

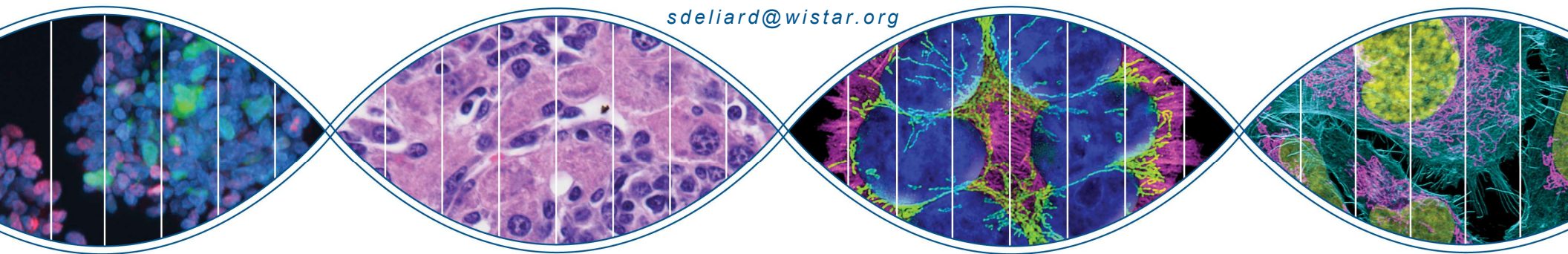
Gene Regulation

Sandra Deliard, PhD

Associate Staff Scientist

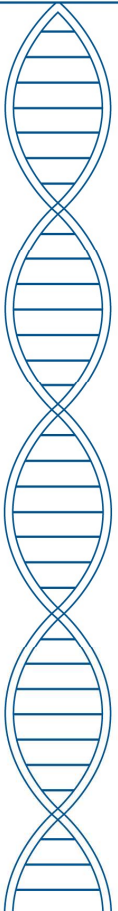
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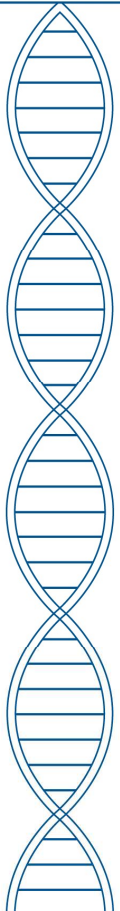
Lecture overview



- Introduction to eukaryotic transcription with RNA Polymerase II
- Transcription Initiation
- Transcription Elongation
- Transcription Termination
- Methods for measuring gene transcription and expression



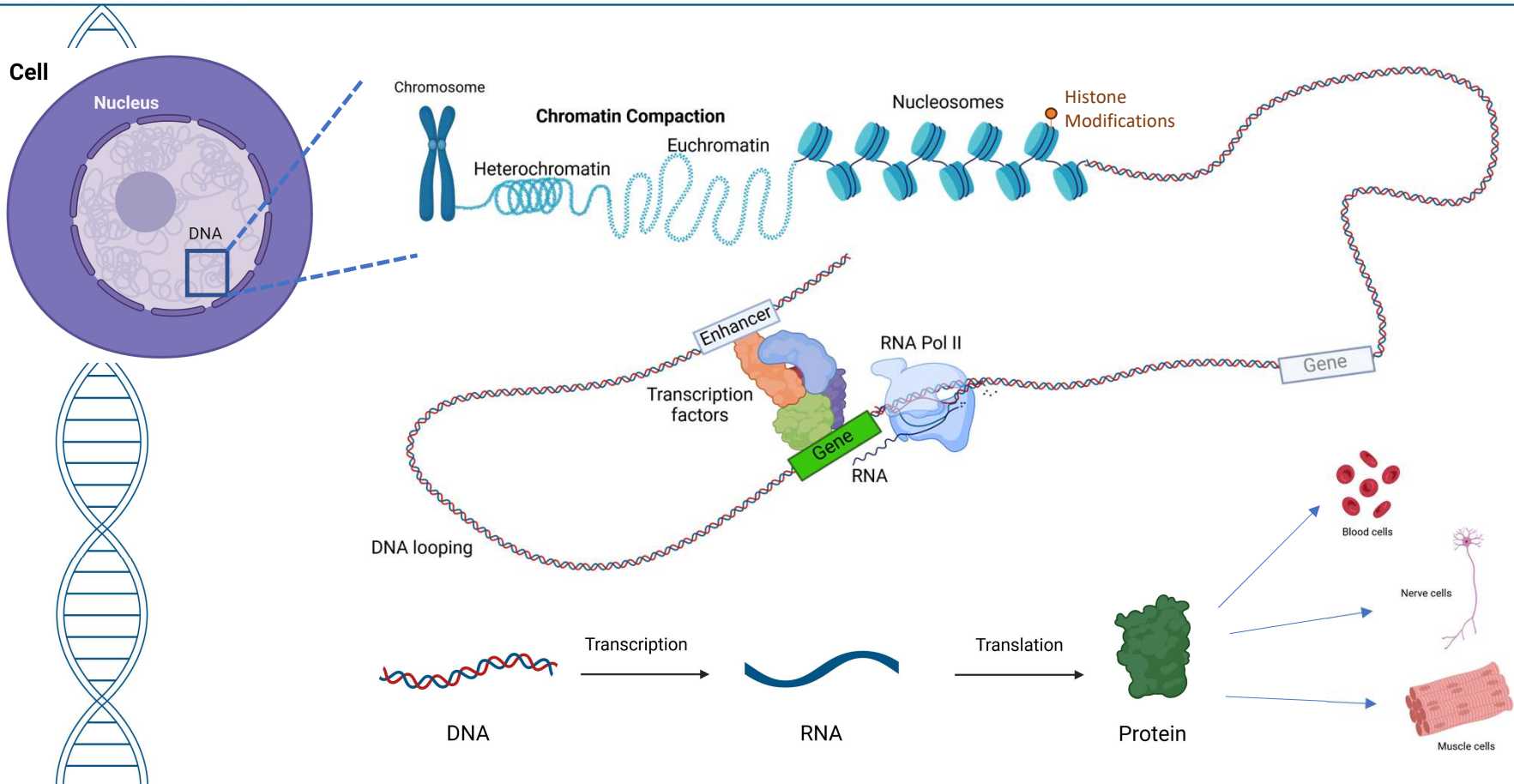
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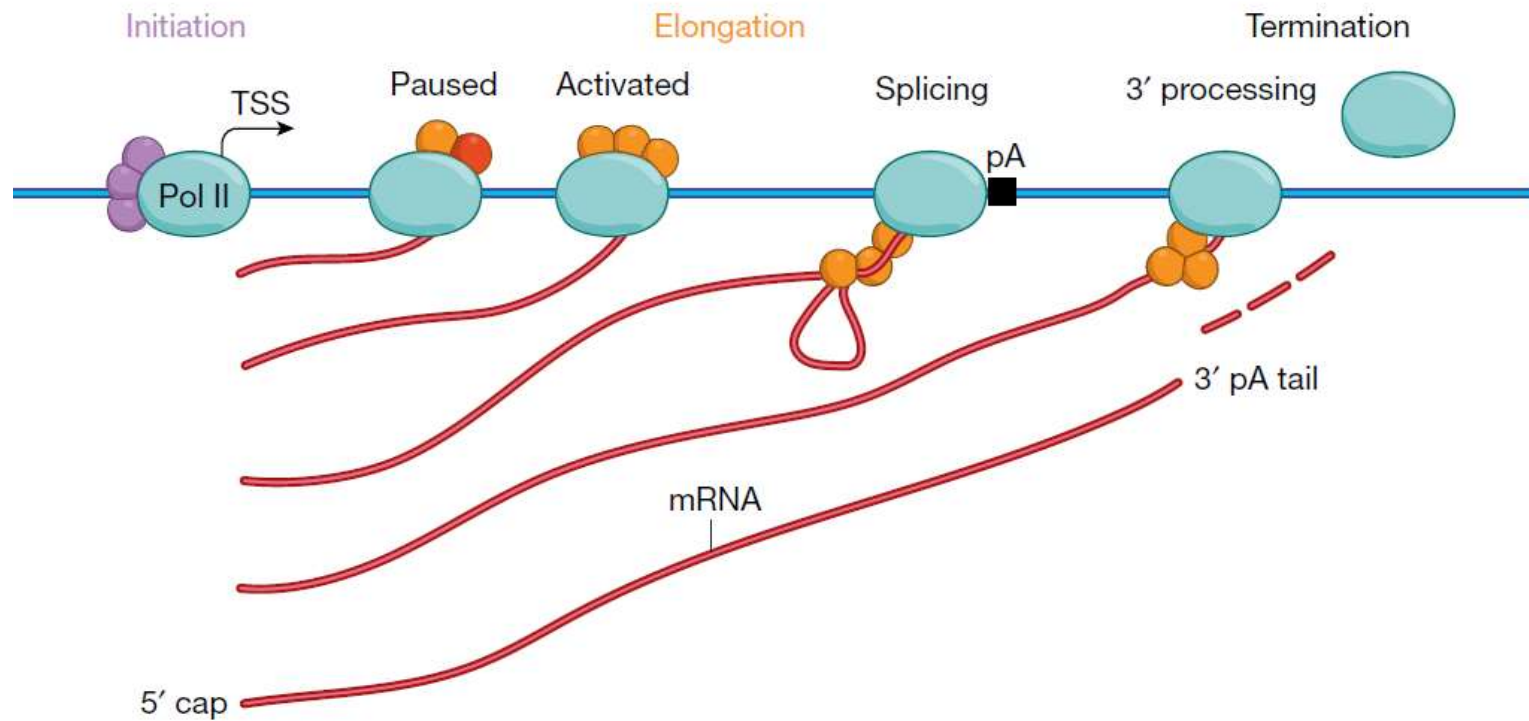
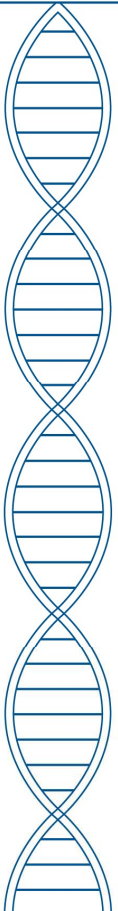


