TRANSCRIPTION & TRANSLATION

GENE EXPRESSION, RNA PROCESSING, GENETIC CODE, PROTEIN SYNTHESIS,

How does information in the sequence of nucleotides in a polynucleotide chain gets converted into the sequence of amino acids in a polypeptide chain?

Transcription
- RNAP mechanisms (ProK vs. EuK)

Genetic code
- only need to understand how it works

RNA processing
- ProK vs EuK

Translation
- ProK vs EuK

Transcription in Prokaryotes

Recall: Transcription is a series of enzyme catalyzed reactions that lead to the formation of preMRNA. by polymerization of RNA.

- Substrates: DNA, rNTPs
- Enzyme: RNAP
- Cofactor: σ-factor
- Coupled with: ATP hydrolysis (multiple SN_2 rxns)

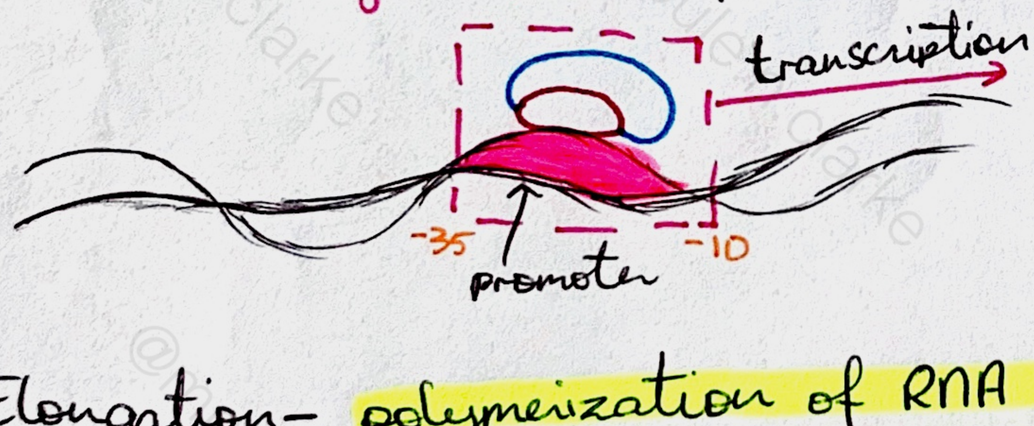
Steps in Transcription in Prokaryotic cells include:

- Initiation - a series of enzyme catalyzed rxn to get the ~~preinitiation complex~~ Holoenzyme to ~~leave the~~ bind to the promoter site and the promoter-polymerase complex (the initial transcribing complex) to ~~leave~~ escape the promoter
  ⇒ Initiation ends & Elongation commences.

Preinitiation complex in prokaryotes

← holoenzyme

Initial Transcribing complex in prokaryotes



- Elongation - polymerization of RNA after the transcribing complex escapes the promoter. with an RNA chain 10+ ~~80~~ long. Rxn catalyzed by RNAP (σ leaves when a short stretch of RNA is initially formed; before the complex escapes the region). A Transcription bubble with the core enzyme, the elongating RNA & the template DNA moves along the template until a termination sequence is encountered.

- Termination – in prokaryotes can be Rho dependent or Rho independent. Both involve a series of reactions that lead to the dissociation of RNAP & the product RNA strand.

Initiation in Prokaryotes

1. Formation of the Pre initiation complex

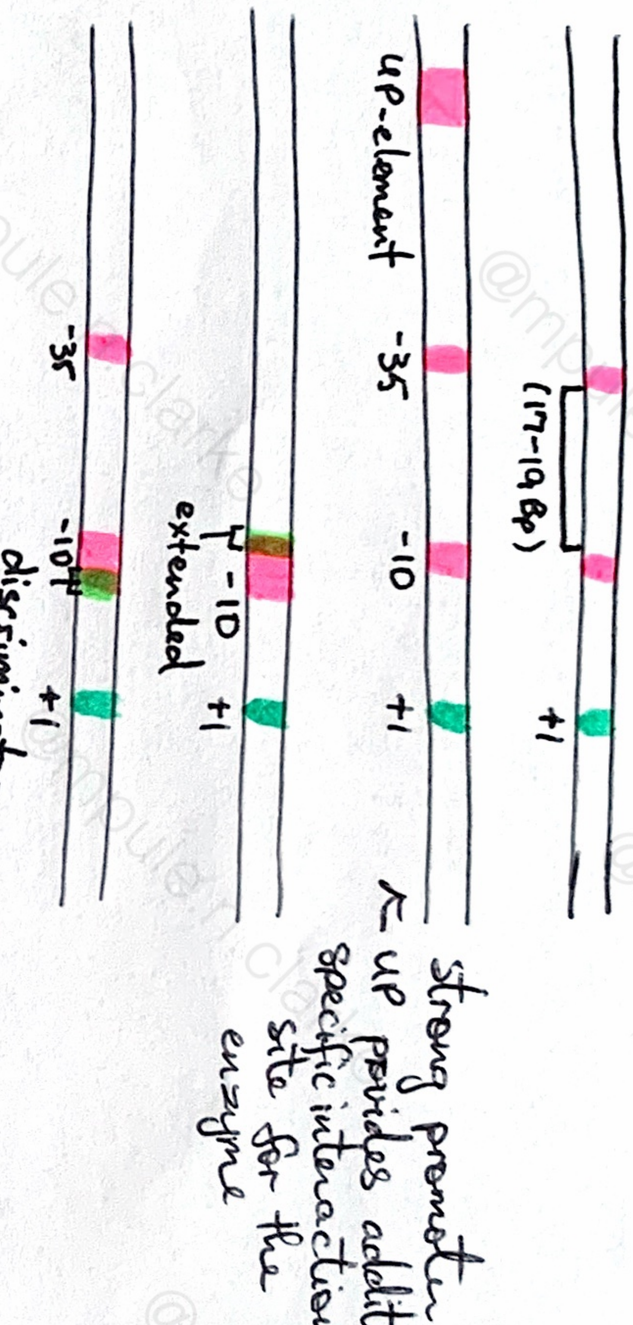
- The type of σ-factor determines where it binds. (multiple promoters are on a sequence)

- Transcription factors are needed to bind the RNAP core enzyme to the promoter region. In E. coli σ^{70} is the GTF utilized. The combined core & GTF is the holoenzyme

- the GTF allows the core enzyme to bind transiently to the promoter region ~~-10~~ spanning ~10Bp & ~-35BP from the target gene. This is an approximation across species. Some promoters are closer to an established consensus sequence which would make them strong promoters.

- The strenght of a promoter is determined by how many transcripts it initiates in a given time ⇒ how long it takes for a transcription complex to escape the promoter as well as how quickly the holoenzyme binds.

