

# **MOLECULAR BIOLOGY**

## **GENE REGULATION IN EUKARYOTES**

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**GENE  
REGULATION  
IN  
EUKARYOTES**

- Gene regulation is the process of controlling which genes in a cell's DNA are expressed.
- It is how a cell controls which genes, out of the many genes in its genome, are "turned on" (expressed).

- Eukaryotic cells differ from bacteria in several ways that affect gene regulation, including, in eukaryotes, the absence of operons, the presence of chromatin, and the presence of a nuclear membrane.

# ROLE OF CHROMATIN

In eukaryotic cells, chromatin structure represses gene expression.

In transcription, chromatin structure may be altered by the modification of histone proteins , including acetylation, phosphorylation, and methylation.

The repositioning of nucleosomes and the methylation of DNA also affect transcription

# CHROMATIN STRUCTURE REPRESSES GENE EXPRESSION

At least three different processes affect gene regulation by altering chromatin structure:

- (1)the modification of histone proteins**
- (2)chromatin remodelling**
- (3)DNA methylation**

# HISTONE MODIFICATION

Histones in the octamer core of the nucleosome have two domains:

(1) a globular domain that associates with other histones and the DNA and

(2) a positively charged tail domain that probably interacts with the negatively charged phosphate groups on the backbone of DNA. The tails of histone proteins are often modified by the addition or removal of phosphate groups, methyl groups, or acetyl groups.



# METHYLATION OF HISTONES

One type of histone modification is the addition of methyl groups to the tails of histone proteins. These modifications can bring about either the activation or the repression of transcription.

A common modification is the addition of three methyl groups to lysine 4 in the tail of the H3 histone protein, abbreviated H3K4me3

# ACETYLATION OF HISTONES

The addition of acetyl groups (CH<sub>3</sub>CO) to histone proteins.

The acetylation of histones usually stimulates transcription.

Eg. H<sub>4</sub> K<sub>16</sub> by HAT

# PHOSPHORYLATION

Addition of phosphate group either activation or repression.

# UBIQUITYLATION OF HISTONES

- H2A on K119 and H2B on K120 .

H2A is associated with transcription repression, whereas H2B is associated with transcription activation.

# CHROMATIN REMODELING

Some transcription factors and other regulatory proteins alter chromatin structure without altering the chemical structure of the histones directly. These proteins are called chromatin - remodeling complexes.

They bind directly to particular sites on DNA and reposition the nucleosomes, allowing transcription factors to bind to promoters and initiate transcription.

# DNA Methylation

Another change in chromatin structure associated with transcription is the methylation of cytosine bases, which yields 5-methylcytosine .

The methylation of cytosine in DNA is distinct from the methylation of histone proteins mentioned earlier.

Heavily methylated DNA is associated with the repression of transcription in vertebrates and plants, whereas transcriptionally active DNA is usually unmethylated in these organisms.

