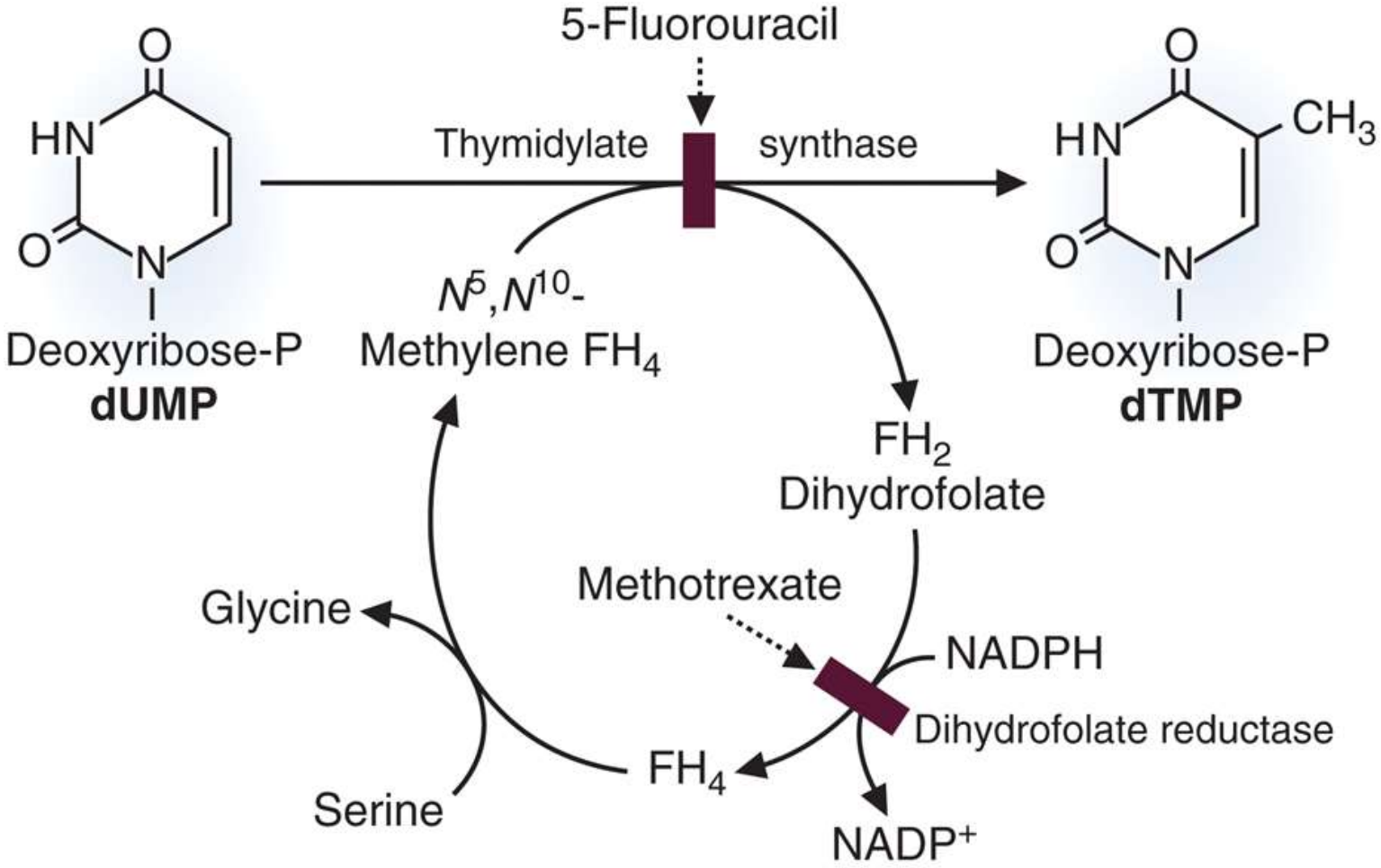
1-Alteration of gene content (regulation through modification to DNA)

- 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.

