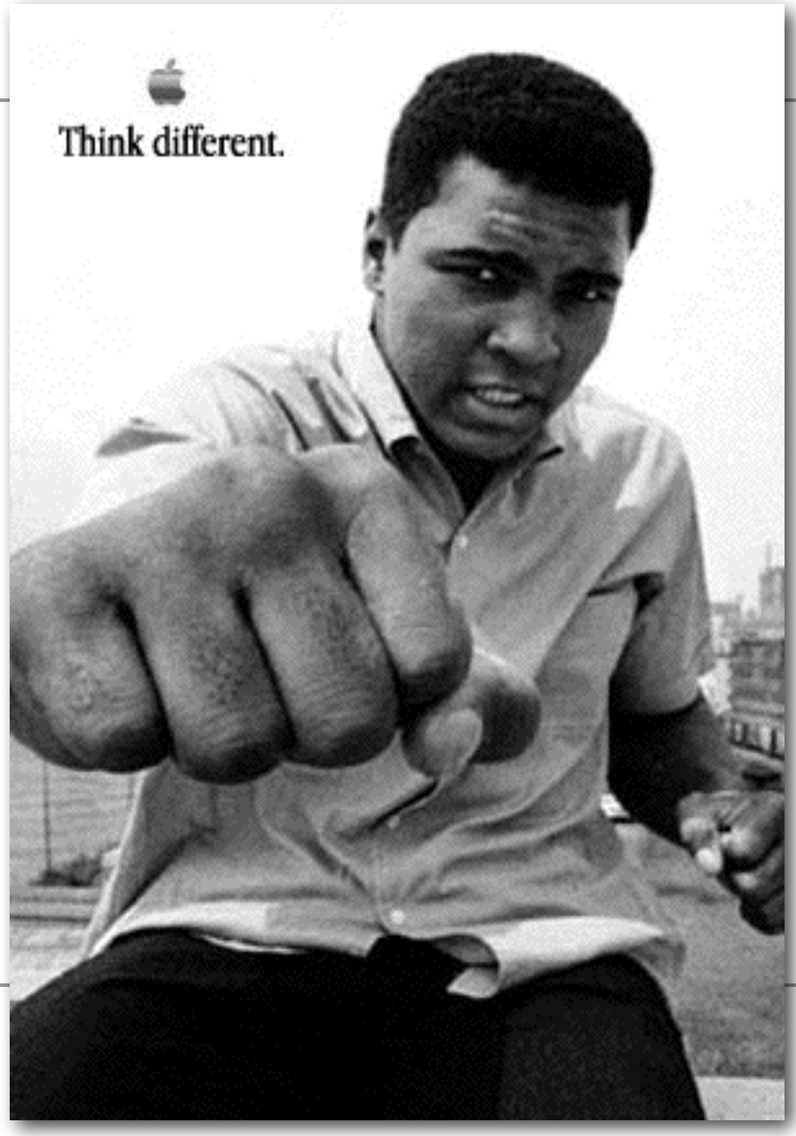
LB145 Spring *2025*



1. **Pick up** Name Folder

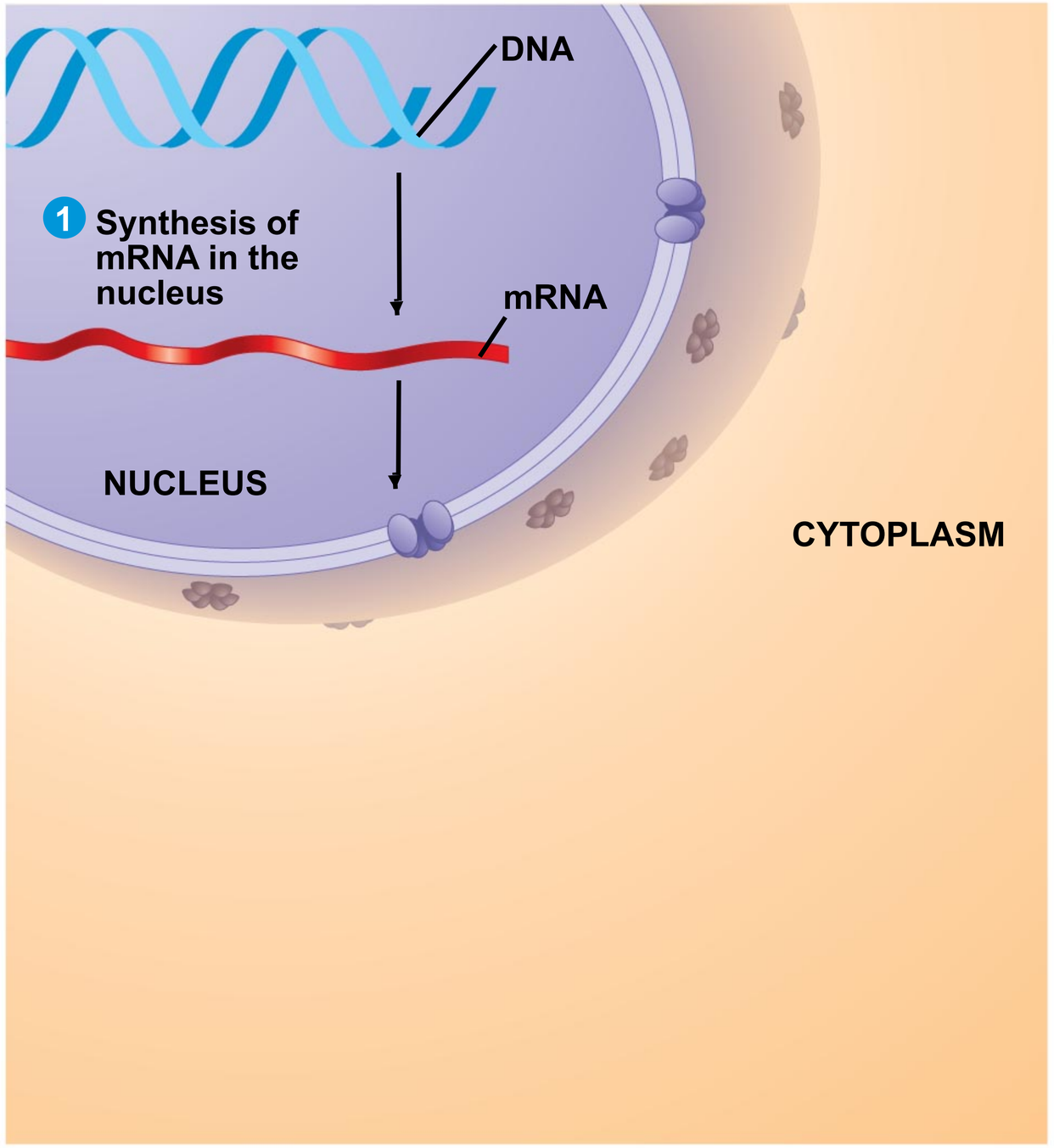
- Pick up name folder and set it up at seat.

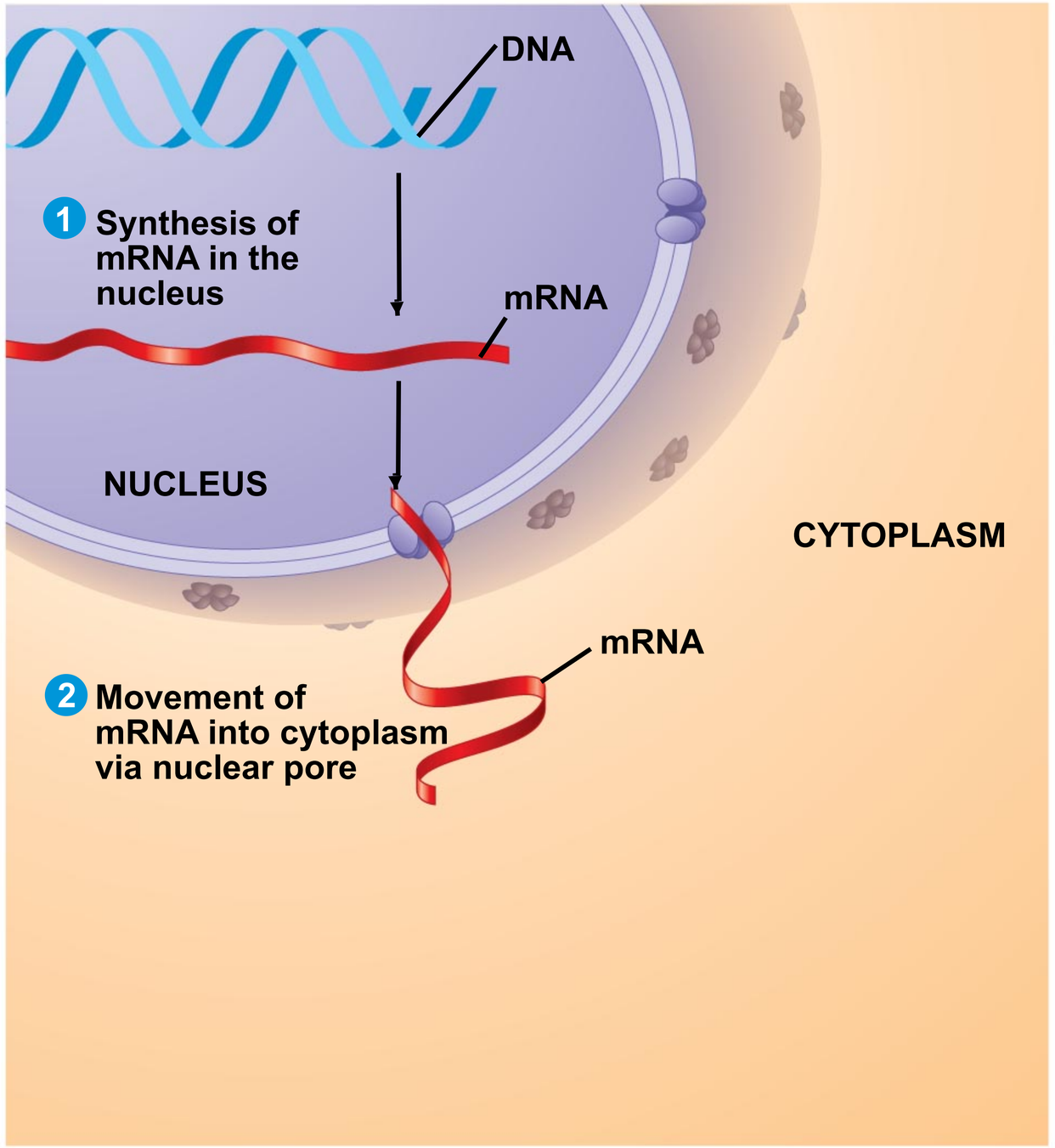
2. **Sit** with your group.

