

## Elongation and Termination of Transcription

### Elongation phase of transcription

- Requires the release of RNA polymerase from the initiation complex
- Highly processive
- Dissociation of factors needed specifically at initiation.
  - Bacterial dissociates from the holoenzyme
  - Eukaryotic TFIID and TFIIA appear to stay behind at the promoter after polymerase and other factors leave the initiation complex

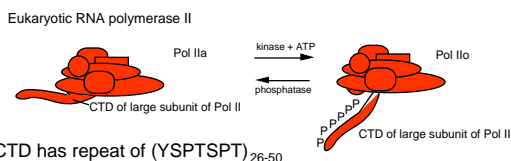
### Proteins implicated in elongation

- P-TEFb
  - Positive transcription elongation factor b
  - Cyclin-dependent kinase
  - Phosphorylates CTD of large subunit, Pol II
- *E. coli* GreA and GreB, eukaryotic TFIIS
  - may overcome pausing by the polymerase
  - induce cleavage of the new transcript, followed by release of the 3' terminal RNA fragment.
- *E. coli* NusG, yeast Spt5, human DSIF
  - Regulated elongation (negative and positive), direct contact with polymerase and nascent transcript
- ELL: increase elongation rate of RNA Pol II
- CSB: Cockayne syndrome B protein, incr. elongation rate

### Phosphorylation of CTD of RNA Pol II is associated with the switch to elongation

- The C-terminal tail of the large subunit of RNA polymerase II has repeats of YSPTSPT that are substrates for protein kinases
- The unphosphorylated form (RNA Pol IIa) is found in paused complexes, while the phosphorylated form (RNA Pol IIo) is in elongating complexes
- Kinases implicated in catalyzing this step :
  - TFIIF
  - P-TEFb

### Model for RNA Polymerase II Phosphorylation

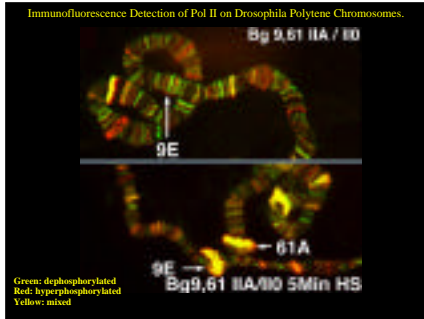


**Model:** Phosphorylation of Pol IIa to make Pol IIo is needed to release the polymerase from the initiation complex and allow it to start elongation.

### The shift from initiation to elongation can be a regulated event.

- **Release from pausing** can be the mechanism for induction of expression.
  - In *Drosophila*, the RNA polymerase can **pause** after synthesizing ~ 25 nucleotides of RNA in many genes.
  - under elevated temperature conditions, the **heat shock** factor stimulates **elongation** by release from pausing.
  - Other possible examples: mammalian *c-myc*, HIV LTR
- This is in addition to regulation at initiation.

Phosphorylated form of RNA Pol II is at sites of elongation after heat shock



Regulation of HIV transcription at elongation

- The human immunodeficiency virus, HIV, is the presumptive cause of AIDS.
- It has an enhancer and a promoter in its long terminal repeat, or LTR.
- RNA polymerase II pauses at about +70 (within the LTR).
- The virally encoded protein Tat is needed to allow elongation past +70.
- Tat binds to an RNA structure centered at about +60, called *tar*.

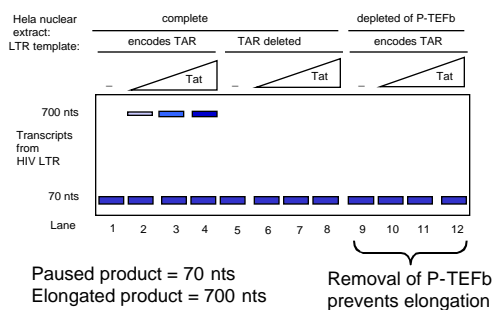
Mechanism of regulation at elongation for HIV: TFIIH

- Elongation requires the CTD of RNA Pol II
- Tat leads to phosphorylation of RNA Pol II CTD
- The kinase in the CDK7 subunit of TFIIH can be used to phosphorylate the CTD of RNA Pol II
- An inhibitor of CDK7 will block Tat-dependent elongation by RNA Pol II

Mechanism of regulation at elongation for HIV: P-TEFb

- Further phosphorylation of CTD of RNA Pol II is catalyzed by the elongation factor P-TEFb, a cellular enzyme.
- The kinase subunit of P-TEFb is CDK9.
- P-TEFb is needed for elongation past *tar* in an *in vitro* assay.
- >100,000 compounds were screened for the ability to block Tat-stimulated transcription of HIV, and all the positive compounds were found to inhibit P-TEFb.

