DNA DAMAGES AND REPAIR.

- DNA is subjected to lots of stress and strain during replication and cell division.
- Also it is exposed to many chemical, physical, and biological agents which enters the cell from environment.
- All such agents inflict a variety of damages on DNA molecule.
- Maintenance of the integrity of DNA is vital for the survival and continuation of species, Hence DNA repair mechanisms to take care of these damages.

CAUSES OF DNA DAMAGE.

- Replication error----

  normally errors are repaired by the proof reading mechanisms of DNA replication and are not transmitted. But when they are increases or are not repaired properly, they can give rise to disease.

CHEMICALS.

- A wide variety of chemicals are present in environment to which all of us are exposed .
- Examples are –
- Insecticide and pesticides.
- Pollutants including gases.
- Oxidising agents, alkylating agents.
- Industrials wastes
- Food adulteration and preservative.
- All the chemicals causes modification of bases, Bulky adduct formation between bases, alteration of bases by deamination, etc and lead to DNA damage.

DEAMINATION OF BASES.

- Cytosine and adenine undergo deamination spontaneous or chemically induced to form uracil and hypoxanthine . The original sequence is thus changed.

Thymus Dimers by UV Light.

- UV light radiations induce the condensation of adjacent thymine bases in DNA strand forming thymine- thymine dimers.
- ***CHAIN BREAKS BY IONIZING RADIATION.***
- X-rays and gamma rays can cause DNA strand in the backbone in single strand or in both the strands.
- ***DAMAGE BY OXYGEN RADICALS.***
-
- ***ADDUCT FORMATION.***

MECHANISM OF DNA REPAIR.

- **BASE EXCISION REPAIR-** modified and altered bases are repaired by base excision repair mechanism.
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- **NUCLEOTIDE EXICION REPAIR-** The repair mechanism involves called Excinuclease. Exinuclease cuts nucleotide, The gap is filed by DNA polymerase.
- **DIRECT THYMINE DIMER REPAIR-** DNA photolyase catalyse the removal of bonds forming thymine dimers and converting them to back to normal in prokaryotes.
- **MISMATCH REPAIR –** specific endonucleases identify the wrong base in newly synthesized strand.

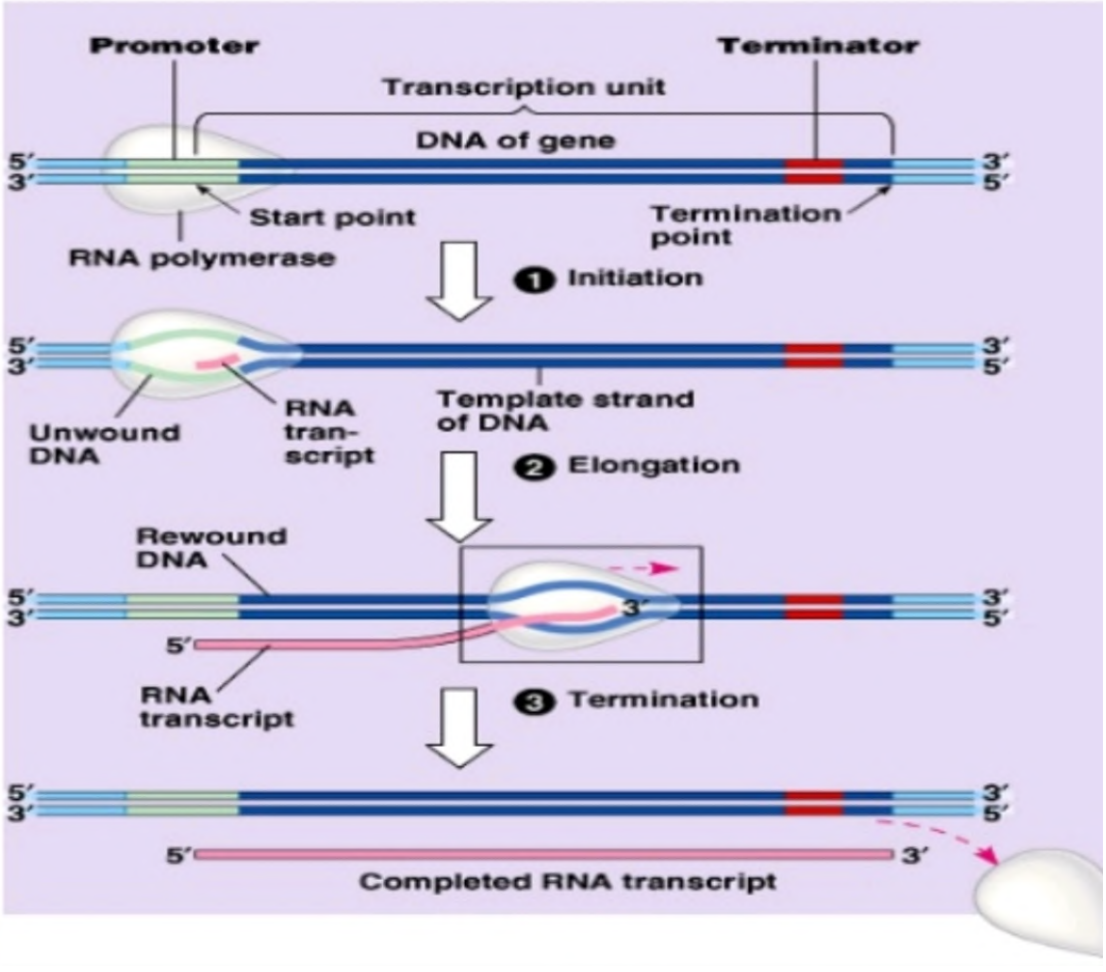
TRANSCRIPTION.

