

# **Modification and processing of eukaryotic pre-mRNAs**

## **3' End Processing-Polyadenylation**

# 3' Ends of Primary Transcripts are Processed by Cleavage and Polyadenylation

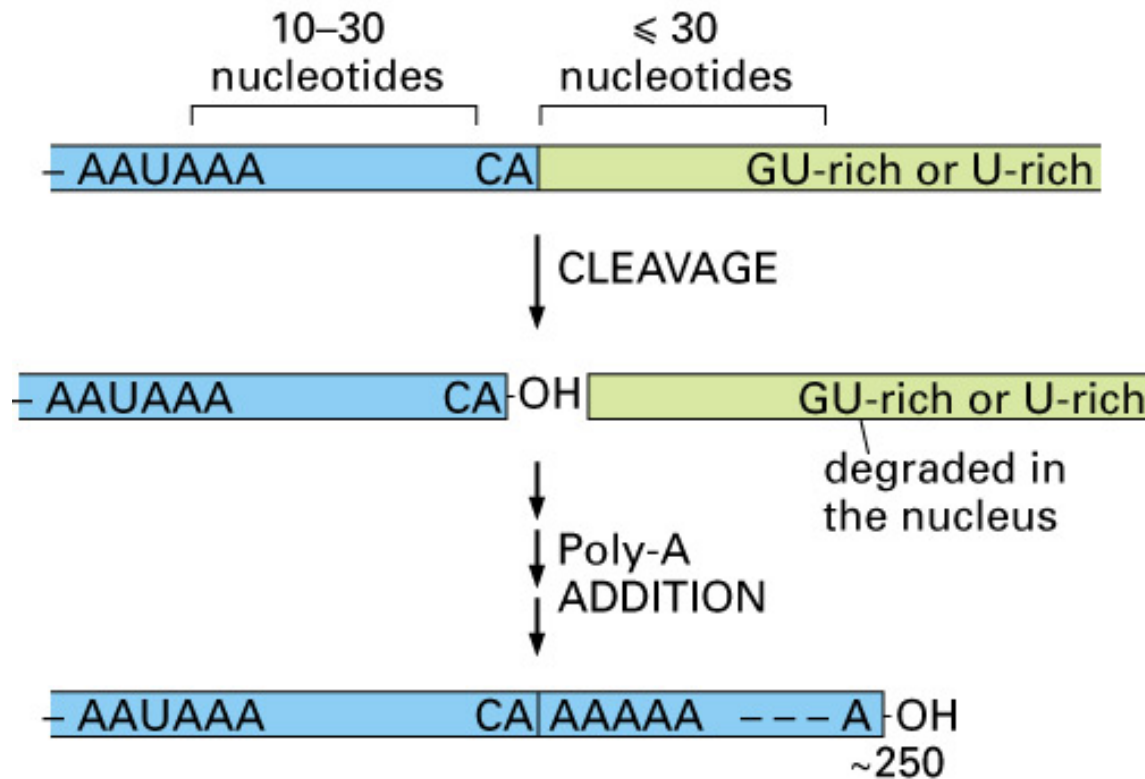


Figure 6-37. Molecular Biology of the Cell, 4th Edition.