Gene expression regulation for cell identity and function





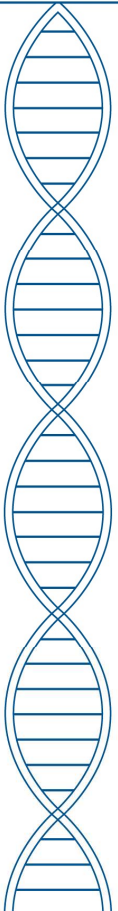
Stages of transcription



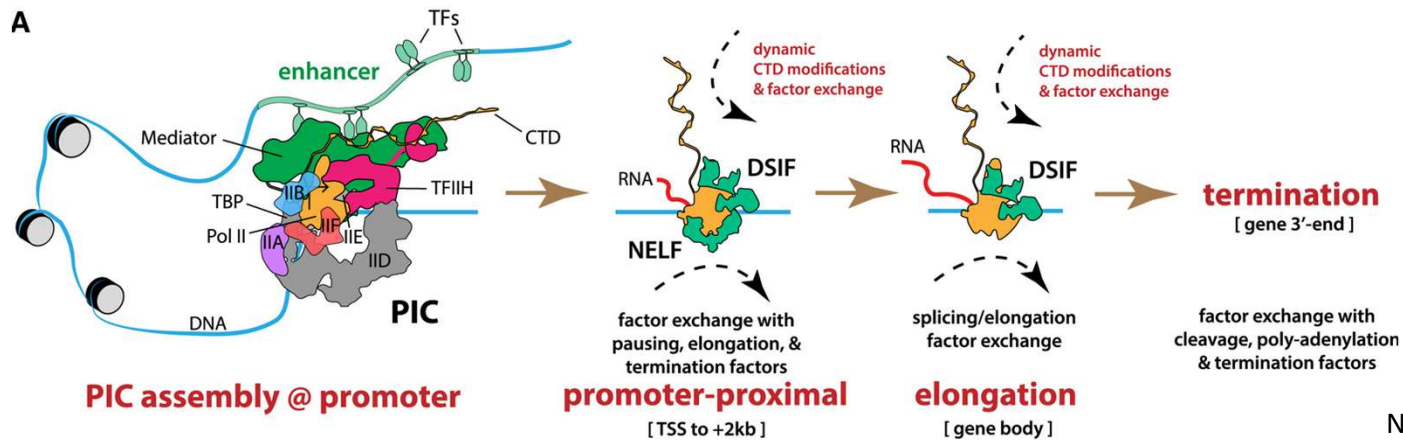
Who are the key regulators in this process?



Key molecules in transcription regulation

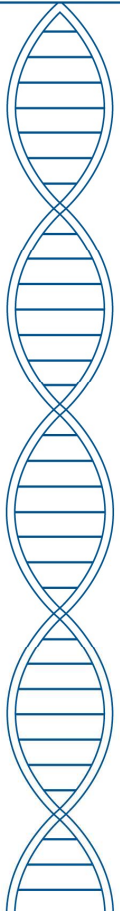


- **RNA Polymerase** – Enzyme that catalyzes transcription of DNA to RNA
- **Transcription Factors (TFs)**
 - General Transcription Factors (GTFs)
 - Sequence Specific Transcription Factors
- **Mediator**
- **Integrator**
- **Elongation Factors**
- **Termination Factors**

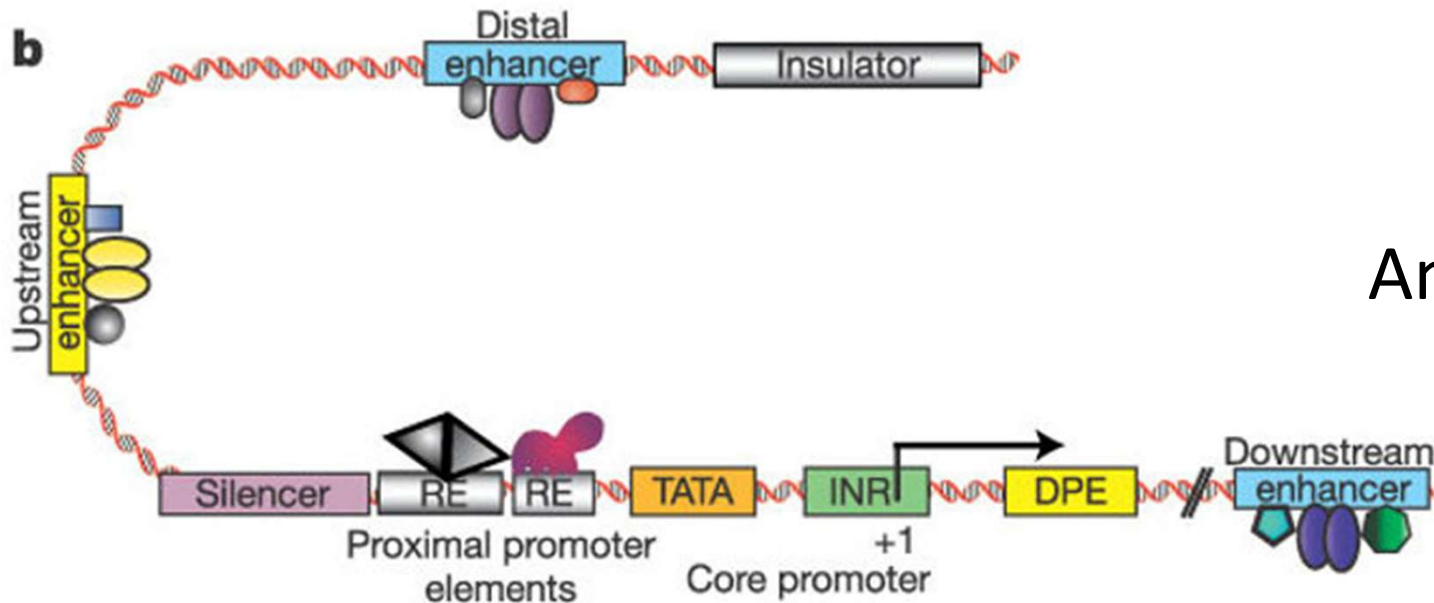




Gene promoter and regulatory elements in Eukaryotes



Yeast



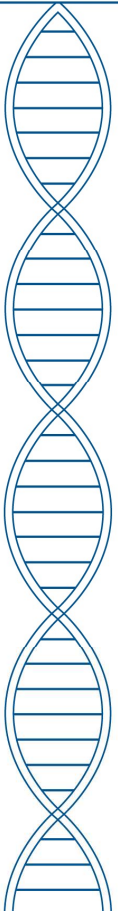
Animals



Adams, Nat Education, 2008



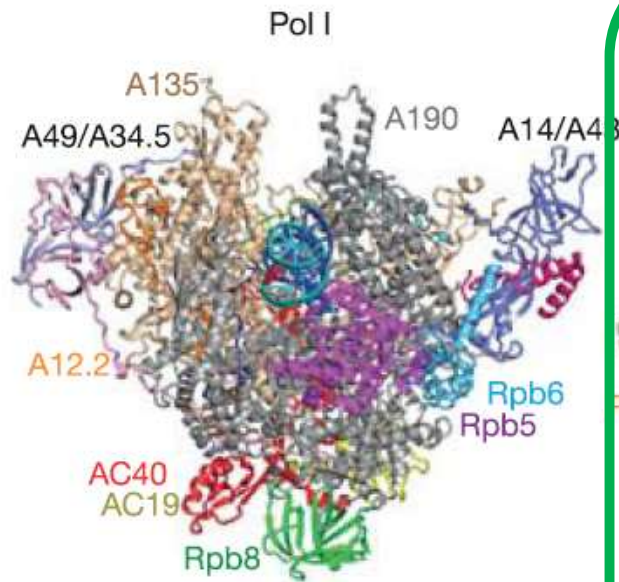
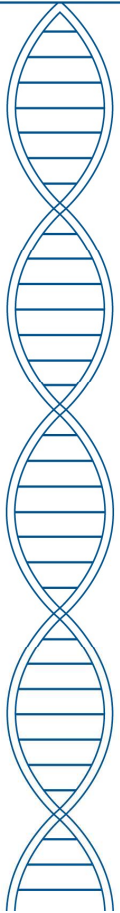
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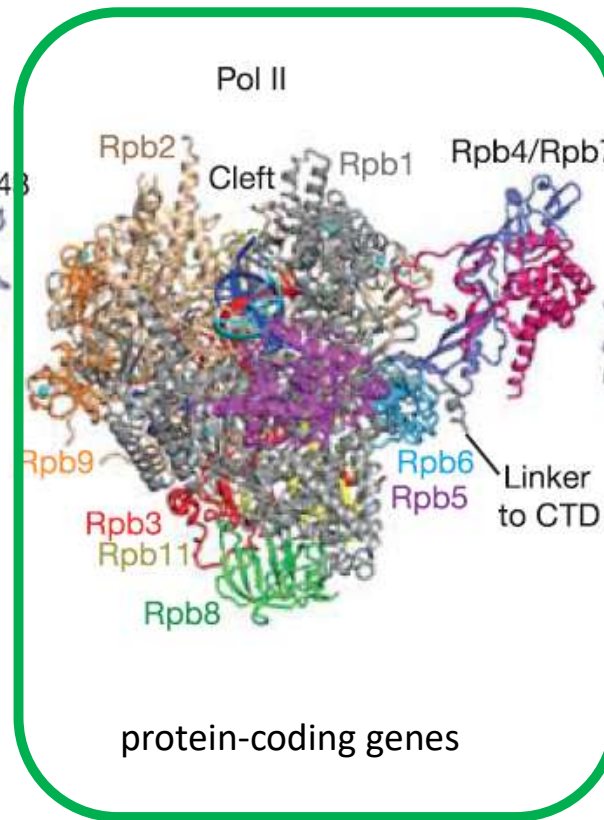
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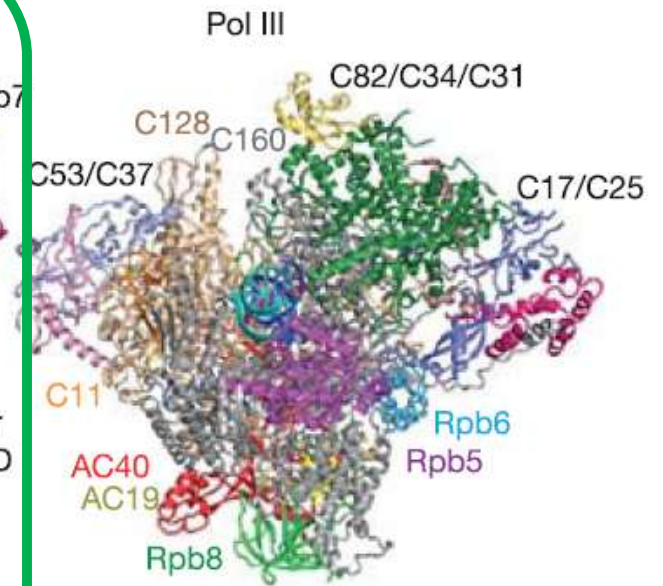
RNA polymerase



