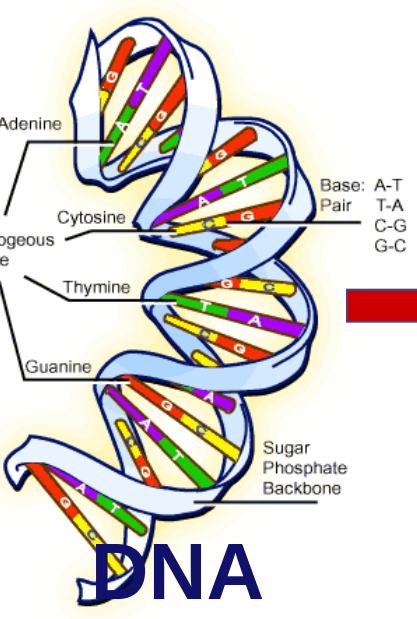
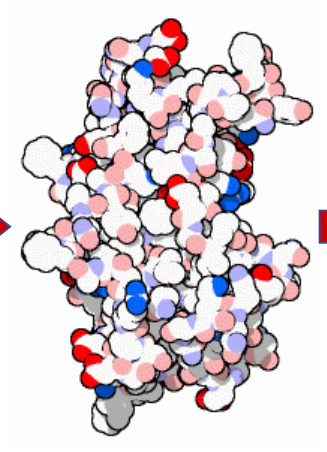
What do genes code for?

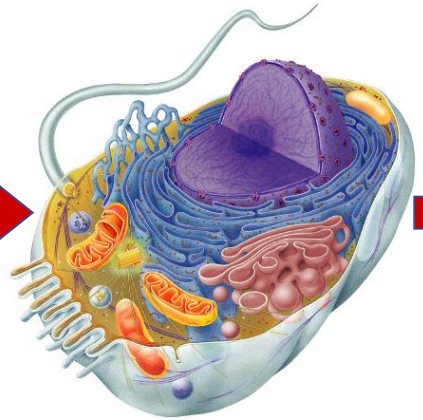
- How does DNA code for cells & bodies?
  - how are cells and bodies made from the instructions in DNA