**ROLE OF ACTIVATORS  
&  
REPRESSORS.**

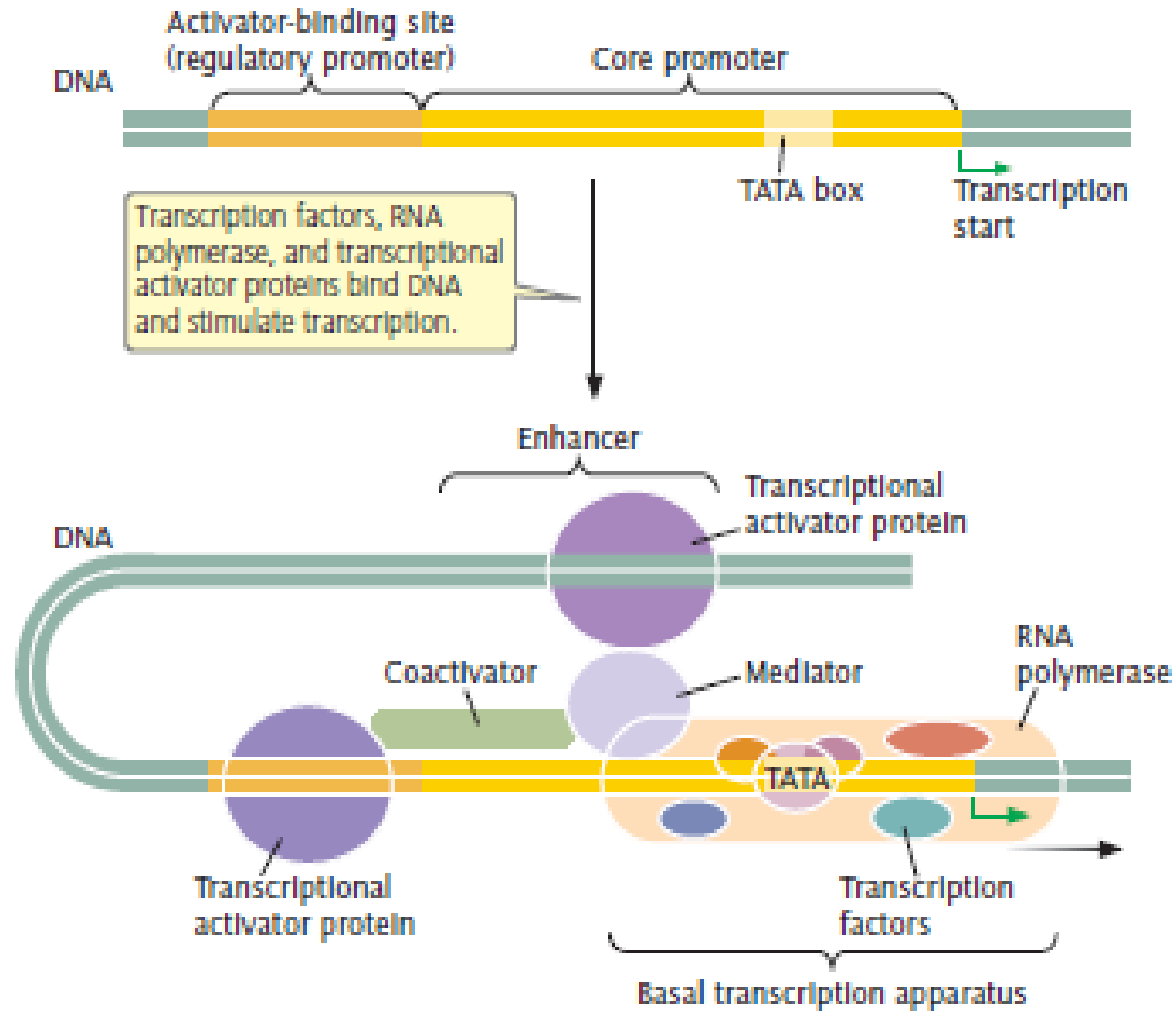
# Transcriptional Activators and Coactivators

- Transcriptional activator proteins stimulate and stabilize the basal transcription apparatus at the core promoter. The activators may interact directly with the basal transcription apparatus or indirectly through protein coactivators.
- Some activators and coactivators, as well as the general transcription factors, also have acetyltransferase activity and so further stimulate transcription by altering chromatin structure.

Transcriptional activator proteins have two distinct functions .

- First, they are capable of binding DNA at a specific base sequence, usually a consensus sequence in a regulatory promoter or enhancer.
- A second function is the ability to interact with other components of the transcriptional apparatus and influence the rate of transcription.





**17.5 Transcriptional activator proteins bind to sites on DNA and stimulate transcription.** Most act by stimulating or stabilizing the assembly of the basal transcription apparatus.

# Transcriptional Repressors

Some regulatory proteins in eukaryotic cells act as repressors, inhibiting transcription. These repressors bind to sequences in the regulatory promoter or to distant sequences called Silencers. Most eukaryotic repressors do not directly block RNA polymerase. These repressors may compete with activators for DNA binding sites: when a site is occupied by an activator, transcription is activated, but, if a repressor occupies that site, there is no activation. Alternatively, a repressor may bind to sites near an activator site and prevent the activator from contacting the basal transcription apparatus. A third possible mechanism of repressor action is direct interference with the assembly of the basal transcription apparatus, thereby blocking the initiation of transcription.