- Transcription-

  cellular process in which RNA synthesized using DNA template known transcription.
- ROLE OF DNA --- only one strand used.
- -Template strand/non –coding strand.

  -non template strand/coding strand.

Transcription.



ROLES OF DNA AND RNA.

- *DNA* is the MASTER PLAN.

RNA is the ***BLUPRINT*** of the Master plan.

Transcription---

process in which RNA is synthesized from DNA.

DNA express through RNA.

Synthesis of RNA occurs in 5'-3' direction.

Nucleotide ATP, GTP,CTP,UTP ARE NECESSORY.

Enzyme and factors.

Eukaryotes	prokaryotes
RNA POLYMERASE	RNA POLYMERASE
RNA polymerase I	Only one type of RNA polymerase
RNA polymerase II	Sigma factor.
RNA polymerase III	

DIFFERENCE BETWEEN REPLICATION ANDTRANSCRIPTION

TEMPLATE	REPLICATION BOTH STRAND	TRANSCRIPTON SINGLE STRAND
PRIMER	YES	NO
ENZYME	DNA POLYMERASE	RNA POLYMERASE
PRODUCT	Ds DNA	ssRNA
BASE PAIR PROOF READING	A -T, G-C, YES	A-U, T-A, G-C NO

DNA dependent RNA polymerase.

- RNA polymerase main enzyme responsible for transcription.
- Five core unit and sixth sigma factor to make holoenzyme.
- Beta subunit has polymerisation activity.
- RNAP reads the template strand in 3'-5' direction and synthesize new RNA in 5'-3' direction and does not require a RNA primer.

RNA polymearase.

RNA POLYMERASES

- RNA polymerase synthesize RNA in the direction of 5'-3' that means DNA template is read in 3'-5' direction.
- Ribonucleotides required -- ATP, GTP, CTP & UTP.
- The prokaryotic RNA polymerase is a multimeric enzyme consisting of six subunits, two identical **α**-subunits, similar but not identical **β** and **β'** and **ω** sixth is **σ** factor.

  2α,β,β'ω -- core enzyme

  2α,β,β'ω + σ --- Holoenzyme

RNA polymerase.

RNA Polymerase of prokaryotes

Subunit	Function
α,α	Determine the DNA to be transcribed
β	Catalyze polymerization
β′	Bind & open DNA template(unwinding)
ω	Function is not known
σ	Recognize the initiation sites called promoter