Ribosomal RNA (rRNA)
precursor



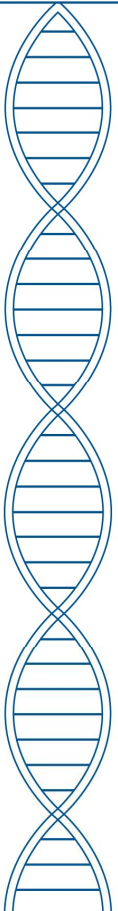
protein-coding genes



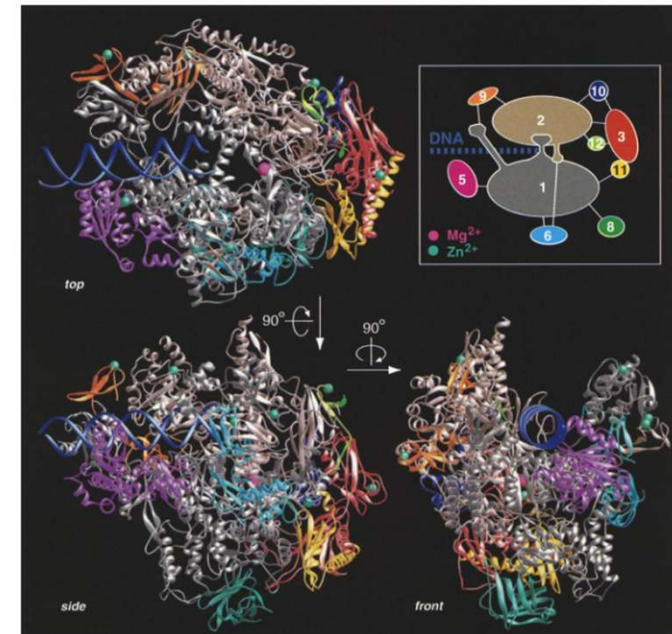
transfer RNAs (tRNAs)
and the small ribosomal RNA



RNA Polymerase II (Pol II)



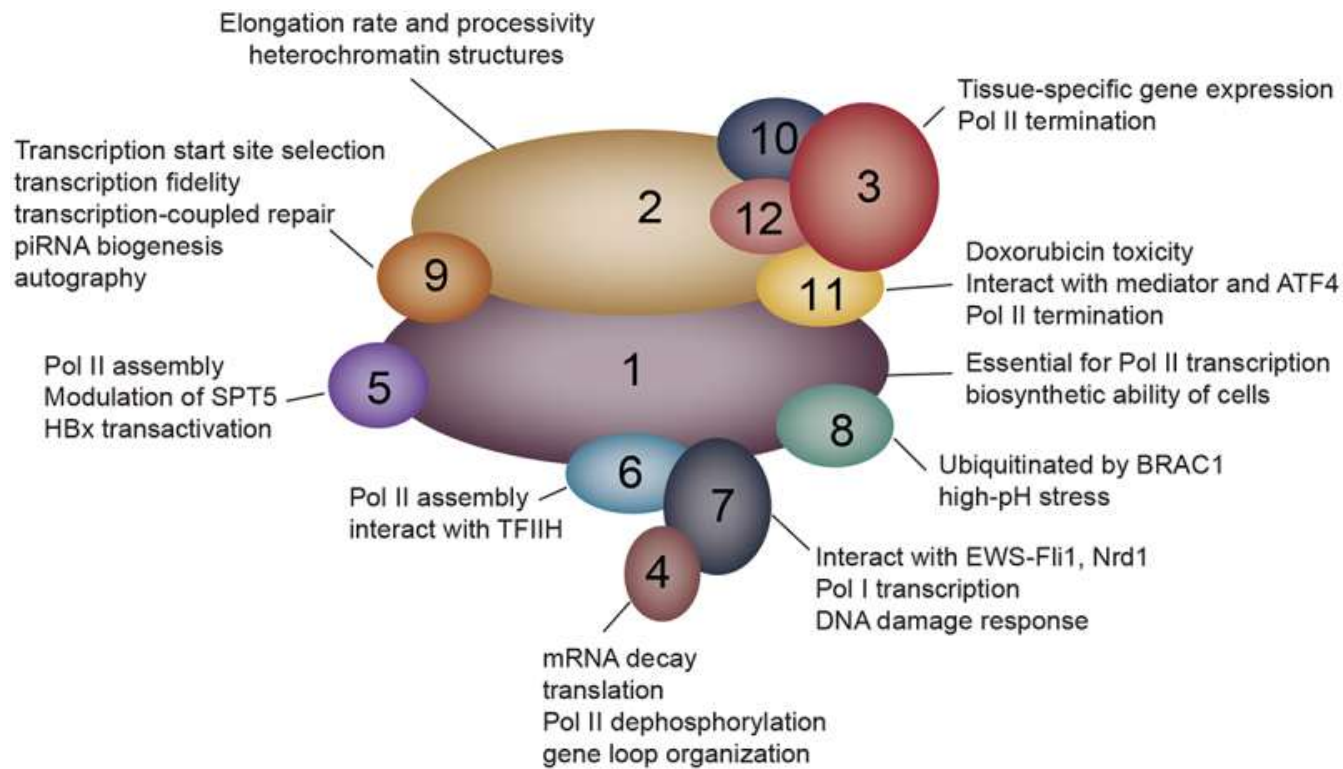
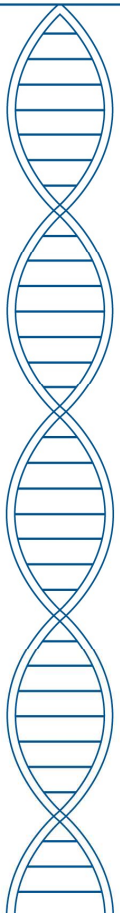
- Purification of Pol II revealed it is a multi-subunit complex in 1970s
- X-ray crystallography and cryo-EM revealed structure to Pol II in 2000
- Transcribes messenger RNA (mRNA) from protein-coding genes
- Operates in the nucleus of eukaryotic cells
- Requires general transcription factors to initiate transcription
- Has a unique C-terminal domain (CTD) with repeats of YSPTSPS
- CTD phosphorylation state controls transcription stages:
 - Ser5-P → initiation
 - Ser2-P → elongation
- Transcription coupled with RNA processing:
 - 5' capping
 - Splicing
 - 3' polyadenylation



Cramer et al, Science, 2000

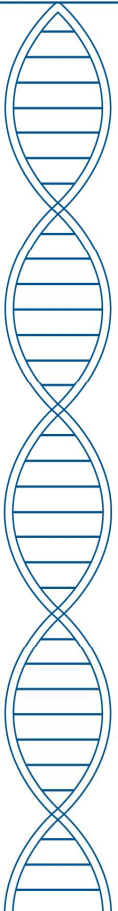


Roles of Pol II subunits

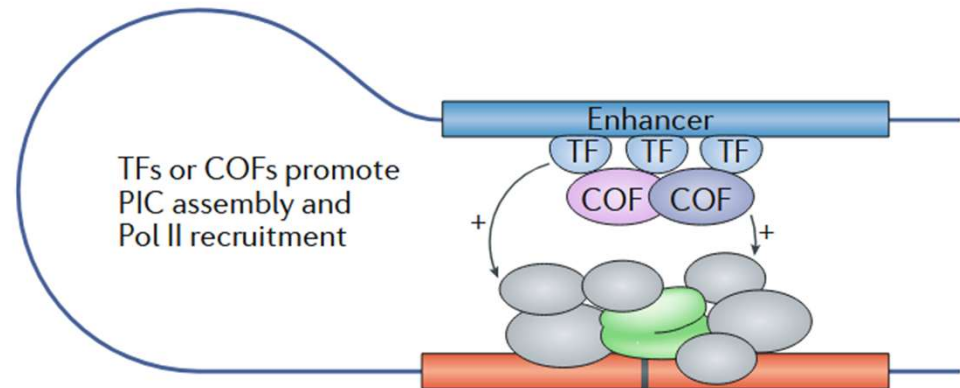
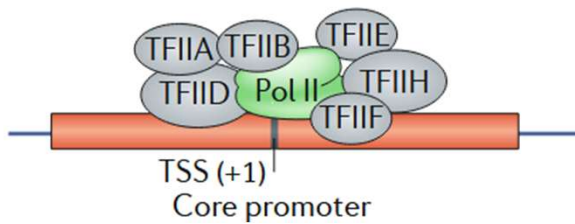




