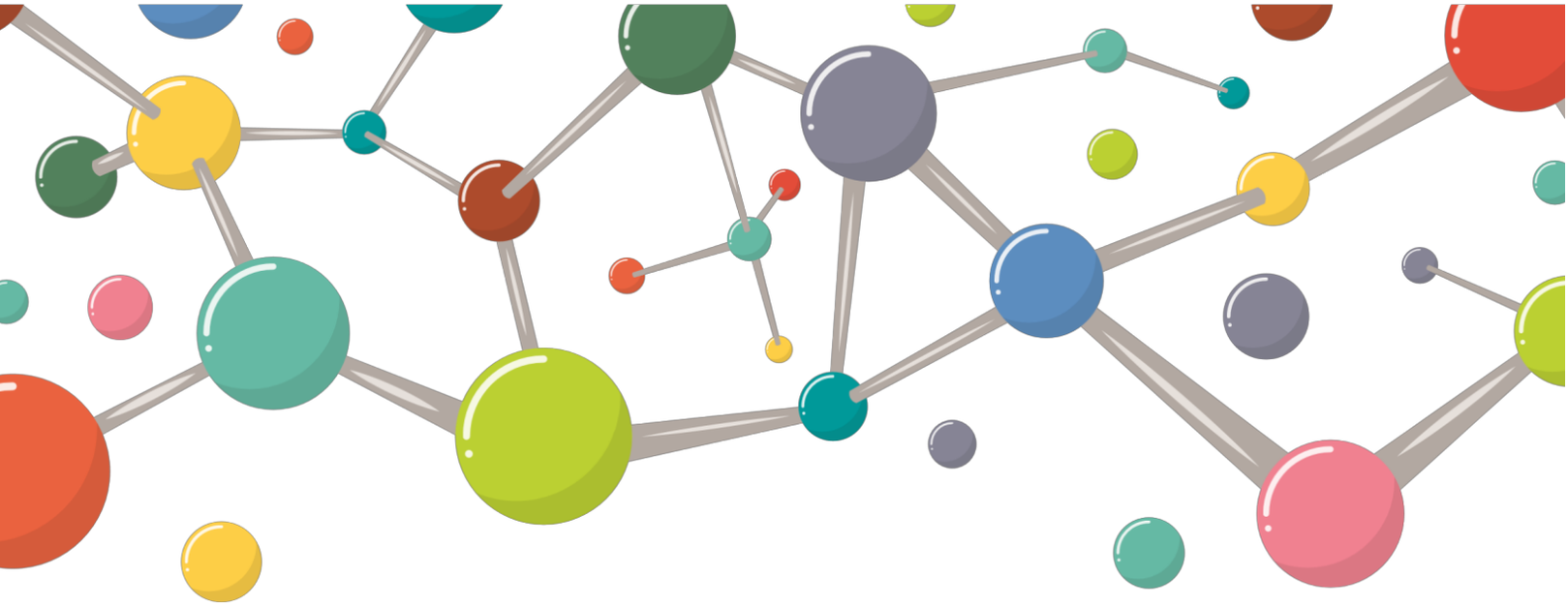

MOLECULAR BIOLOGY

SHEET (8)

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Black color: doctor's explanation, Navy color: slides, Blue color: extra information, Red color: Dr. Khaldun's notes, highlighted sentence: modified, purple color: book notes .

أهلاً دفعتنا، حبينا نعمللكم صفحة تغيير جو هون !!
عيشوا هاي أقصر شيت بالدنيا فإنه قصيدة إهداء للشيت والكم !!!

أَرَاكِ فأُخْلَقُ خَلْقَاً جديداً
كأنِّيَ لَمْ أَبْلُ حربَ الوُجُودْ

ولم أَحتمل فيه عِبئاً ثقيلاً
من الذِّكرياتِ التي لا تَبيدْ

وأَضغاثِ أَيَّامِيَ الغابراتِ
وفيها الشَّقيُّ وفيها السَّعيدْ

ويغْمُرُ روحِي ضِياءٌ رَفيقٌ
مُحلِّلَهُ رائعاتُ الورودْ

وتُسْمِعُني هاتِهِ الكائِناتُ
رقيقَ الأَغاني وحُلْوَ النَّشيدْ

وتَرْقُصُ حَولي أَمانٍ طِرابٌ
وأَفراحُ عُمْرٍ خَوالٍّ سَعيدْ

Eukaryotic Transcription:

Differences Between Bacterial and Eukaryotic Transcription Machinery:

Bacteria use single RNA Polymerase to synthesize ribosomal RNA (rRNA), messenger RNA (mRNA), and transfer RNA (tRNA). In contrast eukaryotes use a specific dedicated nuclear enzyme to synthesis each kind of RNA. So Eukaryotes has 3 types of RNAs: RNA Polymerase I. II, and III. We will focus in our study on the second type since it synthesizes the mRNA which is translated to protein.

