

Advanced Cell Biology. Lecture 18

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Outline

Questions and answers

From DNA to RNA

RNA export

From RNA to protein

Genetic code

Outline

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Previous final question: the answer

How RNA polymerase recognizes the “proper” strand of DNA?

Previous final question: the answer

How RNA polymerase recognizes the “proper” strand of DNA?

- ▶ Asymmetric promoter

Hoskins et al.: spliceosome visualization

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RESEARCH ARTICLE

Ordered and Dynamic Assembly of Single Spliceosomes

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ABSTRACT

The spliceosome is the complex macromolecular machine responsible for removing introns from precursors to messenger RNAs (pre-mRNAs). We combined yeast genetic engineering, chemical biology, and multiwavelength fluorescence microscopy to follow assembly of single spliceosomes in real time in whole-cell extracts. We find that individual spliceosomal subcomplexes associate with pre-mRNA sequentially via an ordered pathway to yield functional spliceosomes and that association of every subcomplex is reversible. Further, early subcomplex binding events do not fully commit a pre-mRNA to splicing; rather, commitment increases as assembly proceeds. These findings have important implications for the regulation of alternative splicing. This experimental strategy should prove widely useful for mechanistic analysis of other macromolecular machines in environments approaching the complexity of living cells.

Spliceosomes without ATP movie

Spliceosomes with ATP movie

From DNA to RNA

RNA export

Selective export of RNA

- ▶ Nuclear pore will allow only “ready” mRNA to be exported into cytoplasm
- ▶ That will not allow the unprocessed RNA to be translated into protein

RNA export from nucleus

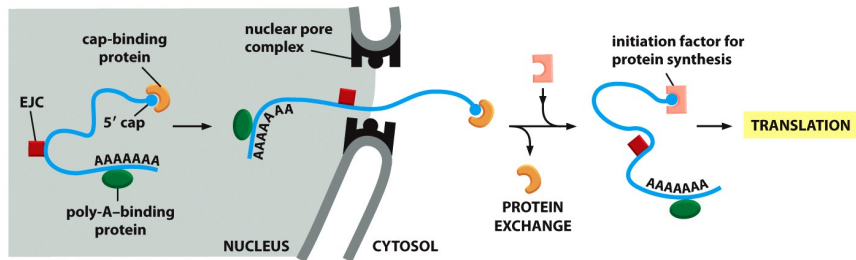


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Degrading of RNA

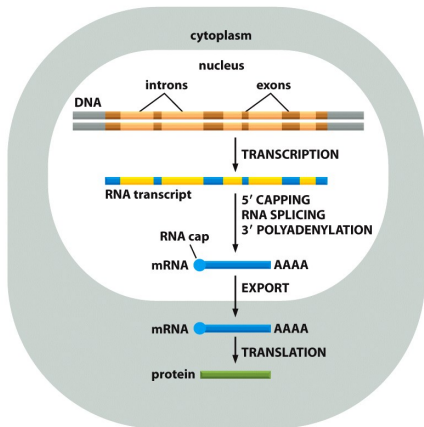
- ▶ Bacterial mRNAs live ≈ 3 min
- ▶ In eukaryotes, mRNA lives longer, and the lifespan depends on how RNA was processed

Origin of introns

- ▶ Introns will increase the flexibility of genome, but lower the speed of cell replication
- ▶ It is therefore possible that prokaryotes are secondary intronless
- ▶ Introns were found in some Archaea

Transcription: eukaryotes vs. prokaryotes

(A) EUCARYOTES



(B) PROCARYOTES

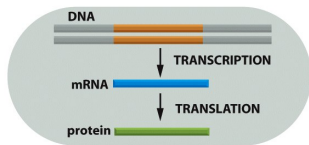


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From RNA to protein

Genetic code

Redundance of genetic code

- ▶ Translation is decoding of codons into amino acids
- ▶ Four nucleotides may encode 64 amino acids
- ▶ As a result, the 3rd position may vary without change of amino acid

Deciphering the code

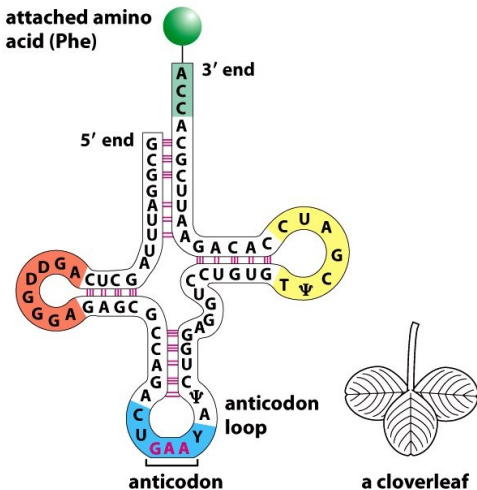
- ▶ First, artificial translation systems (taken from *E. coli* RNAs and enzymes) were created
- ▶ In 1960s, Nirenberg and Leder discovered that trinucleotides may be attached to ribosome and activate the transfer RNAs
- ▶ They produced big amounts of same codons and then analyzed complexes of ribosome-trinucleotide-aminoacyl-tRNAs.

