Lecture 11

*Eukaryotic Transcription
- Gene Organization
- RNA Processing
  - 5' cap
  - 3' polyadenylation
  - splicing

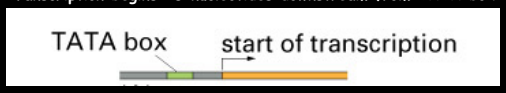
Translation

Initiation of RNA Pol II transcription

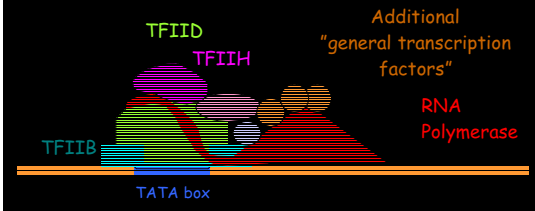
Consensus sequence of promoter

TATA

Transcription begins 25 nucleotides downstream from TATA box



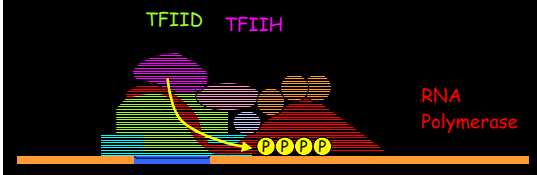
Transcription Initiation Complex



Local distortion of DNA structure

See Fig. 8-10

Transcription Initiation Complex



Phosphorylation of RNA Polymerase II by TFIIH

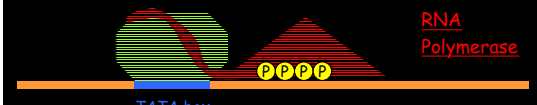
Local distortion of DNA structure

See Fig. 8-10

Transcription Initiation Complex

TFIID

Dissociation of the general transcription factors



Phosphorylation of RNA Polymerase II by TFIIH

Transcription!

See Fig. 8-10

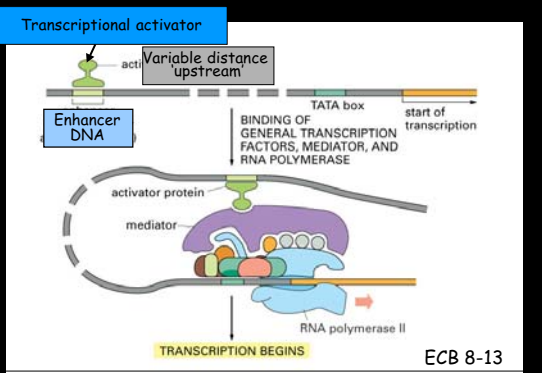
Regulation of transcription in eukaryotes

Assembly of the initiation complex is inefficient

Additional activator proteins are required for high rates of transcription

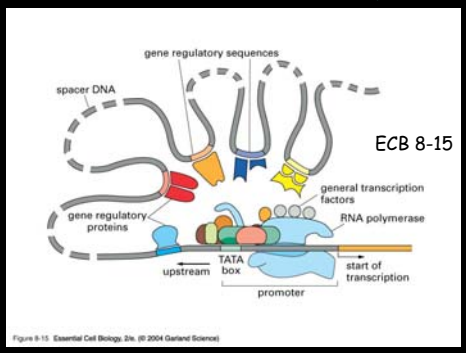
These activator proteins bind to DNA sequences (enhancers) that can be thousands of nucleotides away from gene