- promoter strenght corelation to its sequence location explains why some genes are so heterogenous; expressed more than others



2. Holoenzyme /preinitiation complex binds to the Promote

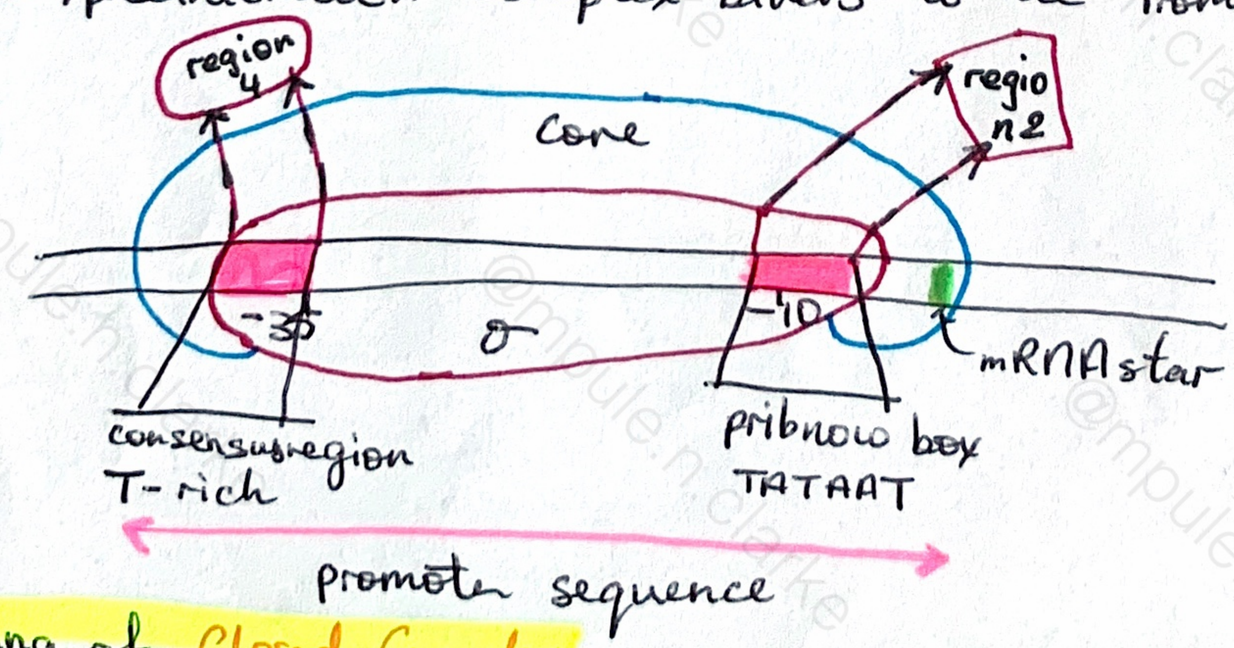


fig. Drawing of Closed Complex

σ^{70} factor in E. coli binds to the -35 & -10 regions of the promoter sequence.

region 4 - contains two helices that form a DNA binding motif called a helix-turn-helix. one of the helices inserts itself into the major grove & interacts with the -35 region; the other helix lies on top of the backbone of the groove. Binding σ to the DNA

region 2 the region is recognized by an α-helix like 4 but the energy provided is more; it melts the DNA & iniates the formation of the open complex.

Initiation in Prokaryotes Ct'd

3. Open Complex formation

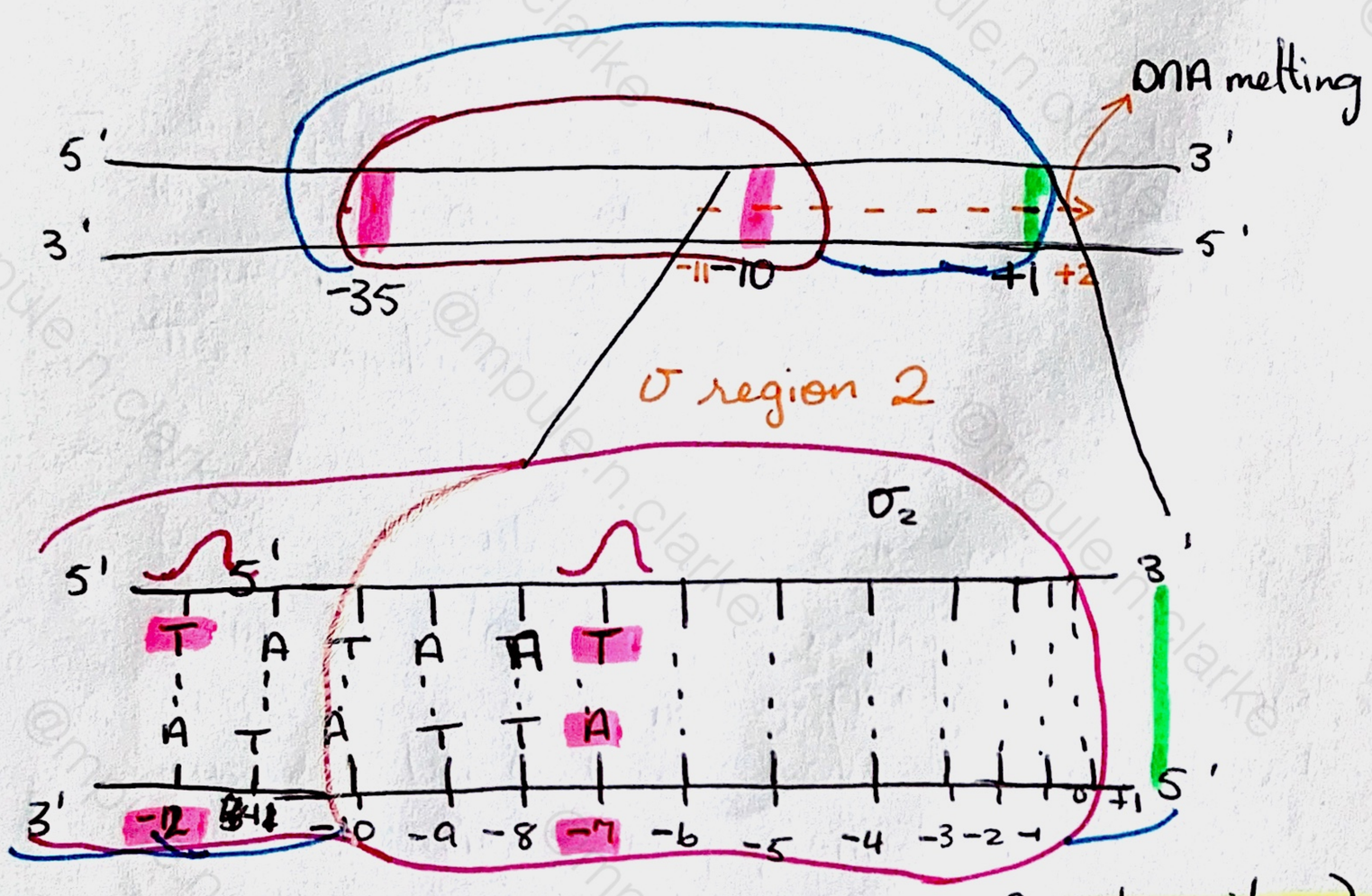


fig. σ detail on closed complex (before transition)