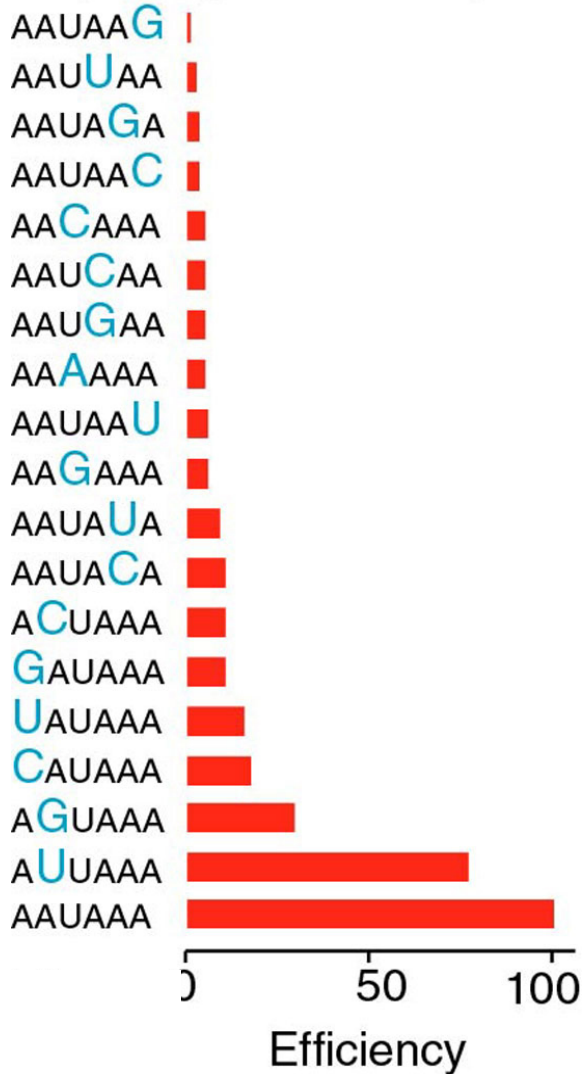
# AAUAAA Signals Polyadenylation

- **AAUAAA sequence is highly conserved, found 20-30 bp before polyadenylation site**
- **Deletions between AAUAAA and normal 3' end shift position of poly(A) tail**
- **Deleting AAUAAA eliminates polyadenylation**
- **Mutations in AAUAAA sequence reduce polyadenylation**

# Consensus



## Polyadenylation activity



# Variation From AAUAAA Reduces Polyadenylation Efficiency

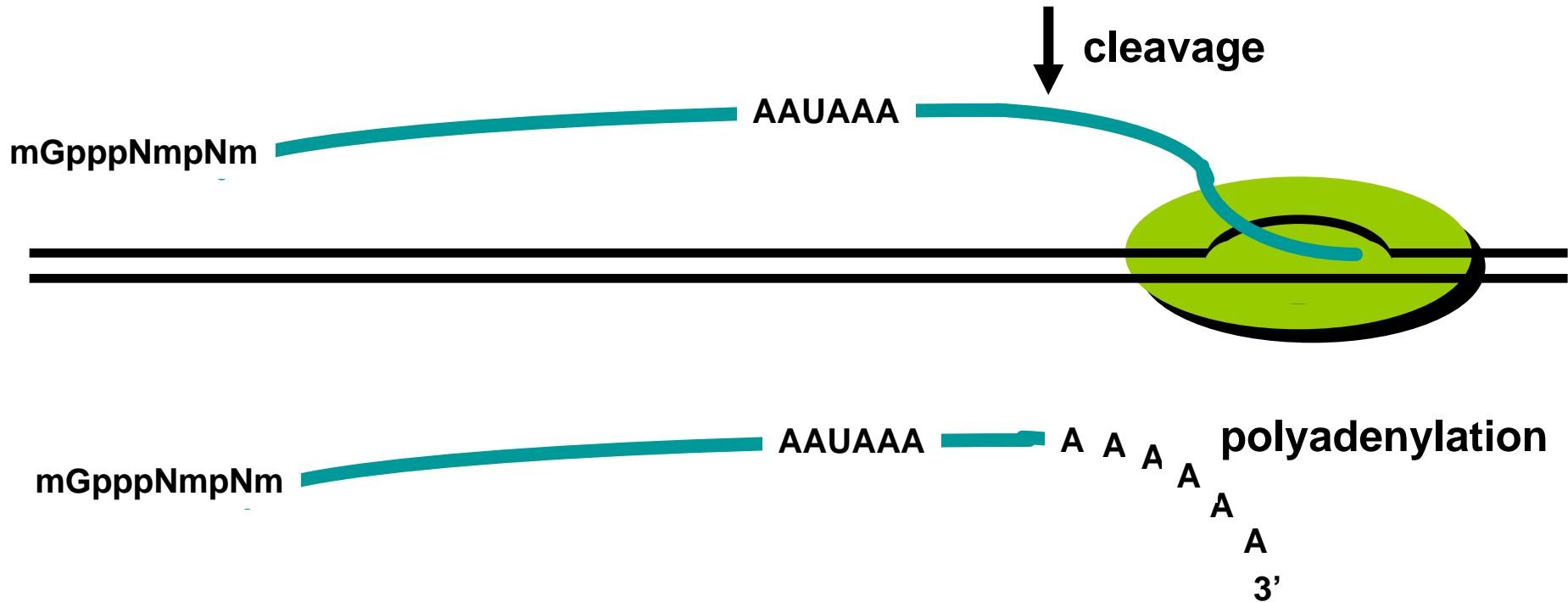
# **AAUAAA Necessary But Not Sufficient**