Transcription initiation

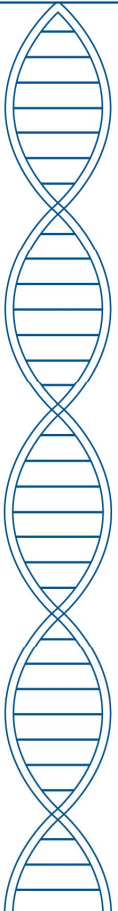


a PIC assembly and Pol II recruitment





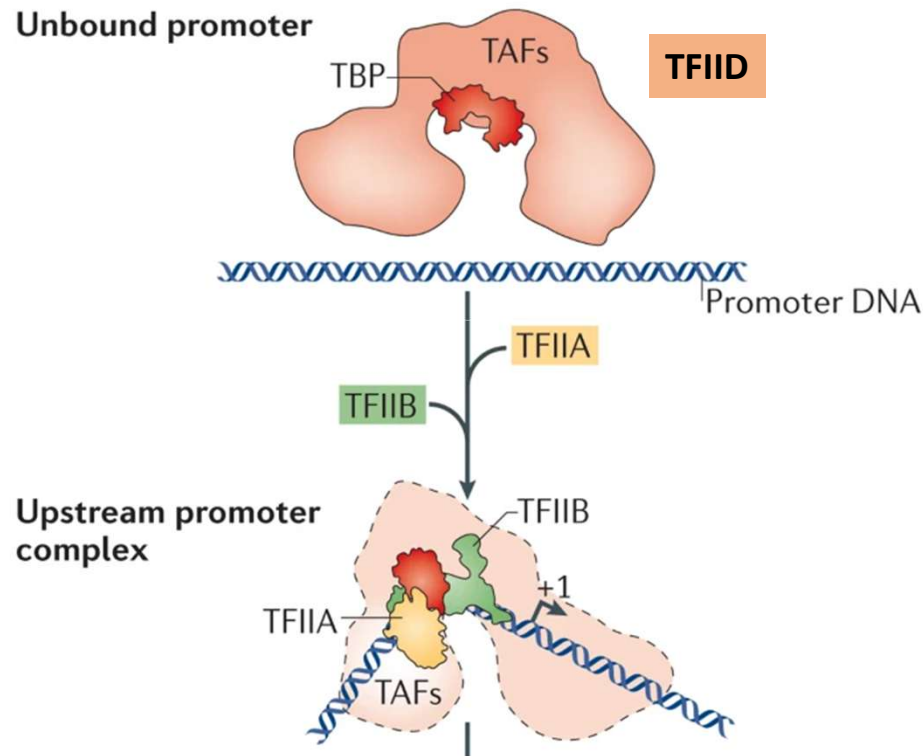
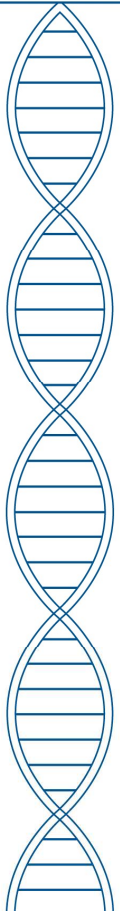
General transcription factors (GTFs) for Pol II recruitment and transcription initiation



- **TFIID**
 - First factor to bind promoter
 - Largest GTF(15 subunits)
 - Contains TATA Binding Protein (TBP) domain and TBP-associated factors (TAFs)
- **TFIIA** - Interacts with TFIID to stabilize binding to DNA
- **TFIIB** - Binds TFIID and acts as bridge for Pol II/TFIIF
- **TFIIF** – Interacts with Pol II to help bind promoter
- **TFIIE** - Recruits TFIIH to complex, transition from initiation to elongation
- **TFIIH** - Final factor to join complex, helicase and kinase activity

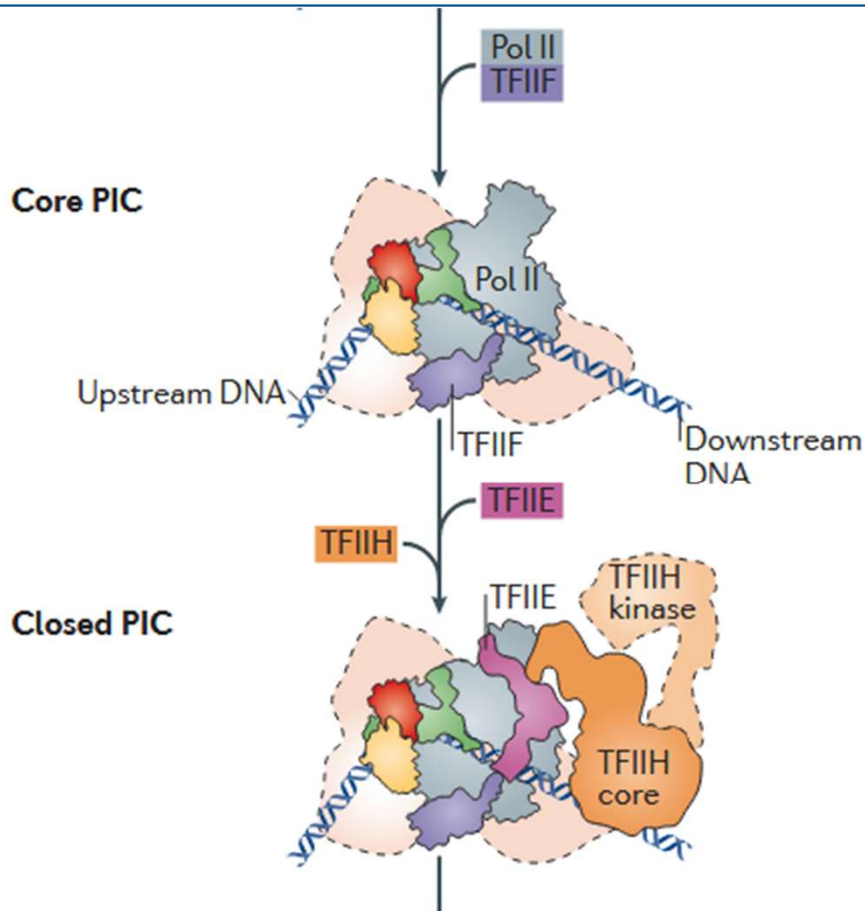
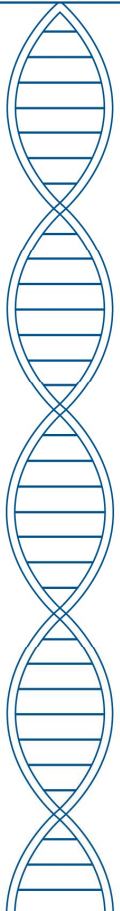


Pre-initiation complex (PIC) assembly: Binding of TFIID, TFIIA, and TFIIB to promoter regulatory elements





Pre-initiation complex (PIC) assembly: Pol II Recruitment and PIC completion

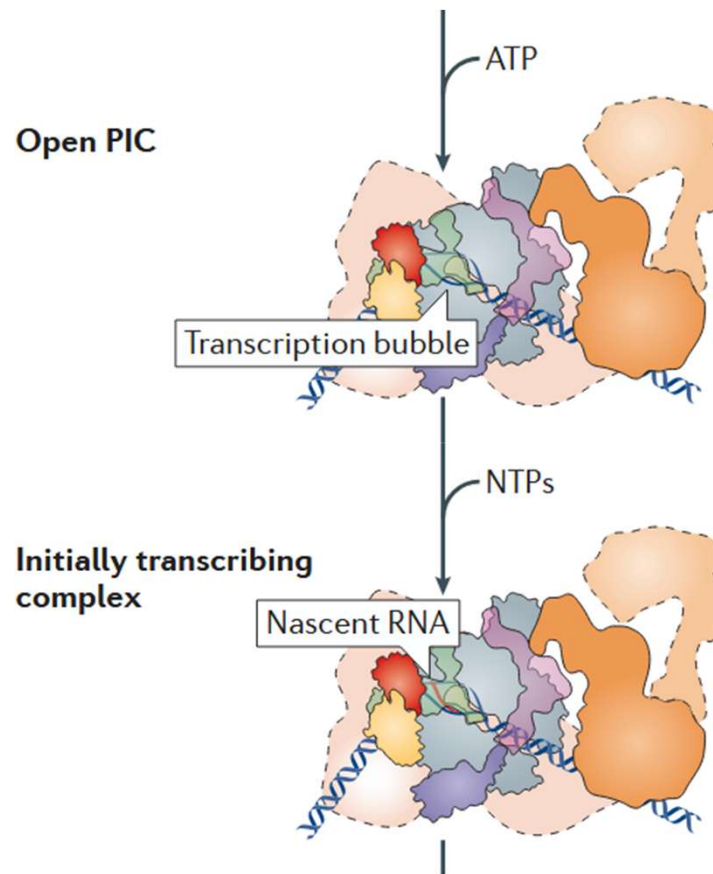
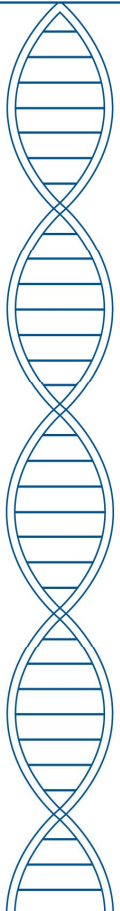