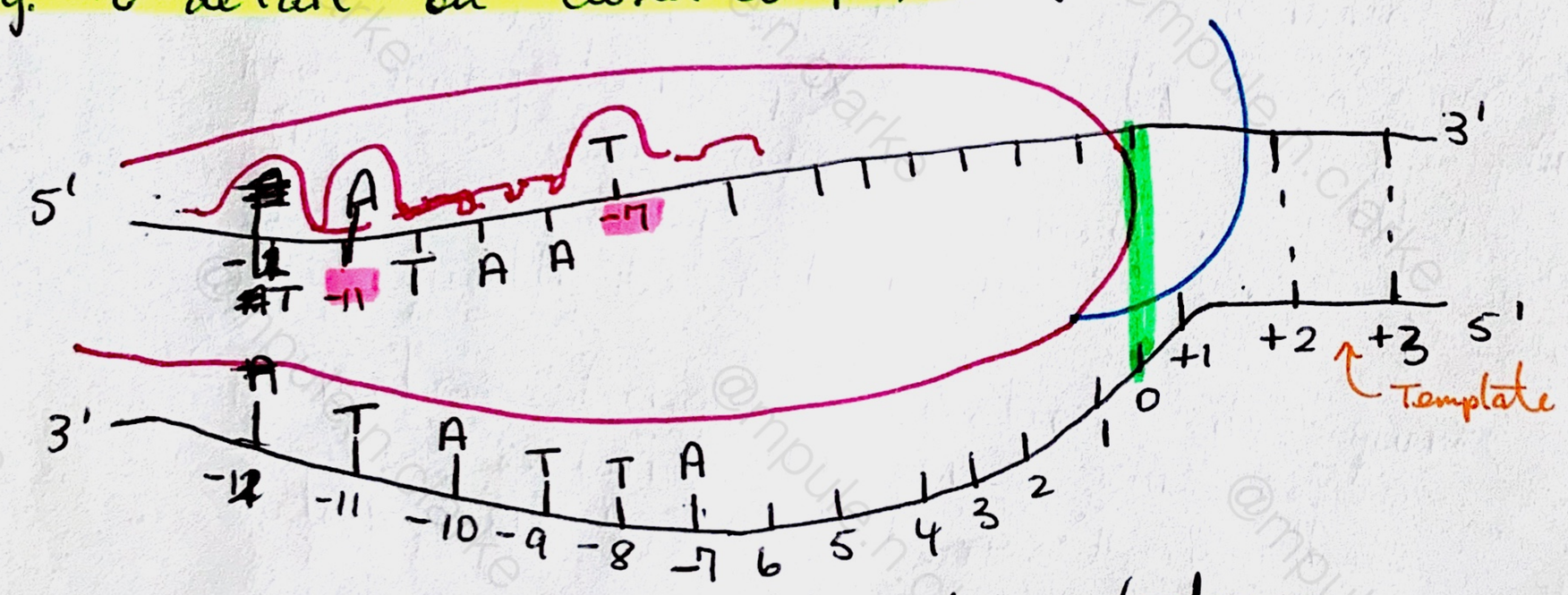


fig. σ_2 detail on open complex initiation

NB

σ-factor region 2 has two pockets that each bind a flipped-out base on the nontemplate strand of the -10 element of the promoter sequence.

The flipping of these two bases is an energetically favoured/spontaneous rxn that occurs when the σ factor region 4 binds to the -35 region of the promoter sequence; making the template strand interact closely with the σ factor.

=> why transcription bubble is 14 bp long

- Region 2 on the σ factor contains a high energy α helix component that forms ~~interactions~~ strong transient interactions with A_{-11} & T_{-7} on the 5' to 3' strand. These strong transient bonds forces the duplex open and triggers polymerization (initiates elongation).

  => because of the spontaneous high energy interactions this transient bond is permanent until the first additions of rNTPs occur & displace the bonds via an Sn_2 Rxn (rNTP nucleophilic attack)

4. Abortive Initiation

Fig.

σ complex diagram from textbook pg 440

- RNA polymerase starts most transcripts with the addition of an adenosine. (Two hydrogen bonds form)
- The region between 3 & 4 on σ organizes the ~~the~~ template strand in the correct orientation for initiation to occur. ⇒ in Orgs lacking this structure higher conc. of the nucleotides are present to increase the chances of accurate collision for bond formation to occur.
- RNA polymerase remains stationary & pulls downstream DNA into itself as rNTPs are added.
  - "Scrunching" reflects what actually happens; DNA downstream of the stationary open complex is melted & pulled into the enzyme for polymerization to occur.

Short transcripts are made repeatedly (enzyme ↺ works back & forth on the promoter) until it Escapes promoter region. ⇒ Abortive Initiation

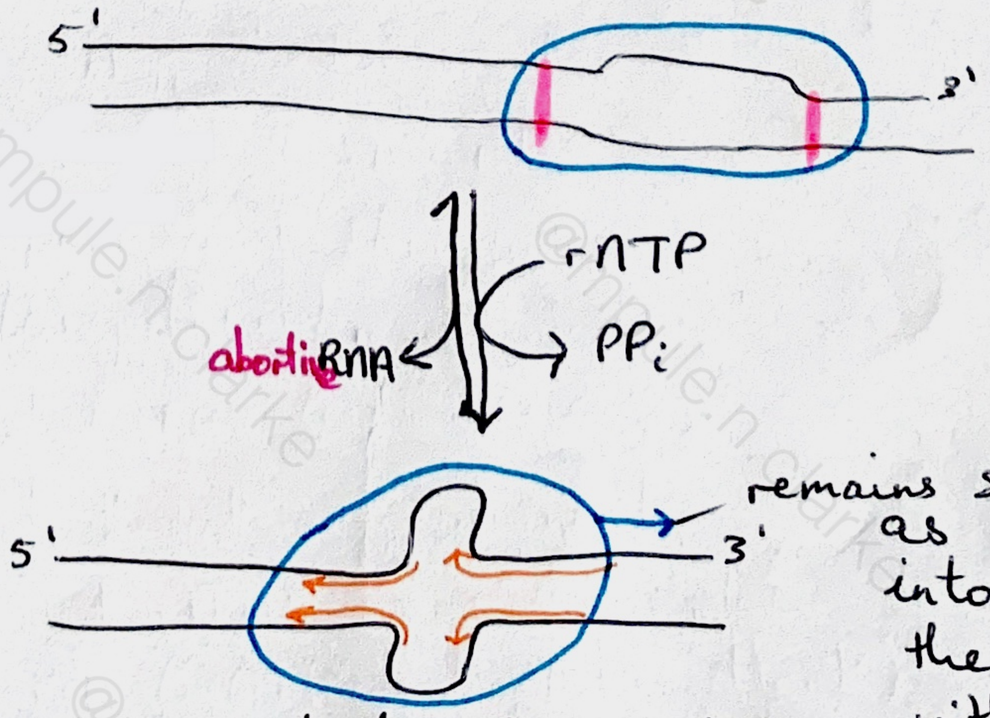


Fig. Demonstrating "Scrunching"

Initiation in Prokaryotes

5. Promoter Escape

- RNAP holoenzyme makes repeated transcripts that are aborted at ~10 RNA bases long. It is unclear why this occurs; the enzyme requires several attempts for all 14 bases on the template DNA to form a 14 nucleotide polymer in the transciption bubble.

- When the 14 nucleotide polymer is made the σ ~~com~~factor dissociates from the DNA but the core enzyme remains in the open complex & continues the elongation of the nucleotide polymer.

- Elongation commences at the instant promoter escape occurs.

Elongation in Prokaryotes

- The RNA core is the elongating enzyme

- DNA enters the elongating enzyme in the same manner that it does in the open complex during initiation.

- only 8 or 9 nucleotides remain "bound" to the template strand at any given time. The remainder of the chain is peeled of and is directed out of the enzyme through the RNA exit channel

- The enzyme adds one nucleotide at a time to grow the RNA transcript. (no scrunching)

- Elongating enzyme moves along the DNA. => the transcription bubble / enzyme-DNA complex is motile &; as 1 bp is separated a head of the enzyme 1 bp is reannealed behind it.

Print fig. 13-11
pg. 444

DNA / RNA Polymerization

Requirements Recall:

- Enzyme: RNA polymerase catalyses the formation of phosphodiester bonds. (SN_2 rxn)
- Substrates: • rNTPs (hydrolyzed & forms phosphodiester bond)
  • DNA Templates 3' → 5' strand
  • dNTPs (DNA synthesis)

⇒ All polymerases share a common ancestral origin so they share the same mechanism for catalysis of polymerization (both DNA & RNA use this mechanism)

⇒ All Pro & EuK polymerases have Asp-Asp residues in their active sites.