Remember: we have different type of genes, some of them will undergo transcription and then translation and some of them will undergo transcription only to produce different types of RNA, so in bacteria we have RNA polymerase for all types of genes but we have specific RNA polymerases for each type in eukaryotes.

Bacterial RNA Polymerase requires the assistance of at most one or two accessory factors to transcribe genes. Eukaryotic RNA Polymerase requires several such factors (Proteins).

Bacterial RNA Polymerase holoenzyme has direct access to its DNA template, whereas the eukaryotic transcription machinery has difficulty in reaching its DNA template because eukaryotic DNA interacts with histones to form nucleosomes, which in turn form more compact chromatin structures. And because of that the cell does chromatin remodeling to make the DNA accessible to the RNA Polymerase. Bacteria has histone-like proteins but they are too few in numbers compared to eukaryotes.

Introduction to Eukaryotic Nuclear RNA Polymerase.

(We called it Nuclear to differentiate it from mitochondrial RNA polymerase).

Each nuclear RNA polymerase has some subunits that are unique to it and some it shares with one or both of the two other nuclear RNA polymerases.

Homologies not only exist among nuclear RNA polymerase subunits but also extend to bacterial and archaeal RNA polymerases (when they compare between subunits in RNA polymerase between bacteria, archaea and eukaryotes, they found that there are some similarities but there is also some differences).

RNA Polymerase II Structure

Nuclear RNA polymerases have limited synthetic capacities (they have limits).

None of the RNA polymerases can initiate transcription from specific start sites within double stranded DNA.

All require assistance of other proteins(transcriptional factors). They help the RNA polymerase with transcription, initiation (binding promoters), elongation and termination.

Each polymerase requires its own specific set of transcription factors.

This table compares the three types of Eukaryotic RNA Polymerases.

Pre-rRNA refers to RNA before modifications.

Remember: **Nucleus** is subdivided to: nucleolus and nucleoplasm

The S refers to the unit of the RNA Sedimentation rate (remember chapter 4). RNAs are differentiated based on their sedimentation rate.

We will talk about snRNAs when we talk about Ribosomes.

Scientists differentiated between 3 types of RNA Polymerases using some toxic materials like Alpha-amanitin and actinomycin D. when we react a substance with a sample that has a number of molecules, same molecules will respond in the same way, so when we have three different responses, that means that we have three different types of molecules and that is the principle which we use here.

Figure 13.F02A: α-Amanitin. (a) Amanita phalloides (also known as the Death Cap). (b) Structural formula of α-amanitin.

Alpha-amanitin is taken from some type of toxic mushroom called Death Cap.

Some book information:

1. Very low a-amanitin concentrations block mRNA synthesis while permitting the continued synthesis of most other kinds of RNA. Higher concentrations also block the synthesis of 5S rRNA, tRNA, and most other small RNA molecules while permitting continued synthesis of other rRNA.
2. A-amanitin binds to the enzymes, but actinomycin D binds to the double stranded DNA , thus prevent it from separation for transcription which is required for transcription and replication. The heterocyclic ring system in Actinomycin D inserts itself between G-C and C-G base pairs.

Core Promoter for Protein-Coding Genes

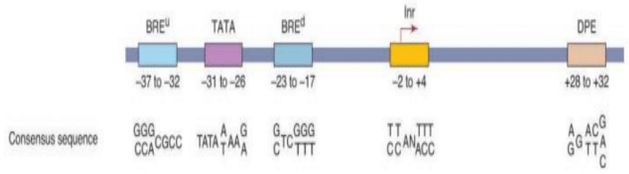
Remember: promoter: DNA sequence where the RNA polymerase binds

Start site: the first nucleotide where we start transcription.