- **AAUAAA not always used – occurs in introns**
- **AAUAAA followed 15 bp later (downstream) by GU or U rich signal**
  - **Test by making deletions at 3' end of gene**
  - **Deleting either GU or U rich signal impairs activity**
  - **Position of GU/U region relative to AAUAAA important**
  - **Spacing between GU and U rich regions important**

# Polyadenylation

cleavage of the primary transcript occurs approximately 10-30 nucleotides 3'-ward of the AAUAAA consensus site

- polyadenylation catalyzed by **poly(A) polymerase**
- approximately 200 adenylate residues are added



- poly(A) is associated with poly(A) binding protein (PBP)

# Polyadenylation Occurs in Two Phases

- Initiation
  - Depends on AAUAAA signal
  - Slow addition of at least 10 As to 3' end of mRNA
- Elongation
  - Independent of AAUAAA
  - Depends on oligoA added during initiation
  - Rapid addition of 200 or more As to 3' end by poly(A) polymerase
  - Requires additional specificity factor: poly(A)-binding protein II (PAB II; one RRM), which enhances processivity of poly(A) polymerase

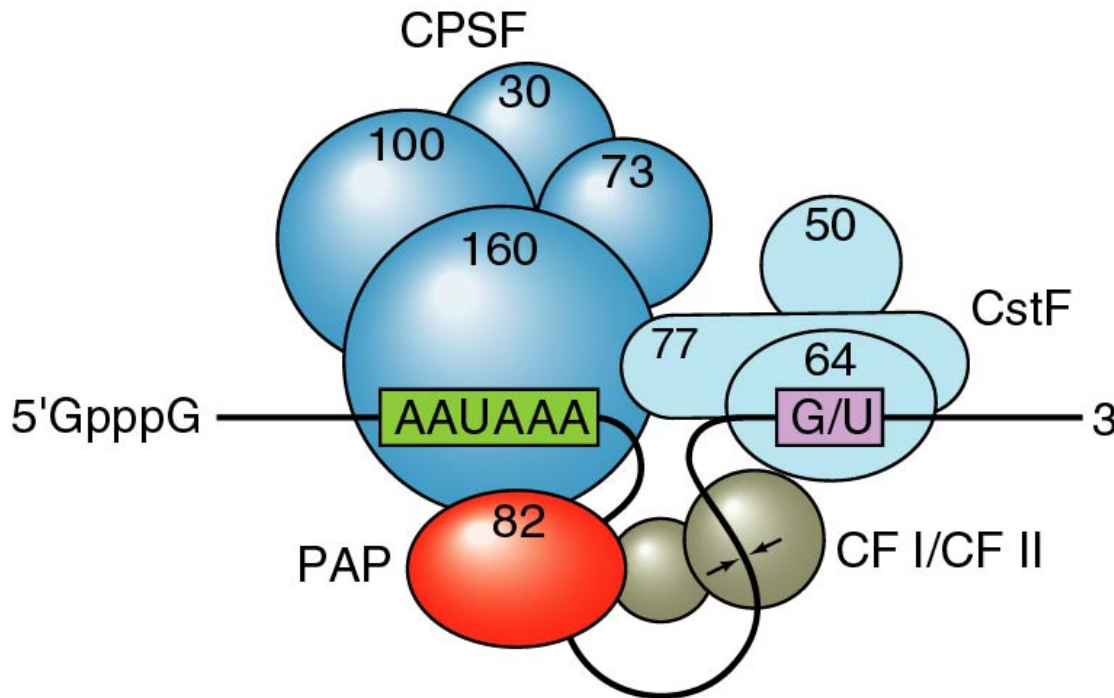
**CPSF/AAUAAA:** schwacher Komplex, wird durch PAP stabilisiert.