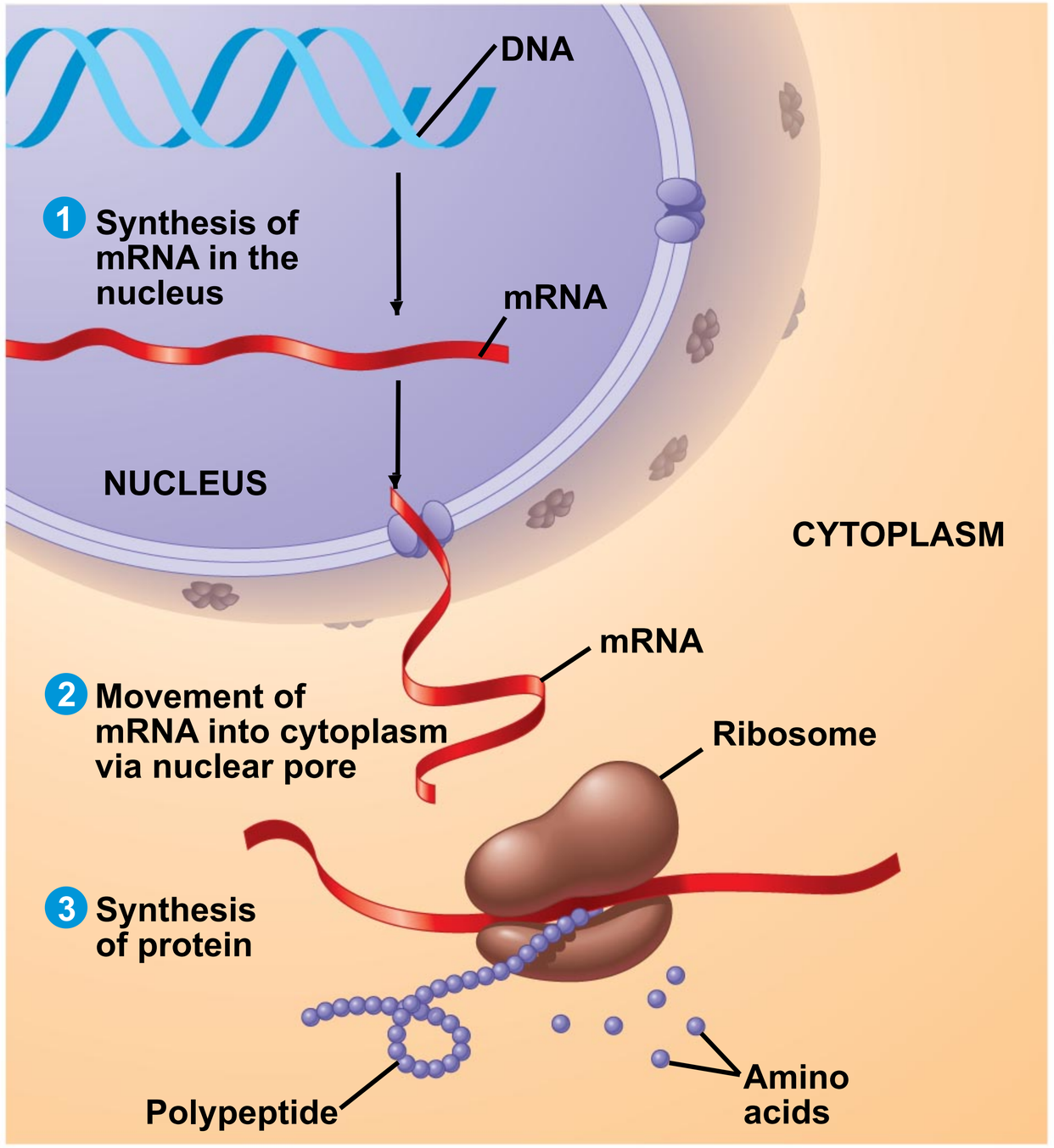
- laptops on outer perimeter (avoid distracting)

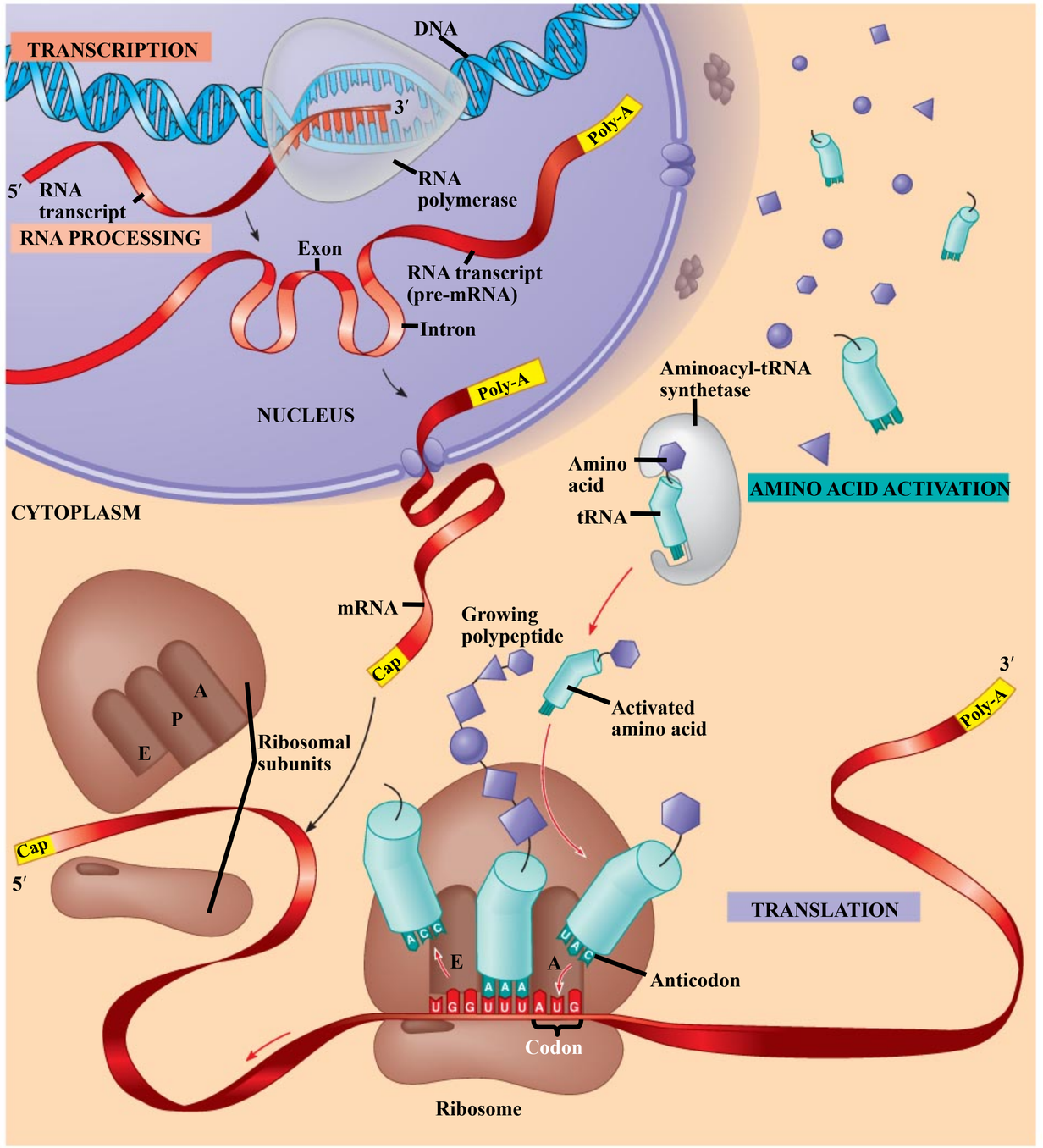
3. **Clicker** Attendance

- Launch your Top Hat, and get ready to click.

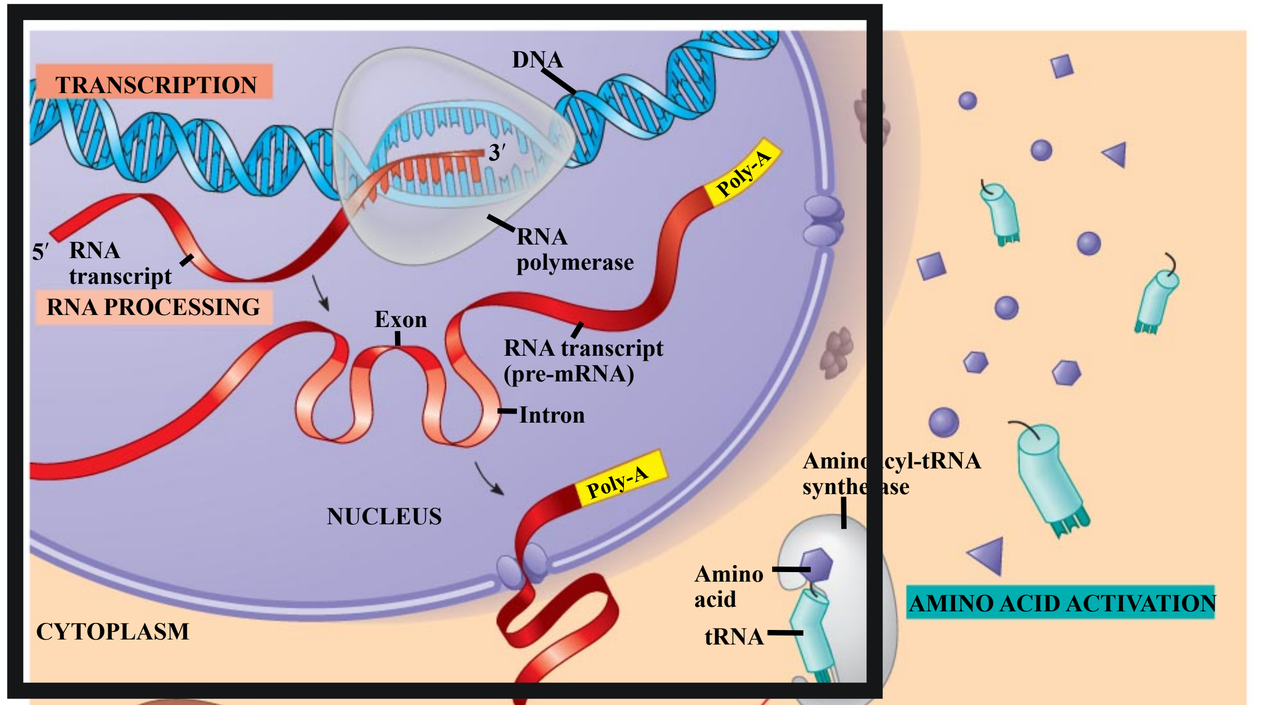


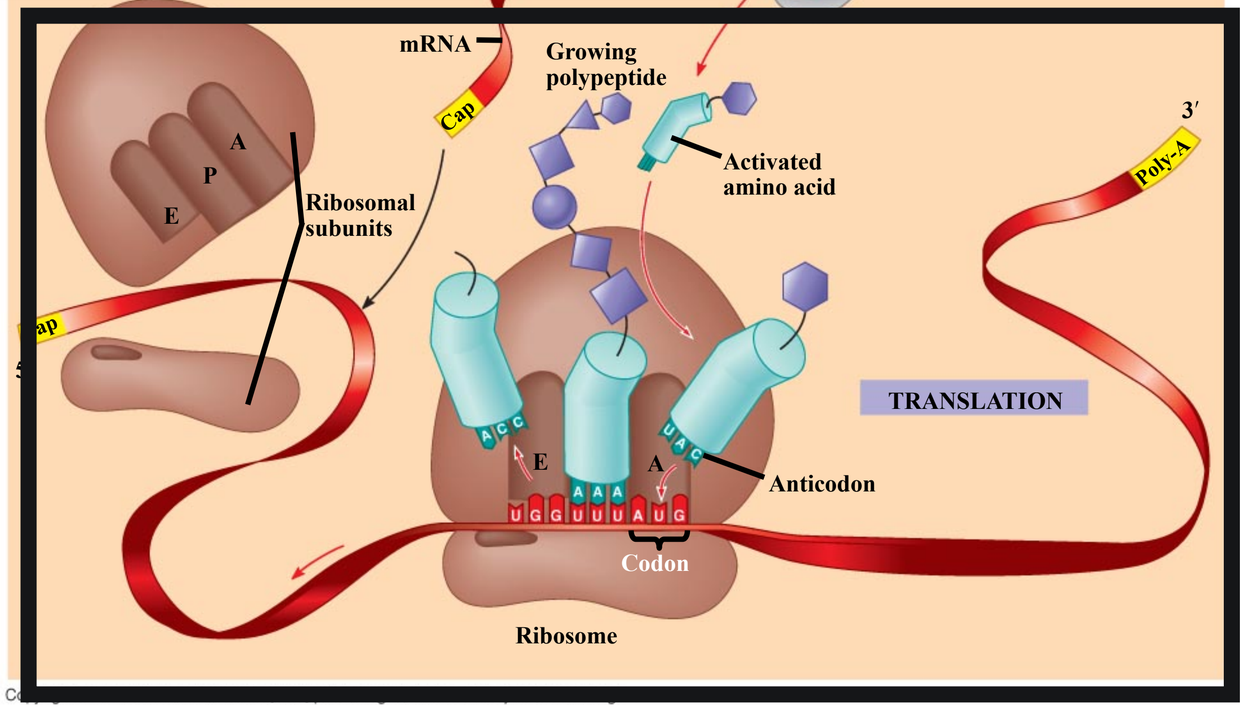






Transcription





Translation

15.3 Eukaryotic Transcription

Summary: By the end of this section, you will be able to:

- List the steps in eukaryotic transcription
- Discuss the role of RNA polymerases in transcription
- Compare and contrast the three RNA polymerases
- Explain the significance of transcription factors

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few key differences. The most important difference between prokaryotes and eukaryotes is the latter's membrane-bound nucleus and organelles. With the genes bound in a nucleus, the eukaryotic cell must be able to transport its mRNA to the cytoplasm and must protect its mRNA from degrading before it is translated. Eukaryotes also employ three different polymerases that each transcribe a different subset of genes. Eukaryotic mRNAs are usually monogenic, meaning that they specify a single protein.