*recall

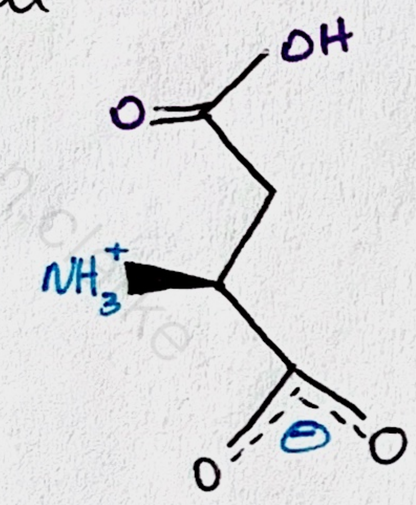


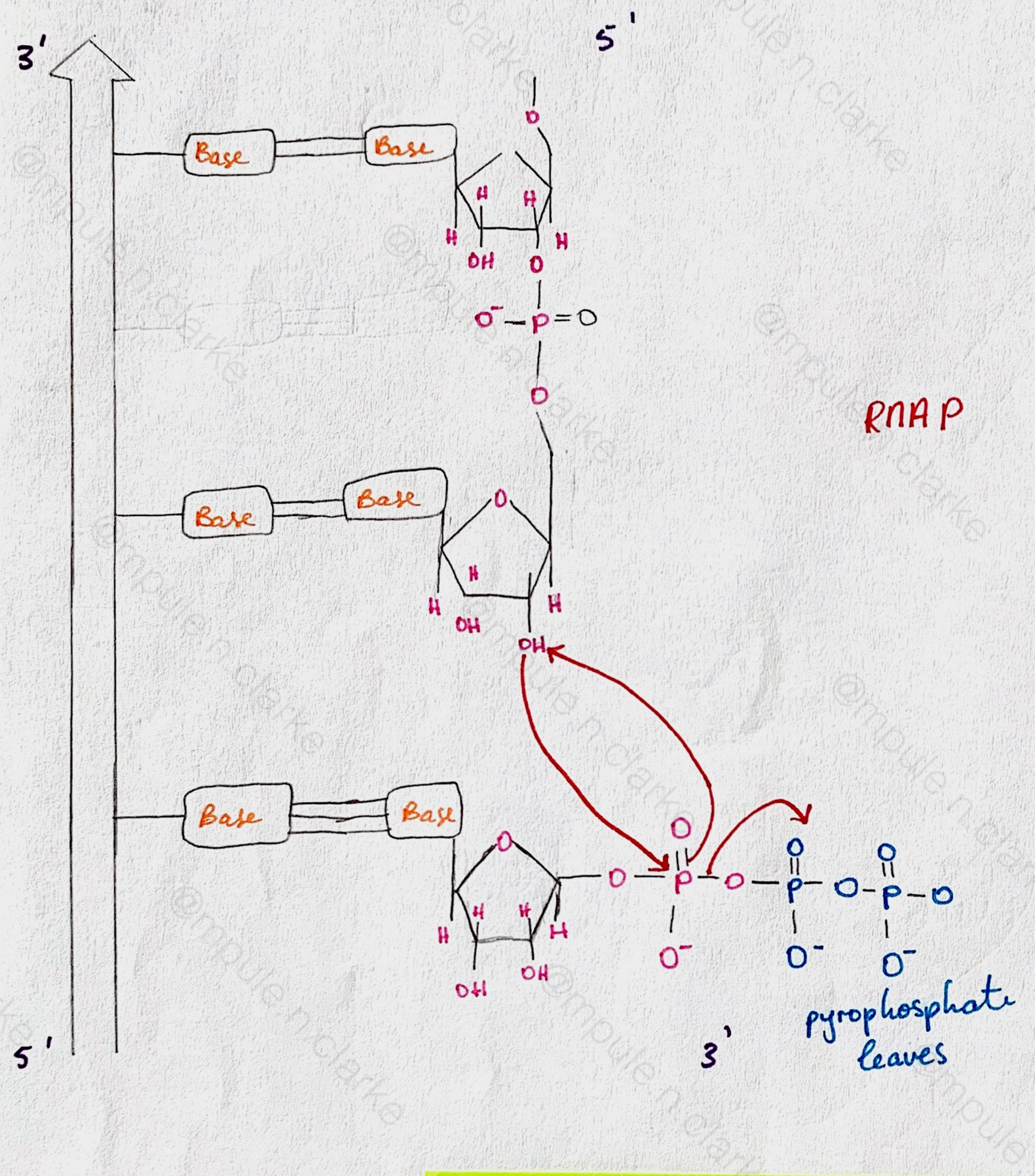
fig. Aspartic Acid (acidic, charged chain amino acid)

Aspartate in the active site of the RNAP along with Mg^{2+} ions (2) stimulates the SN_2 rxn between the incoming rNTP & the growing polymer ⇒ rNTP is hydrolyzed releasing pyrophosphate & a phosphodiester bond is formed

⇒ ~~RAA~~RNAPs & DNAPs are specific to the nucleotides they are polymerizing because of their structures.

- dNTPs are not polymerized by RNAP
- The enzyme collides with all the types of nucleotides & only catalyzes the bonds that will make energetically stable structures e.g Ribonucleotide + Ribonucleotide, dRibonucleotide + dRibonucleotide, A + T, A+U, C+G. any other combinations are not energetically stable & will be removed by proofreading.

RNA/DNA Polymerization



Print Rxn showing mechanism of Mg^{2+} & Asp

Transcription ~~Editing~~ Mechanisms
Proofreading

PyroPhosphorolytic Editing

- recall: elongation mechanism adds one nucleotide at a time to the elongating RNA strand/molecule in the 5'→3' direction & pyrophosphate is hydrolyed from the rNTP that is utilized in the new RNA molecule.

- The enzyme uses its active site to carryout a backward/reverse rxn on the wrong/incorrect nucleotide; pyrophosphate is added to it ~~and~~; the RNA strand is one nucleotide shorter & a rNTP is formed fom the nucleotide & the pyrophosphate.

- This mechanism favors the reverse rxn because of the specificity of the enzyme's active site to the size of the substrates ⇒ an A-G bond is the incorrect size for the active site so the enzyme lags its forward rxn & will activate the reverse rxn to remove it.

Hydrolytic Editing

- The hydrolytic editing mechanism involves the polymerase moving backwards (3'→5') & removing the incorrect sequence (may contain one or more nucleotides).

- hydrolytic editing is stimulated by Gre factors

  ⇒ Gre factors also serve as elongation stimulation factors. in some cells?

Termination in ProKaryotes.

- Termination is triggered by sequences in the transcription bubble. ⇒ The polymerase

Types of Termination in ProKaryotes

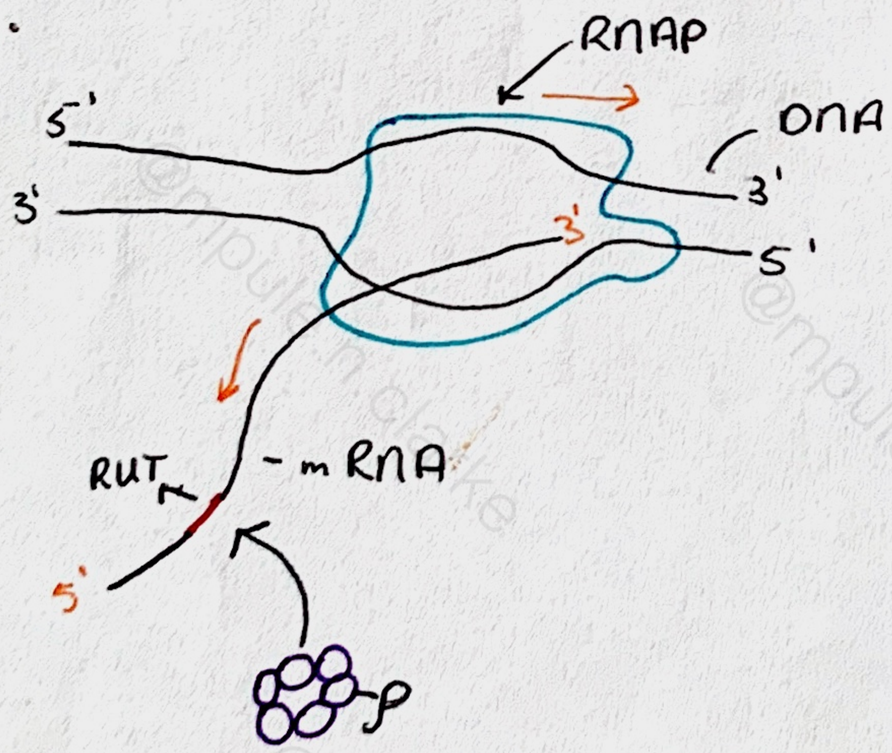
- RNAP arrest - see notes for details
- Rho dependent
- Rho independent

Rho dependent / ℓ ρ

- hexameric helicase protein (Rho) terminates transcription

1. ρ binds to the 'rut' site on the RNA transcript & moves up the strand ⇒ RUT (Rho utilization site)
2. when ρ gets to the polymerase it terminates transcription by dissociating the mRNA & the polymerase from the DNA. (ATP assisted process)
3. DNA reanneals, RNAP dissociates

1.