**PAP/CPSF/PAB II:** macht Polyadenylierung prozessiv, ca. 200 A werden angehängt.

**CPSF:** bindet an RNA-Pol II, Kopplung von Transkription und CTD, 3'Processing.



# Pre-Cleavage Complex



## Formation of a mature mRNA: polyadenylation

- Poly A signals:
- **AAUAAA: CPSF- cleavage & polyadenylation specificity factor**
- Downstream sequence element
- **GU/ U rich: CstF- cleavage stimulatory factor**
- Cleavage site
- **CA: Cleavage factors I and II and poly A polymerase (PAP)**
- Termination of RNA Polymerase transcription is linked to poly A addition.
- Transcription extends beyond -signal.
- Complex proteins assemble to coordinate cleavage and polyA addition.

# Major Steps in 3' End Formation for Primary Transcripts - I

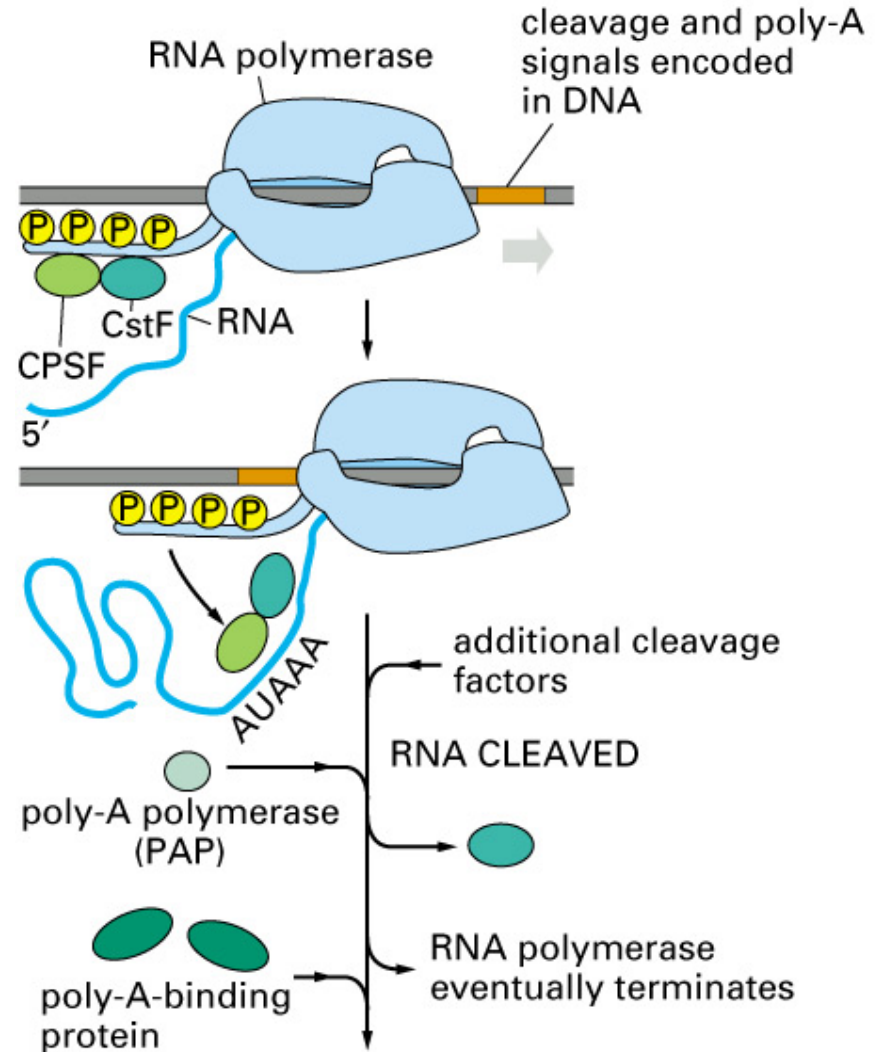
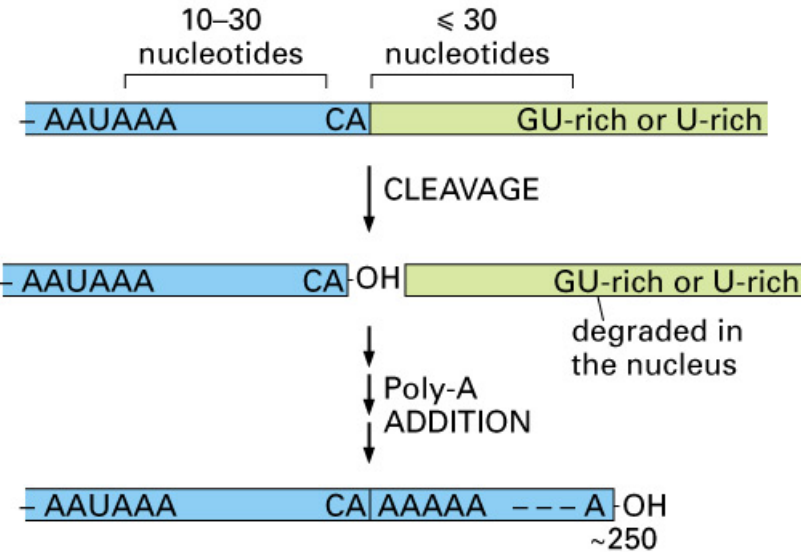