Initiation of Transcription in Eukaryotes

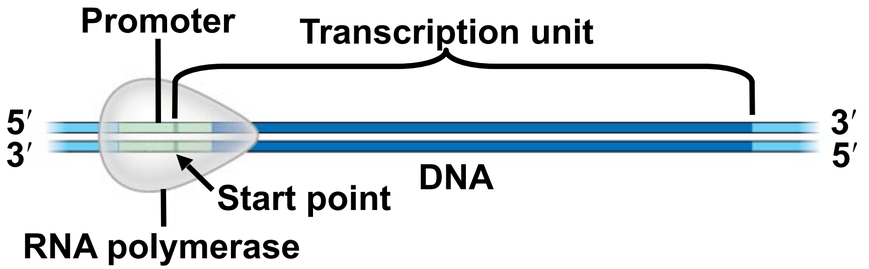
Unlike the prokaryotic polymerase that can bind to a DNA template on its own, eukaryotes require several other proteins, called transcription factors, to first bind to the promoter region and then help recruit the appropriate polymerase.

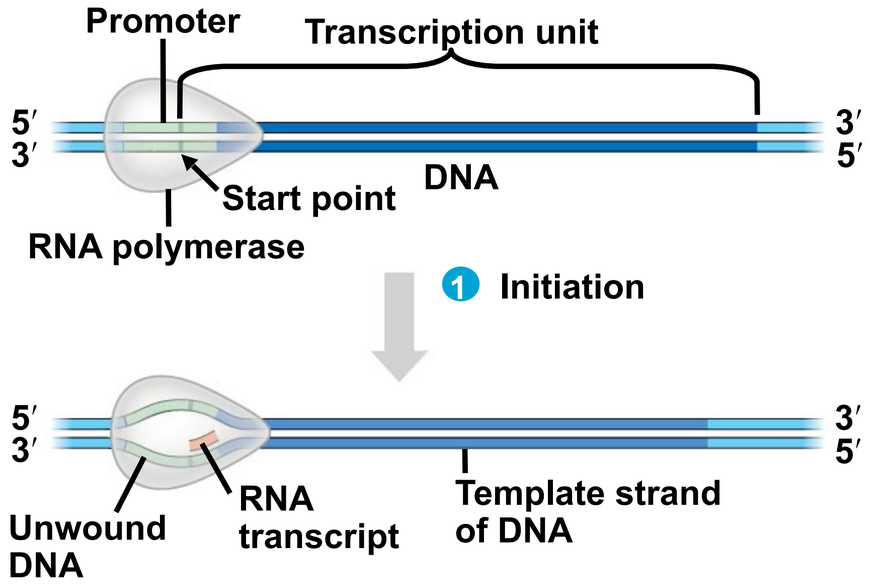
The Three Eukaryotic RNA Polymerases

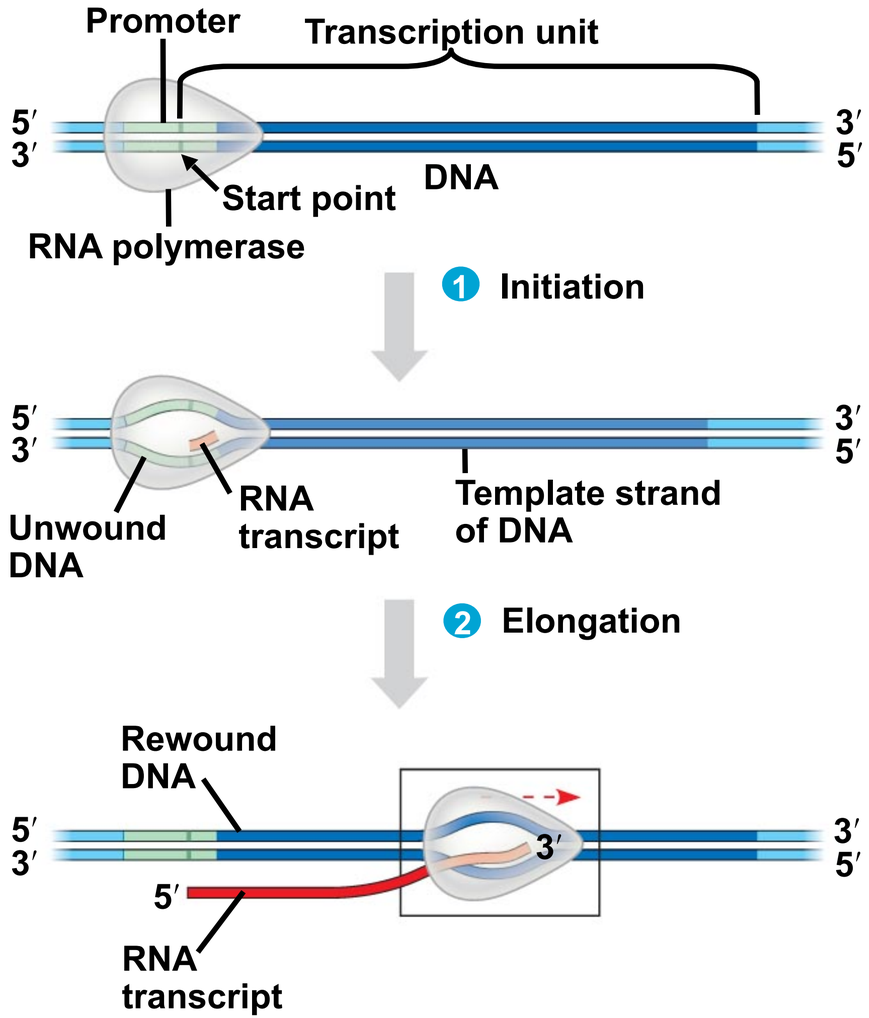
The features of eukaryotic mRNA synthesis are markedly more complex those of prokaryotes. Instead of a single polymerase comprising five subunits, the eukaryotes have three polymerases that are each made up of 10 subunits or more. Each eukaryotic polymerase also requires a distinct set of transcription factors to bring it to the DNA template.

Questions

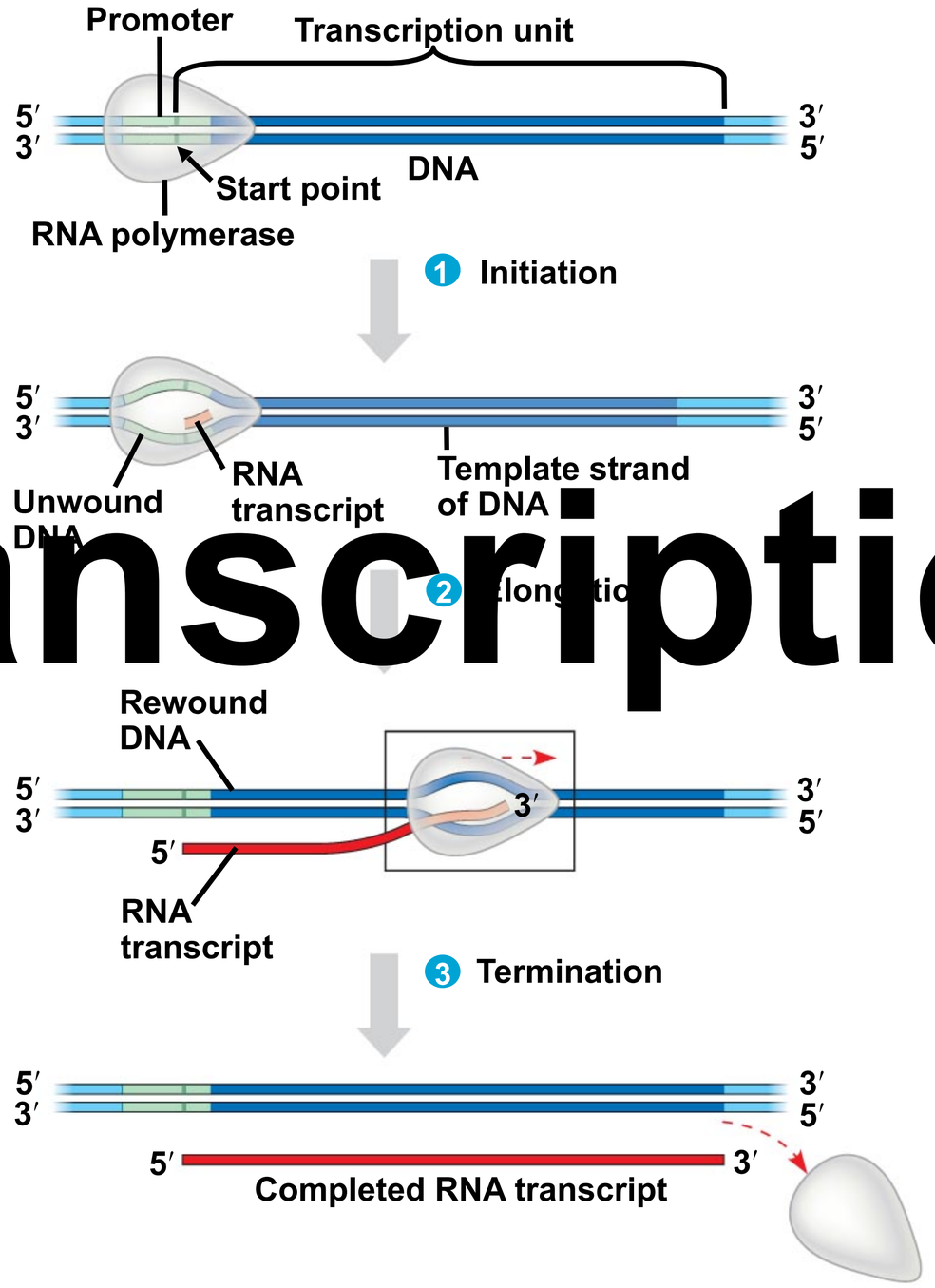
- **What happens when a gene is transcribed?**