TFIIH

- Recruitment completes the PIC
- Unwinds promoter DNA via helicase activity (XPB, assisted by XPD)
- Phosphorylates the Pol II CTD (Ser5) through its CDK7 kinase subunit
- Enables promoter clearance and transition from initiation to elongation
- Also functions in DNA repair



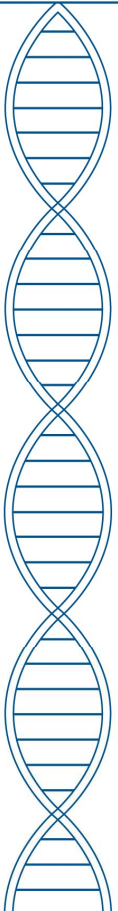
Formation of the transcription bubble for RNA synthesis



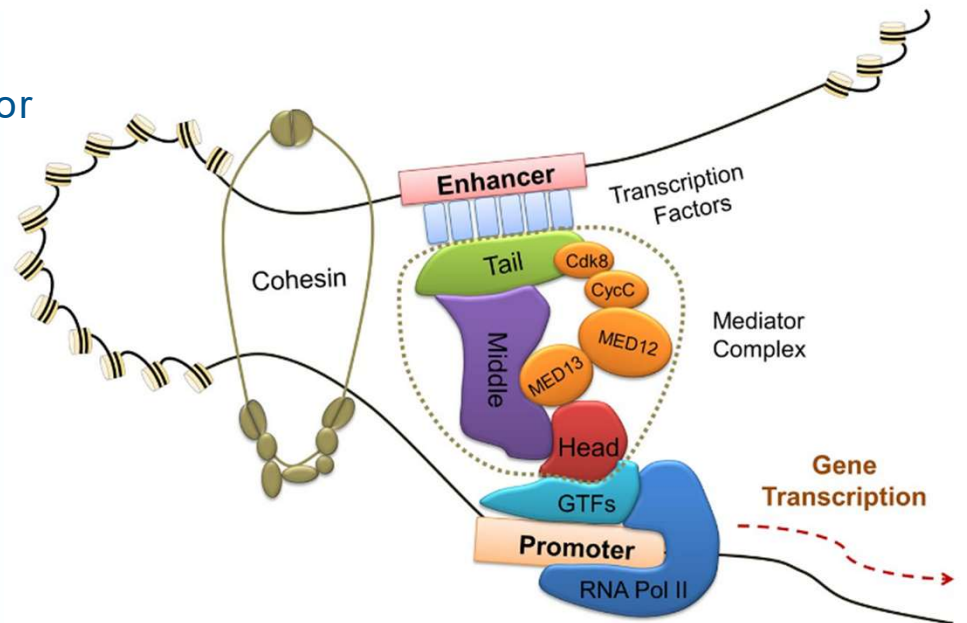
With the addition of ATP, DNA is unwound to expose the single-stranded template DNA, allowing Pol II to read the template and synthesize mRNA



Mediator Complex in transcriptional regulation

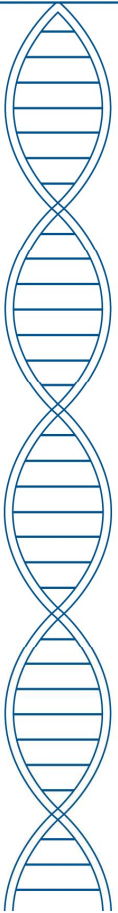


- Large multi-subunit coactivator complex for Pol II
- Bridges enhancer-bound transcription factors to Pol II machinery to promote the formation and stabilization of the PIC by enhancer–promoter looping
- Promotes transcription initiation and re-initiation
- Interacts with TFIID and influences Pol II CTD phosphorylation





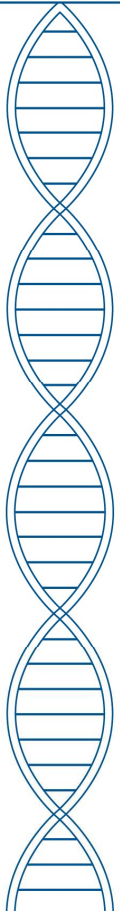
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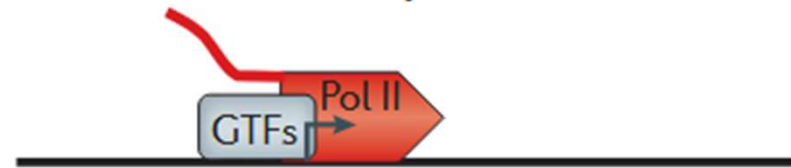


Promoter-proximal pausing of Pol II

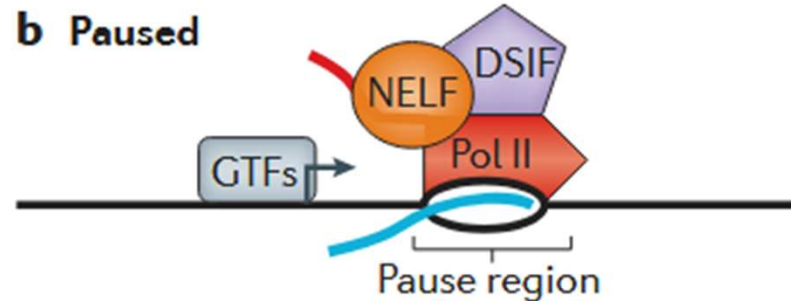


- Pol II pauses ~20–60 nt downstream of the TSS
- Occurs after initiation but before productive elongation
- Stabilized by:
 - DSIF** - positive elongation factor
 - NELF** – Negative elongation factor (causes dissociation)
- Keeps genes primed for rapid activation
- Adds a major regulatory checkpoint

a Pre-initiation complex

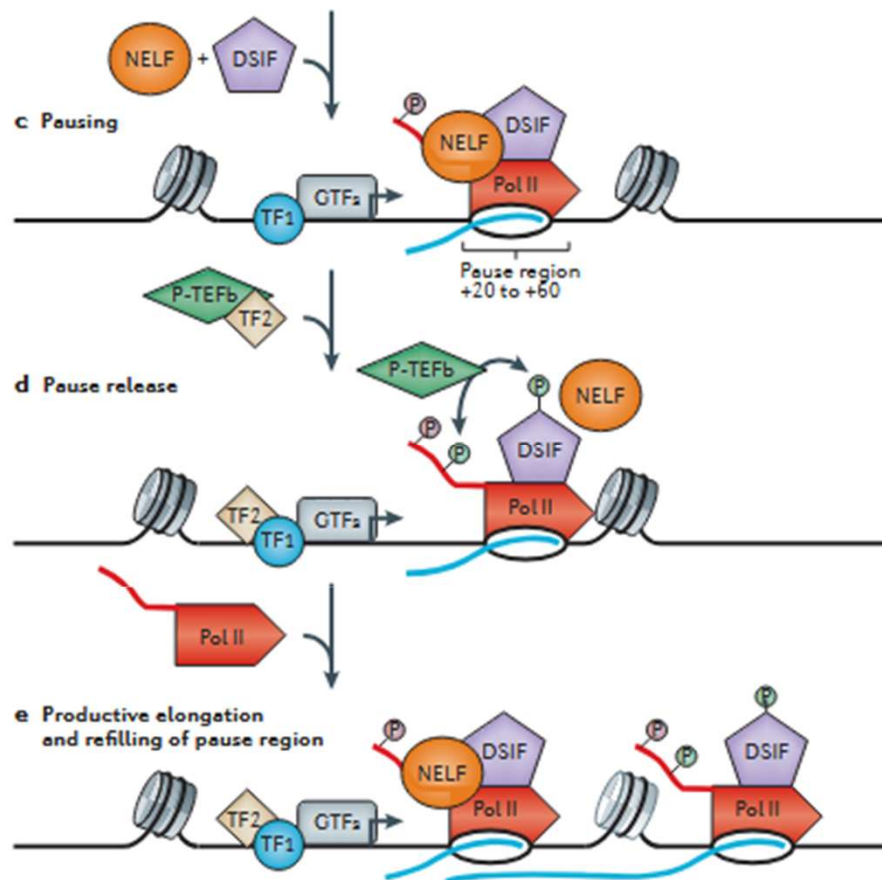
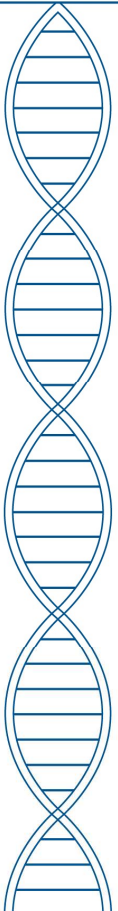