Enhancer + activator stimulates transcription



ECB 8-13

Combinatorial control of transcription



ECB 8-15

Lecture 11

Eukaryotic Transcription

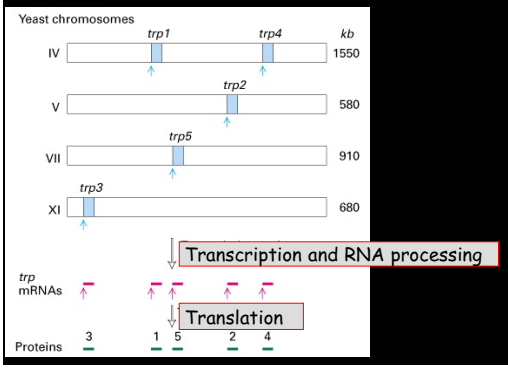
***Gene Organization**

RNA Processing

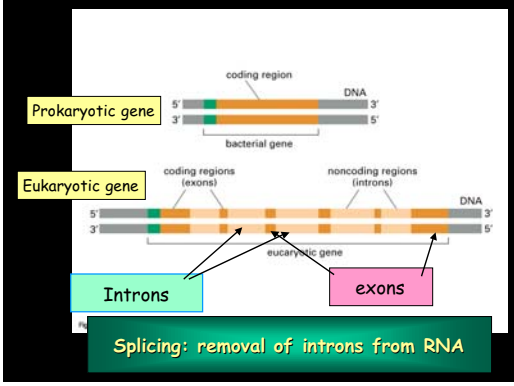
- 5' cap
- 3' polyadenylation
- splicing

Translation

Eukaryotic gene organization - no operons



Eukaryotic genes contain introns and exons



Splicing: removal of introns from RNA

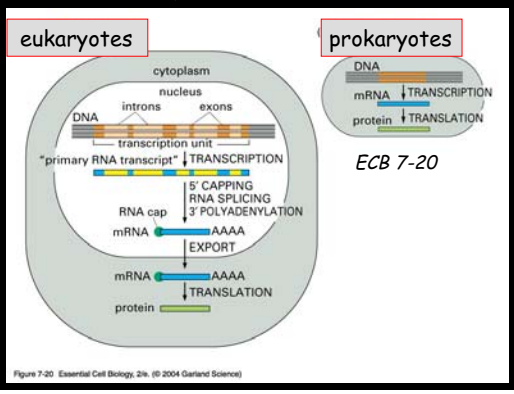
Lecture 11

Eukaryotic Transcription

- Gene Organization
- *RNA Processing
  - 5' cap
  - 3' polyadenylation
  - splicing

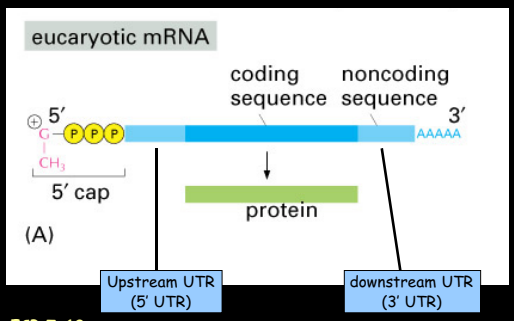
Translation

RNA processing; overview



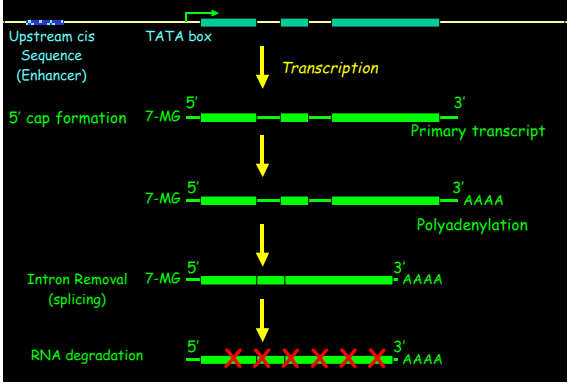
ECB 7-20

Mature mRNA in a eukaryotic cell

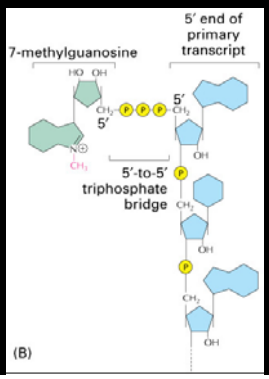


ECB 7-12

The life of a eukaryotic mRNA



5′ cap = 7 me G bonded 5′ to 5′



Only on mRNA

ECB 7-12B

mRNA cleavage and polyadenylation

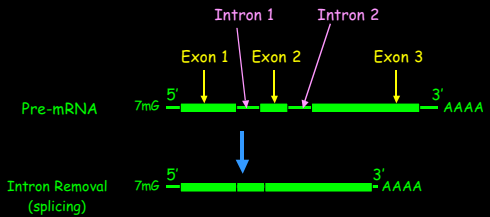
Cleavage and polyadenylation

Sequence on mRNA signaling cleavage downstream

AAUAAA

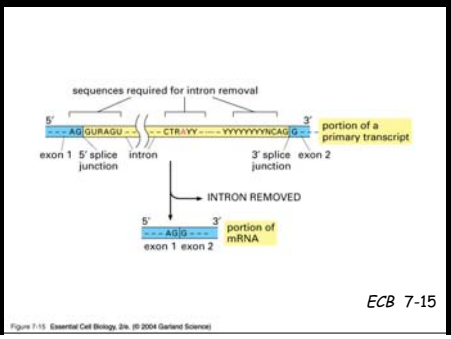
~~AAAAAA~~AA AAAAAAAAAAAAA

Called a poly-A tail; added by poly A polymerase



Splicing following addition of 5′ cap and 3′ poly A
(still in nucleus)