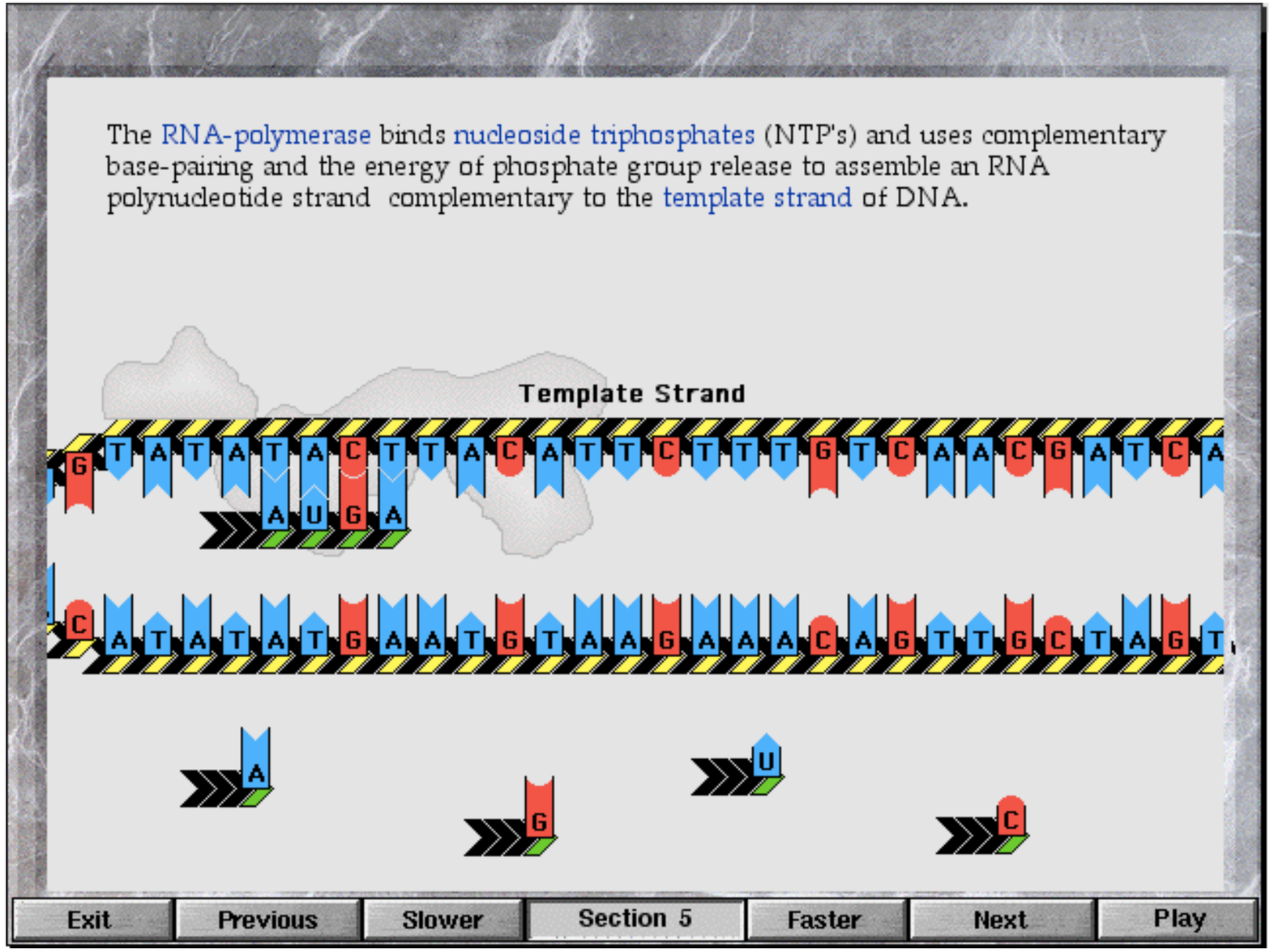




Transcription



Transcription animation course website



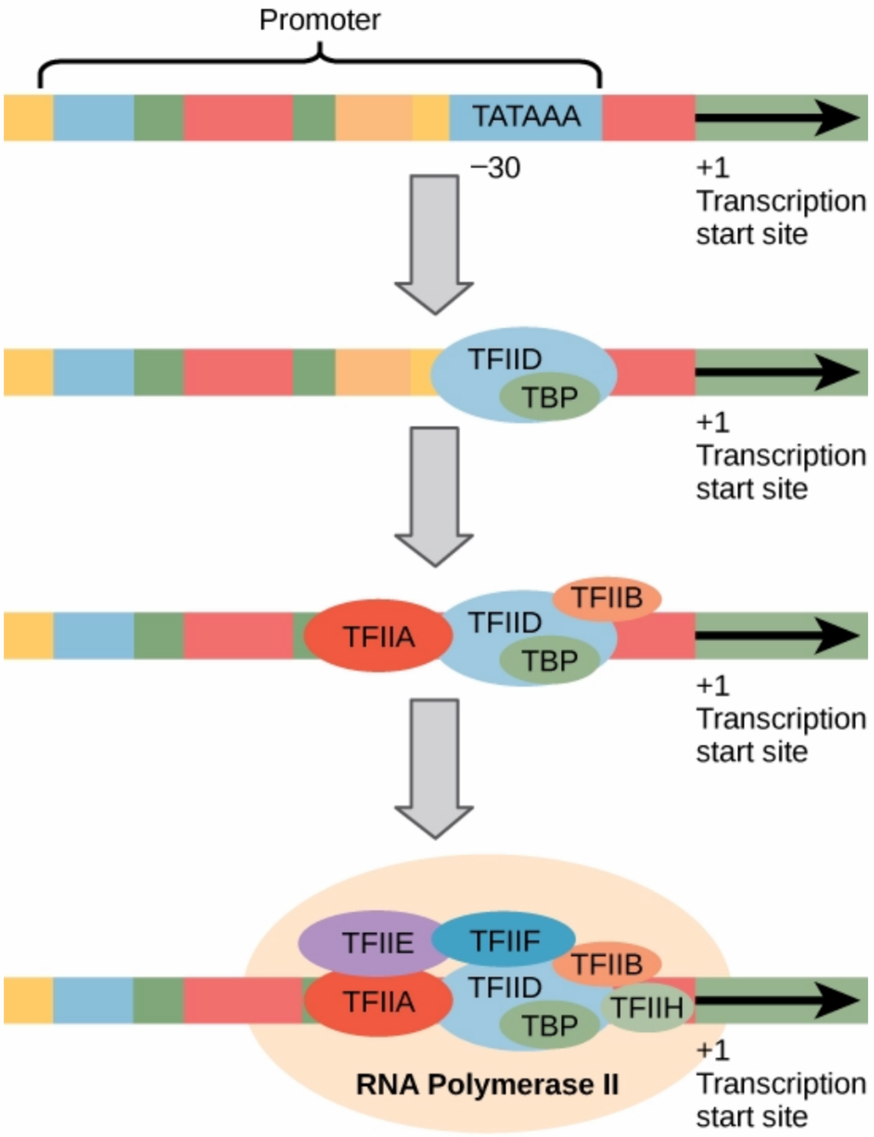


Figure 1: A generalized promoter of a gene transcribed by RNA polymerase II is shown. Transcription factors recognize the promoter. RNA polymerase II then binds and forms the transcription initiation complex.

Trifecta exercises

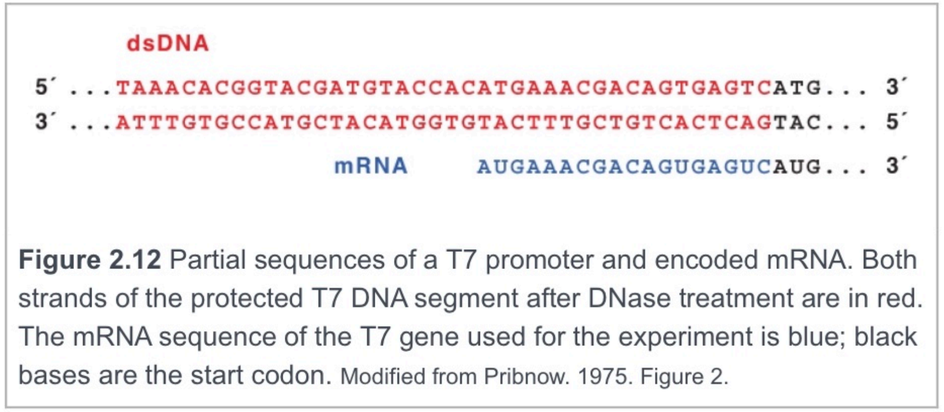


Figure 2.12 Partial sequences of a T7 promoter and encoded mRNA. Both strands of the protected T7 DNA segment after DNase treatment are in red. The mRNA sequence of the T7 gene used for the experiment is blue; black bases are the start codon. Modified from Pribnow. 1975. Figure 2.

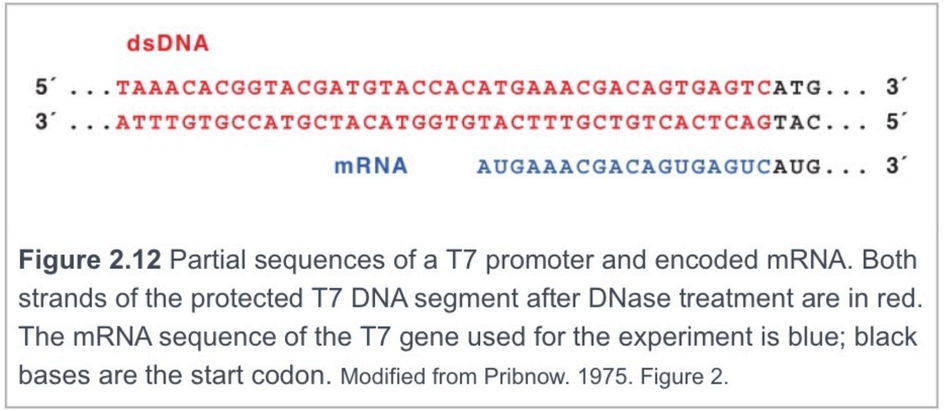
Pribnow was building on the work of many others when he started his project to define a consensus promoter sequence. He mixed *E. coli* RNA polymerase with DNA containing the promoter for a virus called T7. Like all viruses, T7 is especially adept at stealing the host's central dogma machinery to make more viruses. For this reason, choosing a viral promoter was clever because T7 DNA has an exceptionally high affinity for *E. coli* RNA polymerase. Pribnow mixed T7 DNA with *E. coli* RNA polymerase and then added DNase, which digests any DNA not covered by the RNA polymerase protein. RNA polymerase shielded the T7 promo ed this protec discov

Trifecta: purpose, methods, findings

Pribnow knew that other investigators had isolated segments of DNA containing several different promoters, but no one was sure exactly where the RNA polymerase paused before beginning transcription. He aligned the DNA sequences from other promoters with the T7 promoter that he had carefully mapped with DNase. He wanted to know whether any sequences were conserved across the mixture of bacterial and viral promoters (Figure 2.13). Back in the 1970s, this sort of alignment was performed manually and required a lot of patience. Now, we have **bioinformatics** tools to automate sequence alignment. Bio-

Figure 2.13 Alignment of DNA sequences containing promoters. Sequences from viral (V) and bacterial (B) genes are aligned so that conserved bases are in the boxed region and the first transcribed bases (underlined) are nearby. Modified from Pribnow. 1975. Figure 4.

Prepare to **Explain**: Purpose, Methods, Findings (trifecta)



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Years after the experiment in Figure 2.14, a team of Canadian molecular biologists wanted to determine how transcription factors bind to eukaryotic promoters and initiate transcription (Figure 2.15). Do they bind all at once, or do they each bind independently? To perform their experiment, the investigators produced many copies of a radioactive promoter, which would always be detectable as a black band when exposed to x-ray film. In the presence of a radioactive promoter, they mixed every possible combination of RNA polymerase and three transcription factors called PAR 74, TFIIB, and TBP. The

Trifecta: purpose, methods, findings

did not cause molecular interactions to be broken. Any proteins bound to the promoter would stay bound to the DNA during electrophoresis. The resulting gel was placed next to an x-ray film to expose the film to the radioactive DNA. The film turned black only where the radioactive promoter was located in the gel. In Figure 2.15, plus signs above the developed x-ray film indicates the presence of a particular transcription factor, and empty boxes indicate which transcription factors were omitted from the experiment for each lane. The first lane on the left contained a negative control so that the researchers could see how big the promoter was in the absence of any added transcription factors. Before you answer Integrating Questions about Figure 2.15, consider an additional experiment designed to map the full extent of a gene's promoter.