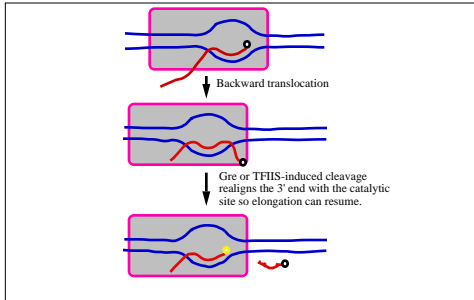
An *in vitro* transcription elongation assay shows that both P-TEFb and Tat are needed for elongation



HIV regulation via elongation : Summary

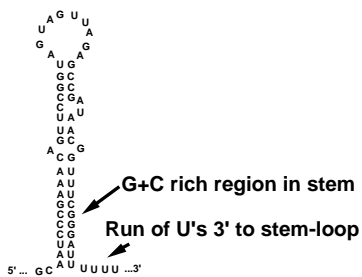
- Tat-dependent activation works through both kinases to phosphorylate the Pol II CTD.
  - TFIIH - perhaps for promoter clearance
  - T-TEFb - for full elongation
- HIV LTR is **also** regulated at **initiation** by a large number of transcription factors that bind upstream of the core promoter, all within the LTR.

### Elongation factor-dependent realignment of the 3' RNA end



### Termination of transcription

### Termination of transcription in *E. coli*: Rho-independent site



### Termination of transcription in *E. coli*: Rho-dependent site

5' ...AUCGCUACCUCUAUAUCCGCACCUCCUCAAAACGCUACCUCGACCAGAAAGGGCUCUCUU

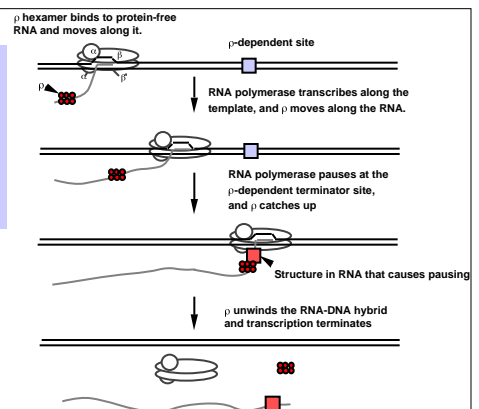
Termination occurs at one of these 3 nucleotides.

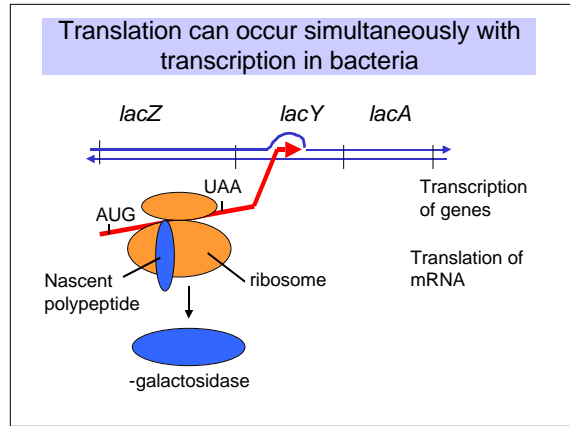
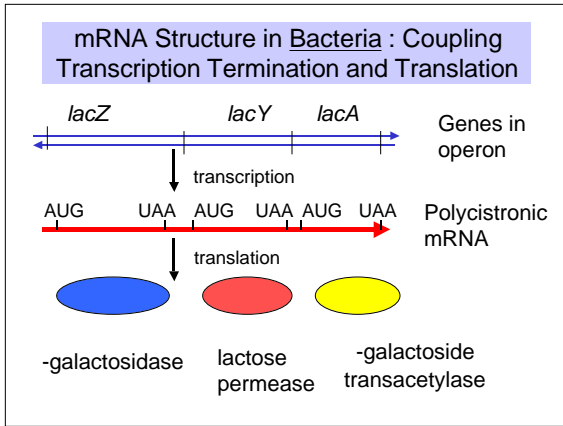
- Little sequence specificity: rich in C, poor in G.
- Requires action of rho ( ) *in vitro* and *in vivo*.
- Many (most?) genes in *E. coli* have rho-dependent terminators.