Figure 6-38 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

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# Major Steps in 3' End Formation for Primary Transcripts - II

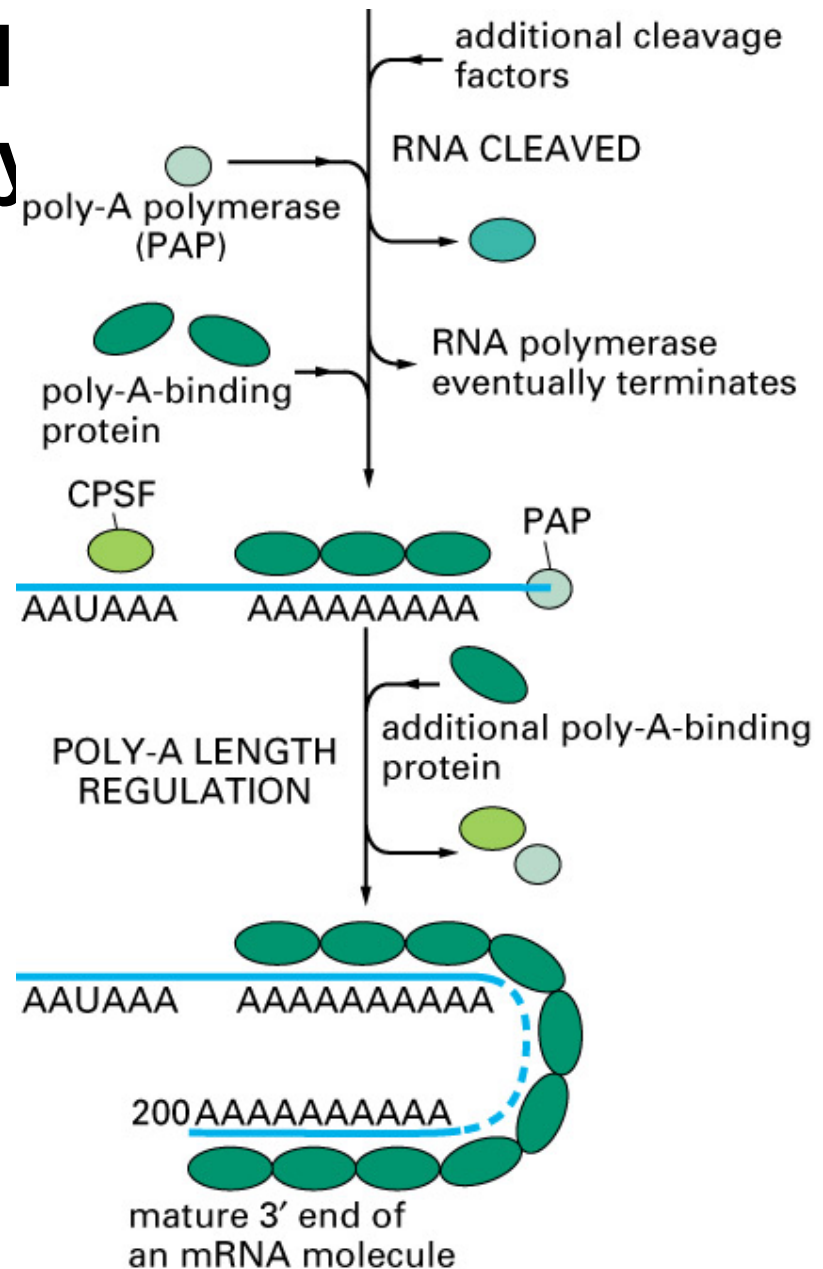


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# Functions of polyA tail

- **Protects RNA from degradation**
- **Required for mRNA export**
- **Stimulates translation of mRNAs**
- **Nature's gift to molecular biologists: facilitates separation of mRNA from total RNA**

## **Connection of mRNA 3'end formation and splicing**

Direct interaction of 3' splice site recognition factor U2AF<sup>65</sup> and poly(A) polymerase  
Definition of the last exon and splicing of the last intron