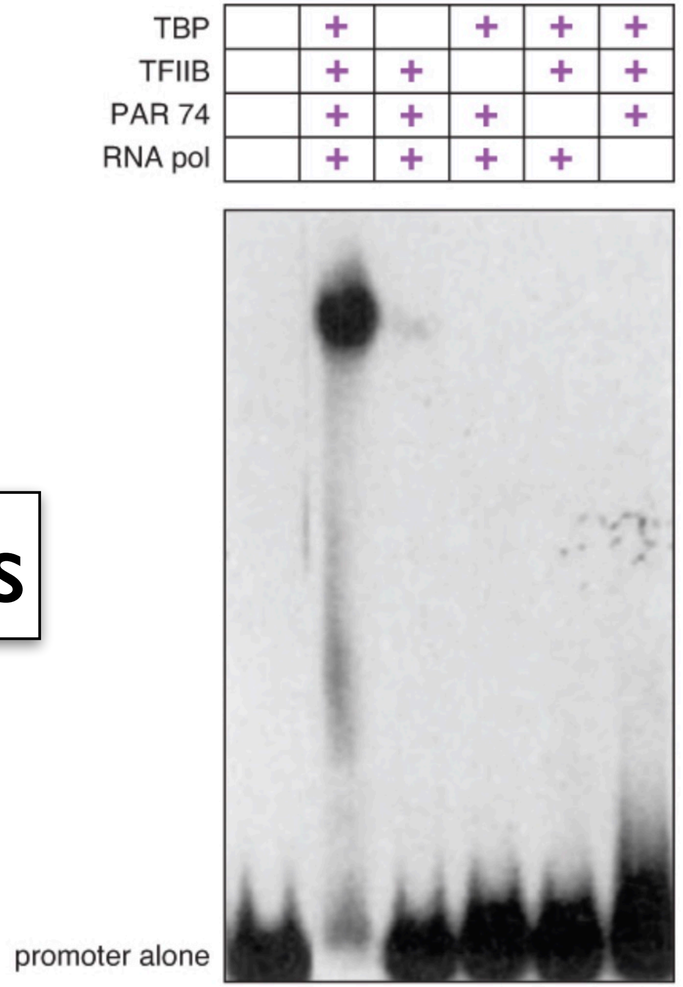

Figure 2.15 X-ray film shows the results of a promoter-binding experiment. A radioactive DNA promoter sequence was incubated under six different conditions; combinations contained three transcription factors and RNA polymerase (RNA pol). Samples were loaded on the gel and separated by electrophoresis; promoter alone was loaded in the far left lane as a negative control. Plus signs indicate which components were incubated with the radioactive promoter. From Killeen *et al.*

Prepare to **Explain**: Purpose, Methods, Findings (trifecta)

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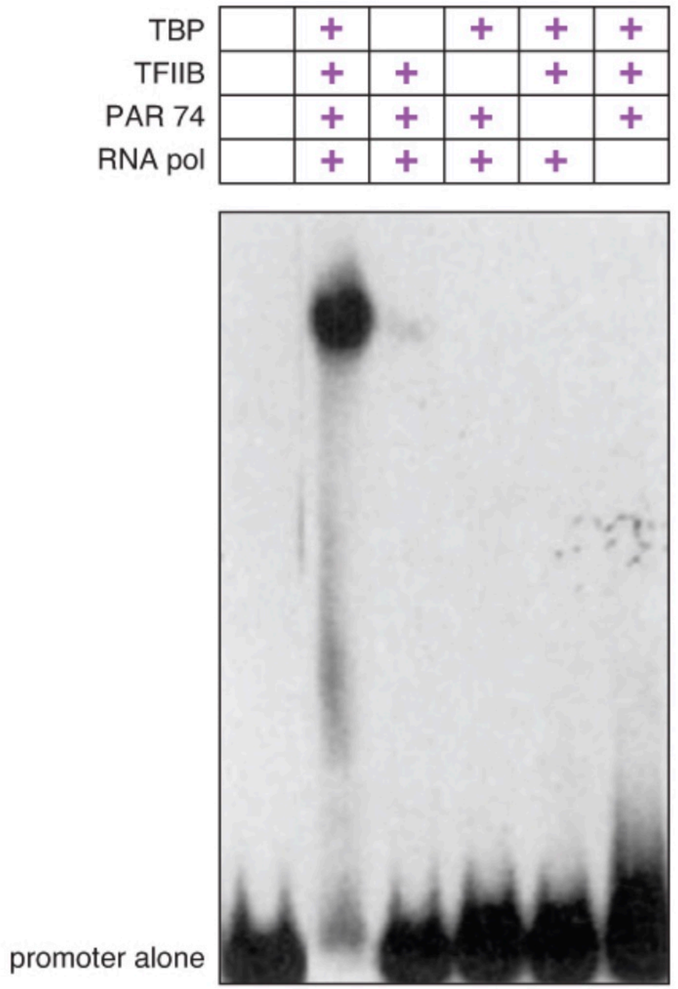

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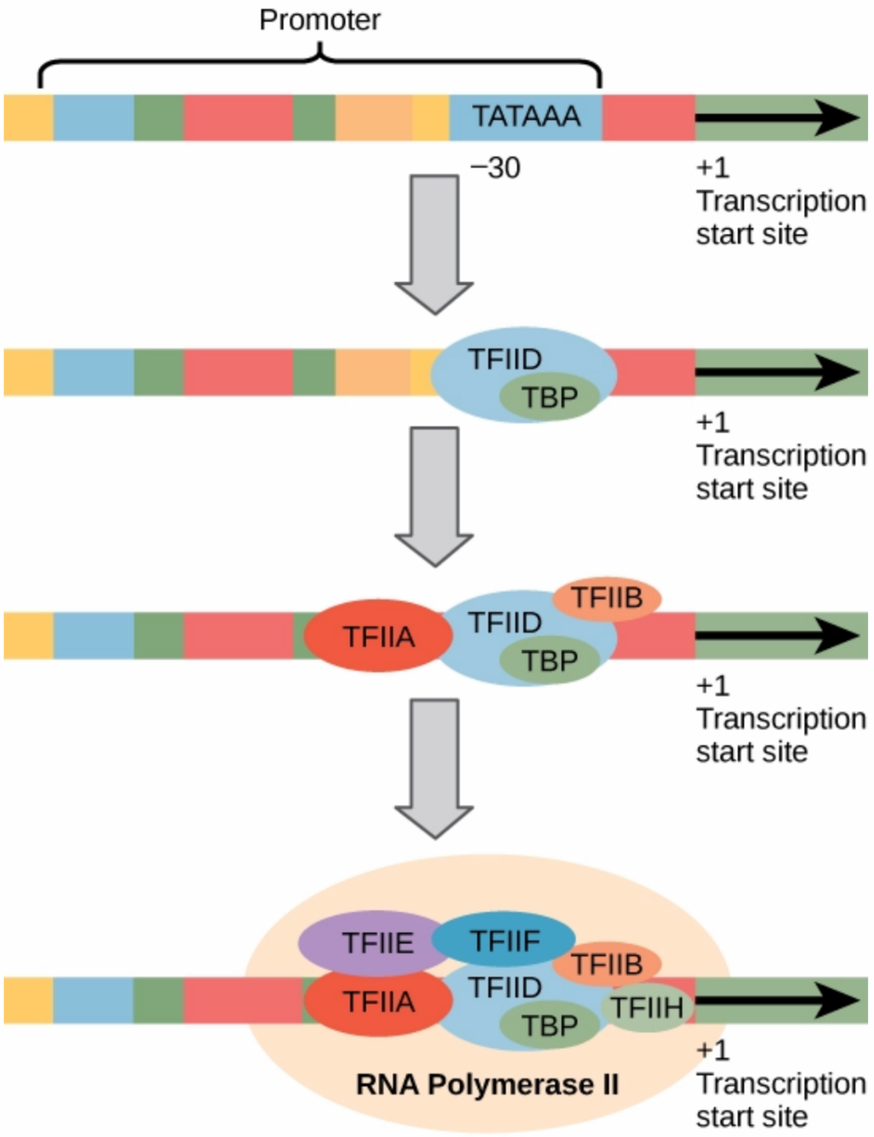