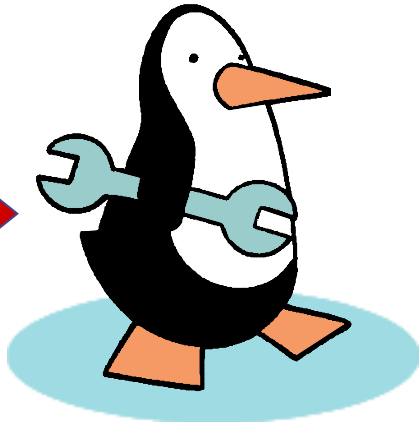
DNA



proteins

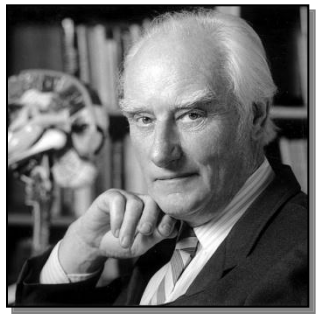


cells

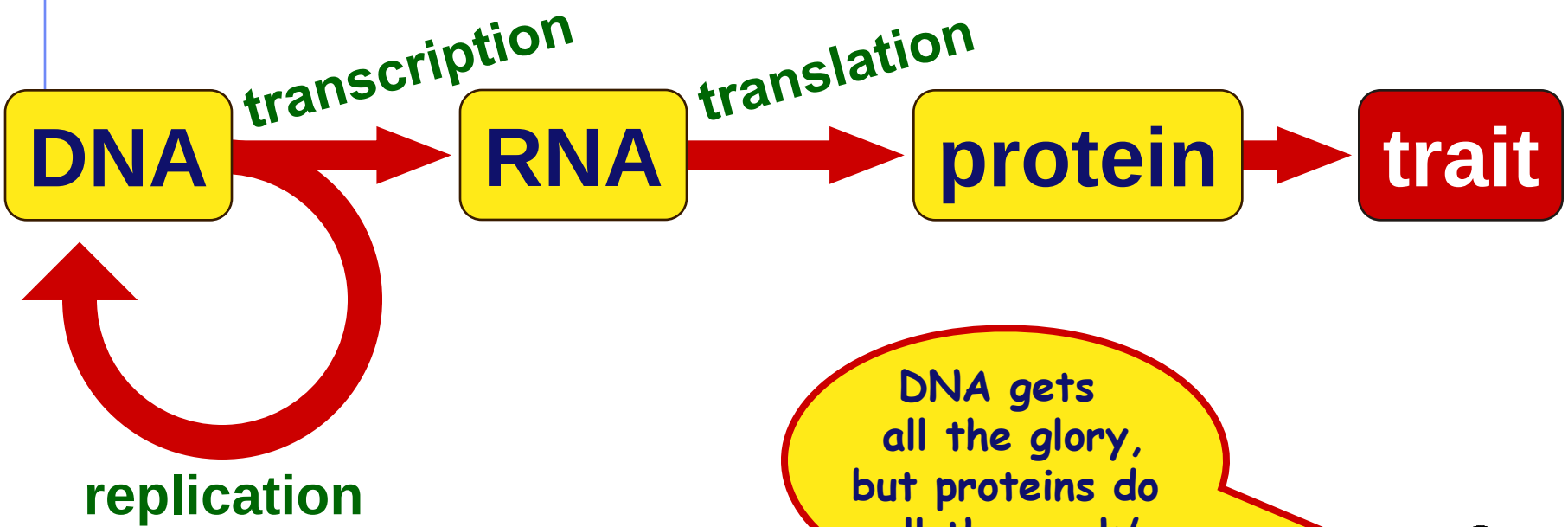


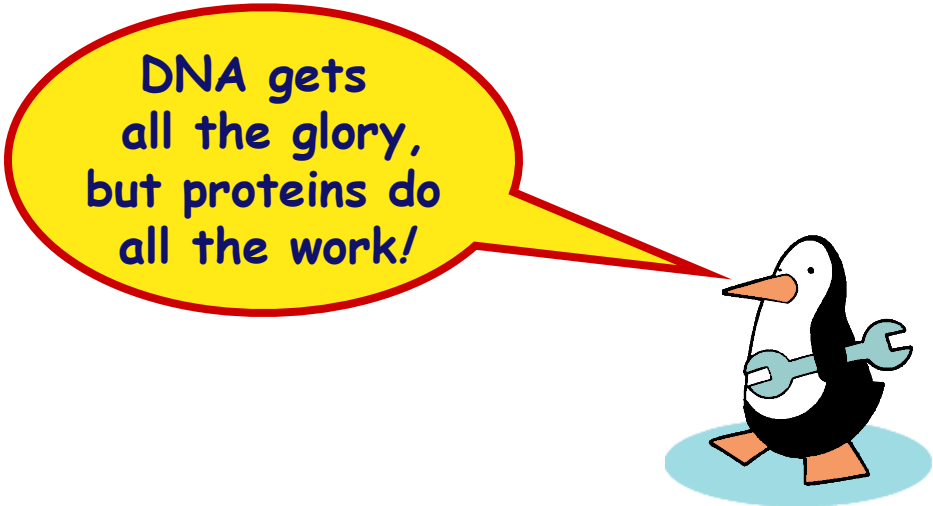
bodies

The “Central Dogma”



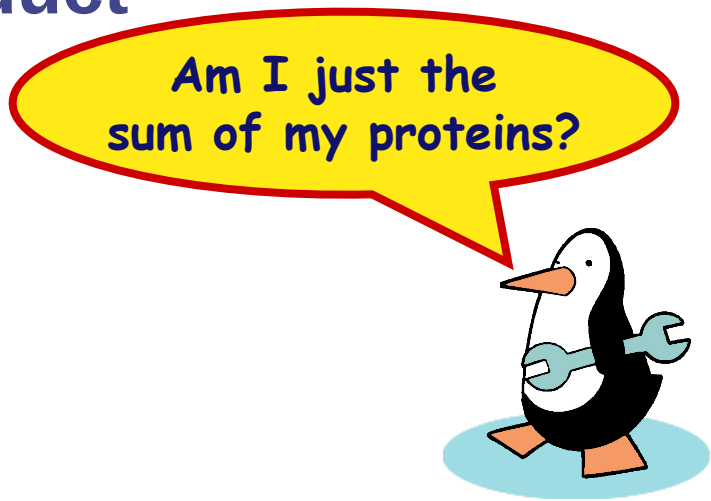
- Flow of genetic information in a cell
  - How do we move information from DNA to proteins?

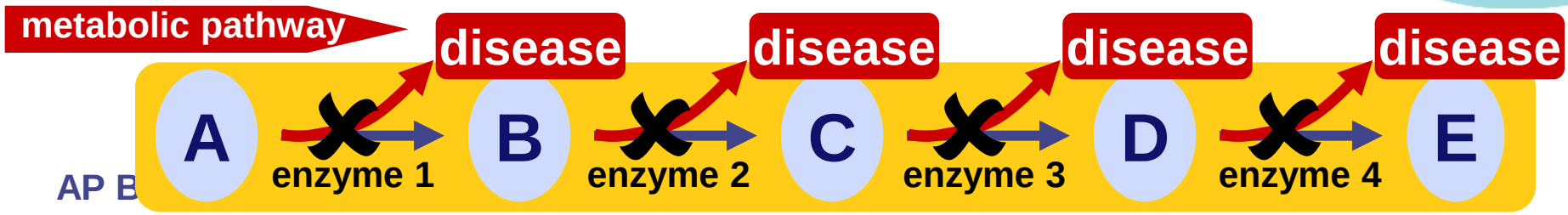




Metabolism taught us about genes

- Inheritance of metabolic diseases
  - suggested that genes coded for enzymes
  - each disease (phenotype) is caused by non-functional gene product
    - lack of an enzyme
    - Tay sachs
    - PKU (phenylketonuria)
    - albinism