### Rho factor, or

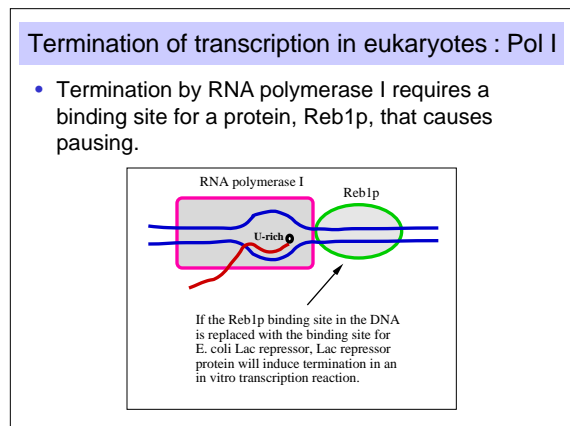
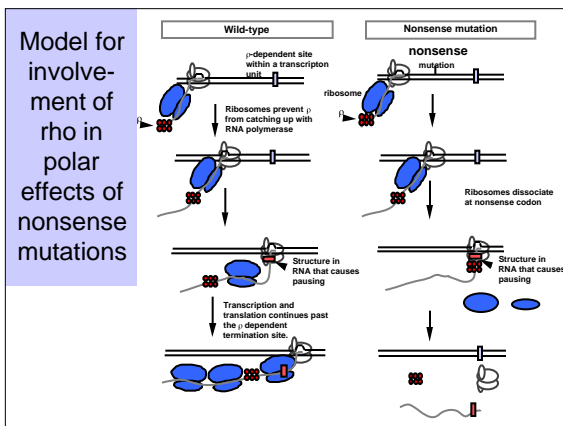
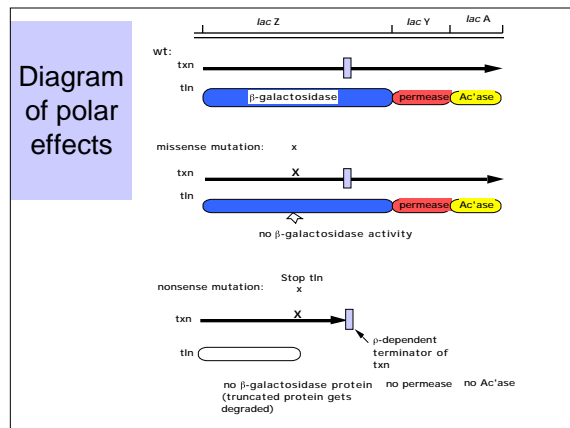
- Rho is a hexamer, subunit size is 46 kDa
- Is an RNA-dependent ATPase
- Is an essential gene in *E. coli*
- Rho binds to **protein-free RNA** and moves along it (tracks)
- Upon reaching a paused RNA polymerase, it causes the polymerase to dissociate and unwinds the RNA-DNA duplex, using ATP hydrolysis. This terminates transcription.

### Model for action of rho factor





- ### Polarity
- Polar mutations occur in a gene early in an operon, but affect expression of both that gene and genes that follow in the operon.
  - Usually affect **translation** at the beginning of an operon, and exert a negative effect on the **transcription** of genes later in the operon.
    - Usually are nonsense (**translation termination**) mutations in a 5' gene that cause **termination** of **transcription** of subsequent genes in the operon.
  - Rho mutants can suppress polarity.



### Termination of transcription in eukaryotes : Pol II and Pol III

- RNA polymerase III terminates in a run of 4-5 T's on the nontemplate strand, surrounded by G+C-rich DNA.
- No clear evidence for a discrete terminator of transcription by RNA polymerase II.
- The 3' end of the mRNA is made by cleavage and polyadenylation.

### Eukaryotic mRNA structure