b Paused





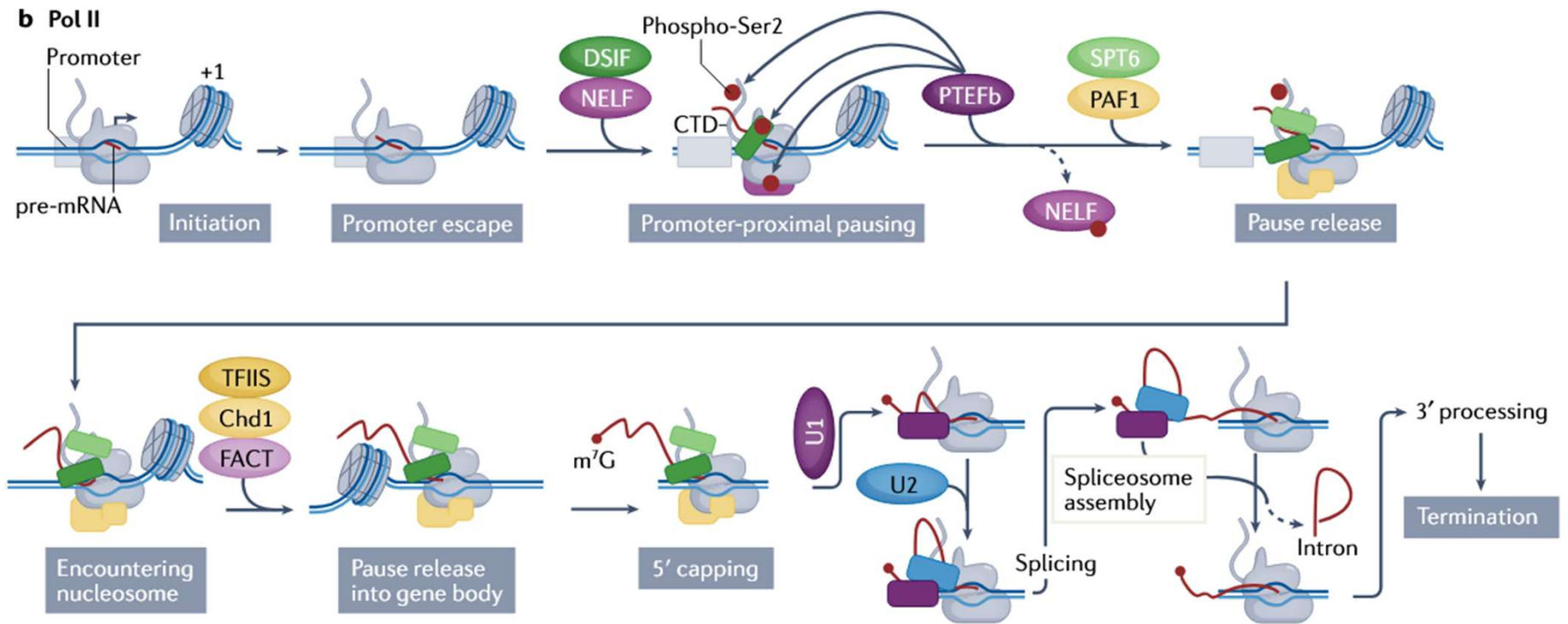
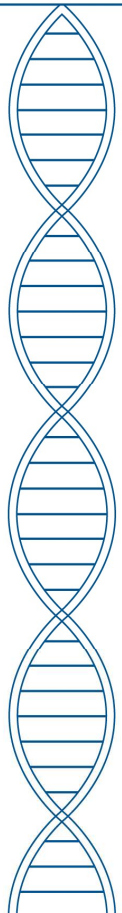
Promoter-proximal pausing and release of Pol II for transcription elongation



- Pause release is triggered by the recruitment of the **P-TEFb kinase**
- P-TEFb phosphorylates the DSIF–NELF complex to release paused Pol II, and also targets the Pol II CTD (Ser2)
- DSIF switches to positive elongation factor and NELF dissociates

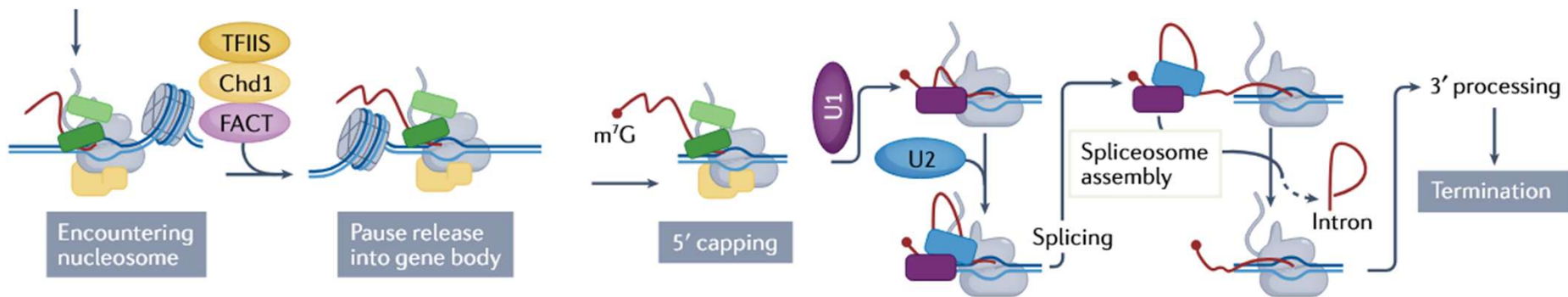
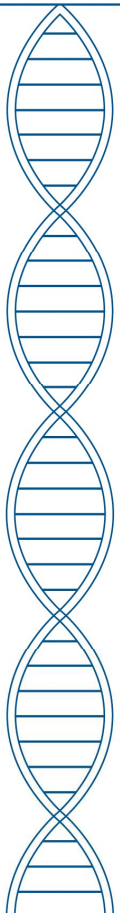


Transcription pause release into elongation





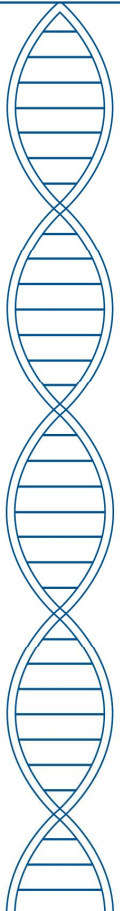
Transcription elongation



- Accessory factors TFIIIS, Chd1, and FACT for nucleosome passage
- RNA is processing (5' capping and splicing) happens co-transcriptionally
 - U1 snRNP binds the Pol II elongation complex
 - U2 snRNP promotes release of nascent RNA from Pol II for spliceosome assembly



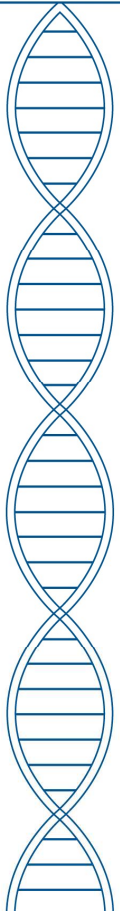
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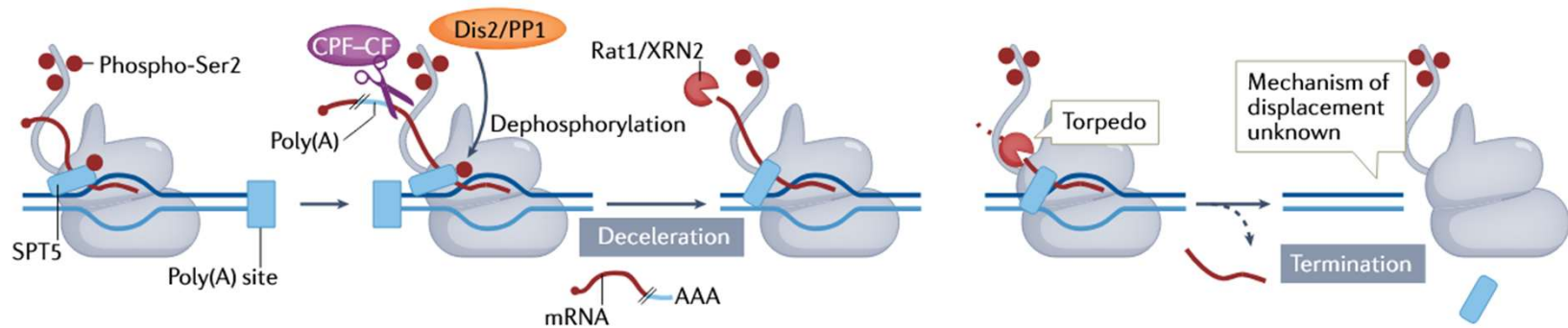
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Transcription termination at protein coding genes



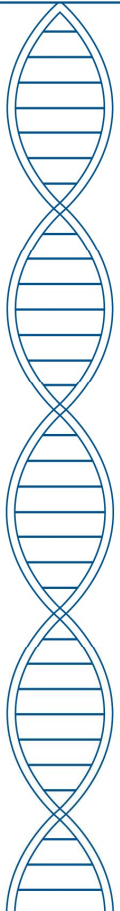
Protein coding genes



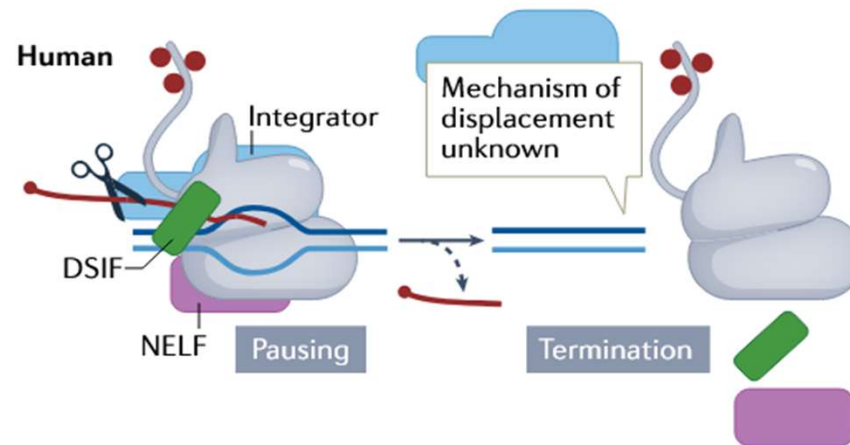
- CPF–CF complex recognizes Ser2-phosphorylated Pol II CTD and cleaves mRNA at the poly(A) site
- Dis2/PP1 dephosphorylates Pol II-bound SPT5(DSIF), slowing Pol II
- ~200–250 adenine residues (poly-A tail) added to 3' end
- RNA cleavage creates an entry site for Rat1/XRN2
- Rat1/XRN2 degrades downstream RNA and catches Pol II, triggering termination and Pol II release