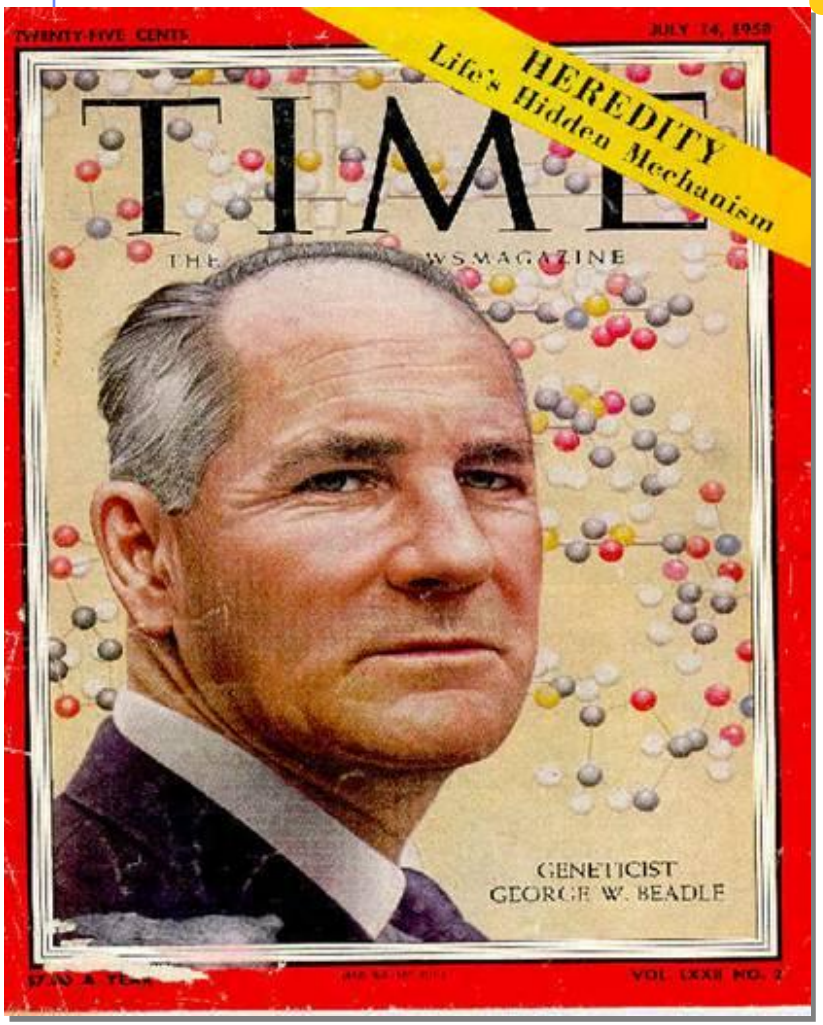


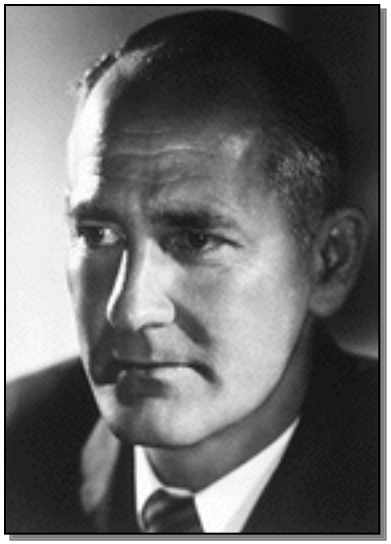


Beadle & Tatum

1941 | 1958

one gene : one enzyme hypothesis