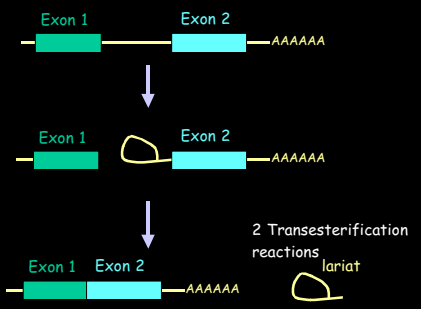
Question: how are intron sequences distinguished from exon sequences?

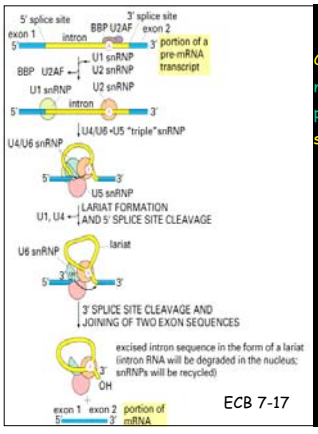


ECB 7-15

Figure 7-15 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Intron removal involves formation of lariat





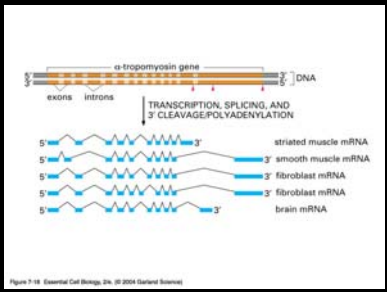
ECB 7-17

Splicosome

Complex containing snRNPs (small nuclear ribonucleoprotein particles); U1, U2, U5, and U4/U6 snRNPs contain RNA and proteins

See movie on ECB CD

Alternative splicing example = tropomyosin mRNA



Modular design of a gene allows mixing and matching of domains

Half life of mRNAs in selected cells

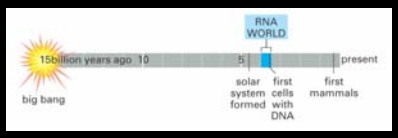
Cell Type	Cell Generation time	Average mRNA half-life	mRNA half-life range
E. coli	20 - 60 min.	3-5 min	2 - 10 min
Yeast (*Saccharomyces cerevisiae*)	3 Hrs	22 min	4 - 40 min
Human or Rodent cells (cultured)	16 - 24 hrs	10 Hrs	20 min - 24 hrs

Poly A tail protects mRNA from degradation; loss of tail results in exonuclease cleavage and destruction of mRNA

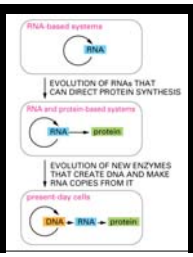
Regulation of Gene Expression:

1. Transcriptional Regulation (inducible and repressible operons, combinatorial regulation in eukaryotes)
2. RNA Splicing (alternative splicing, tropomyosin)
3. RNA Stability
4. Protein Synthesis (Translational regulation) won't discuss

Primitive cells thought to be RNA based



ECB 7-38

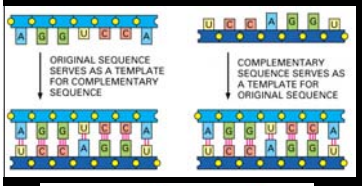


In this earliest cell RNA must have been able to replicate itself

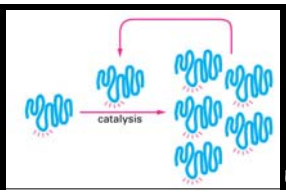
ECB 7-42

Evidence for RNA world

ECB 7-39



RNA can serve as a template for it own replication



RNA can serve as an enzyme (ribozyme) and perhaps could catalyze own synthesis

ECB 7-41