Transfer RNAs

- ▶ “Cloverleaf” of ≈ 80 nucleotides
- ▶ Three loops and (optionally) amino acid on 3' end (the arm, which also contains specific nucleotides)
- ▶ Top loop contain anticodon
- ▶ There are 31 kinds of tRNAs because many of them can tolerate a mismatch in 3rd position (wobble)

- └ From RNA to protein
- └ Genetic code

Cloverleaf and tRNA



Transfer RNA movie

Linkage between amino acid and tRNA

- ▶ Aminoacyl-tRNA-synthetase is specific to every kind of amino acid
- ▶ Aminoacyl-tRNA-synthetase can recognize tRNA arm and anticodon
- ▶ Bond between amino acid and tRNA is highly-energetic

aminoacyl-tRNA-synthetase

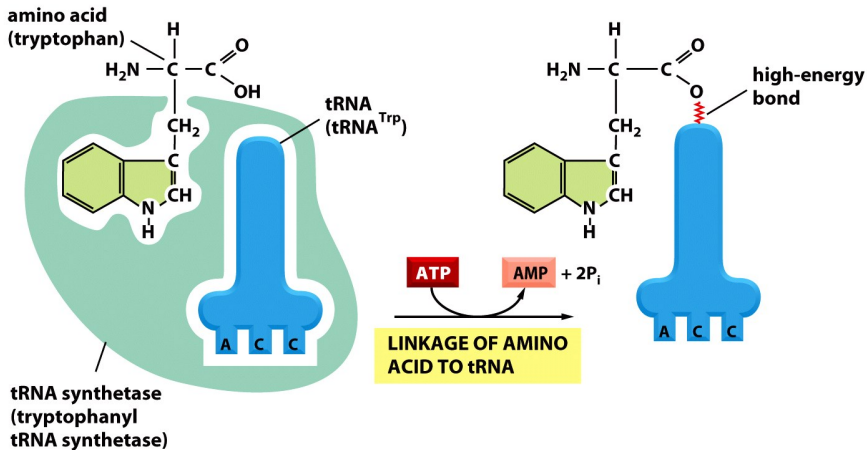


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Ribosome

- ▶ Prokaryotes: 70S ribosomes
 - ▶ Small subunit: 30S
 - ▶ 16S RNA
 - ▶ 21 proteins
 - ▶ Large subunit: 50S
 - ▶ 5S RNA + 23S RNA
 - ▶ 34 proteins
- ▶ Eukaryotes: 80S ribosomes
 - ▶ Small subunit: 40S
 - ▶ 18S RNA
 - ▶ 33 proteins
 - ▶ Large subunit: 60S
 - ▶ 5S RNA + 28S RNA + 5.8S RNA
 - ▶ 49 proteins

Structure of eukaryotic ribosome

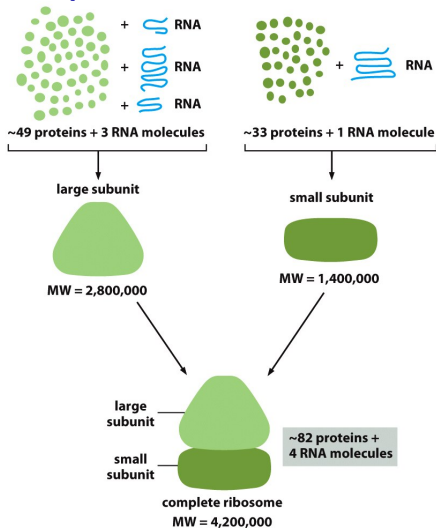


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Ribozyme

- ▶ Has three binding sites, A, P and E (for aminoacyl-tRNA, peptidyl-tRNA and exit)
- ▶ RNAs, not proteins are responsible for ribosome conformation and activity
- ▶ Ribosome is a RNA-enzyme, ribozyme

└ From RNA to protein

└ Genetic code

Final question (1 point)

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What is bigger, eukaryotic or prokaryotic ribosome?

Summary

- ▶ Transcription processes are seriously different between prokaryotes and eukaryotes
- ▶ Transcription factors, RNA splicing, and exporting from the nucleus are key features of eukaryotic transcription
- ▶ For 61 meaningful codons, there are 31 tRNAs and 20 amino acids
- ▶ Ribosome is a ribozyme

For Further Reading



A. Shipunov.

Advanced Cell Biology [Electronic resource].

2011—onwards.

Mode of access: [http:](http://)

[//ashipunov.info/shipunov/school/biol_250](http://ashipunov.info/shipunov/school/biol_250)



B. Alberts et al.

Essential Cell Biology. 3rd edition.

Garland Science, 2009.

Chapter 7.