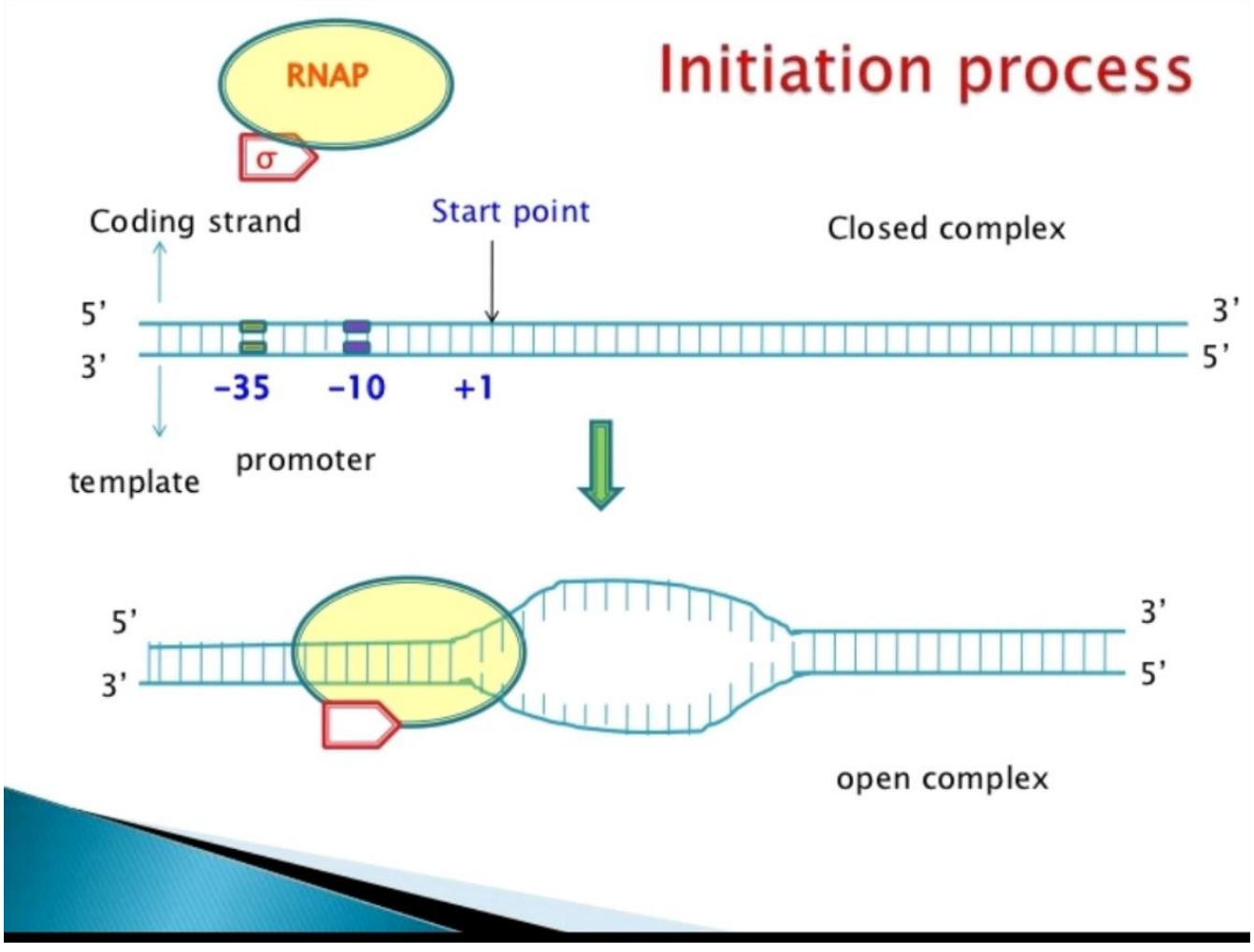
STEPS OF TRANSCRIPTION.

- Initiation.
- Elongation.
- Termination.

- ***Initiation*----**
- RNAP binds to a region of gene DNA called promoter with the help of sigma factor.
- RNA polymerase binds to DNA promoter.
- Initiation require special DNA sequence.

- Recognise DNA strand.
-

Transcription.



INITIATION OF TRANSCRIPTION.

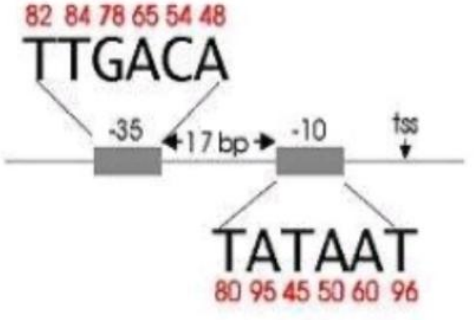
- RNAP binding–
- initiation begins with the binding of RNAP to the promoters of the DNA to form pre initiation complex.
- RNAP has an associated unbinding activity.
- Binding is followed by a change in RNAP conformation. This places Beta subunit of RNAP on the first nucleotide of the initiation site.
- The RNAP then catalyzes polymerisation of the first nucleotide with the second nucleotide according to the template strand sequence.

Promoter region.

Structure of bacterial prokaryotic promoter region

Pribnow box

- This is a stretch of 6 nucleotides (5'- TATAAT-3') centered about 8-10 nucleotides to the left of the transcription start site.



-35 Sequence

- A second consensus nucleotide sequence (5'- TTGACA-3'), is centered about 35 bases to the left of the transcription start site.