# Alternative Poly(A) Site Selection: Role in Antibody Production - I

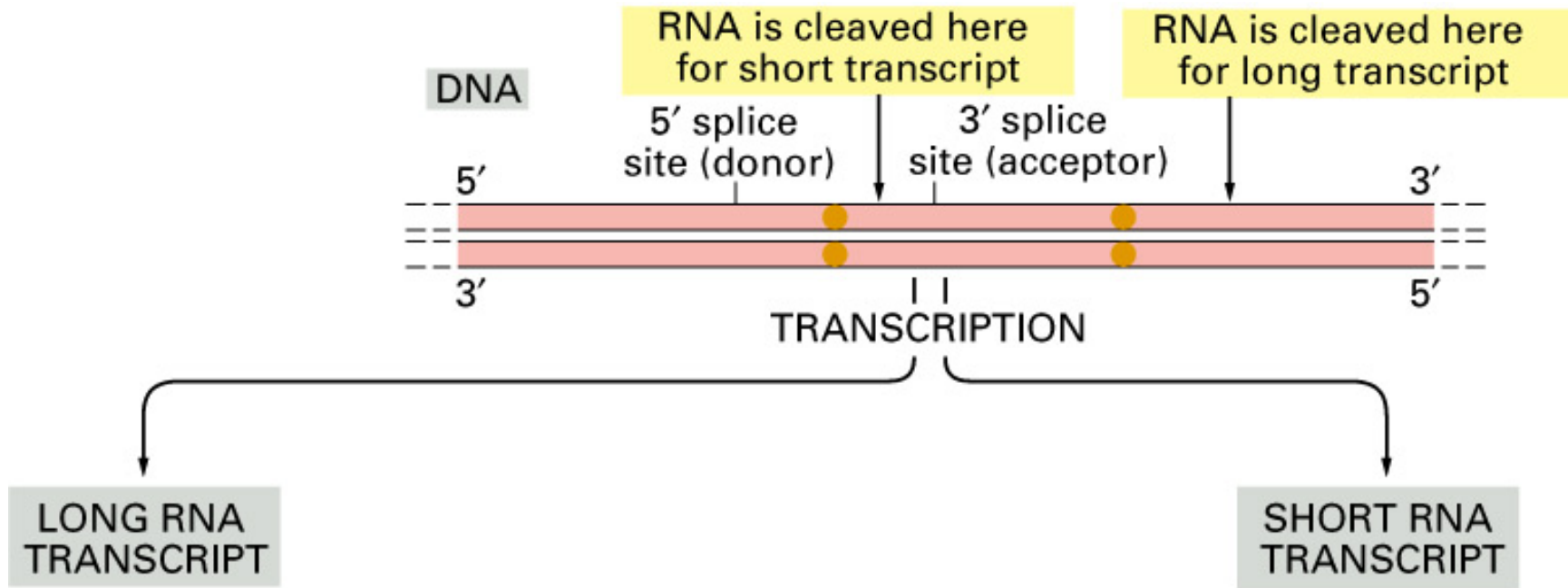


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# Alternative Poly(A) Site Selection: Role in Antibody Production - II