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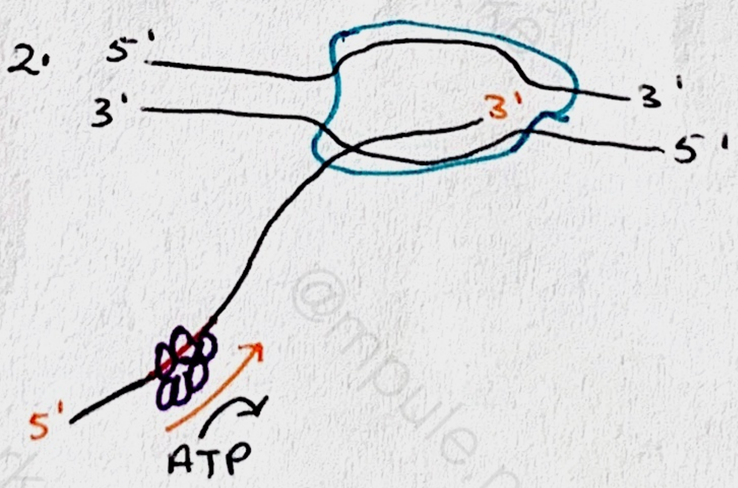
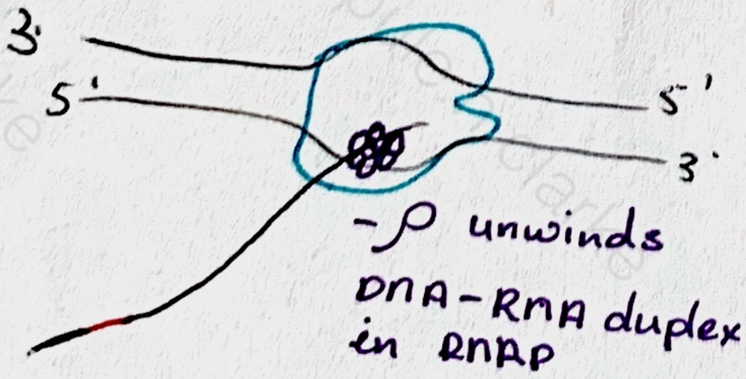
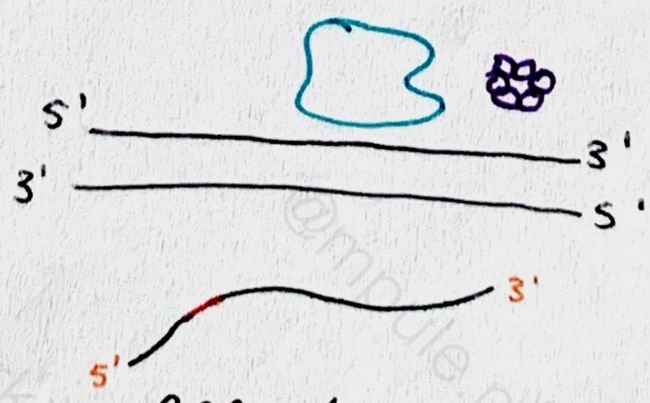




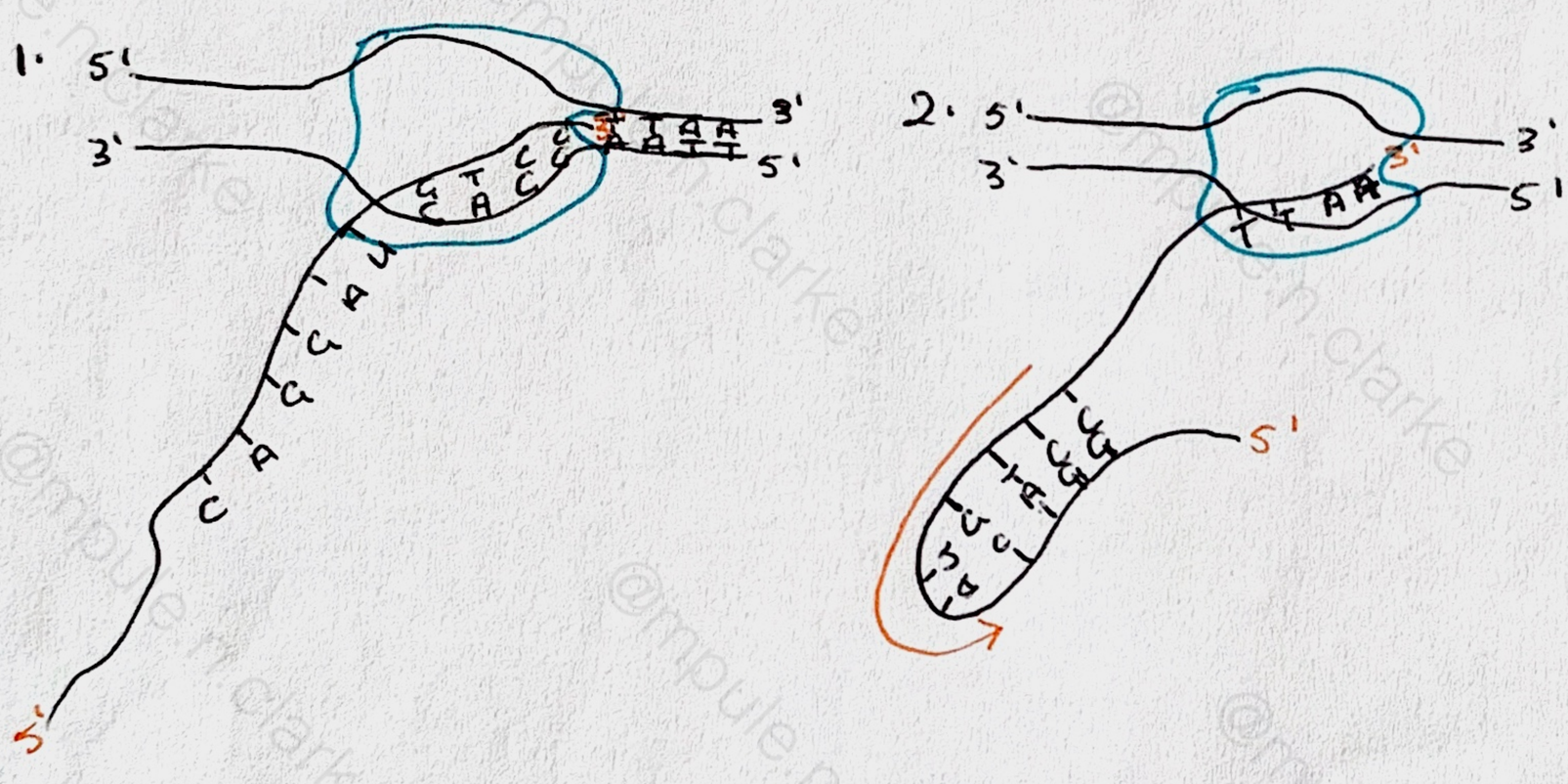
- ρ unwinds DNA-RNA duplex in RNAP



- RNAP dissociates
- DNA reanneals

Rhoindependent ~~Transcription~~ Termination / Intrinsic Termination

- does not involve a protein or ATP.
  - Consists of two sequence elements:
    - a short G-C rich inverted repeat sequence ~20 nucleotides long that folds on itself to form a hairpin loop on the mRNA
  - An A-T rich region that dissociates easily from the DNA in the polymerase when the hairpin loop forms & pulls the mRNA.