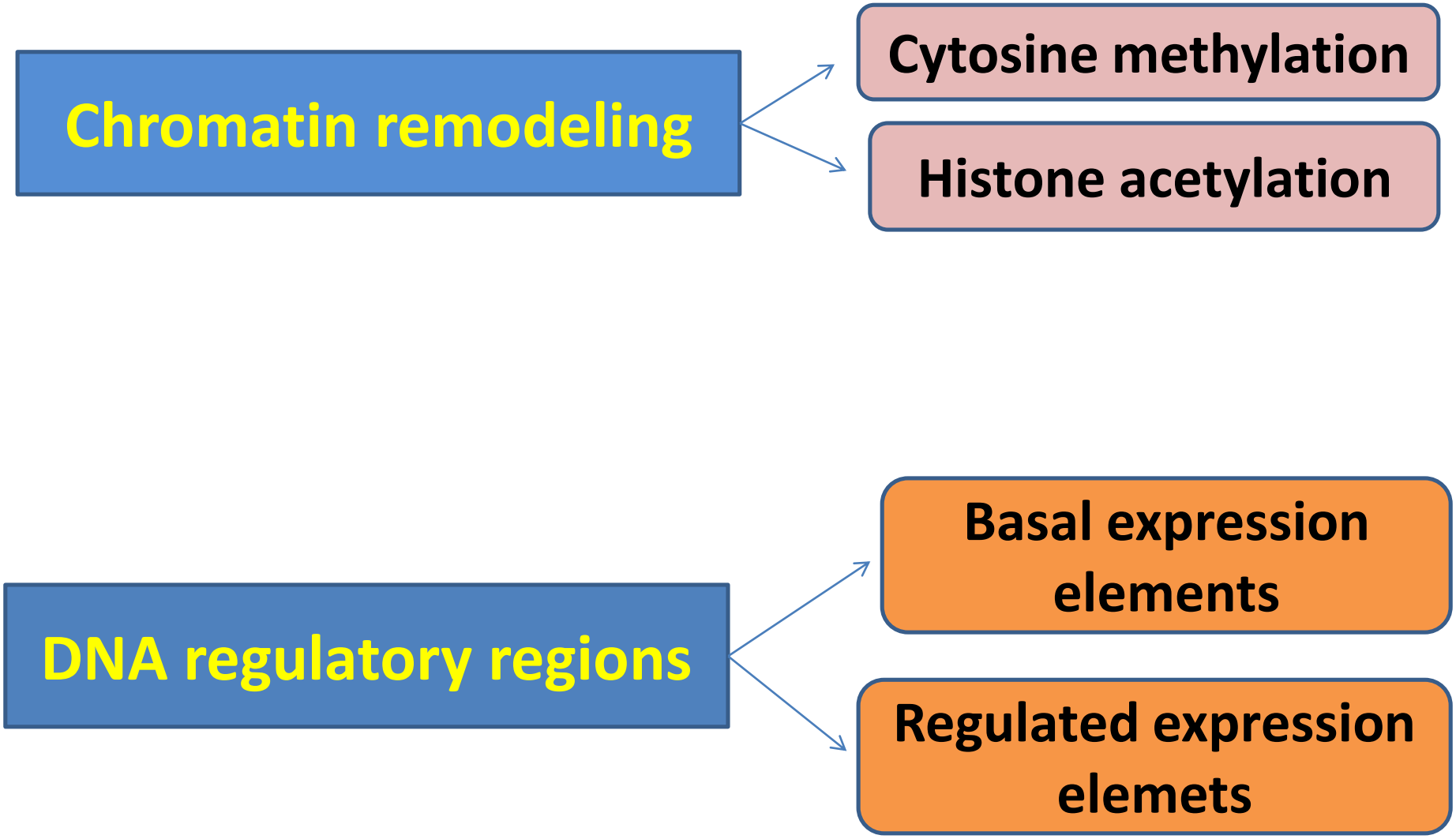
- Dihydrofolate (FH2 is a derivative of folic acid) is reduced to tetrahydrofolate (FH4) by dihydrofolate reductase.
- Methylene-FH4 is required for conversion of dUMP to dTMP which is utilized for DNA synthesis.
- It has been demonstrated in patients receiving methotrexate (an inhibitor of FH2-reductase) 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