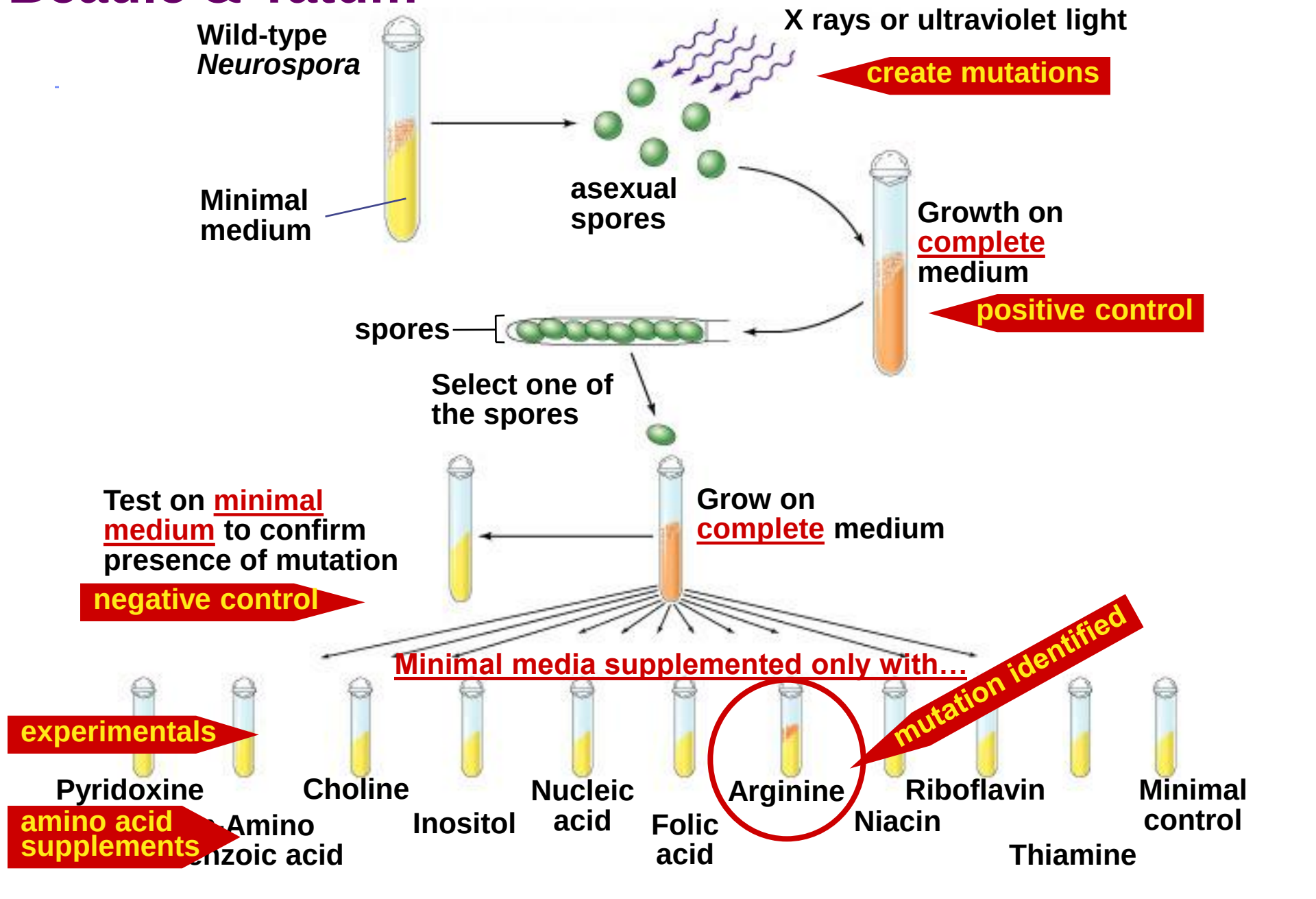


George Beadle

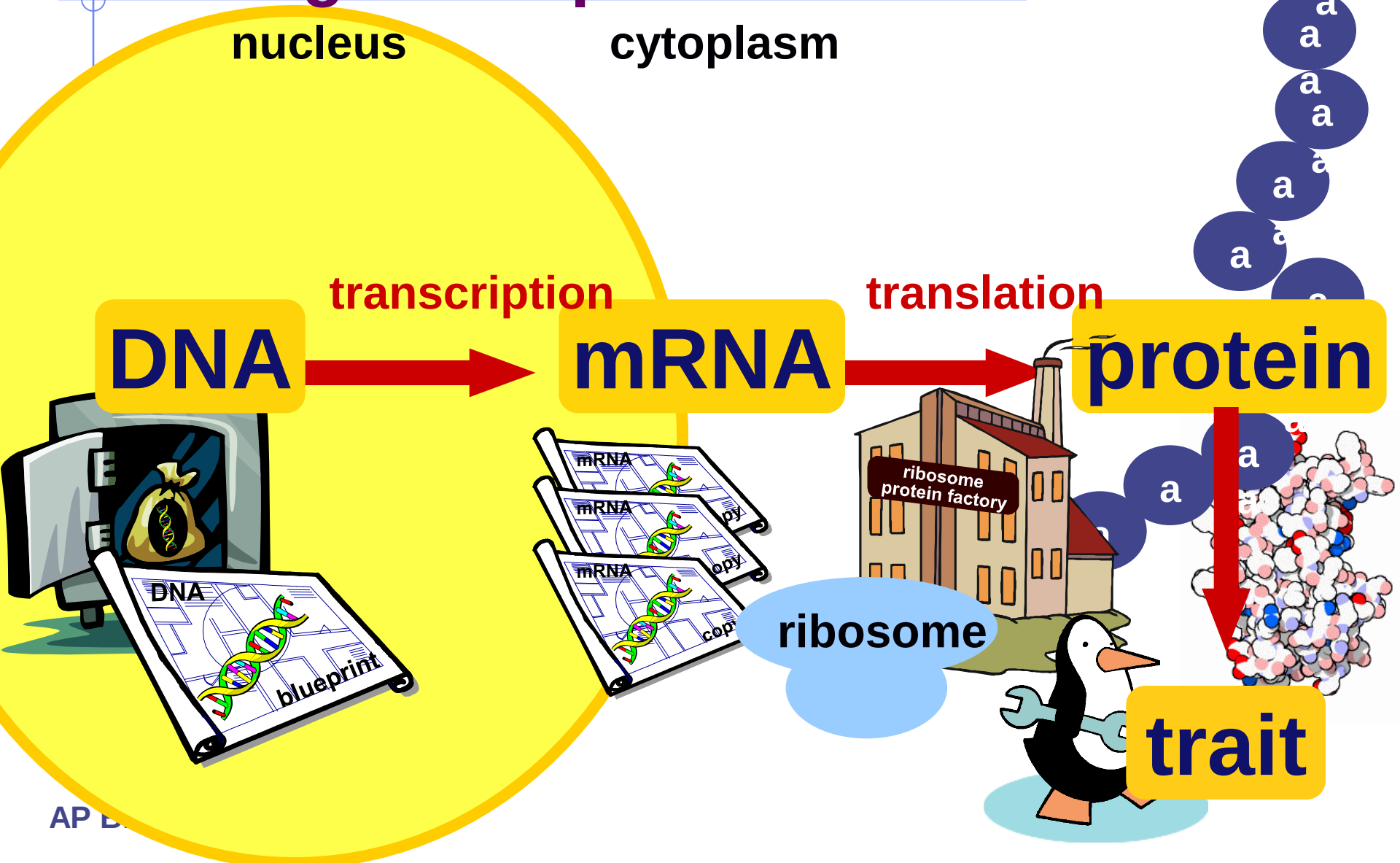
Edward Tatum

"for their discovery that genes act by regulating definite chemical events"