Some regulatory proteins bind to **enhancers**, which are regulatory elements that are distant from the gene whose transcription they stimulate.

**Insulators** are DNA sequences that block the action of enhancers.

# REGULATION OF TRANSLATION BY GENE REARRANGEMENT

- Explain immunoglobulin genes.
- Enhancers in regulation of immunoglobulin genes. Enhancers are certain DNA sequences, that increase the transcription of genes, although this is not the only characteristic of DNA segment to be called enhancer.
- These enhancers have been discovered in immunoglobulin genes and have been found to work only in cells that make immunoglobulins. In this manner, enhancer is a remarkable regulatory sequence that is cell specific.

# REGULATION OF ALTERNATIVE TRANSCRIPTS BY SPLICING

**Alternative splicing** is a regulated process during gene expression that results in a single gene coding for multiple proteins.

- It is a process of alternate skipping of exons.
- It increases diversity.

Alternative splicing regulates gene expression at the level of RNA processing and provides a mechanism by which a single gene can encode two or more related proteins.

- In the fruit fly, for example, the developmental pathway that leads an embryo into becoming a male or female is determined by alternative splicing of the transcripts from certain genes.

Sex differentiation in *Drosophila* arises from a cascade of gene regulation.

- When the ratio of X chromosomes to the number of haploid sets of autosomes is 1, a female-specific promoter is activated early in development and stimulates the transcription of the sex-lethal (Sxl) gene .
- The protein encoded by Sxl regulates the splicing of the premRNA transcribed from another gene called transformer (tra). The splicing of tra pre-mRNA results in the production of the Tra protein.
- Together with another protein (Tra-2), Tra stimulates the female-specific splicing of pre-mRNA from yet another gene called doublesex (dsx). This event produces a female-specific Dsx protein, which causes the embryo to develop female characteristics.

In male embryos, which have an X : A ratio of 0.5 the promoter that transcribes the Sxl gene in females is inactive; so no Sxl protein is produced.

In the absence of Sxl protein, tra pre-mRNA is spliced at a different 3' splice site to produce a nonfunctional form of Tra protein.

In turn, the presence of this non-functional Tra in males causes dsx pre-mRNAs to be spliced differently from that in females, and a male-specific Dsx protein is produced. This event causes the development of male-specific traits.

*In summary, the Tra, Tra-2, and Sxl proteins regulate alternative splicing that produces male and female phenotypes in Drosophila.*