Transcription termination at non-coding genes in Humans



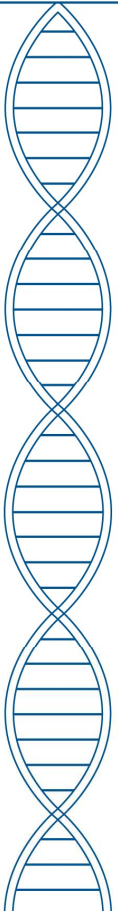
Non-coding genes



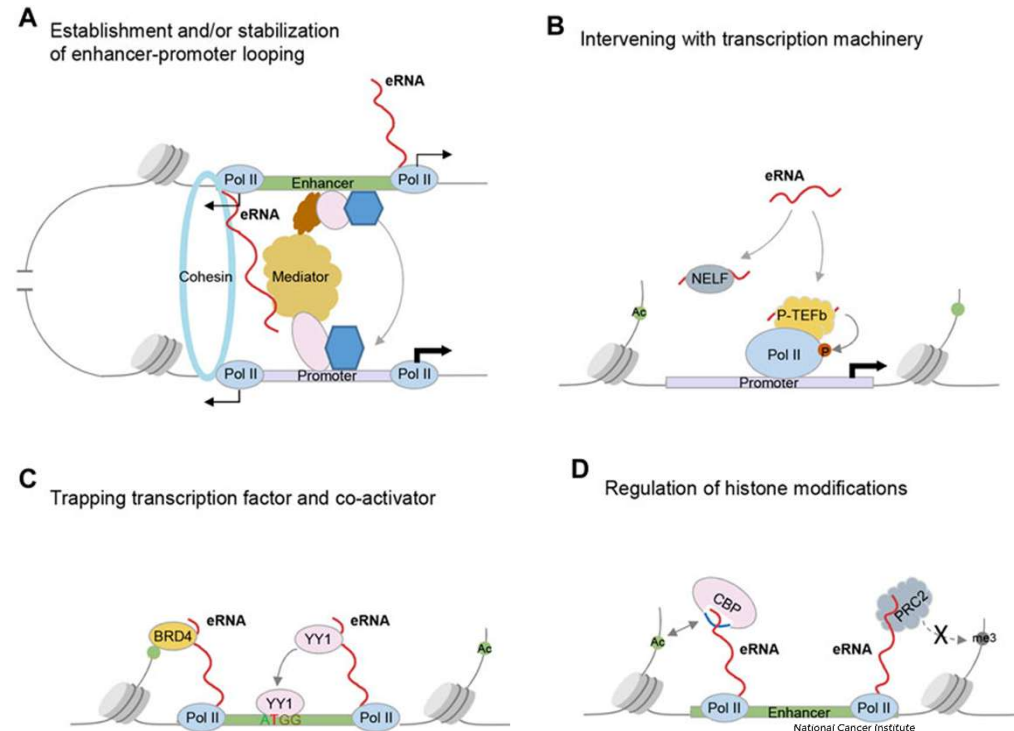
Non-coding RNA gene transcription termination requires association of the Integrator complex with paused Pol II, DSIF, and NELF, followed by RNA cleavage by Integrator



Enhancer RNA (eRNA) Transcription



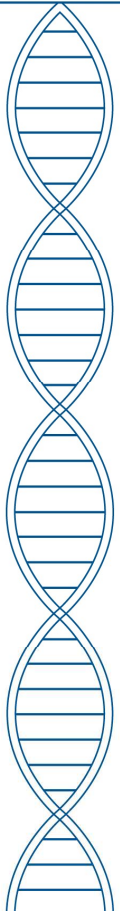
- Enhancers are actively transcribed by RNA polymerase II
- Short, non-coding RNAs produced
- Often unstable and non-polyadenylated
- Bidirectional transcription
- Initiation involves TFIID, Mediator, and Pol II
- Integrator complex mediates cleavage and termination of eRNAs
- eRNA transcription correlates with active enhancers



Chen et al., Front in Cell and Dev Bio, 2023



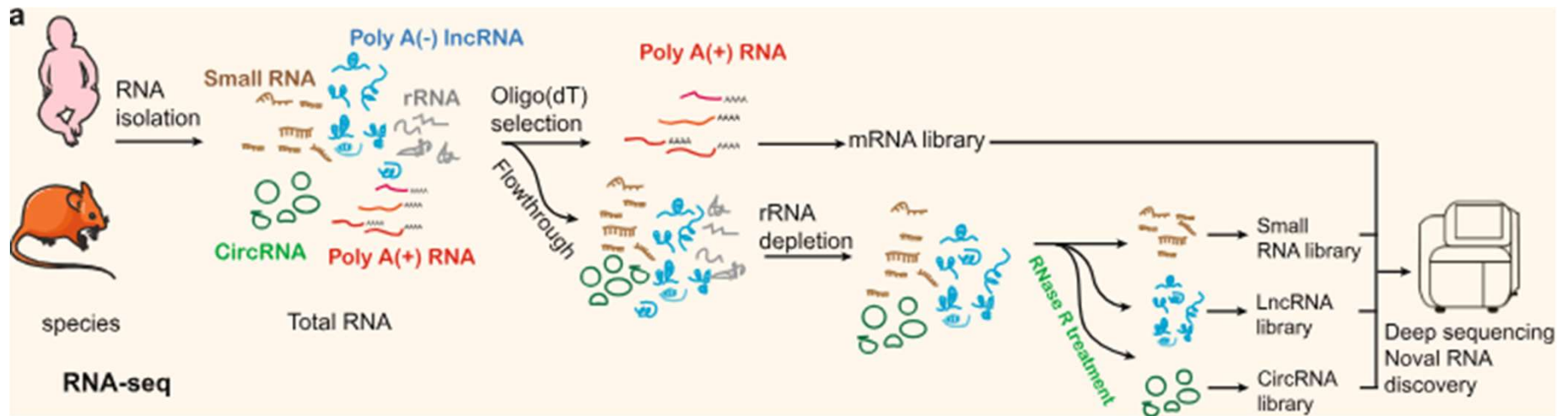
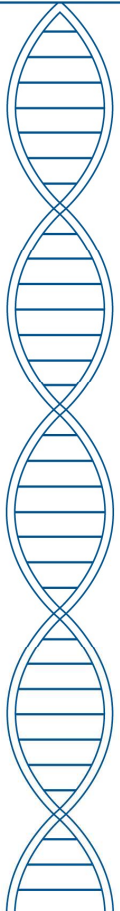
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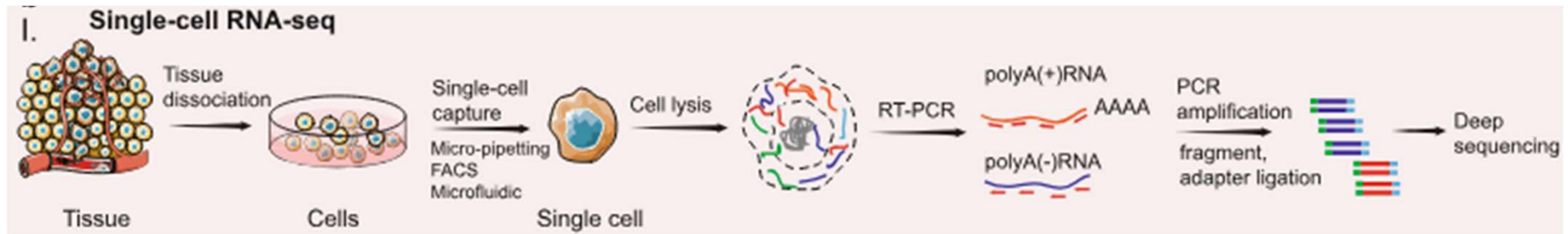
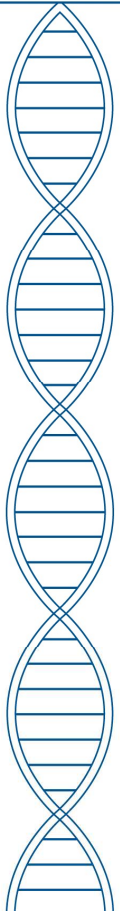


Measuring transcribed genes by RNA sequencing (RNA-seq)



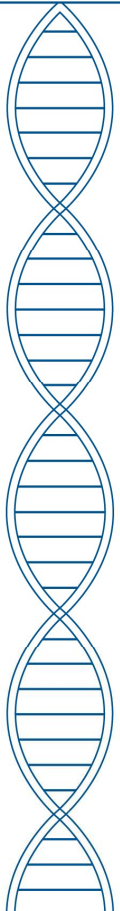


Measuring transcribed genes by RNA sequencing (RNA-seq)





Short read sequencing



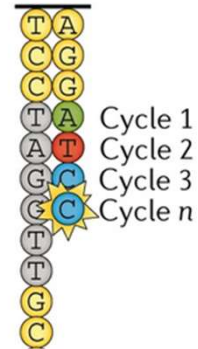
- Most common and affordable platform for RNA-Seq
- Read length: 50–300 bp (paired-end or single-end)
- Strengths: High accuracy, throughput, cost-effective