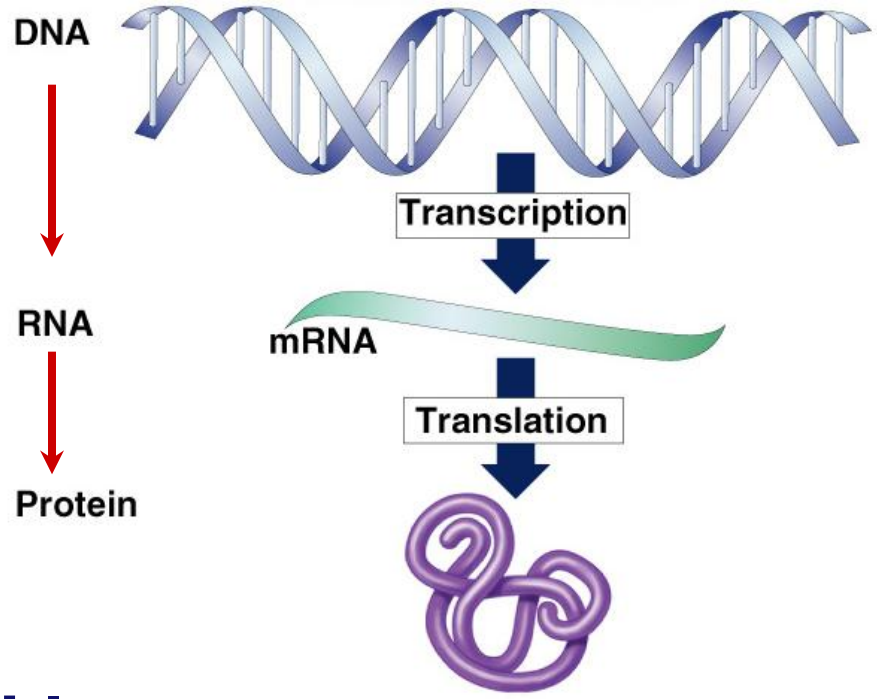
Beadle & Tatum



From gene to protein



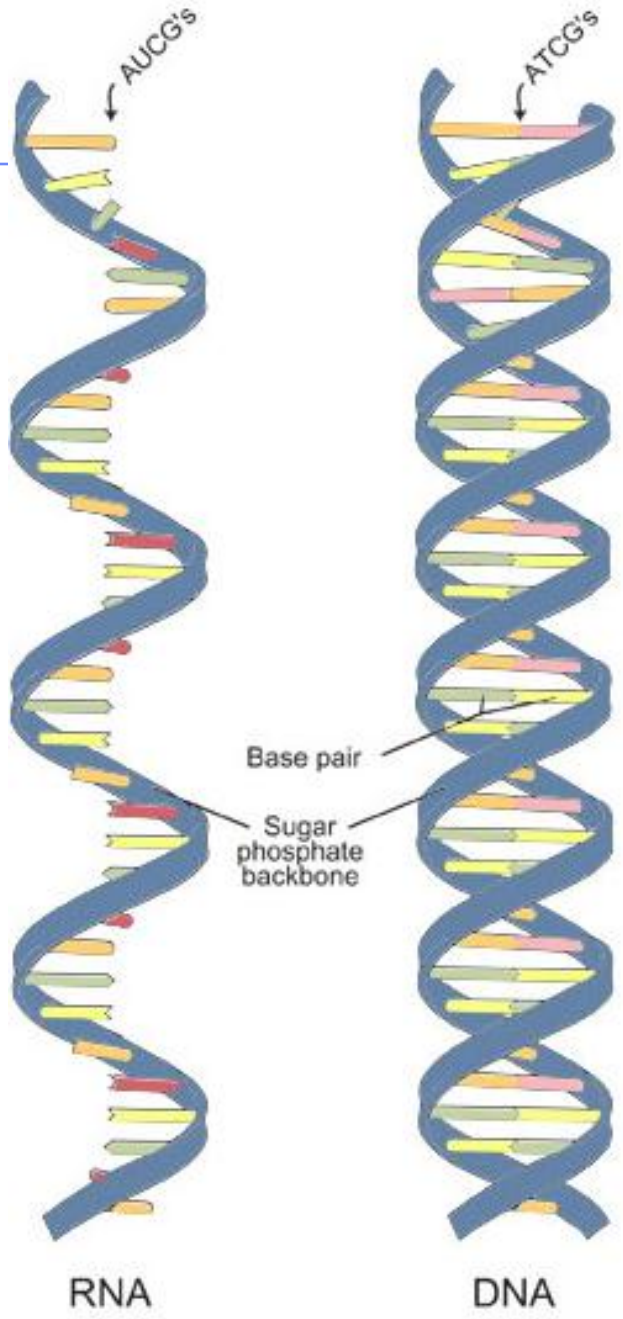
Transcription



from
DNA nucleic acid language
to
RNA nucleic acid language

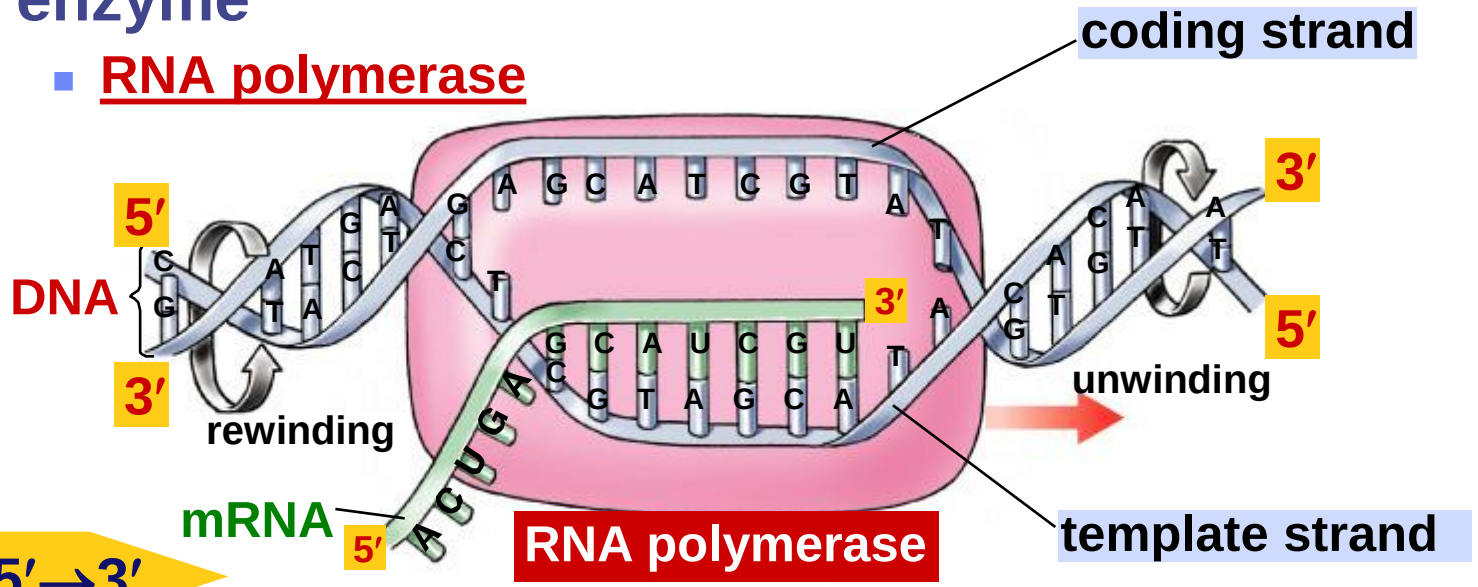
RNA

- ribose sugar
- N-bases
  - uracil instead of thymine
  - U : A
  - C : G
- single stranded
- lots of RNAs
  - mRNA, tRNA, rRNA, siRNA…



Transcription

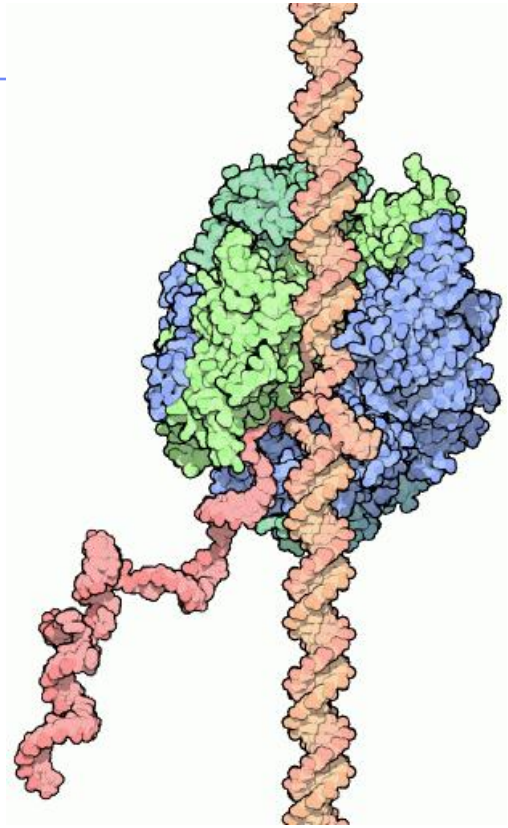
- **Making mRNA**
  - transcribed DNA strand = <u>template strand</u>
  - untranscribed DNA strand = <u>coding strand</u>
    - same sequence as RNA
  - synthesis of complementary RNA strand
    - transcription bubble
  - enzyme
    - <u>RNA polymerase</u>



