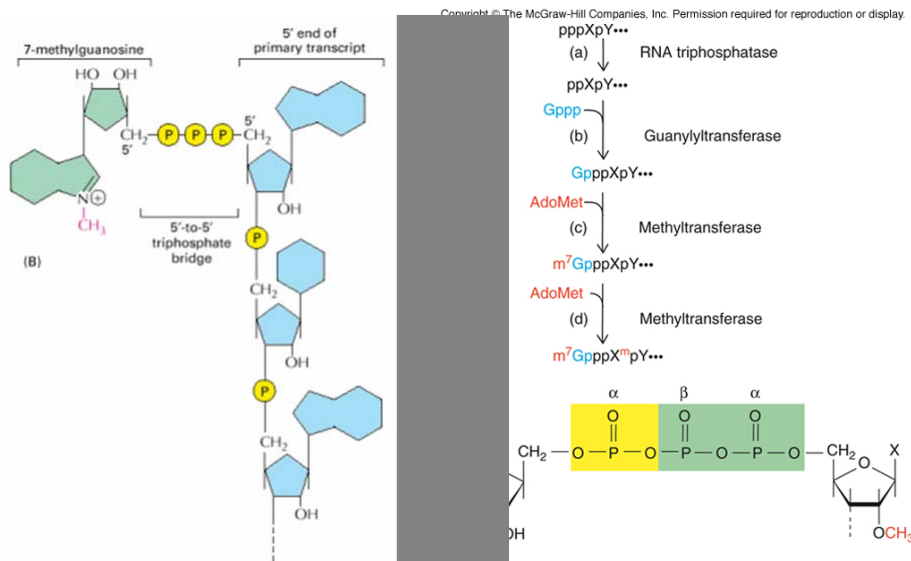


# mRNA Processing

- Capping
- Polyadenylation
- Termination

## Cap Structure



## Which RNAs are G-capped?

### G-Capped:

All known RNA pol-II transcripts, including:

- mRNAs
- Certain snRNAs (including U1, U2, U4, U5)

### Not G-capped:

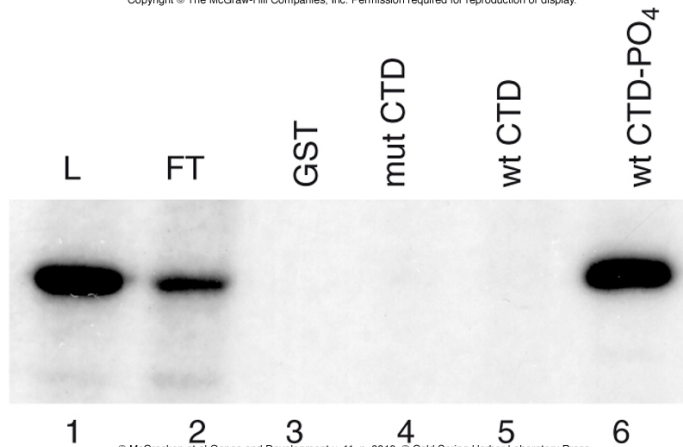
RNA pol-I and pol-III transcripts

## Capping complex binds CTD

Affinity Chromatography

Assay guanylyl transferase activity

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.



# Function of Cap

- Translational Initiation (>50X increase)
- Stability - Protection
- Export from Nucleus
- Splicing

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

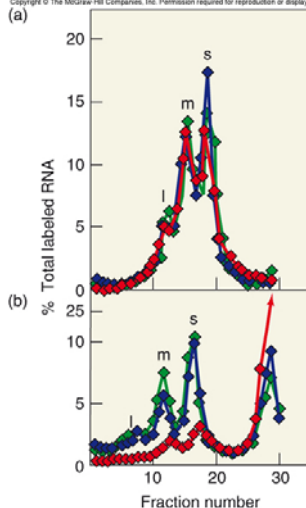
**Table 15.1 Synergism Between Poly(A) and Cap during Translation of Luciferase mRNA in Tobacco Protoplasts**

mRNA	Luciferase mRNA Half-Life (min)	Luciferase Activity (light units/mg protein)	Relative Effect of Poly(A) on Activity	Relative Effect of Cap on Activity
Uncapped				
Poly(A) <sup>-</sup>	31	2941	1	1
Poly(A) <sup>+</sup>	44	4480	1.5	1
Capped				
Poly(A) <sup>-</sup>	53	62,595	1	21
Poly(A) <sup>+</sup>	100	1,331,917	21	297

Source: Galile, D.R., The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency, *Genes & Development* 5:2108-2116, 1991. Copyright © Cold Spring Harbor, NY. Reprinted by permission.