TABLE 13.1 Comparing the Three Eukaryotic RNA Polymerases

Enzyme	Location	RNA Products	Sensitivity to α-amanitin	Sensitivity to actinomycin D
RNA polymerase I	Nucleolus	Pre-rRNA (leading to 5.8S, 18S, and 28S rRNA)	Resistant	Very sensitive
RNA polymerase II	Nucleoplasm	Pre-mRNA and some small nuclear RNAs (snRNAs)	50% inhibition at 0.02 μg/mL	Slightly sensitive
RNA polymerase III	Nucleoplasm	tRNA, 5S rRNA, U6 snRNA (spliceosomal RNA), and 7SL RNA (signal recognition particle RNA)	50% inhibition at 20 μg/mL	Slightly sensitive

The core promoter for protein-coding genes extends from 40bp upstream of the transcription start site to 40bp downstream from this site. In this type of DNA there are some segments on the promoter where transcription factors bind on called Modules. And it's important because Eukaryotic transcription relies heavily on them.



A consensus TATAXAX (where X is an A or T) is present 25 to 30 bases upstream of transcription start sites for RNA Pol II genes. Known as the TATA box.

Found primarily in highly expressed gene promoters.

– TATA-less promoters have other short sequences that replace the TATA box. There are types of promoters other than the TATA box.

The DNA region that includes these elements (we call them regulatory segments and they bind specific factors) is the core promoter.

Other components of the core promoter:

– Initiator (Inr) element: flanks (besides) the start site. Flanks means surrounding it on both sides. Downstream and Upstream.

– Downstream promoter element (DPE)

– TFIIB recognition elements (BRE). (TF= transcription factor), and has two types:

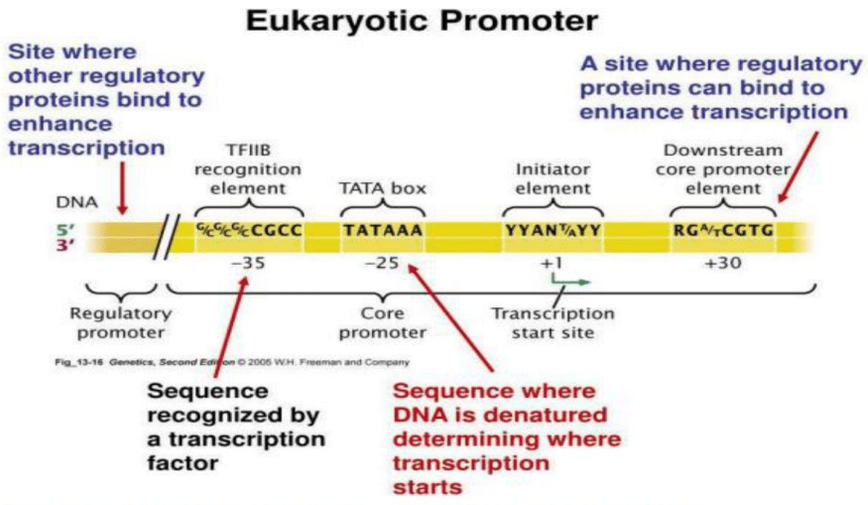
BRE^u (u = upstream) and BRE^d (d=downstream) flank the TATA box.

In ≈10,000 known human core promoters

- Inr is present in about half.
- DPE and BRE present in about a quarter.
- TATA boxes present in about one-eighth.

***Initial efforts to characterize the eukaryotic promoter concentrated on highly expressed protein-coding genes, such as those that code for hemoglobin, histone and ovalbumin, because these genes were easiest to study at the time.*

***RNA polymerase has 12 subunit and a magnesium ion.*



General Transcription Factors: Basal Transcription.

Basal because it allows for the base minimum of transcription

RNA polymerase II requires the assistance of general transcription factors to transcribe naked (where the DNA is not packed by histones) DNA from specific transcription start sites.

RNA polymerase II, acting together with general transcription factors, comprises the minimum transcription machinery required for correct transcription initiation of a linear duplex with a core promoter that has a TATA box. This minimum transcription machinery determines the transcription start site and the direction of transcription.
Because the level of transcription catalyzed by RNA polymerase II together with the general transcription factors is much lower than that observed in the cell, it is called basal transcription. For this reason some investigators prefer to use the term basal transcription factor instead of general transcription factor. RNA polymerase I and general (basal) transcription factors assemble at the transcription start site to form a preinitiation complex.