build RNA 5′→3′

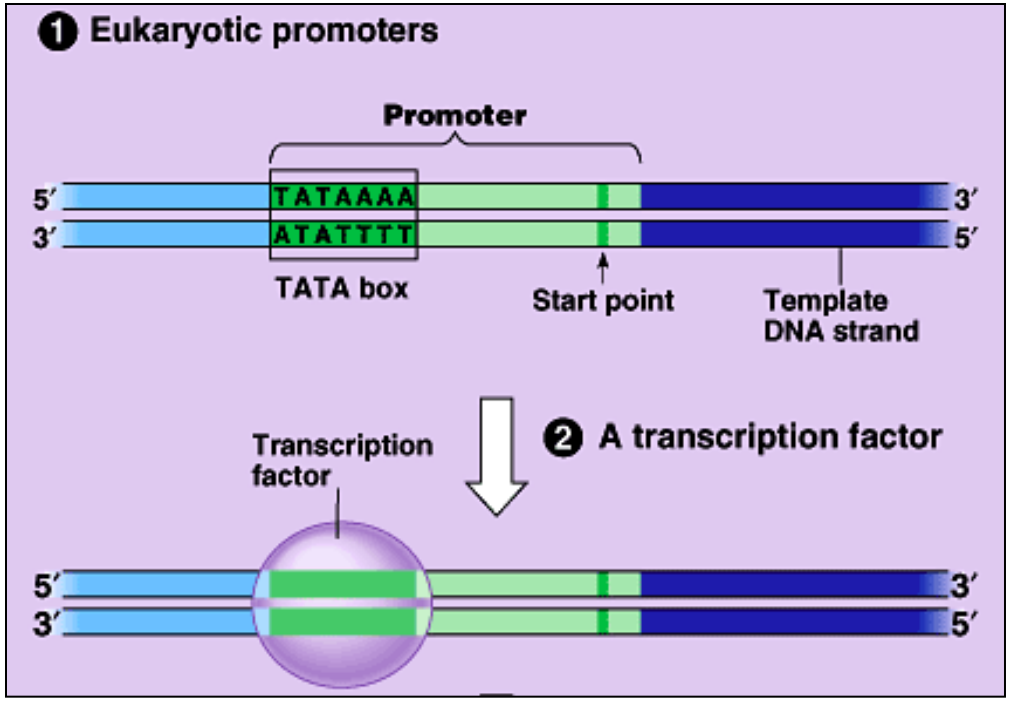
RNA polymerases

- 3 RNA polymerase enzymes
  - RNA polymerase 1
    - only transcribes rRNA genes
    - makes ribosomes
  - **RNA polymerase 2**
    - transcribes genes into mRNA
  - RNA polymerase 3
    - only transcribes tRNA genes
  - each has a specific promoter sequence it recognizes



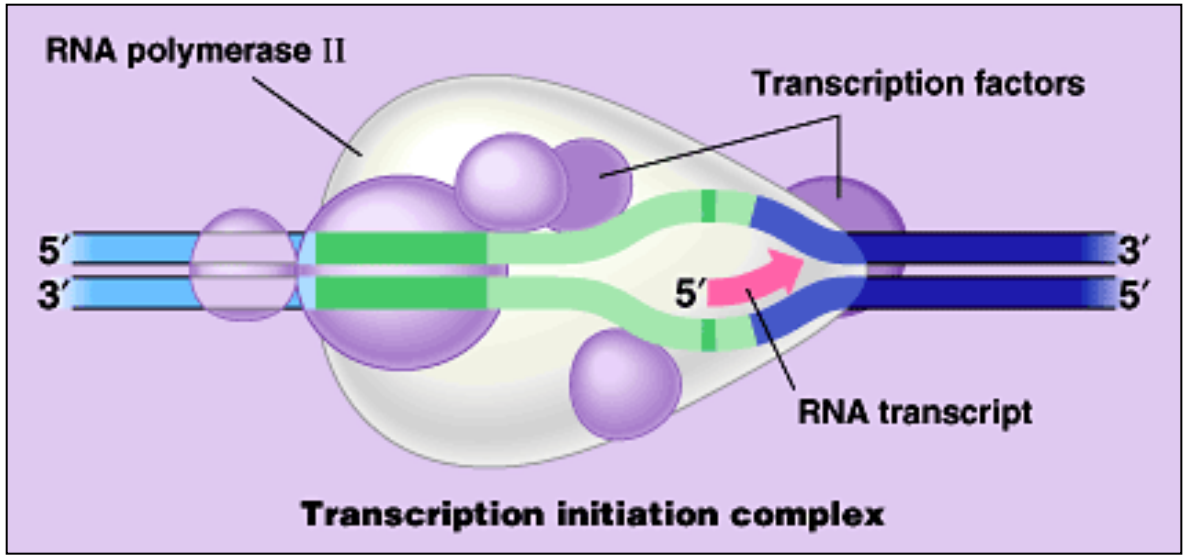
Which gene is read?

- **Promoter region**
  - binding site before beginning of gene
  - **TATA box binding site**
  - binding site for RNA polymerase & transcription factors
- **Enhancer region**
  - binding site far upstream of gene
    - turns transcription on HIGH