Transcription in ~~Prokaryotes~~ Eukaryotes

recall: Transcription is a series of enzyme catalyzed rxns that lead to the formation of pre mRNA. by polymerization of RNA.

- Substrates: DNA, rNTPs
- Enzyme: RNAP (I, II, III)
- Cofactors: TfIID, A, B, f, E, H
- Coupled with: ATP hydrolysis

STEPS in Transcription in Eukaryotic cells include:

- Initiation - a series of enzyme catalyzed rxns to get all of the factors along with the RNAP to bind to their respective regions on the promoter to form the preinitiation complex which must then escape the promoter for Elongation to occur.

- Elongation - Same as prokarya

Initiation in EuKaryotes

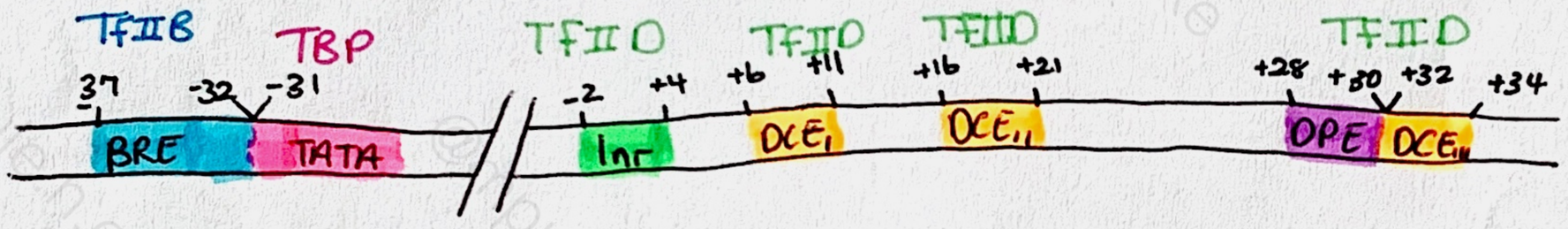
The **Promoter Region** is vastly different to that of the ProKaryotic cell's DNA. Eukaryotic ~~cell~~ promoter region comprise of:

- TFIID binding sites / sequences
- TFIIB binding site / sequence
- TBP binding site / sequence (located on TFIID)

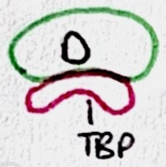
if TFIID does not bind, intiation will <u>not</u> occur.

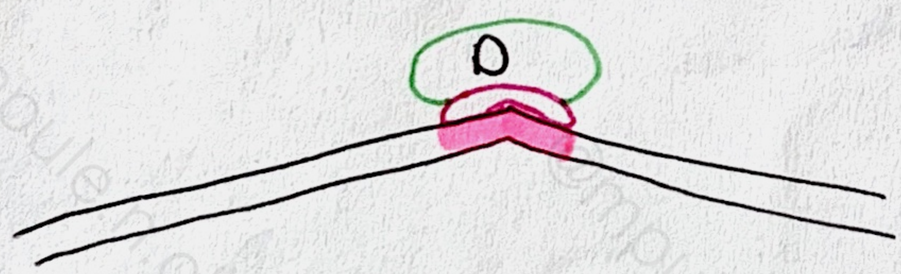
1. **RNAP Recruiting Proteins** commence the initiation of of transcription in EuKaryotes; RNAP cannot bind to the promoter region & initiate translation on its own (effeciently) without the cofactors.
   ⇒ They are called Transcription factors / Regulatory Proteins.

- the strongest binding is between ~~TFIID~~ TBP & TATA ⇒ most efficient transcription cycles.



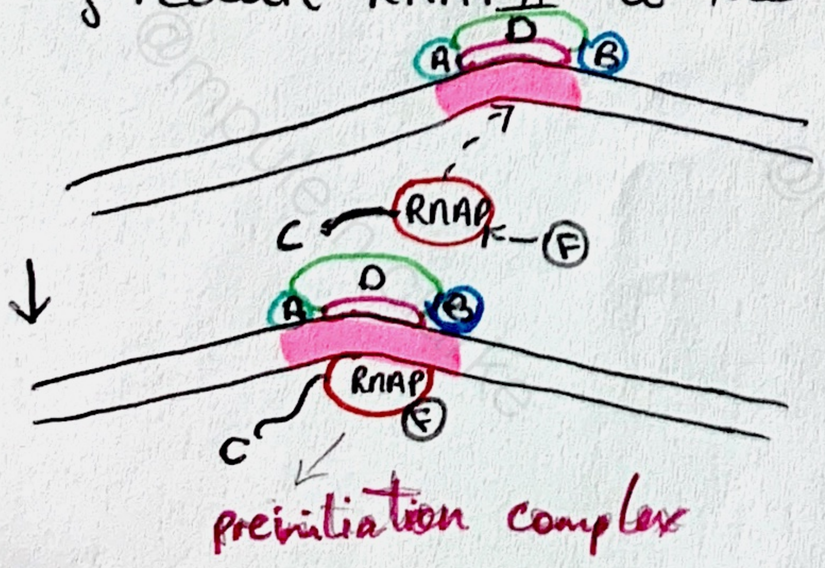
2. TFIID has TBP on it; TFIID binding of TFIID bends the DNA





- TFIID is a multisubunit complex that binds to DNA to initiate transcription in EuKaryotic cells.
- The <u>other subunits (TAFs)</u> <u>on TFIID</u> are what recognize the other core promoter elements such as Inr, DCE (I, II & III) & DPE

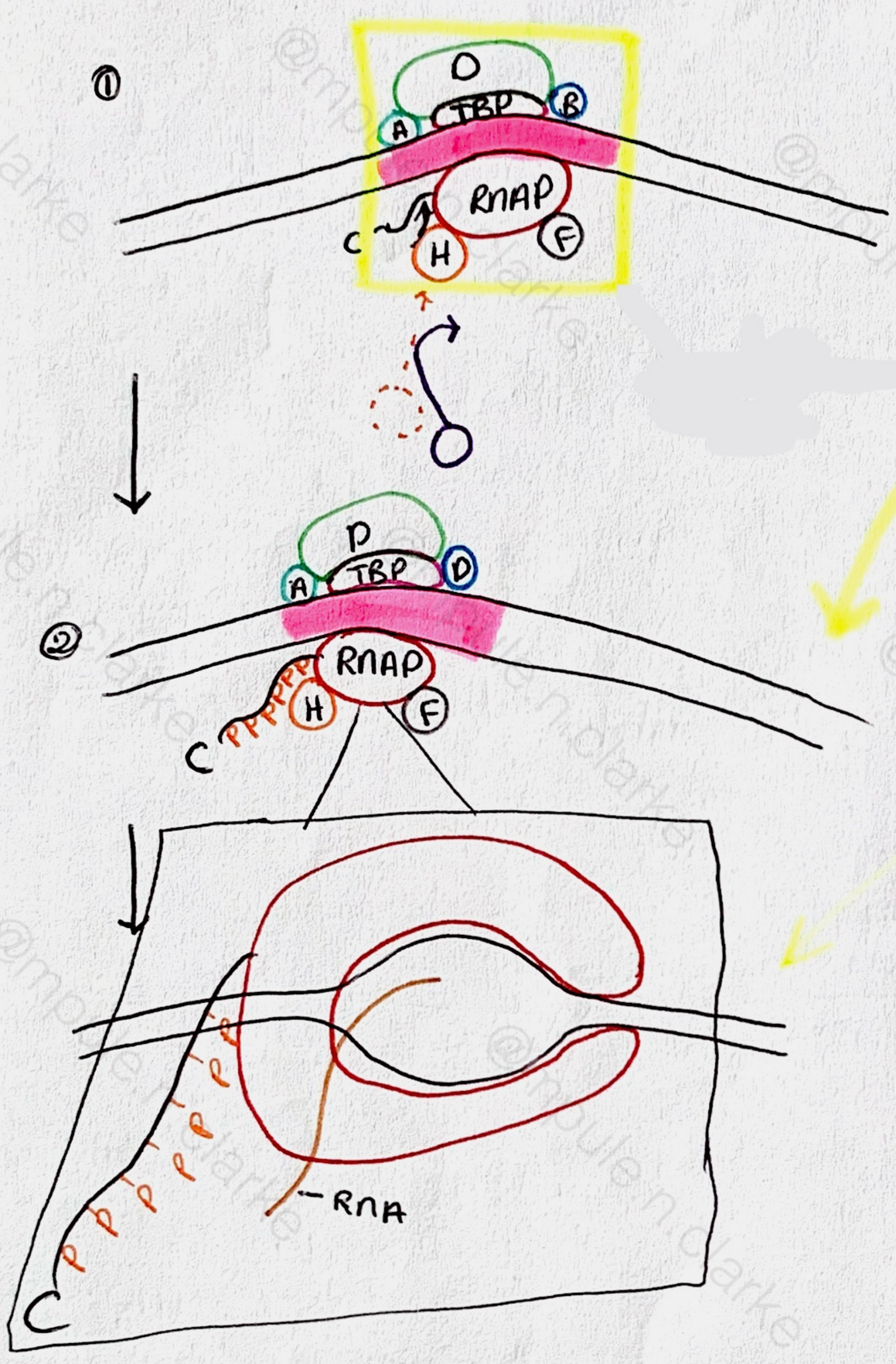
3. The bent DNA signals for TFIIA & B to attach to the DNA
   - they recruit RNAPII to the DNA