Elongation.

The RNA polymerase core enzyme progresses along DNA molecule to continue elongation.

The elongation continues till the termination signals are encountered.

TERMINATION--

Two types of termination signals are present.

Rho- factor dependent signals.....

Rho- factor independent signals.....

Termination

Eukaryotes	Prokaryotes
Polyadenylation signal(AAUAAA) transcribed. Reaches to -35 bp downstream	Rho-factor dependent..... Rho- factor independent.....
RNA transcript released.	

INITIATION.

- INITIATION.
- The process of RNA synthesis in bacteria begins with the binding of RNA polymerase molecule on the DNA.. RNAP recognises the transcription start site on the template strand of DNA with the help of certain specific region on the DNA known as promoter element.
- PE region identified in bacteria---
- 1.Pribnow box
- 2.The '-35' sequence

ELONGATION.

- ELONGATION---The elongation of the RNA molecule occurs from the 5’ to its 3’ end, antiparallel to the template strand. RNAP assembles ribonucleotides in a complementary sequence to the tepmlate strand by watson – crick base pairing rule.

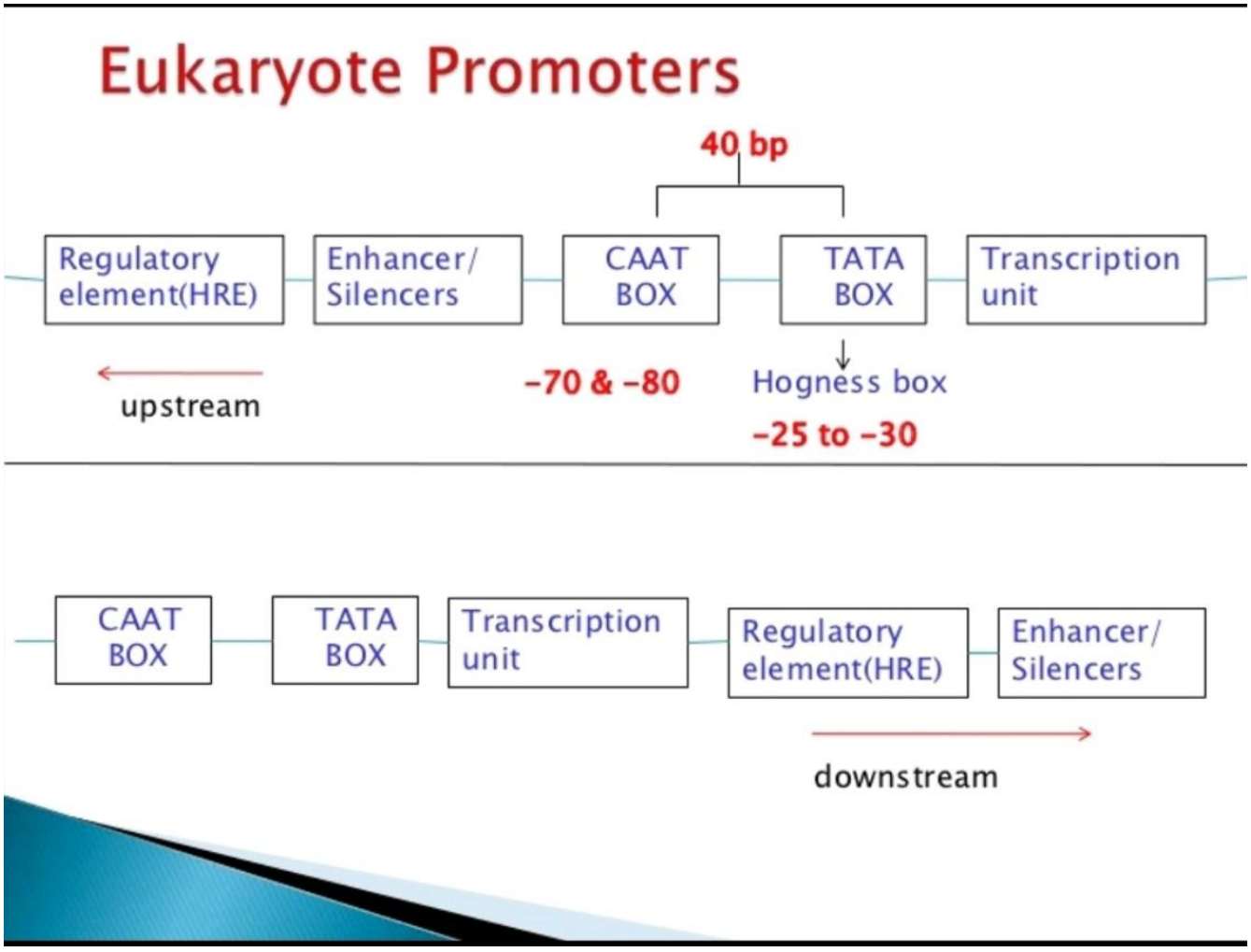
TERMINATION.