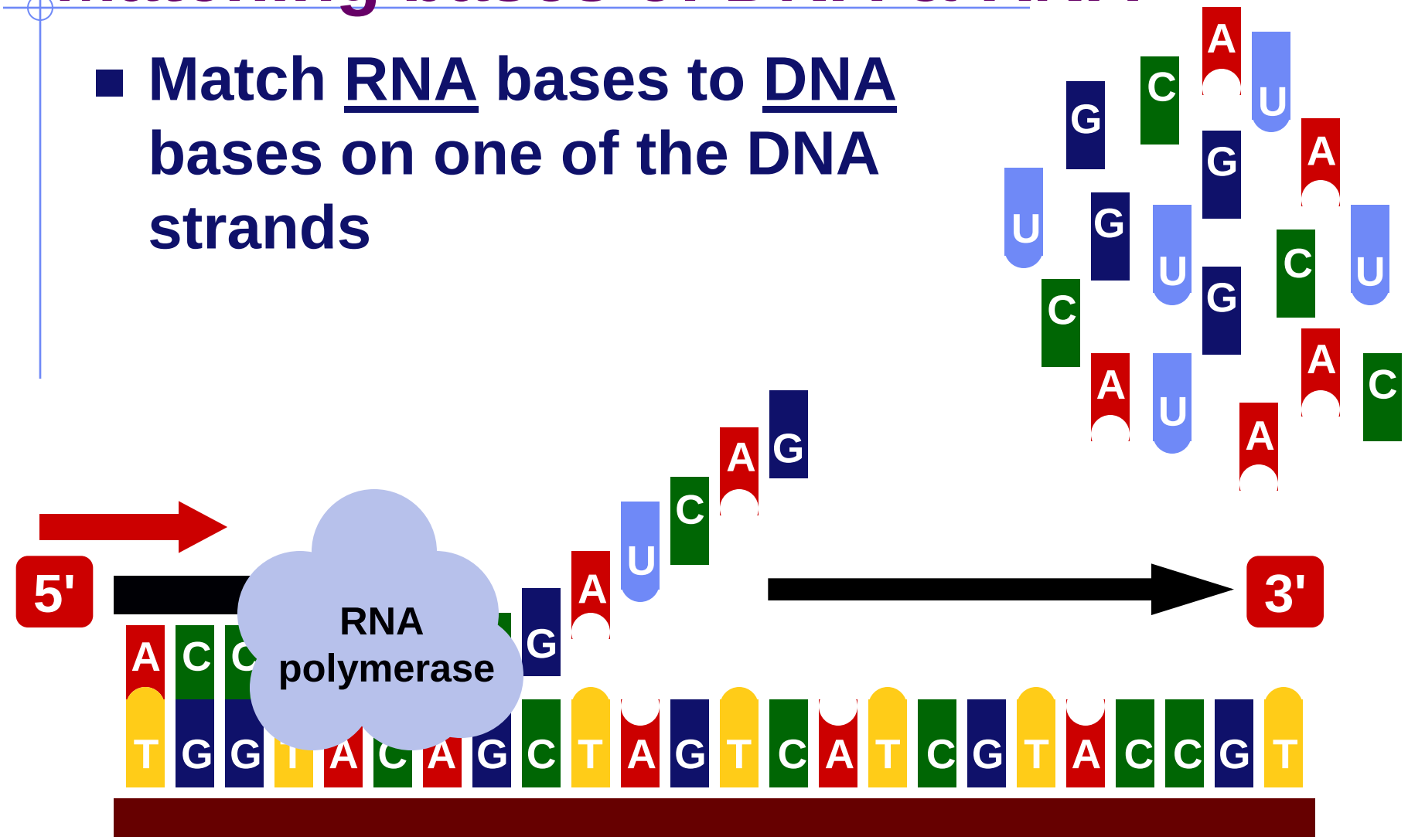
Transcription Factors

- **Initiation complex**
  - **transcription factors** bind to **promoter region**
    - suite of proteins which bind to DNA
    - hormones?
    - turn on or off transcription
  - trigger the binding of RNA polymerase to DNA



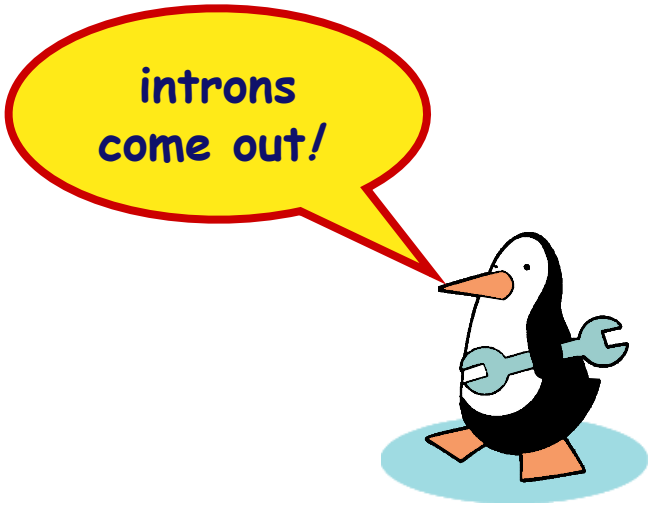
Matching bases of DNA & RNA

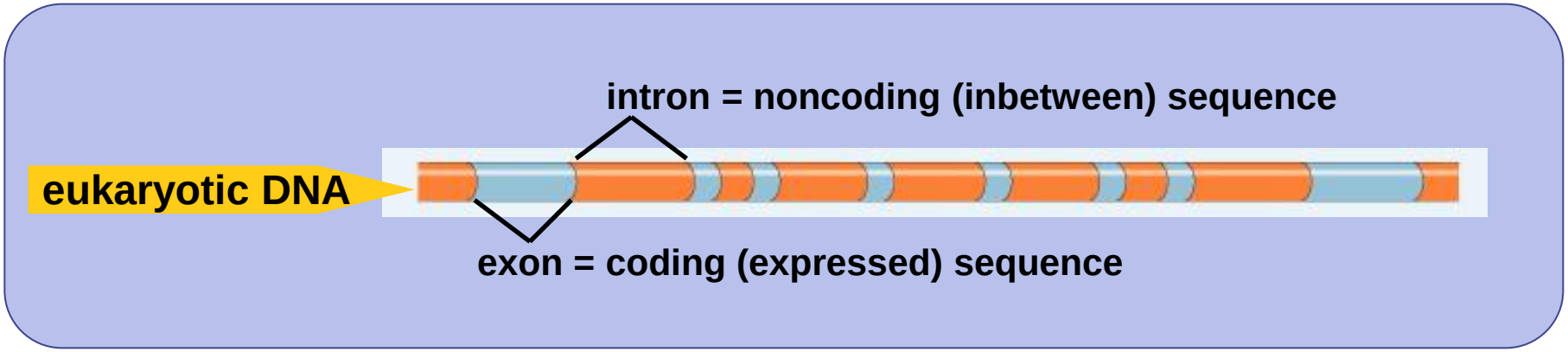
- Match RNA bases to DNA bases on one of the DNA strands



Eukaryotic genes have junk!