Remember: DNA tightly packed with histons= heterocromatin , and we cannot transcribe it because there is no accessibility on it by RNA polymerase.

The general transcription factors: TFIIA, TFIIB, TFIID, TFIIF and TFIIH
- The first two letters TF indicate general transcription factor
- Roman numeral II signifies RNA polymerase II
- Final letter based on protein fractionation scheme

G and C are missing because later studies showed the proteins originally assigned these letters are not transcription factors for RNA.

الله يقوّيكم تحفظوه

TBP = TATA Box binding Protein

TAF 1 and other proteins inhibit the transcription. So, it (TFIIA) inhibits the inhibitor.

EF = Elongation Factor

Note: TFIIE is important to the denaturation of promoters, although we are in initiation, some nucleotides will be synthesized in this stage

A good link:

TABLE 13.3 General Transcription Factors

Factor	No. of Subunits	Functions
TFIIA "Regulatory protien"	2	Stabilizes TBP and TFIID binding. Blocks the inhibitory effects of TAF1 and other proteins.
TFIIB	1	Stabilizes TFIID-promoter binding. Contributes to transcription start site selection. Helps recruit RNA polymerase II • TFIIF complex to the core promoter.
TFIID (TBP and TAFs)	14	Binds to the TATA box, Inr, and DPE. It can deform promoter DNA and serve as a platform for the assembly of TFIIB.
TFIIE	2	Helps to recruit TFIIH to the core promoter and is required for promoter melting.
TFIIF	3	Binds RNA polymerase II and is involved in recruiting the polymerase to the pre-initiation complex. Required to recruit EFIIE and EFIIH to the pre-initiation complex.
TFIIH	10	Functions in transcription and DNA repair. It has kinase and helicase activities and is essential for open complex formation.

https://youtu.be/EMDuf_kBJcs

*****Further study showed that TBP is the TATA binding subunit in TFIID. The additional proteins present in TFID, called TBP-associated factors (TAFs), are required to transcribe genes that lack a TATA box as well as for the high levels of transcription that occur within the cell.***

From the book

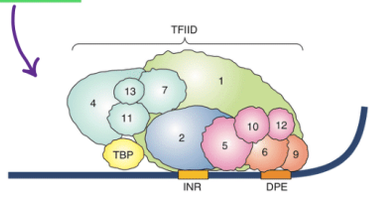


FIGURE 13.11 Schematic composite showing the interactions of various TFIID subunits with the core promoter. Several TFIID subunits have been implicated in binding to the core promoter, including TBP (yellow), which binds to the TATA box when it is present, TAF2 (blue), which interacts with the initiator (Inr), and TAF6 (orange) and TAF9 (orange), which interact with the downstream promoter element (DPE). Many, but not all, of the 13 highly conserved TAFs are shown in this figure. (Adapted from Näär, A. M., Lemon, B. D., and Tijian, R. 2001. *Annu Rev Biochem* 70:475–501.)

Transcription elongation

The C-terminal domain of the largest RNA polymerase subunit must be phosphorylated for chain elongation to proceed. RNA polymerase constitute of many subunits, the largest one among the largest subunit has N terminal domain and C terminal domain (At the end of the polypeptide). At the C terminal domain, a phosphate group must bind (phosphorylation) so the RNA polymerase can shift from the initiation to the elongation stage.

- **The carboxyl terminal domain (CTD) contains tandem repeats of unusual heptapeptide.** When they sequenced this CTD, they found this sequence of amino acids which is conserved in all RNA polymerases.
- **Tyr-Ser-Pro-Thr-Ser-Pro-Ser**
  (3 Ser, 2 pro, 1 Thr, 1 Tyr)
- **Five of the seven residues** (amino acids) **can be phosphorylated .**
  By protein kinases ←
- **Must be dephosphorylated for Pol II to assemble into the pre-initiation complex .**

RNA polymerase II must be dephosphorylated after each round of transcription is completed before the enzyme can reassemble into an initiation complex to begin the next round of transcription.

Specific protein phosphatases catalyze the dephosphorylation.