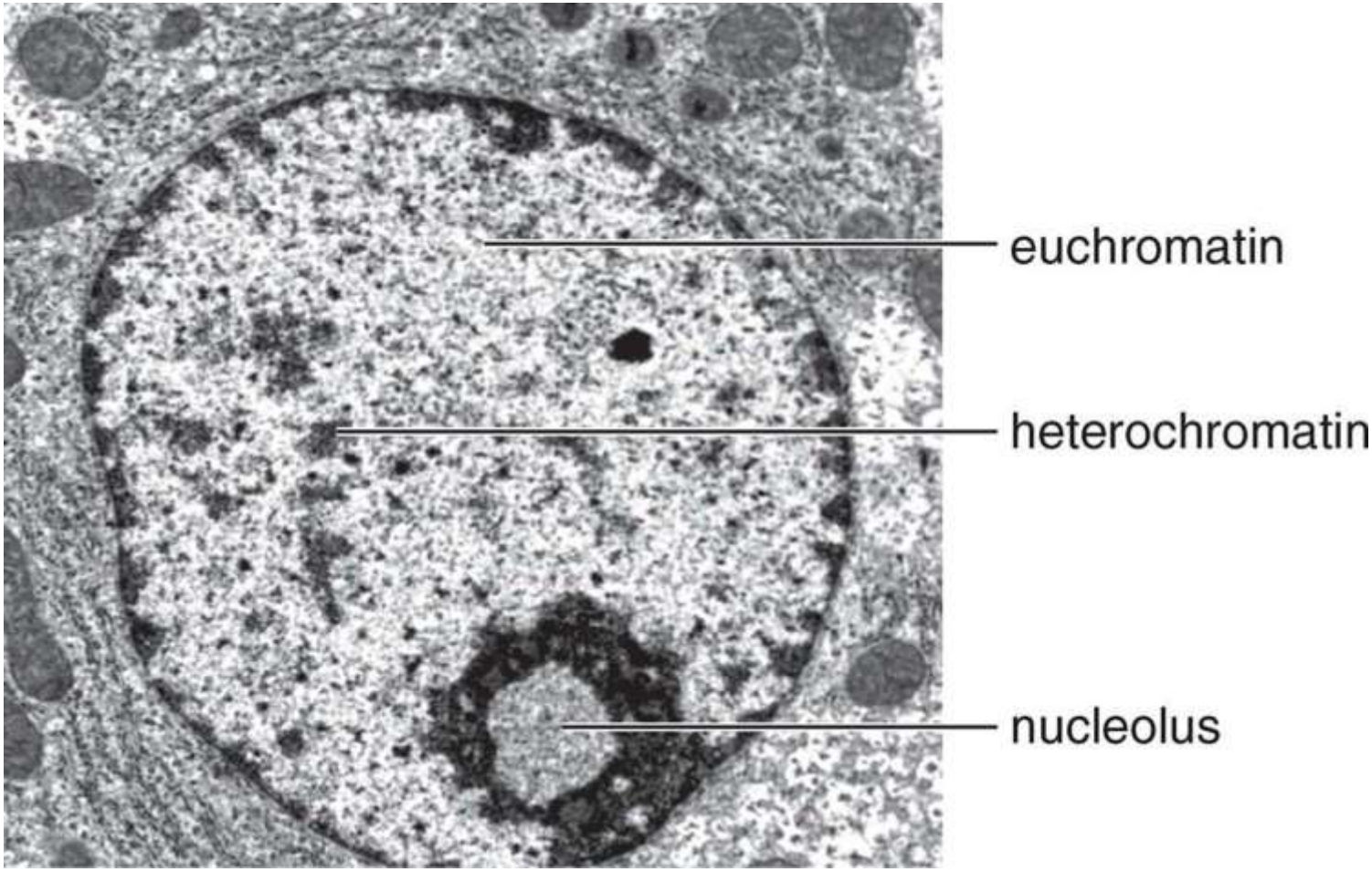
- **Many mammalian genes have CG- rich regions upstream of the coding region, that provide multiple sites for methylation.**

- **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.