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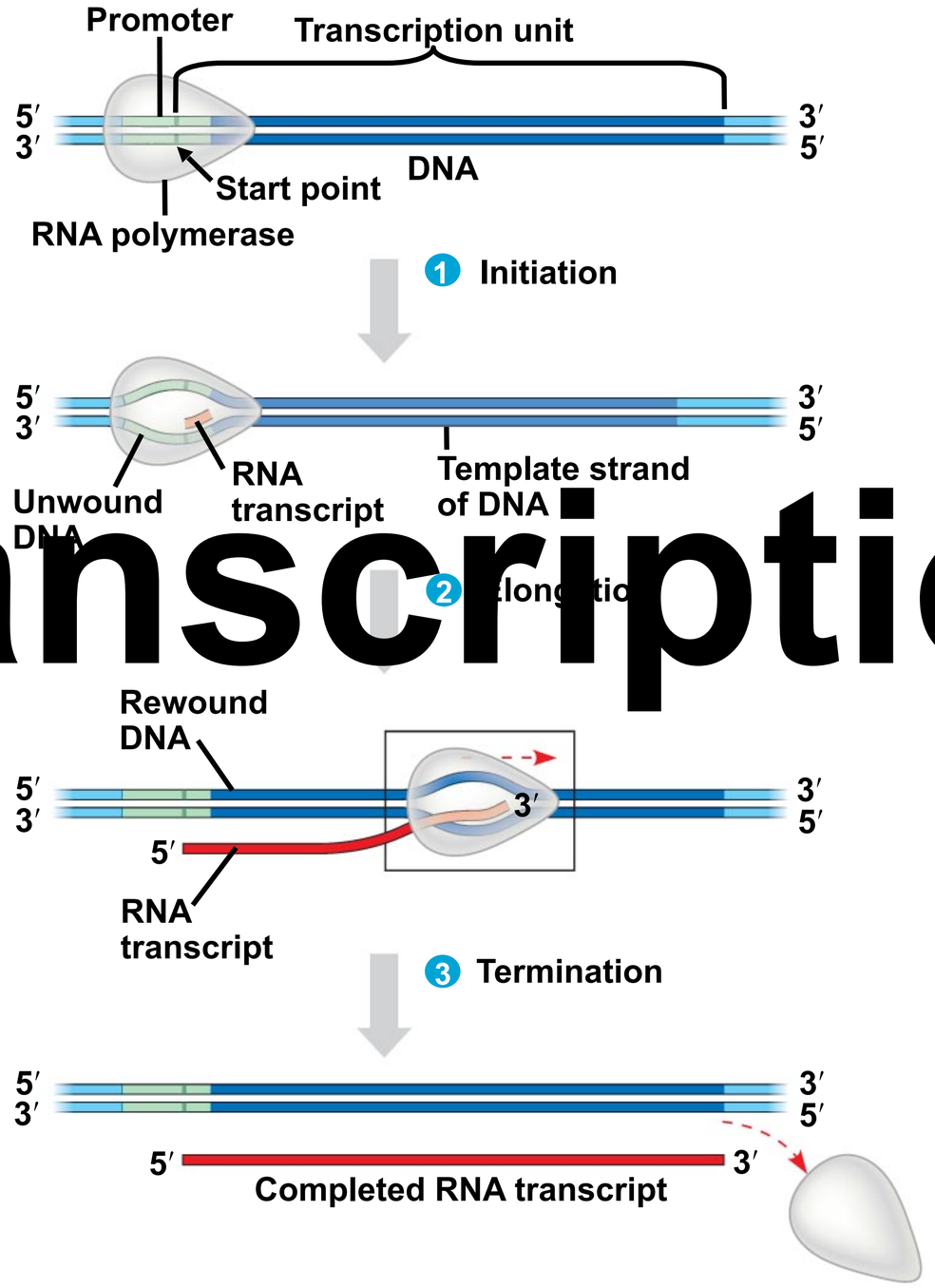
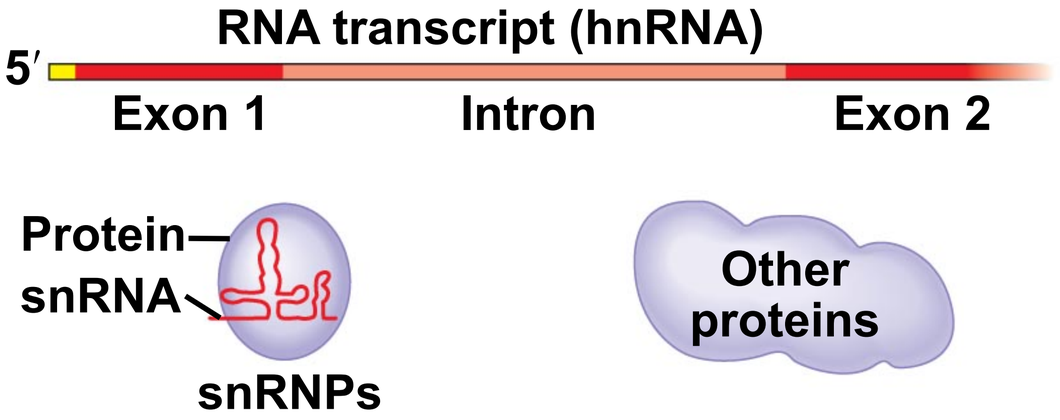
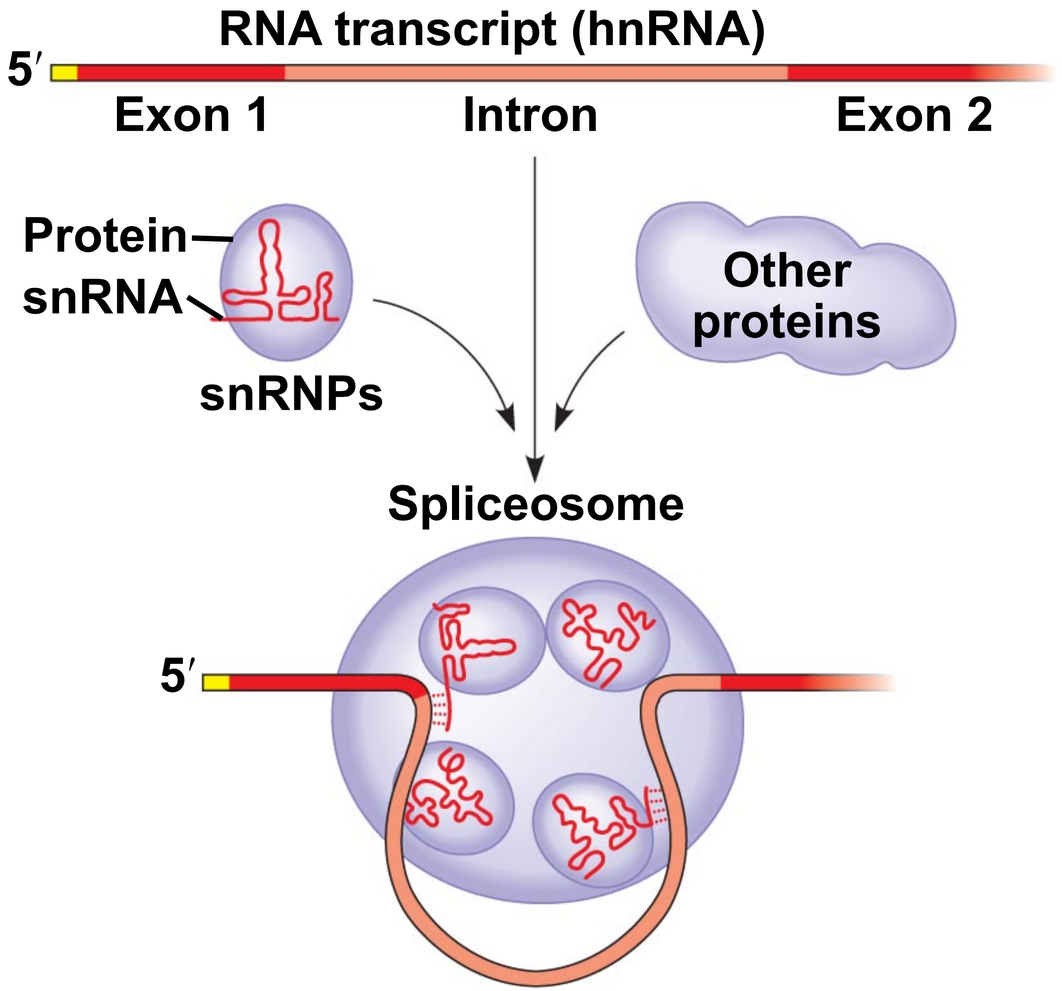
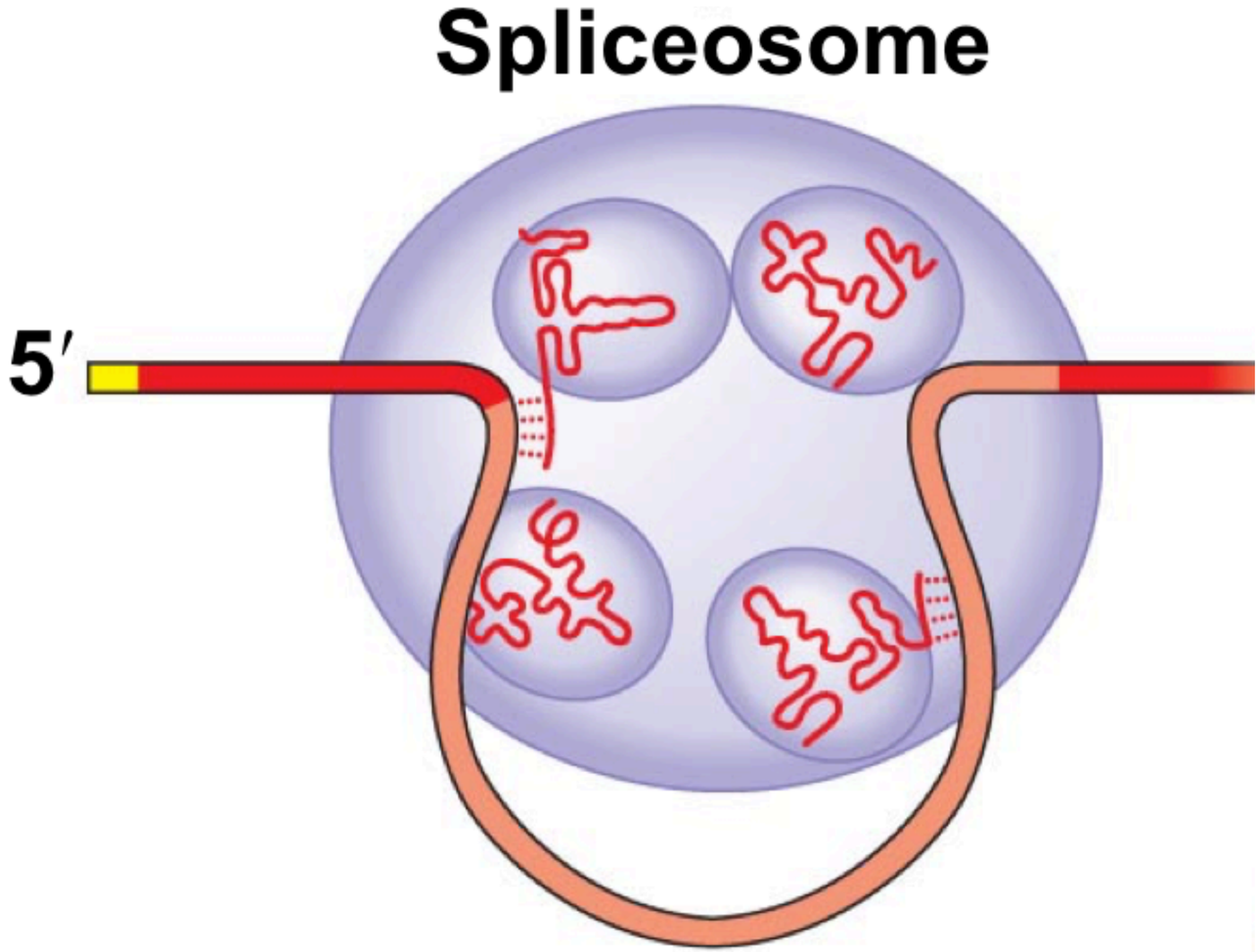
Transcription