So phosphorylation is for elongation and dephosphorylation for initiation .

- **Phosphorylation is necessary for elongation**
- **TFIIH phosphorylates Ser-5 permitting promoter clearance** (moving from the promoter to the coding region). This step is important for the RNA polymerase to move from the promoter and continues elongation downstream.

Ser-2 and Ser-7 residues are phosphorylated during the elongation stage.

General Transcription Factors:

Basal transcription

* **TFIIE : interacts with RNA polymerase || "jaws" placing it in a position to interact with promoter DNA 25bp downstream of the start site.**
* **TFIIH: acts as a helicase and a cyclin-dependent protein kinase** (has the ability to phosphorylate).

Transcription Elongation

A variety of transcription elongation factors helps to suppress(prevent it from pausing) transient pausing (resuming of RNA polymerase at some sequences (beco seconds)) during elongation.

- **Reverse movement of Pol II occurs called backtracking .**
- **In the paused state (2-4 nucleotides) it can return to the elongation stage without assistance of transcription factors.**

Elongin, stimulates transcription by stabilizing the active conformation of RNA polymerase II, increasing the rate at which the inactive form is converted back to the active form, or both methods.

Other transcription elongation factors, such as the members of the ELL family, may act in a similar way.

- **In the arrested state (7-14 nucleotides) TFIIS is required to reactivate transcription (back from the arrested state).**
- **When an error occurs the polymerase backtracks.**
- **The mismatch is removed (3' to 5') and transcription can resume.**

***Pausing in both eukaryotes and prokaryotes allow the RNA polymerase to proofread (3' to 5') backtracking.**

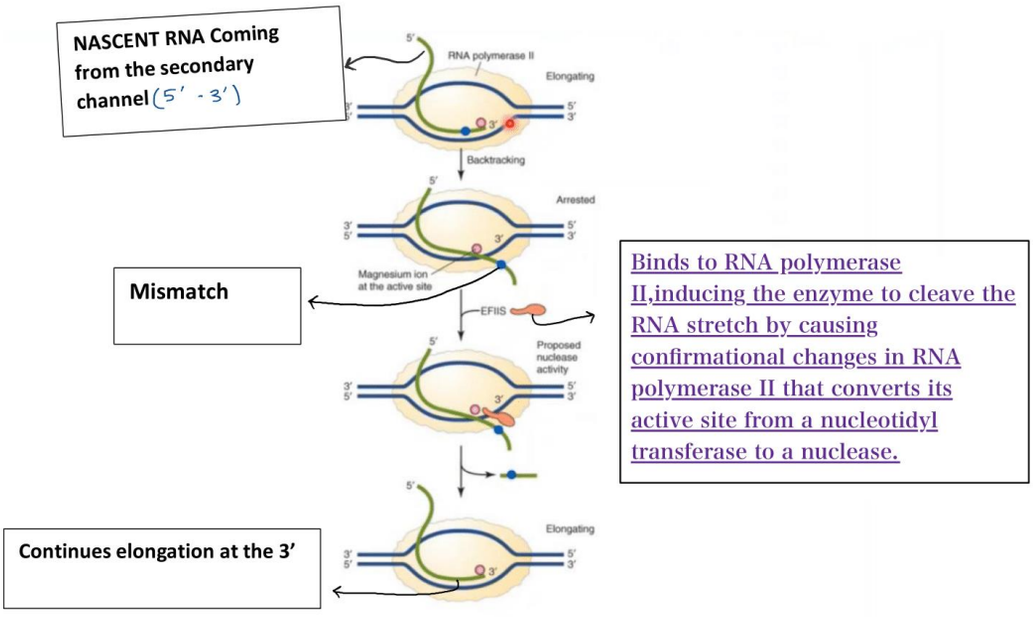


Figure 13.F15: Backtracking and TFIIS action. RNA polymerase II attaches the wrong nucleotide (blue circle) to the growing RNA chain, producing a base pair mismatch.