Limitation: Cannot resolve full-length isoforms easily



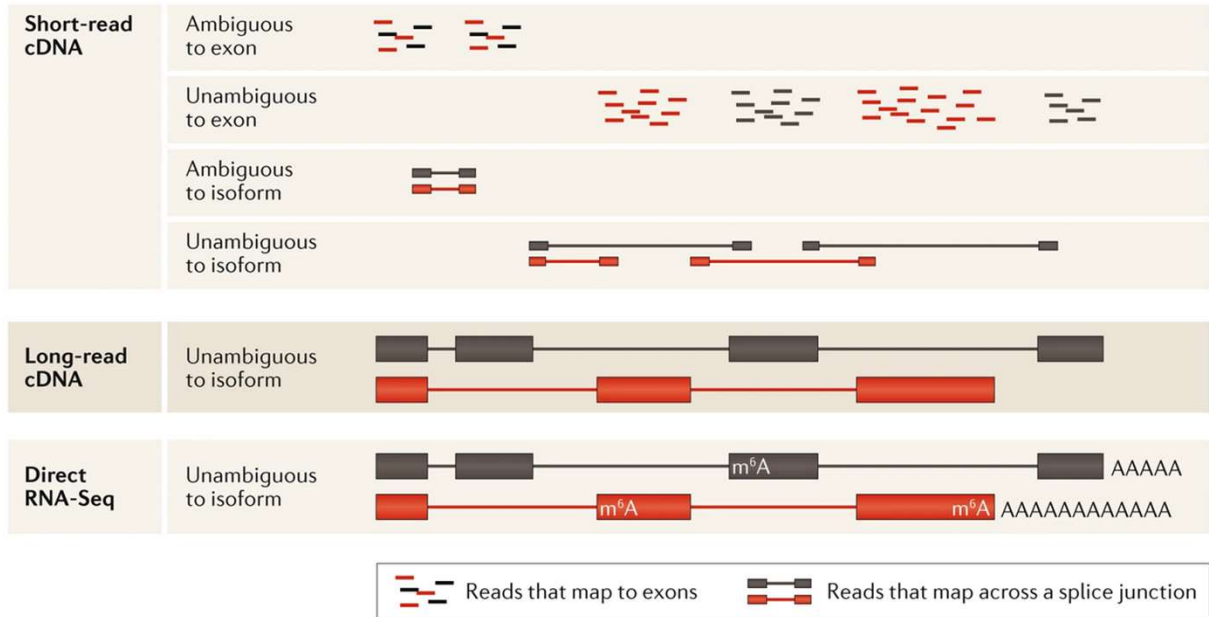
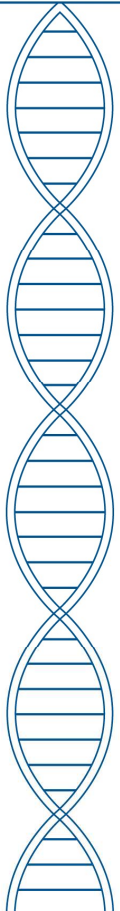
Illumina

Flowcell





Long read sequencing



- **Long read cDNA(Nanopore, PacBio)**

Full-length transcript sequencing (no assembly needed)

Strength: Isoform diversity, alternative splicing

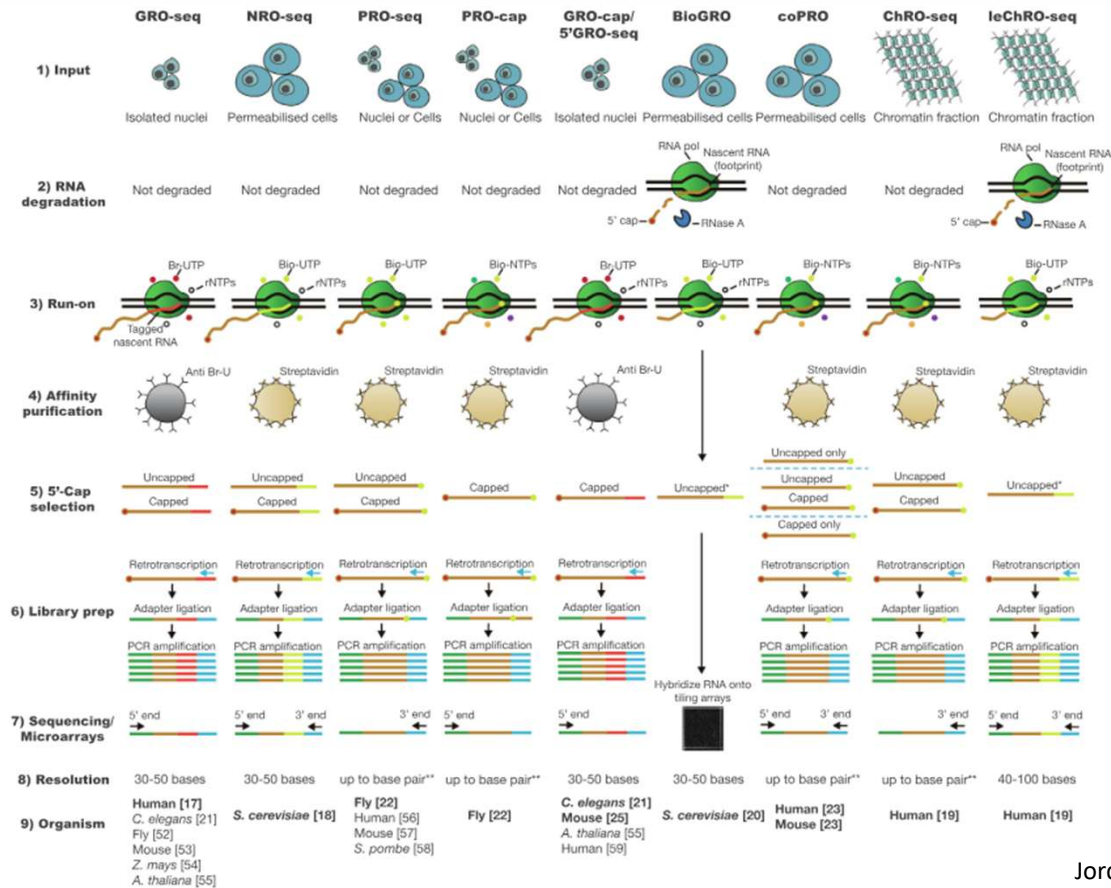
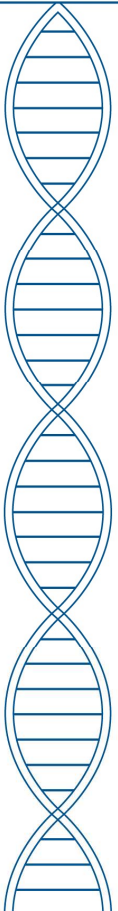
- **Long read Direct RNA(Nanopore)**

Direct RNA sequencing (no reverse transcription)

Detects modifications (e.g., m6A) in native RNA

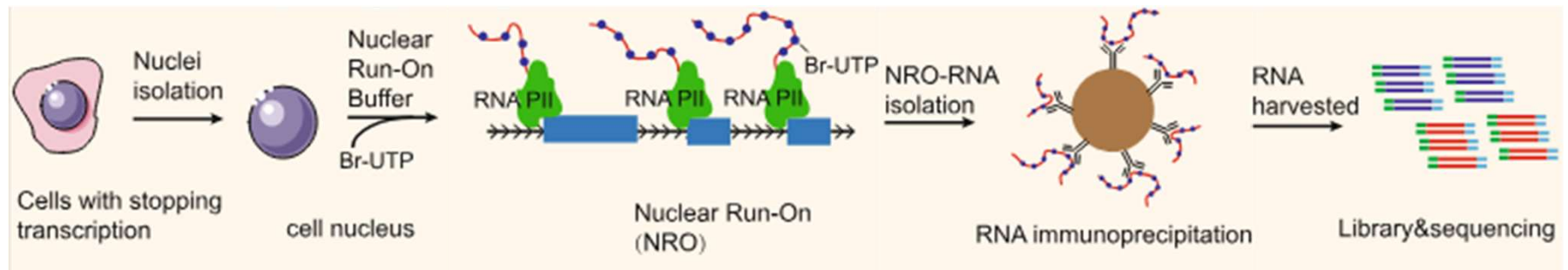
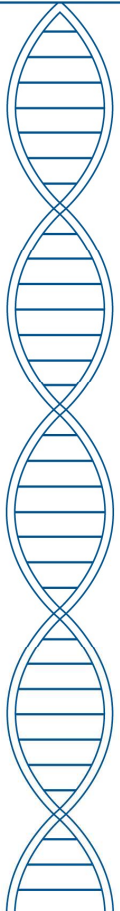


Run-on sequencing assays for analyzing transcription





Global Run-On sequencing (GRO-seq)



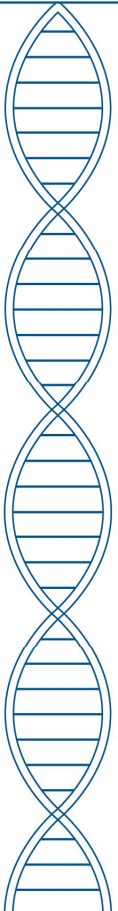
- Based on a nuclear run-on assay with labeled nucleotides
- Measures nascent transcription by capturing RNA made by engaged RNA polymerases
- Detects active transcription, not steady-state RNA levels

Identify and measure:

- Promoter-proximal pausing
- Elongation rates
- Bidirectional transcription of eRNAs



Summary



- **Key steps**

- Initiation

- Pol II recruited to DNA at gene promoters by transcription factors

- Elongation

- Pol II move DNA template strand producing an RNA molecule. Coupled with 5' capping and splicing

- Termination

- Polymerase encounters termination signals, cleaves, and releases the RNA transcript

- Regulation of gene expression can occur at initiation, elongation (e.g., Pol II pausing), or termination.

- Controlled by:

- Transcription factors
 - Regulatory regions of DNA promoters, enhancers, repressors
 - Chromatin state (histone modifications, DNA methylation)