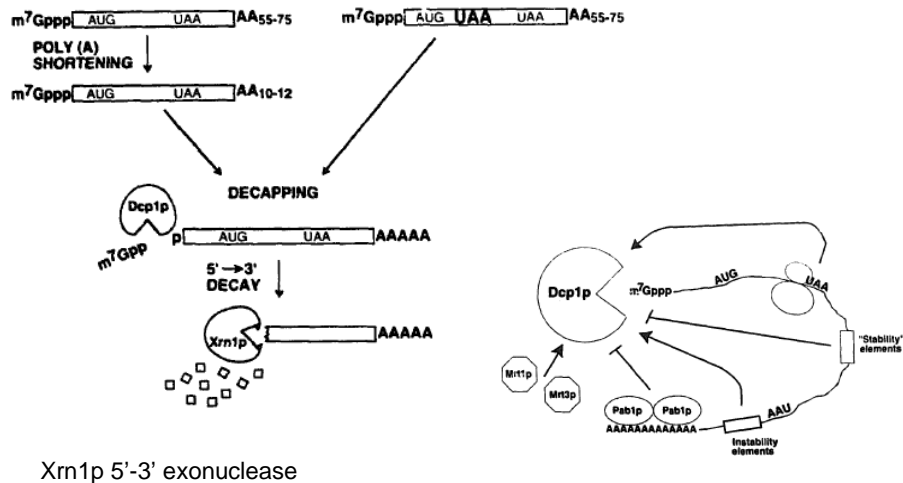
## Cap and Stability

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

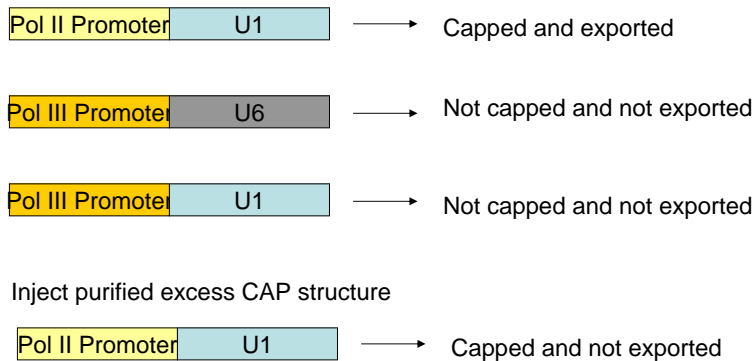


- Synthesize reovirus mRNA – three size classes
- Prepare one class
  - capped green
  - blocked blue
  - uncapped red
- Inject into oocyte
- Recover after 8 hours and analyze