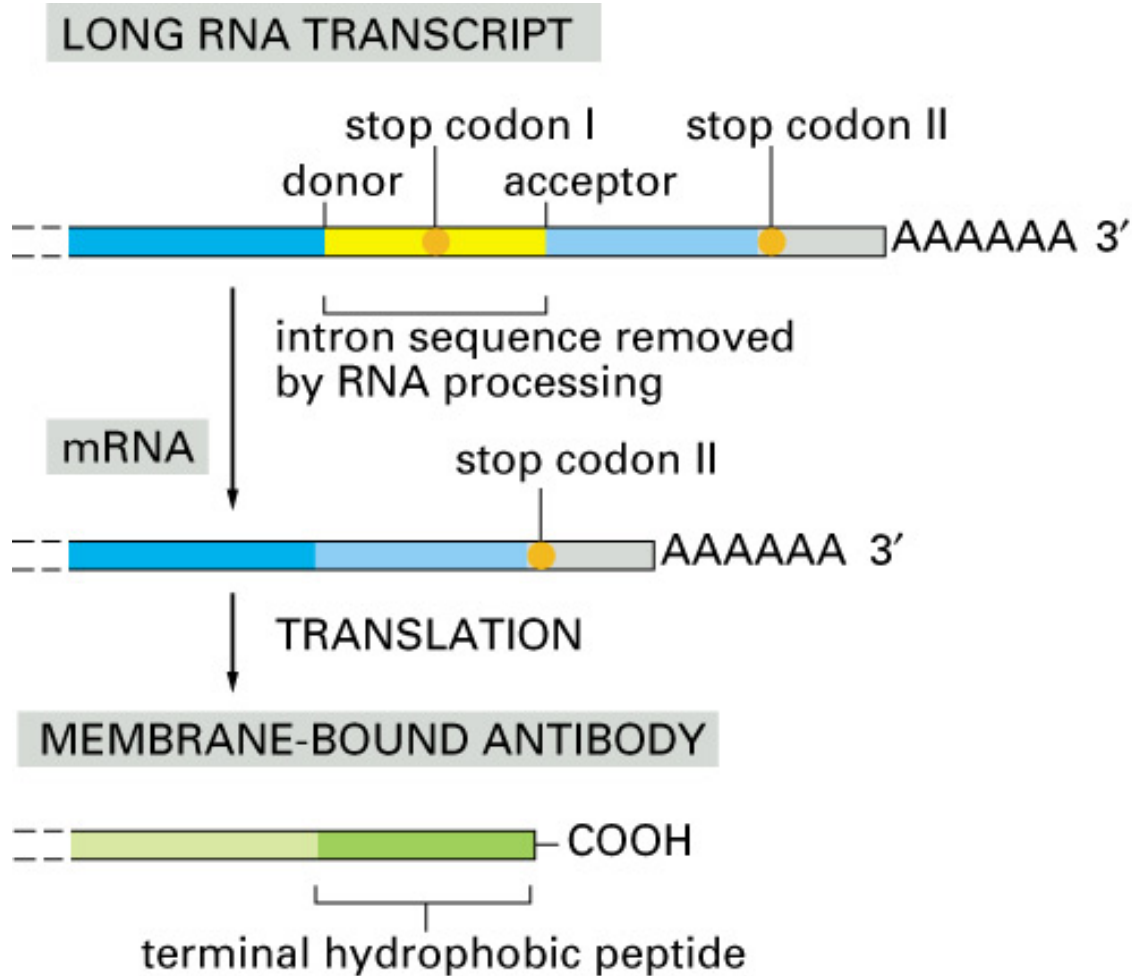


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