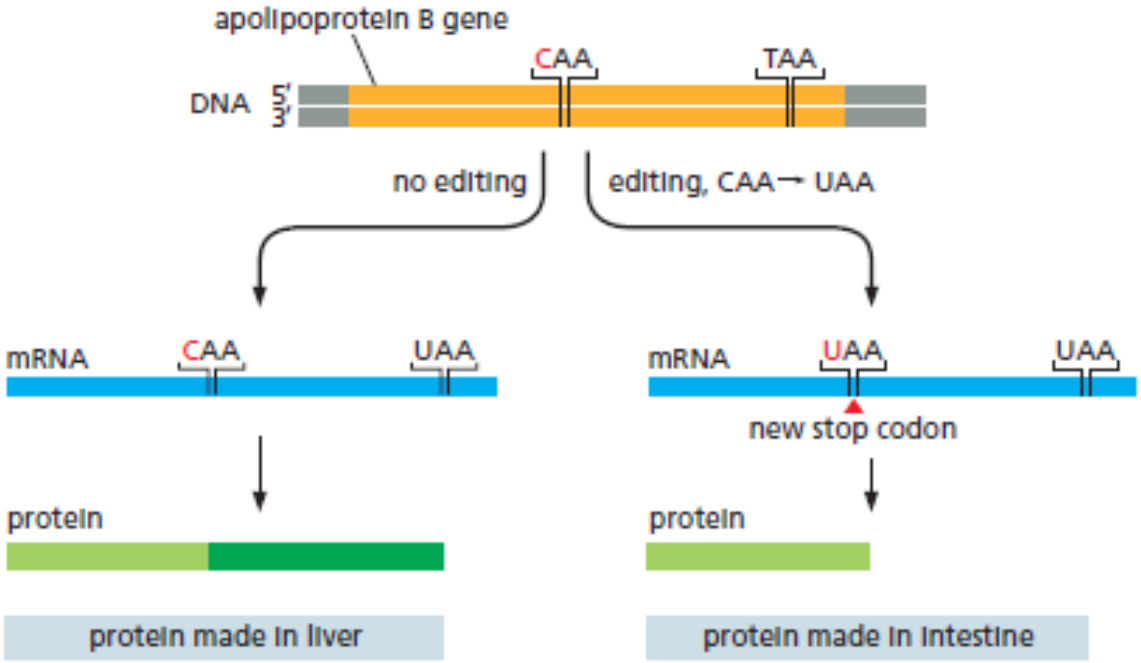
❖**<u>Regulation of RNA stability</u>**:

- 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:

- An 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.**


apolipoprotein B gene
CAA
TAA
DNA
5′
3′
no editing
editing, CAA → UAA
mRNA
CAA
UAA
protein
protein made in liver
mRNA
UAA
UAA
new stop codon
protein
protein made in intestine

REGULATION OF GENE EXPRESSION BY NONCODING RNAs

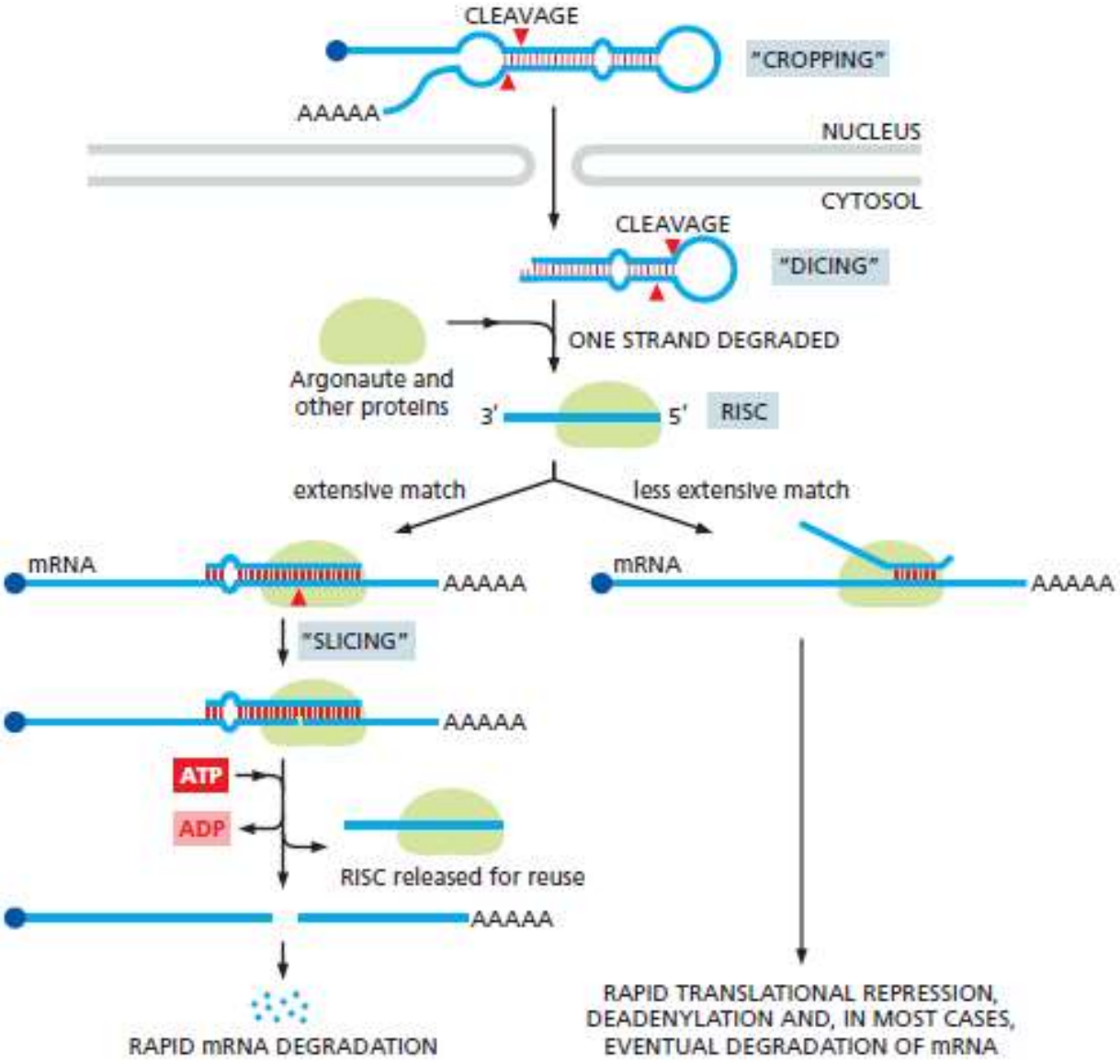
The noncoding RNAs include:

- The rRNA and tRNA molecules, which are responsible for reading the genetic code and synthesizing proteins.
- The RNA molecule in telomerase serves as a template for the replication of chromosome ends
- snRNAs direct RNA splicing.
- **Short RNAs that carry out RNA interference (RNAi)**. Here, short single-stranded RNAs (20–30 nucleotides) serve as guide RNAs that selectively bind—through complementary base-pairing—other RNAs in the cell. When the target is a mature mRNA, the small noncoding RNAs can inhibit its translation or catalyze its rapid destruction. If the target RNA molecule is in the process of being transcribed, the small noncoding RNA can bind to it and direct the formation of repressive chromatin on its attached DNA template to block further transcription

- Three classes of small noncoding RNAs work in this way— ***microRNAs (miRNAs)***, ***small interfering RNAs (siRNAs)***, and ***piwi-interacting RNAs (piRNAs)***. Although they differ in both the way the short pieces of single-stranded RNA are generated and in their ultimate functions, all three types of RNAs locate their targets through RNA–RNA base pairing, and they generally cause reductions in gene expression.

miRNAs Regulate mRNA Translation and Stability

- More than 1000 different microRNAs (miRNAs) are produced from the human genome. Once made, miRNAs base-pair with specific mRNAs and fine-tune their translation and stability.
- The miRNA precursors are synthesized by **RNA polymerase II** and are **capped** and **polyadenylated**. They then **undergo a special type of processing**, after which the miRNA (typically 23 nucleotides in length) is **assembled with a set of proteins** to form an ***RNA-induced silencing complex, or RISC***. Once formed, the RISC seeks out its target mRNAs by searching for complementary nucleotide sequences



The precursor miRNA, through complementary base pairing between one part of its sequence and another, forms a double-strand structure. This RNA is “cropped” while still in the nucleus and then exported to the cytosol, where it is further cleaved (“diced”) by the **Dicer** enzyme to form the miRNA proper.

Argonaute, in conjunction with other components of RISC, initially associates with both strands of the miRNA and then cleaves and discards one of them. The other strand guides RISC to specific mRNAs through base-pairing.

- In animals, the extent of base-pairing is typically at least seven nucleotide pairs, and this pairing most often occurs in the 3′ UTR of the target mRNA. Once an mRNA has been bound by an miRNA, several outcomes are possible.
- **If the base-pairing is extensive** (which is unusual in humans but common in many plants), the mRNA is cleaved (*sliced*) by the Argonaute protein, effectively removing the mRNA's poly-A tail and exposing it to exonucleases.
- After cleavage of the mRNA, the RISC with its associated miRNA is released, and it can seek out additional mRNAs. Thus, a single miRNA can act catalytically to destroy many complementary mRNAs. These miRNAs can thus be thought of as guide sequences that repeatedly bring destructive nucleases into contact with specific mRNAs.

- If the base-pairing between the miRNA and the mRNA is less extensive (as observed for most human miRNAs), Argonaute does not slice the mRNA; rather, translation of the mRNA is repressed by the recruitment of deadenylase enzymes—which shorten the poly-A tail—and other proteins that directly block access of the mRNA to the proteins needed to translate it