# Regulated decapping by Dcp1p

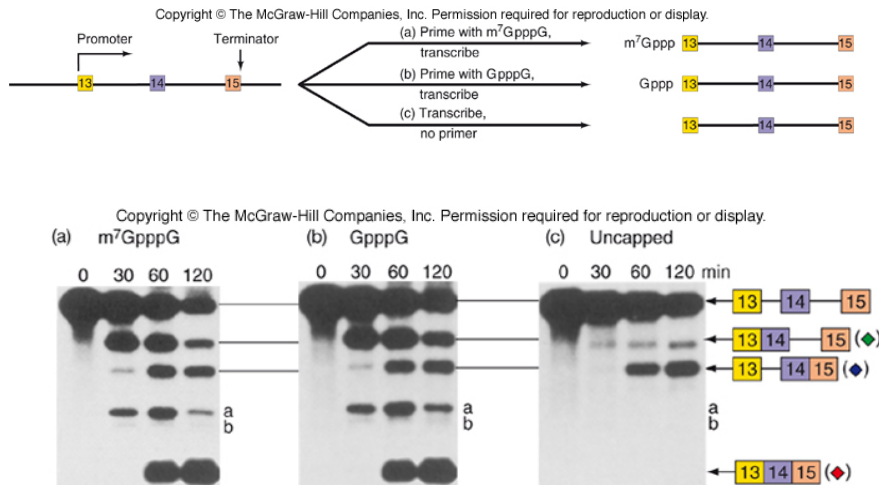


# Cap and Export from nucleus

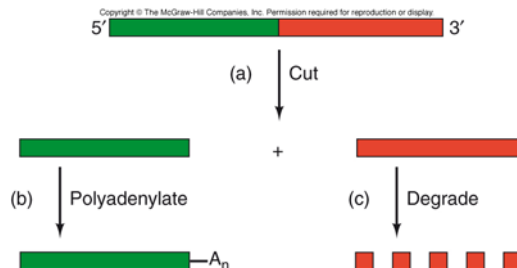


CBC – Nuclear cap binding complex

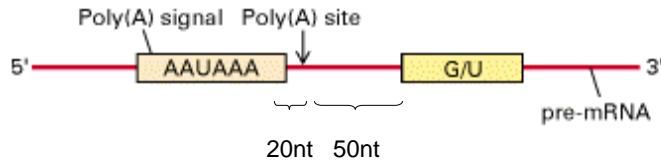
# Cap and Splicing



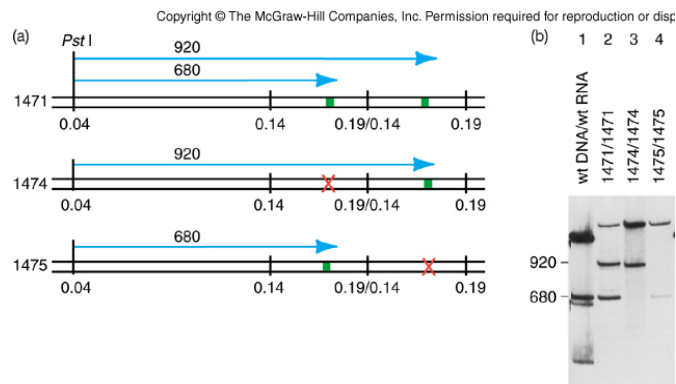
## Mechanism of Polyadenylation 2 Step Process



# Polyadenylation Signal



## Role of polyA signal AAUAAA



© Fitzgerald, M. and T. Shenk, The sequence 5'-AAUAAA-3' forms part of the recognition site for pol of late SV40 mRNAs. "Cell" 24 (April 1981) p. 257, f. 7. Reprinted by permission of Elsevier Science Publishing Co., Inc.

**Consensus sequence:**

A<sub>98</sub>A<sub>86</sub>U<sub>98</sub>A<sub>98</sub>A<sub>95</sub>A<sub>96</sub>  
 U<sub>12</sub>

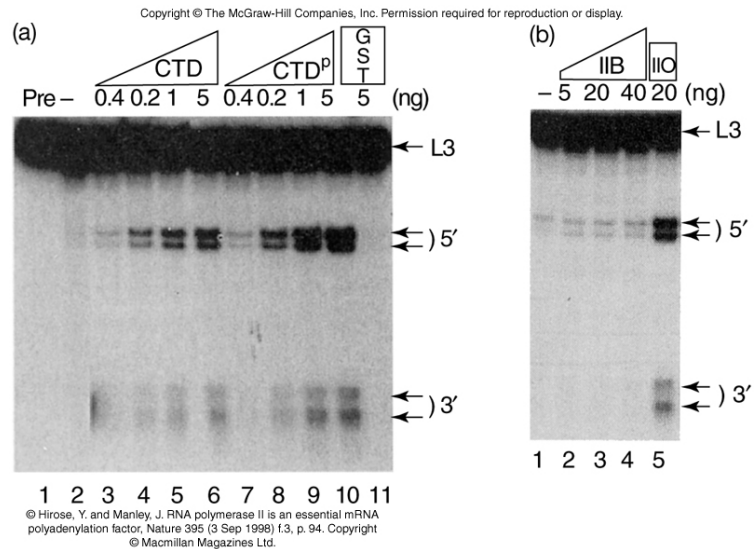
Sequence	Polyadenylation efficiency (%)
AAUAAG	~2
AAUUAA	~3
AAUUGA	~4
AAUAGA	~5
AAUAAC	~6
AACAAA	~7
AAUCAA	~7
AAUCAA	~7
AAA AAA	~7
AAUAUU	~7
AAGAAA	~7
AAUAUA	~10
AAUACA	~11
ACUAAA	~11
GUAUAA	~11
UAUAAA	~15
CAUAAA	~17
AGUAAA	~28
AUUAAA	~85
AAUAAA	~100