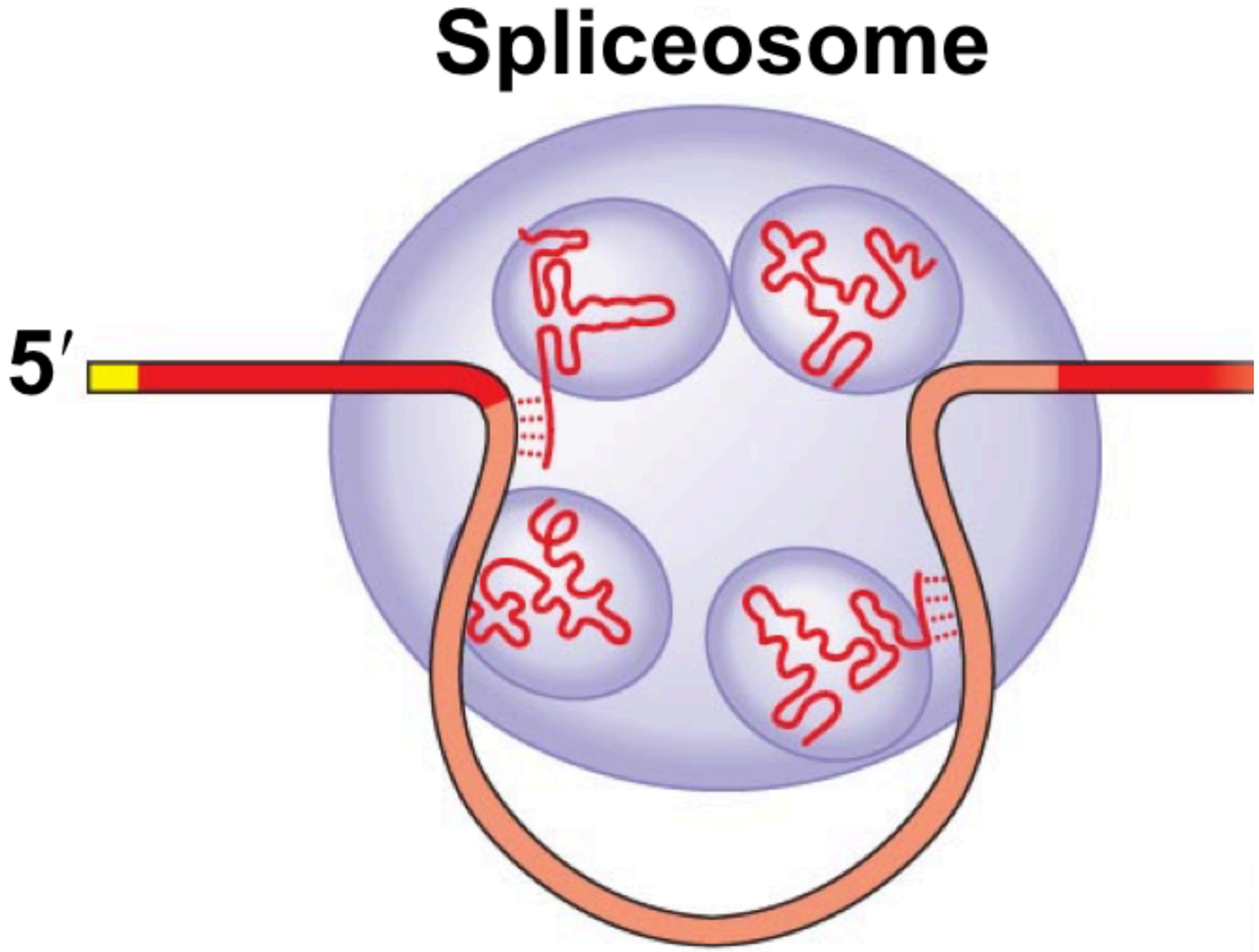


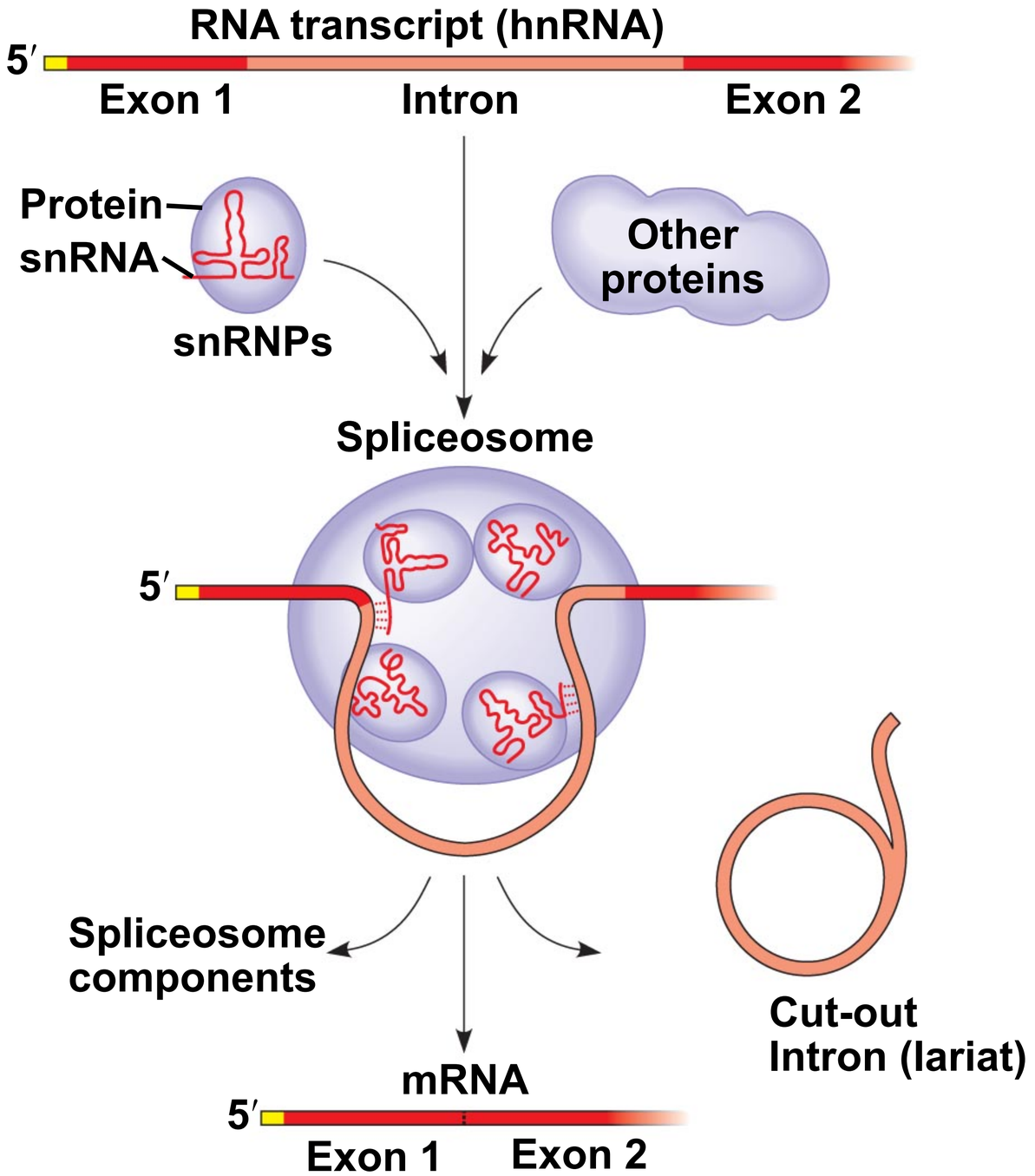
Predict: What role does snRNA (red) serve?

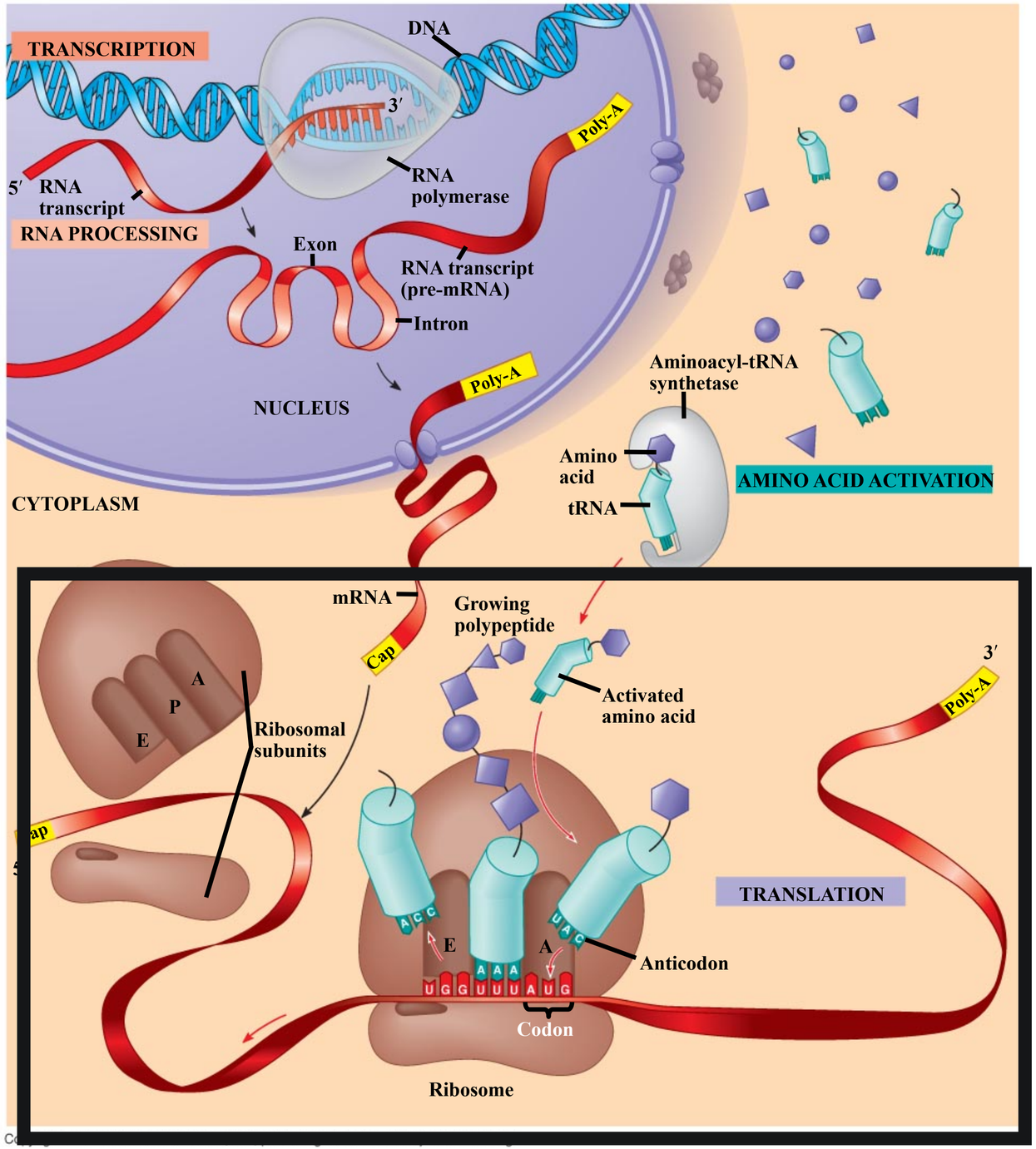


snRNAs work with a complex of proteins to form snRNPs

(pronounced "snurps")







Translation

Questions

Take a second…

- **Be ready to explain Figure 2 aloud to class.**

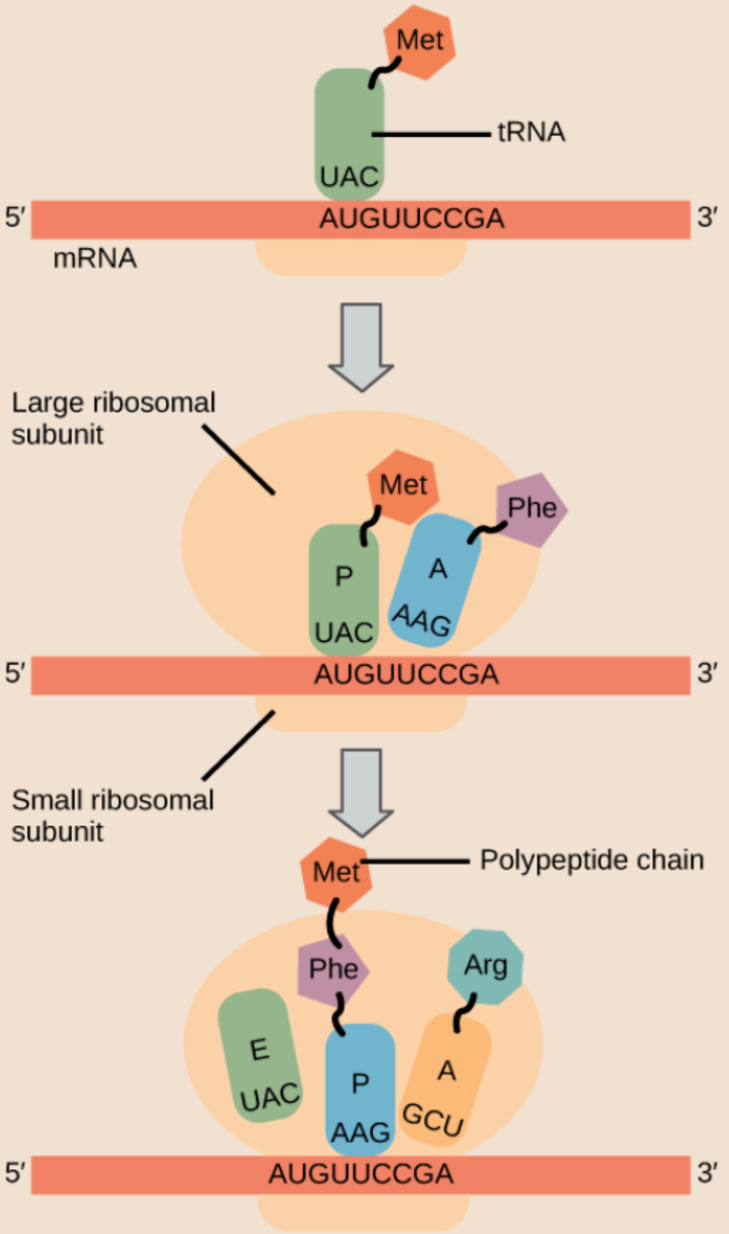


Figure 2: Translation begins when an initiator tRNA anticodon recognizes a codon on mRNA. The large ribosomal subunit joins the small subunit, and a second tRNA is recruited. As the mRNA moves relative to the ribosome, the polypeptide chain is formed. Entry of a release factor into the A site terminates translation and the components dissociate.