# siRNA & miRNA IN GENE REGULATION



# RNA Interference Is an Important Mechanism of Gene Regulation

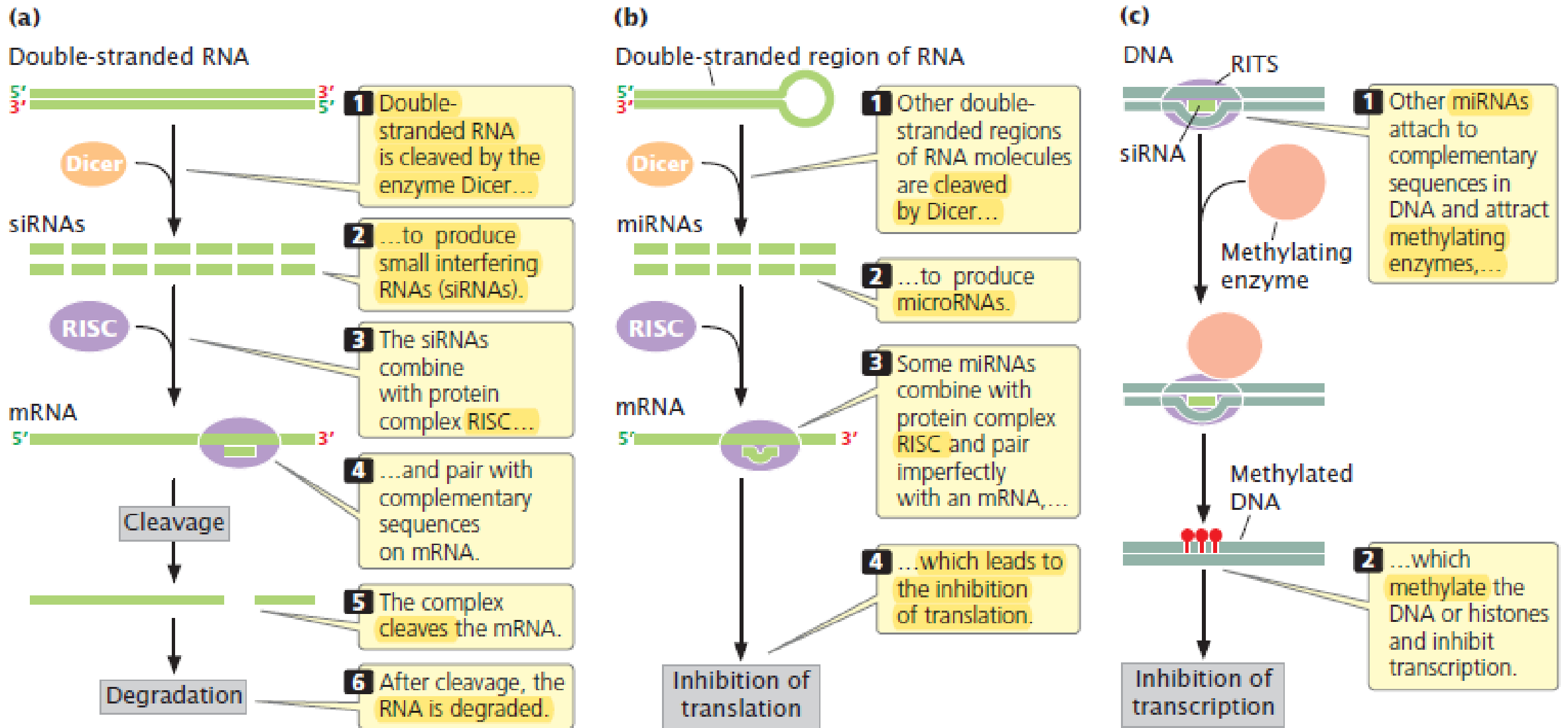
The expression of a number of eukaryotic genes is controlled through RNA interference, also known as RNA silencing and posttranscriptional genesilencing.

RNA interference is widespread in eukaryotes, existing in fungi, plants, and animals.

# Mechanisms of Gene Regulation by RNA Interference

Small interfering RNAs and microRNAs regulate gene expression through at least four distinct mechanisms:

- (1) cleavage of mRNA,
- (2) inhibition of translation
- (3) transcriptional silencing
- (4) degradation of mRNA.



**17.13 RNA silencing leads to the degradation of mRNA or to the inhibition of translation or transcription.** (a) Small interfering RNAs (siRNAs) degrade mRNA by cleavage. (b) MicroRNAs (miRNAs) lead to the inhibition of translation. (c) Some small interfering RNAs (siRNAs) methylate histone proteins or DNA, inhibiting transcription.

## Small Interfering RNAs and MicroRNAs

RNA interference is triggered by microRNAs (miRNAs) and small interfering RNAs (siRNAs), depending on their origin and mode of action. An enzyme called Dicer cleaves and processes double-stranded RNA to produce siRNAs or miRNAs that are from 21 to 25 nucleotides in length and pair with proteins to form an RNA-induced silencing complex (RISC). The RNA component of RISC then pairs with complementary base sequences of specific mRNA molecules, most often with sequences in the 3' UTR of the mRNA. Small interfering RNAs tend to base pair perfectly with the mRNAs, whereas miRNAs often form less-than-perfect pairings.

# ANTISENSE RNA

- Some small RNA molecules are complementary to particular sequences on mRNAs and are called antisense RNA. They control gene expression by binding to sequences on mRNA and inhibiting translation.
- Antisense RNA is complementary to other RNA or DNA sequences. In bacterial cells, it can inhibit translation by binding to sequences in the 5' UTR of mRNA and preventing the attachment of the ribosome.

- Antisense RNAs are complementary to sequences in mRNA and may inhibit translation by binding to these sequences, thereby preventing attachment or progress of the ribosome.
- An example of naturally occurring mRNA antisense mechanism is hok/sok system of E.coli R1 plasmid

THANK YOU..