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

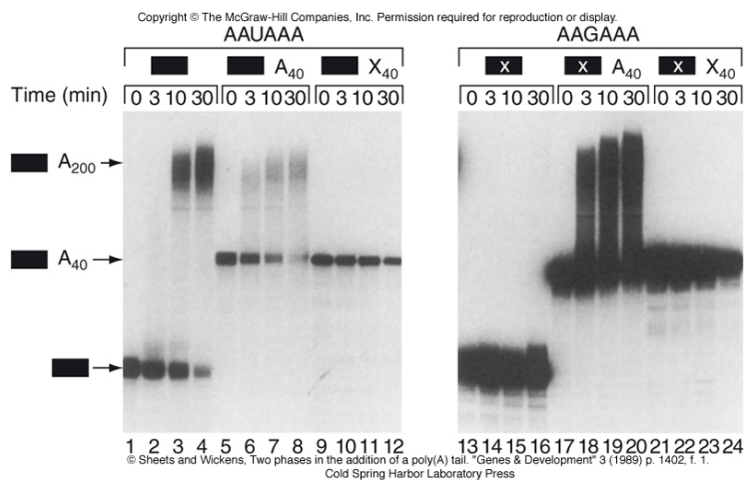


- 7

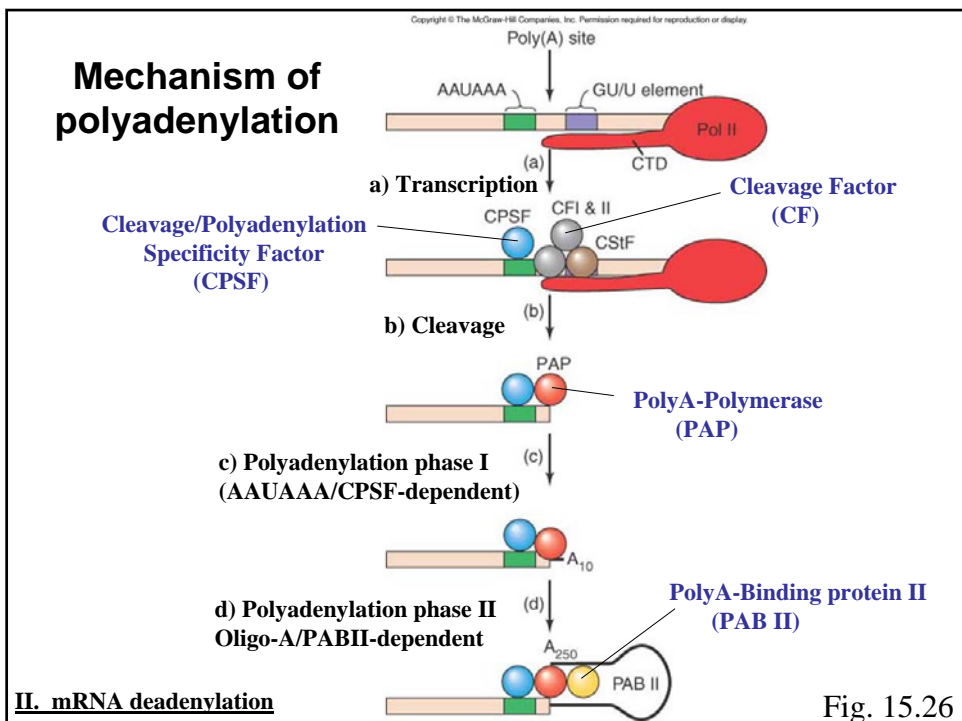
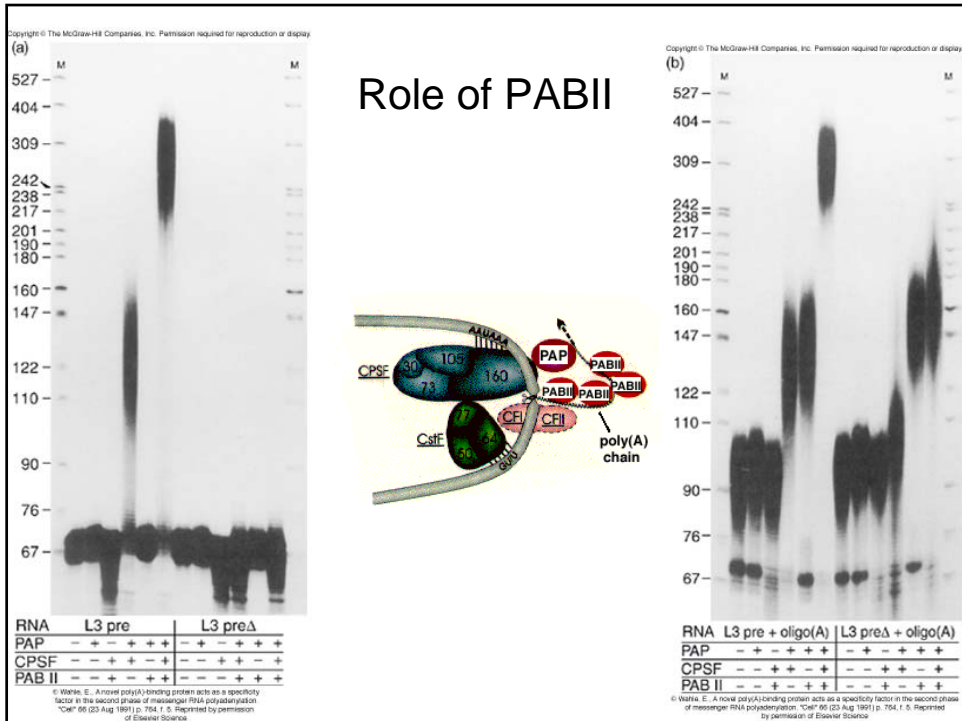
# CTD stimulates