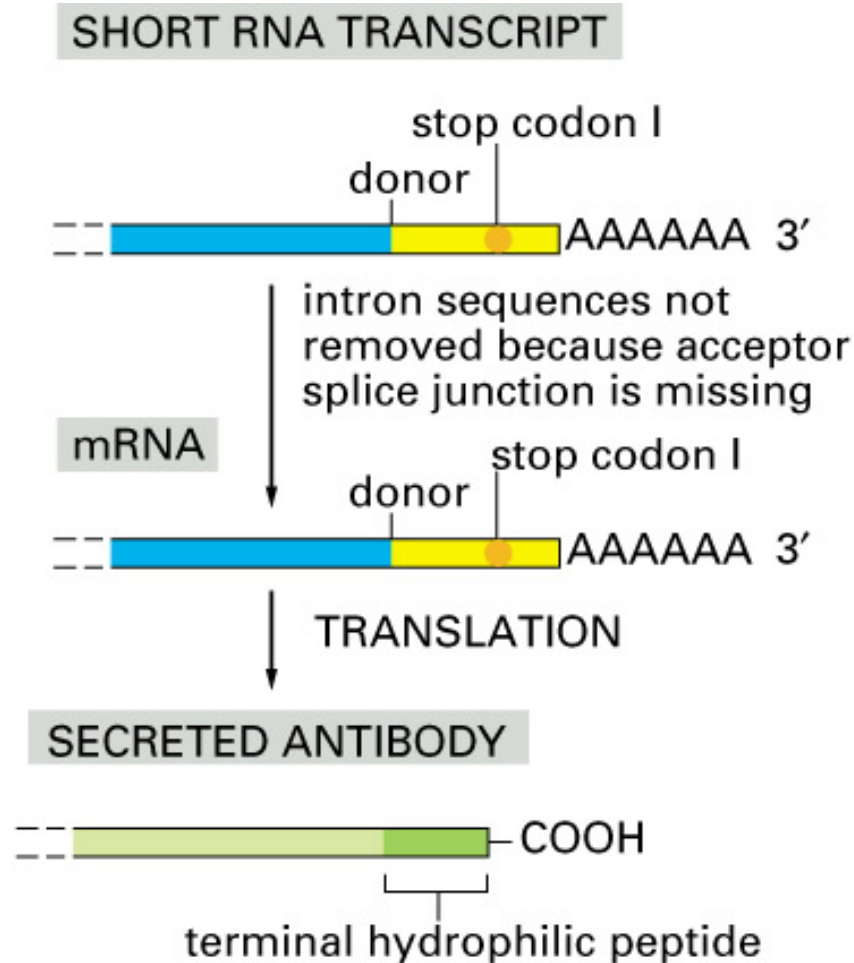


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