- place the RNA pol exactly where it needs to be
- TFIIF attaches to <u>RNAPII</u> & escorts it to the ~~Promoter~~ region.

Initiation in Eukaryotes

4. TfIIE recruits TfIIH to the polymerase; is formed.



- TfIIH has both Kinase & helicase activity.
  ⇒ the C ~~term~~ terminal of RNAP is phosphorylated.
  ⇒ DNA is melted to start elongation.
- TfIIH phosphorylates the Cterminus of RNAP II - does not attach to polymerase.
- TfIIH melts the DNA & the DNA-RNA+TF complex is open.

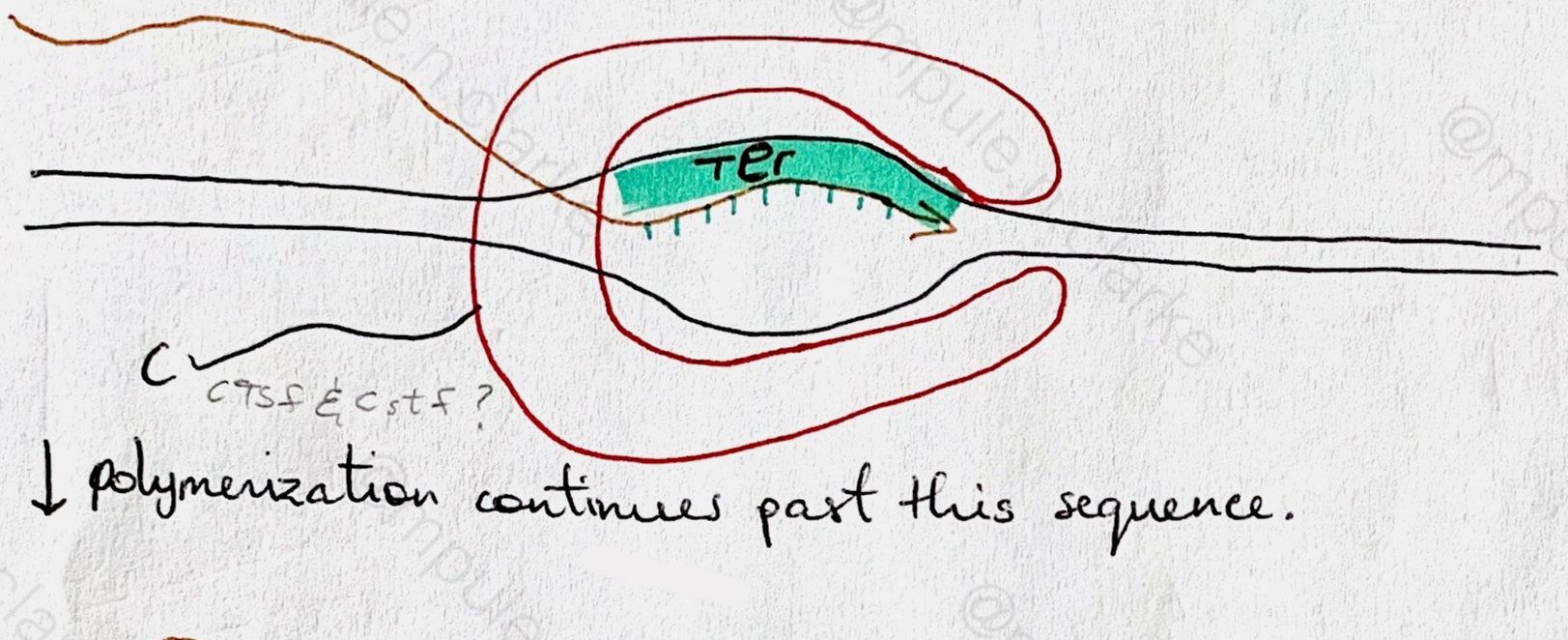
is it that the phosphorylation of the C-terminal destabilizes the energy composition of the RNAP so it melts the DNA as a means of acheiving equilibrium ⇒ rNTPs rush in form transient DNA-RNA bonds that stabilizes the open duplex ⇒ RNAP is offset as these addition occur so they move down the strand 3'→5' until it is terminated (reads a seq. that results in its dissociation

⇒ Initiation is regulated by Activators & repressors that either bind directly to RNAP or to a downstream sequence that cause the DNA to loop.

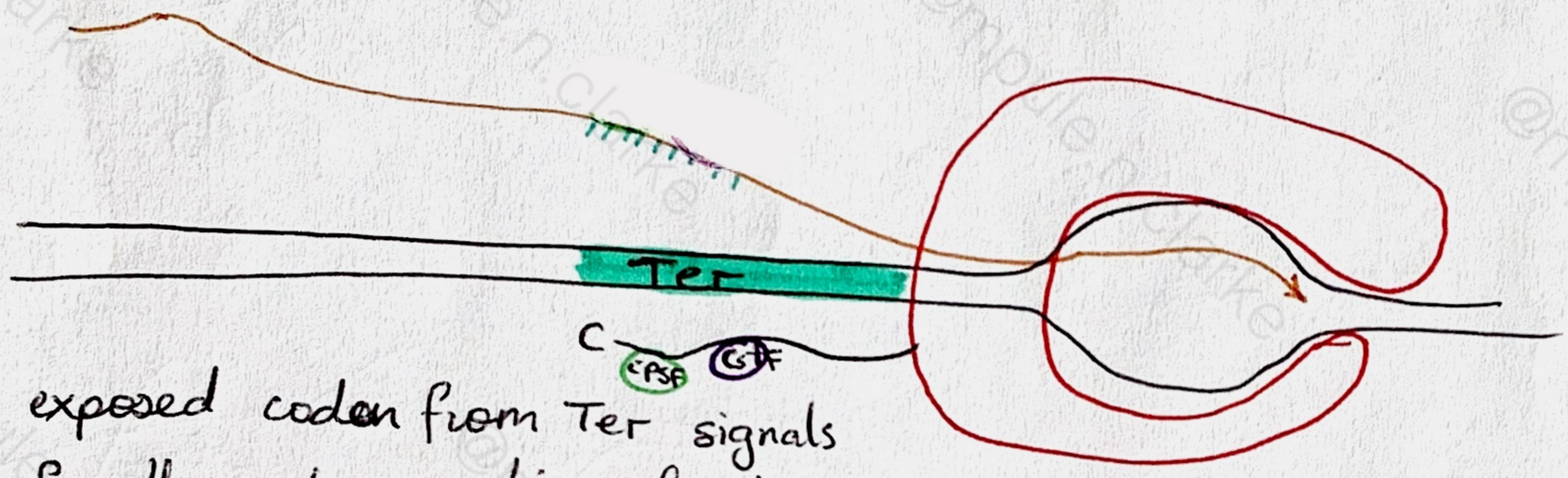
Regulation is specific to the gene that the cell is signalling to be expressed.