Regulatory Promoters(important for the binding of transcription factors) , Enhancers and Silencers Linker-

scanning mutagenesis reveals the regulatory promoter's presence just upstream from the core promoter

- The core promoter is very inefficient by itself
- The regulatory promoter is between 50-200 bp upstream of the initiation site

The doctor skipped this figure

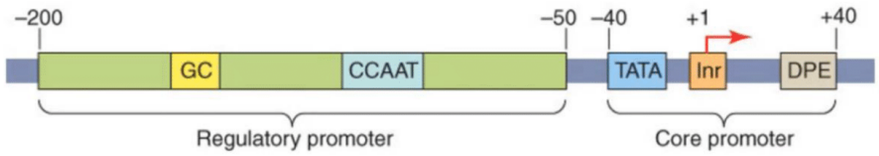


Figure 13.18: The regulatory promoter.

Enhancers stimulate transcription and silencers block transcription

- Enhancers (increases transcription) are distance (away from the promoter) and orientation independent (5' – 3' OR 3' – 5')

Regulatory DNA segments

- First identified in SV40 early gene
- 72 bp repeat stimulates transcription by 100-fold in in-vitro studies
- Can work even when located 1,000s of bp upstream or downstream
- Can work inserted forwards or backwards

- **Two features distinguish Enhancers from other regulatory sequences:**

1. **Enhancers stimulate transcription from the transcription site even if they are located several thousand bp.**
2. **Work in either orientation.**

Enhancers range 50 b to 1.5 kb. They have cluster of modules, like regulatory promotors.

Eukaryotes also have silencers that repress (decrease) transcription. "Sequence specific elements".Any specific human cell type may have about 50,000 functional enhancers

Most function independently of distance and orientation, but some silences are position dependent (unlike enhancers, if position was altered, they won't work).

A cell must have transcription activator proteins capable of binding to the modules within an enhancer or a regulatory promoter for full gene expression to occur.Silencers are binding sites for negative transcription factors that prevent a nearby activator from binding to its DNA-binding site, or in few cases blocking preinitiation complex formation.

The upstream activating sequence regulates genes in yeast.

Epigenetic Modifications
Cells remodel (make it loose and disrupt its attachment to histones to expose DNA for binding to RNA polymerase) or modify chromatin to make the DNA in chromatin accessible to the transcription machinery

Eukaryotic cells use ATP-dependent chromatin remodeling complexes (to make RNA polymerase able to recognise and bind to the DNA)

- **Reposition nucleosomes**
- **Eject nucleosomes** (cleave its subunits)
- **Unwrap nucleosomes**
- **Exchange or eject histone dimers** (disassociate them from DNA to make it free)

Histone modification influences transcription of protein-coding genes

- **Active genes have acetylated histones** (Acetyl groups are large so they separate histones)
  - **Histone acetyltransferase (HAT)**
  - **Acetylation takes place on specific lysine** (amino acid). **Acetylation prevents chromatin compaction** (to turn genes on) .
- **Chromatin remodeling complexes respond to advancing transcription by:**

1) displacing histones from DNA onto chaperones (proteins that help in polypeptide folding to produce a 3d structure)

- Reassemble after the DNA region has been transcribed (returning the structure to its original state)

2) Only one H2A •H2B heterodimer needs to be removed (By chaperones)

DNA methylation plays an important role in determining whether chromatin will be silenced or actively expressed in vertebrates methylated ←

- **Many vertebrate core promoters are in CpG islands (CG repeats)**
- **Methyl transferase add methyl groups to specific lysine and arginine (or it can add more than one methyl group on the same amino acid). Methylation of C in these regions can lead to gene silencing**
- **Methyl-CpG prevents transcription factor binding (CpG regions after they have become methylated, prevent the transcription factor from binding as their chemical nature have changed)**
- **Methyl-CpG acts as a signal for histone modification (affects negatively)**
- **Many genes are silenced by this mechanism (as DNA is condensed or compacted)**

So methylation can either prevent transcription factors' binding or prevent histones' remodelling and modification.

They found that the best affecting mechanism in methylation is the HISTONE's MODIFICATION.

Epigenetics is the study of inherited changes in phenotype caused by changes in chromatin other

than changes in DNA sequence (And here we're not talking about the DNA sequence, we're talking about the methyl groups that are present on the amino acids or even on the Sequence of DNA)

- **Patterns of histone modification and DNA methylation are inheritable**
- **Since these patterns determine which DNA segments will be transcriptionally active and silent, this is a form of inheritance that is not directly determined by**
- **DNA sequence**
- **Epigenetics is term used to describe these patterns of inheritance**

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