- ***TERMINATION***—
- Two type of such signals are are identified in prokaryotes.
- 1.Rho factor– it is a protein that binds either to the growing RNA or template strand of DNA and dissociate the the RNAP from DNA, thus terminating the synthesis of RNA. The released RNAP associates with the sigma factor(which was released earlier during initiation) and is recycled.

TRANSCIRIPTION IN EUKARYOTES.

- Much more complicated.
- Three different RNA polymerase.
- Required many transcription factor protein.
- Transcription initiation needs promoter and upstream regulatory regions.
- Enhancer /silencer --are DNA sequences that regulate the rate of initiation of transcription by RNA polymerase II.

PROMOTERS.



TRANSCRIPTION IN EUKARYOTES.

- Eukaryotes transcription is more complex compound to that of prokaryotes. There exist three distinct RNA polymerases transcribing different type of RNAs compared to the single RNAP in prokaryote.
- RNAP I ----for r RNA.
- RNAP II ---for m RNA.
- RNAP III-- for t RNA and small r RNA.

- **INITIATION---**
- Transcription starts with the RNAP recognising the promoter site.
- TATA box---similar to the pribnow box in prokaryotes a highly conserved nucleotide base sequence (TATAAA) occurs about -25 bp upstream to the TIS in eukaryotes. This sequences is also called as Goldberg- Hogness box.
- ENHANCER SEQUENCE— increases the rate of transcription.
- SILENCER SEQUENCE--- Decreases the rate of transcription.
- HORMONE RESPONSE ELEMENT— are the sites used by the hormones to regulate the transcription.
-

INITIATION COMPLEX.

- CAAT box—Further upstream to the TIS (-70 bp), there occurs another sequence (GGCCAATCT) known as CAAT box.
- ***PRE-INITIATION COMPLEX*** –
- RNA polymerase of eukaryotes (e.g RNAP II) can not recognise TATA and CAAT boxes directly. A group of protein factor play a role in recognising the promoter regions of DNA and called as transcription factor. TF bind to the promoter on DNA sequentially along with the enzyme to form a pre-initiation complex.

TRANSCRIPTION IN EUKARYOTES.

- *ELONGATION--*
- *Elongation of the nascent m RNA occurs similar to that of prokaryotes.*
- *TERMINATION– once transcription is completed , it is terminated and RNAP-II is dephosphorylated.*

POST-TRANSCRIPTIONAL MODIFICATIONS.

- *The mRNA in prokaryotes is fully functional as soon as it is synthesised. on the contrary , eukaryotic RNA produced by transcription as primary transcript are non-functional. They undergo extensive structural alteration to produce the mature functional molecule.*

Messenger RNA.

- The primary transcript of mRNA synthesised by RNA polymerase II in eukaryotes is often called heterogeneous nuclear RNA. hn RNA is subjected to extensive processing in the nucleus to produce mature mRNA, which then enters the cytoplasm to take part in protein synthesis.

Messenger RNA processing.

- The cap-

  The 5' terminal of mRNA is crowned with a 7-methylguanosine cap.

- The tail-

  The 3' end of mRNA is attached by a number of adenylate residues .This is known as poly-A tail.

Splicing-

During processing introns are removed and exons are spliced together to form mature RNA.

MICRO RNAs(miRNA) AND SMALL INTERFERING RNA(siRNA).

- *Micro RNA and small interfering RNA are a class of small RNA involved in gene silencing.*
- *They form complimentary hybrid with the target DNA and lead to either its degradation or inhibition of translation.*
- *Micro RNA- Long single stranded precursor RNAs.*
- *INSIDE the nucleus, primary miRNA transcript is first processed by a nuclease called DROSHA to produce a smaller double stranded hairpin loop like pre-miRNA.*
- *This is exported out of the nucleus to cytoplasm and trimmed by nuclease enzyme DICER to produce double stranded miRNA duplex.*
- *Duplex miRNA unbound and one of the strand is selected, The selected miRNA is loaded into the RNA induced silencing complex(RISC) to form a mature functional mi RNA.*
- *It is used to silence silence target mRNA------TRANSLATIONAL ARREST.*