Termination in Eukaryotes

1. RNAP II reaches the 'ter.' sequence that codes for the polyA tail polymerization. Polyadenlylation Identification

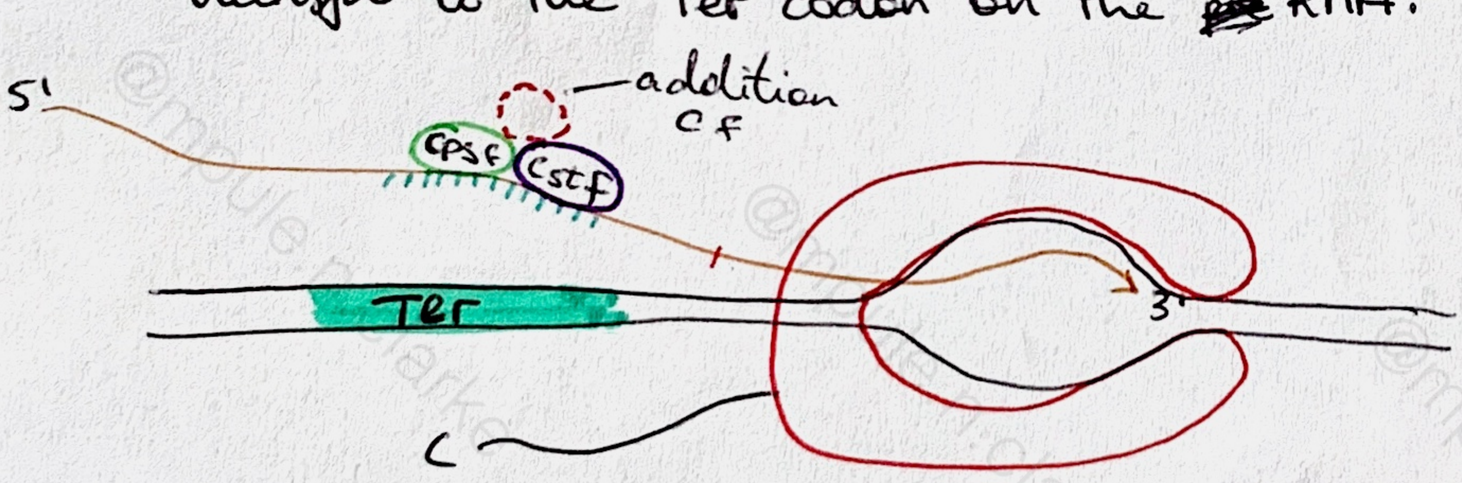


↓ polymerization continues past this sequence.



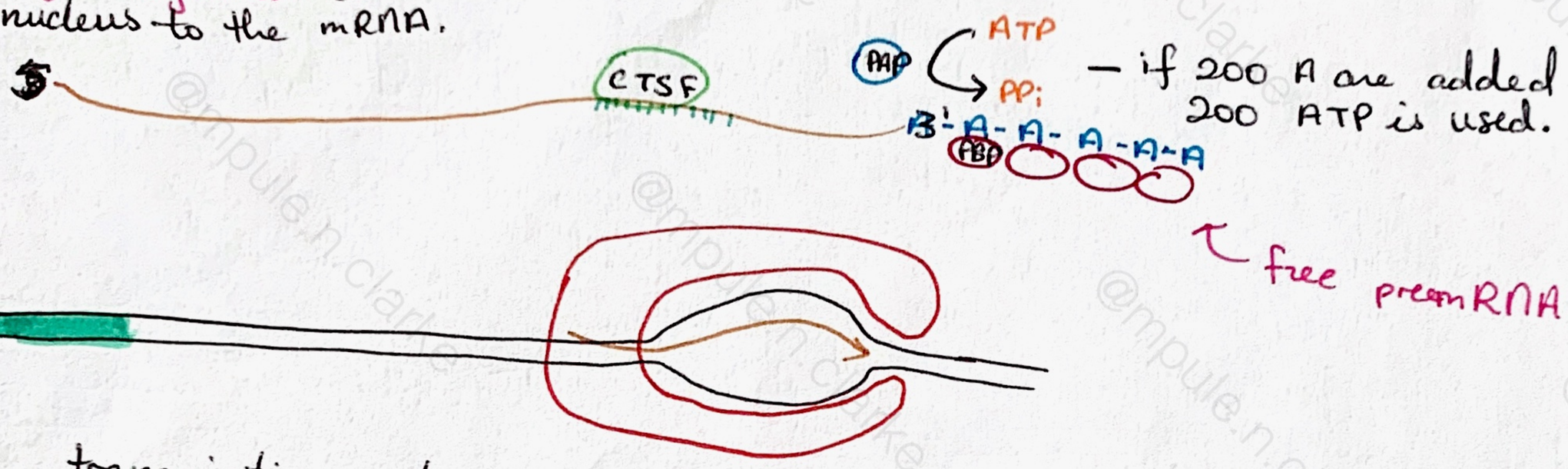
exposed codon from Ter signals for the polymerization of adenine from free ATP to the ~~DNA~~ RNA right after the Ter codon.

2. CPSF & CstF bind to the Cterminal of the RNAP. transfer to the Ter codon on the ~~DNA~~ RNA.



CPSF & CstF on the RNA attract endonucleases called additional cleavage factors. => they cleave the RNA right after the Ter codon; resulting in the removal of CstF.

3. Poly A Polymerase adds Adenine from free ATP in the nucleus to the mRNA.



- if 200 A are added 200 ATP is used.

transcription continues on the DNA strand after the mRNA has been cleaved from the elongating complex.

PolyA binding proteins attach to the PolyA chain to prevent the formation of double stranded RNA ⇒ these are single strand binding proteins.

4. It is believed that RNA pol dissociates from the strand after a while due to the change in the complex after the mRNA is cleaved & a 5' cap is no longer on the elongating strand.

Prokaryotic mRNA Translation

Prokaryotic mRNA is polycistronic: it contains two or more Open Reading frames (ORF)s to code for multiple proteins on the same mRNA molecule. This way the organism makes efficient use of its biochemistry relative to its size.

e.g. P_R codes for CIII, Int, Xis & N during early transcription of phage DNA in *E. coli*.

Prokaryotic mRNA contain Ribosome binding site sequences (3-9 bp long) upstream of the start codon on the ORFs. The RBS is also called the Shine-Dalgarno sequence. This sequence is recognized by 16s rRNA; it is complementary to a sequence on the 3' end of rRNA. 16s rRNA is on the small subunit of the ribosome.

RBS $5'$ AGGAGG $3'$
16s rRNA $3'$ UCCUCC $5'$

why the ribosome reads mRNA in the $5' \rightarrow 3'$ & adds tRNA $3' \rightarrow 5'$

ORF activity depends on spacing between RBS & the start codon $\Rightarrow$ RBS closer to AUG $\rightarrow$ more efficient