(notebook)

Translation: 3 parts

- **Language** (AUG!)
- **Translators** (tRNA & synthetase)
- **Factory** (Ribosome subunits)

Language (AUG!)

What words are hidden in this mRNA sentence?

5’ 3’

UUAAGUAAGGAUGGGUGUAAUAAGUCAGCUGA UUG A

What additional *information* do you need?

????????? | THE | DOG | AND | MAN | EAT | HAM | STOP

Frameshift mutation (delete O in DOG)

???????? | THE | DGA | NDM | ANE | ATH | AMS | TOP

Language (AUG!)

What words are hidden in this mRNA sentence?

5’ 3’

UUAAG ❙ AUG ❙ GGG ❙ UAU ❙ AAG ❙ UCA ❙ ACC ❙ UUG A

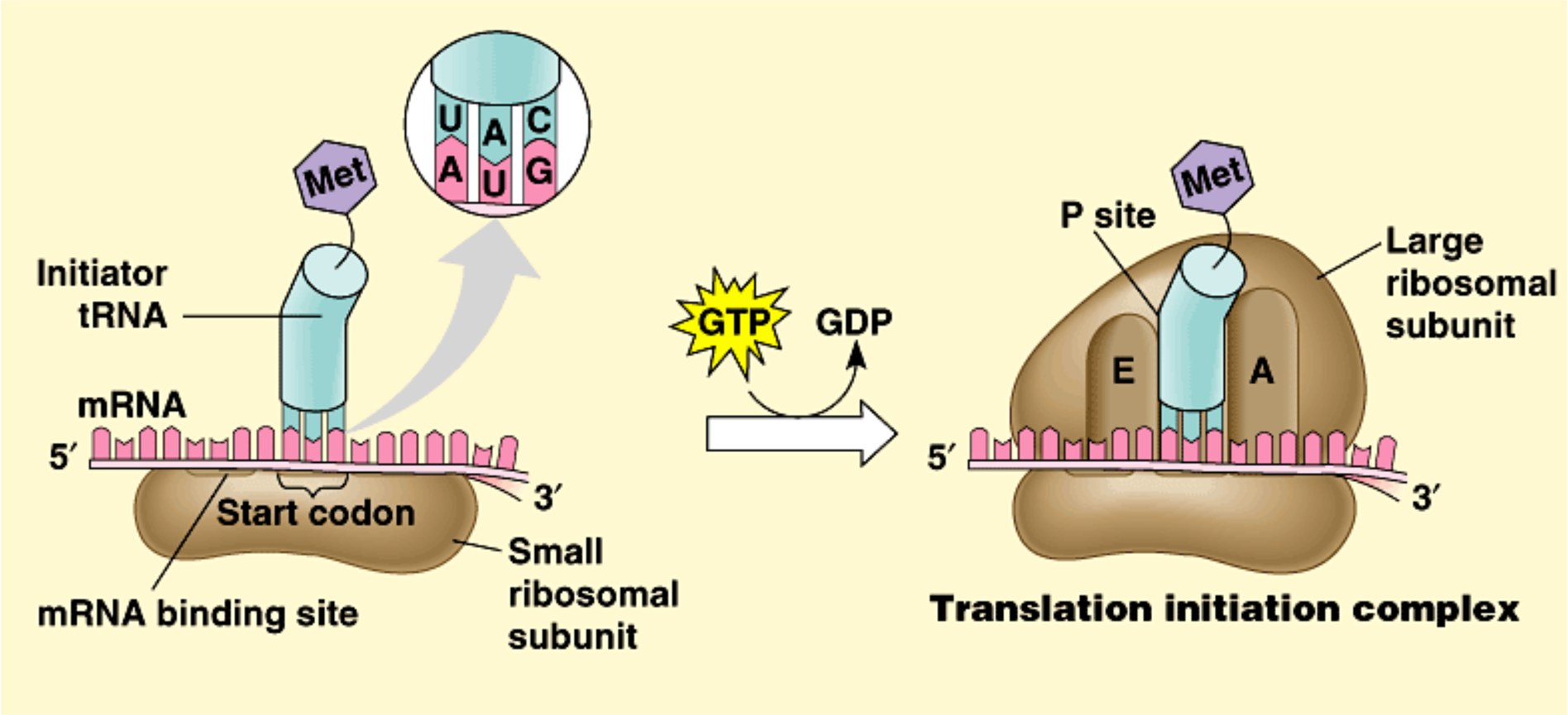
What additional *information* do you need?

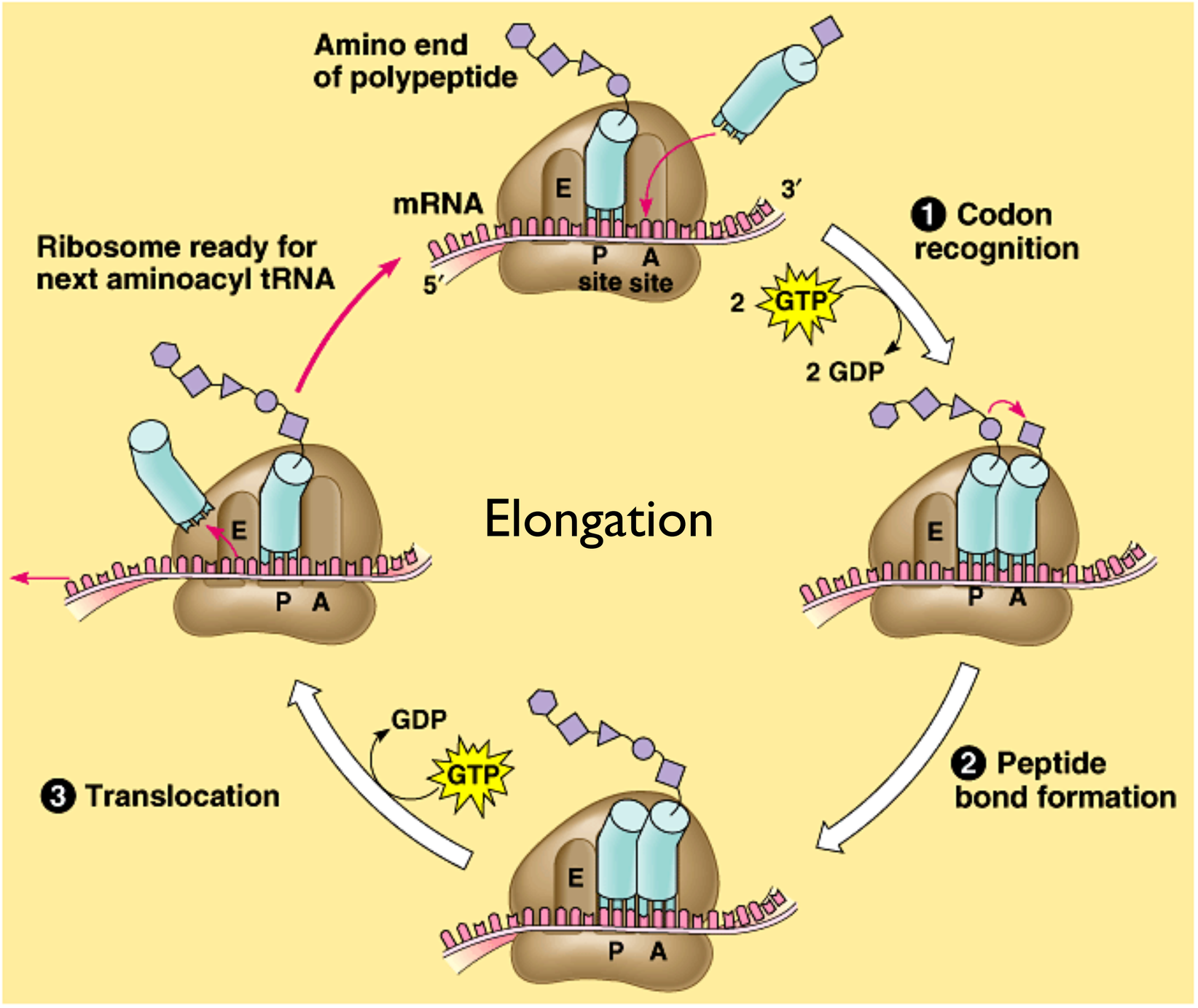
?????????? ❙ THE ❙ DOG ❙ AND ❙ MAN ❙ EAT ❙ HAM ❙ STOP

In-frame mutation (delete AND)

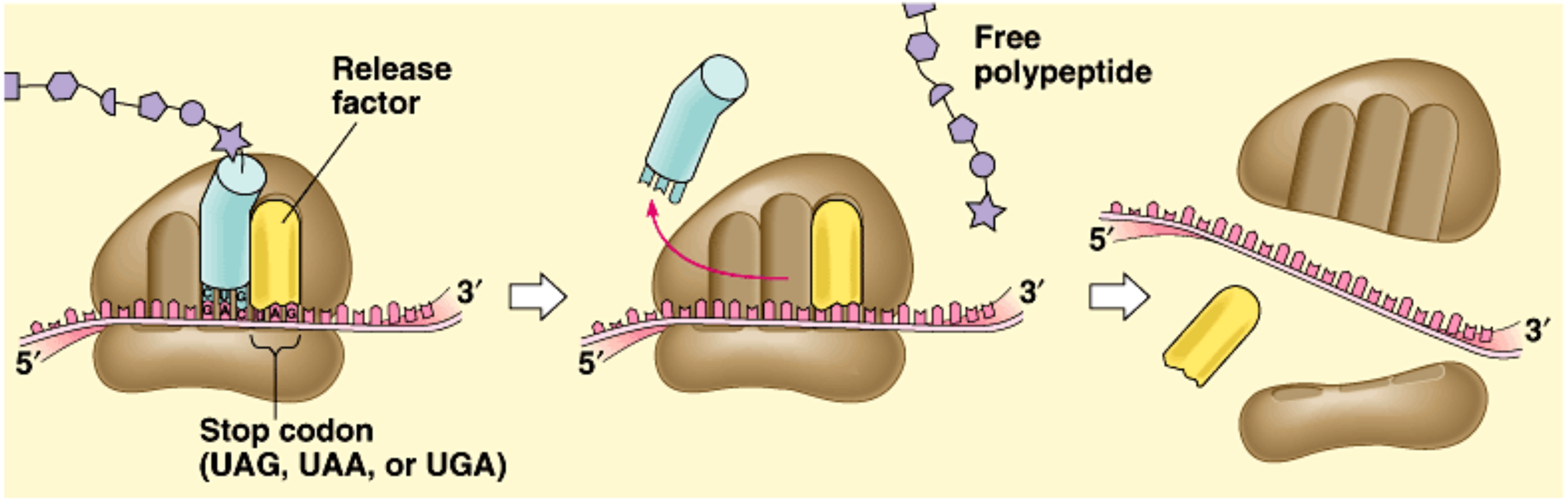
?????????? ❙ THE ❙ DOG ❙ MAN ❙ EAT ❙ HAM ❙ STOP

Initiation


U A C
A U G
Met
Initiator tRNA
mRNA
5′
3′
Start codon
mRNA binding site
Small ribosomal subunit
GTP
GDP
Met
P site
Large ribosomal subunit
E
A
5′
3′
Translation initiation complex


Amino end
of polypeptide
mRNA
5′
3′
E
P
site
A
site
1 Codon
recognition
2
GTP
2 GDP
2 Peptide
bond formation
GDP
GTP
3 Translocation
Ribosome ready for
next aminoacyl tRNA
Elongation

Termination



Translation steps