# Two phases of Polyadenylation







# Function of Polyadenylation

1. Translation
2. Protection
3. Splicing
4. Transport
5. Termination

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.  
**Table 15.1 Synergism Between Poly(A) and Cap during Translation of Luciferase mRNA in Tobacco Protoplasts**

mRNA	Luciferase mRNA Half-Life (min)	Luciferase Activity (light units/mg protein)	Relative Effect of Poly(A) on Activity	Relative Effect of Cap on Activity
Uncapped				
Poly(A) <sup>-</sup>	31	2941	1	1
Poly(A) <sup>+</sup>	44	4480	1.5	1
Capped				
Poly(A) <sup>-</sup>	53	62,595	1	21
Poly(A) <sup>+</sup>	100	1,331,917	21	297

Source: Gallo, D.R., The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency, *Genes & Development* 5:2108-2116, 1991. Copyright © Cold Spring Harbor, NY. Reprinted by permission.

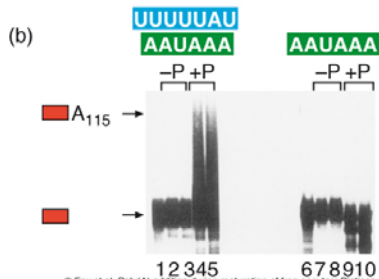
# Cytoplasmic Polyadenylation

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

UAAUUUUUUAAGCUGCAAUAAA CAAGUUAACAACCUCUAG<sub>OH</sub>

UAACCAUUAUAAGCUGCAAUAAA CAAGUUAACAACCUCUAG<sub>OH</sub>

(a)

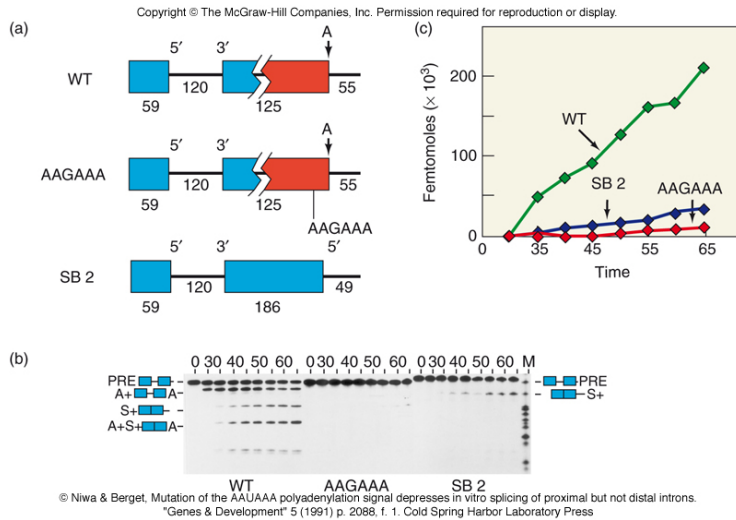


PolyA length is a balance between cytoplasmic exonuclease activity and cytoplasmic polyadenylation.

The polyA tail length is steady state.

© Fox et al. Poly(A) addition during maturation of frog oocytes. Distinct nuclear and cytoplasmic activities and regulation by the sequence UUUUUUAU. *Genes & Development* 3 (1989) p. 2154.15. Cold Spring Harbor Laboratory Press

# Polyadenylation and Splicing



## Termination "Torpedo Model"