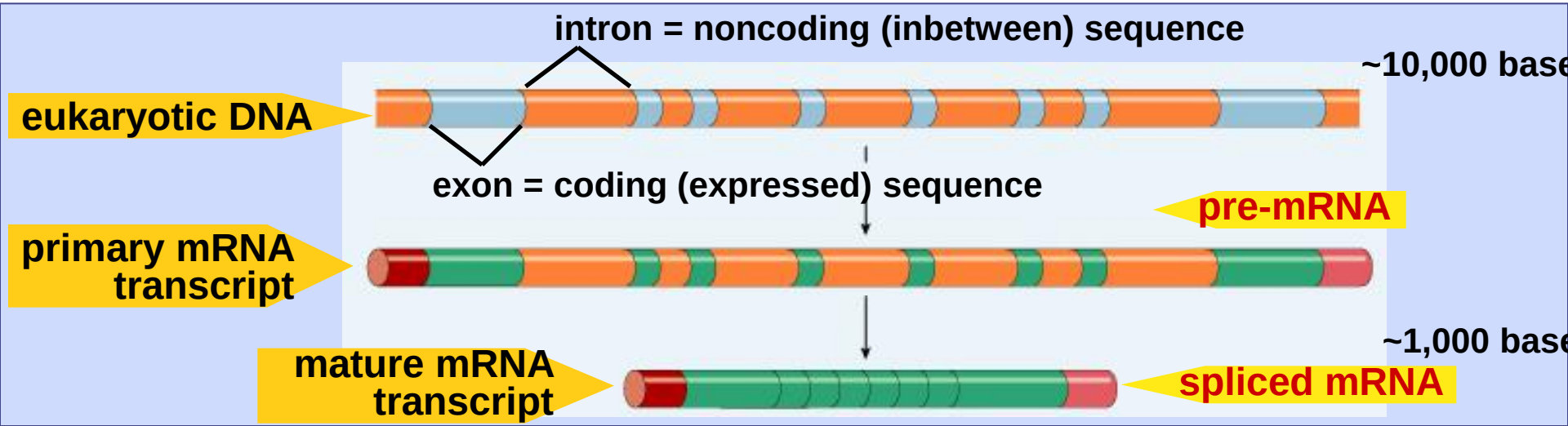
- Eukaryotic genes are not continuous
  - exons = the real gene
    - expressed / coding DNA
  - introns = the junk
    - inbetween sequence





mRNA splicing

- **Post-transcriptional processing**
  - eukaryotic mRNA needs work after transcription
  - primary transcript = pre-mRNA
  - mRNA splicing
    - edit out introns
  - make mature mRNA transcript

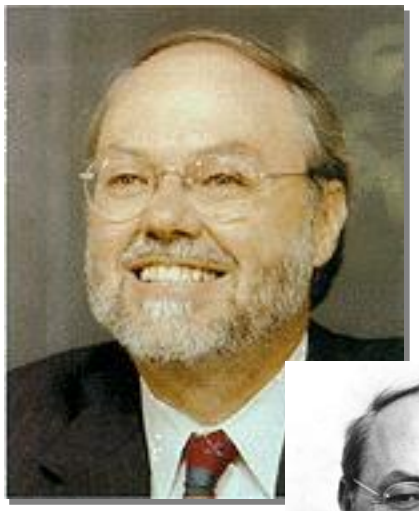


Discovery of exons/introns

1977 | 1993

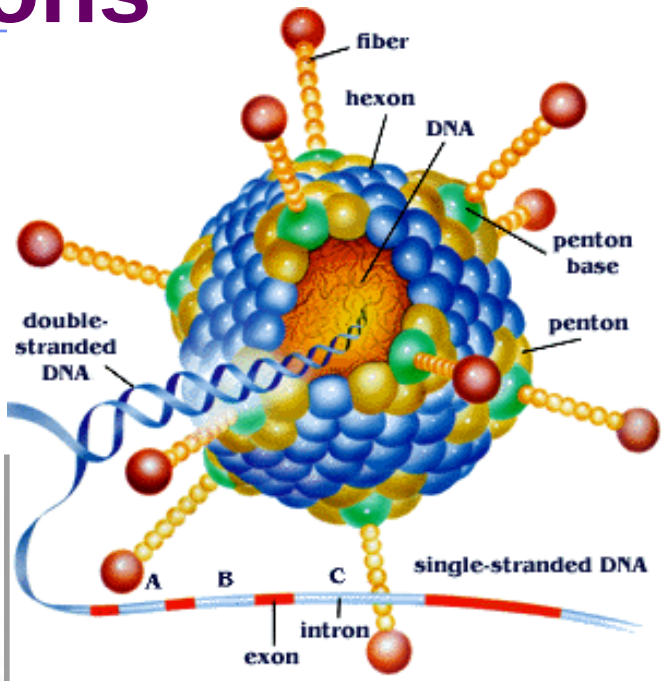
Richard Roberts

CSHL



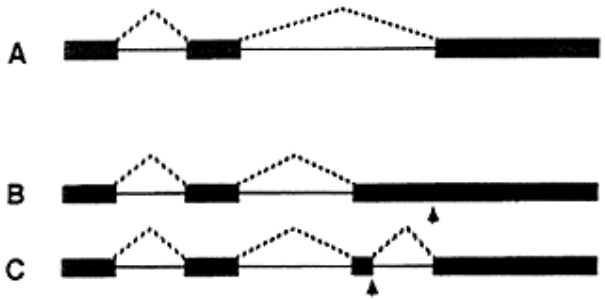
Philip Sharp

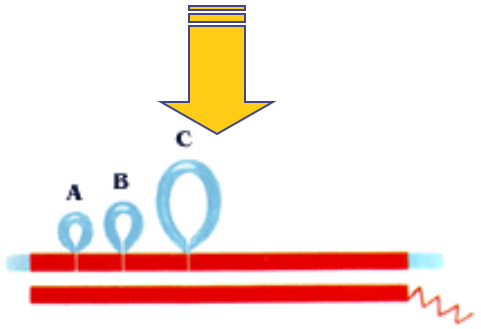
MIT



adenovirus

common cold





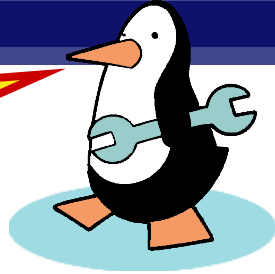
Splicing must be accurate

- No room for mistakes!
  - a single base added or lost throws off the reading frame

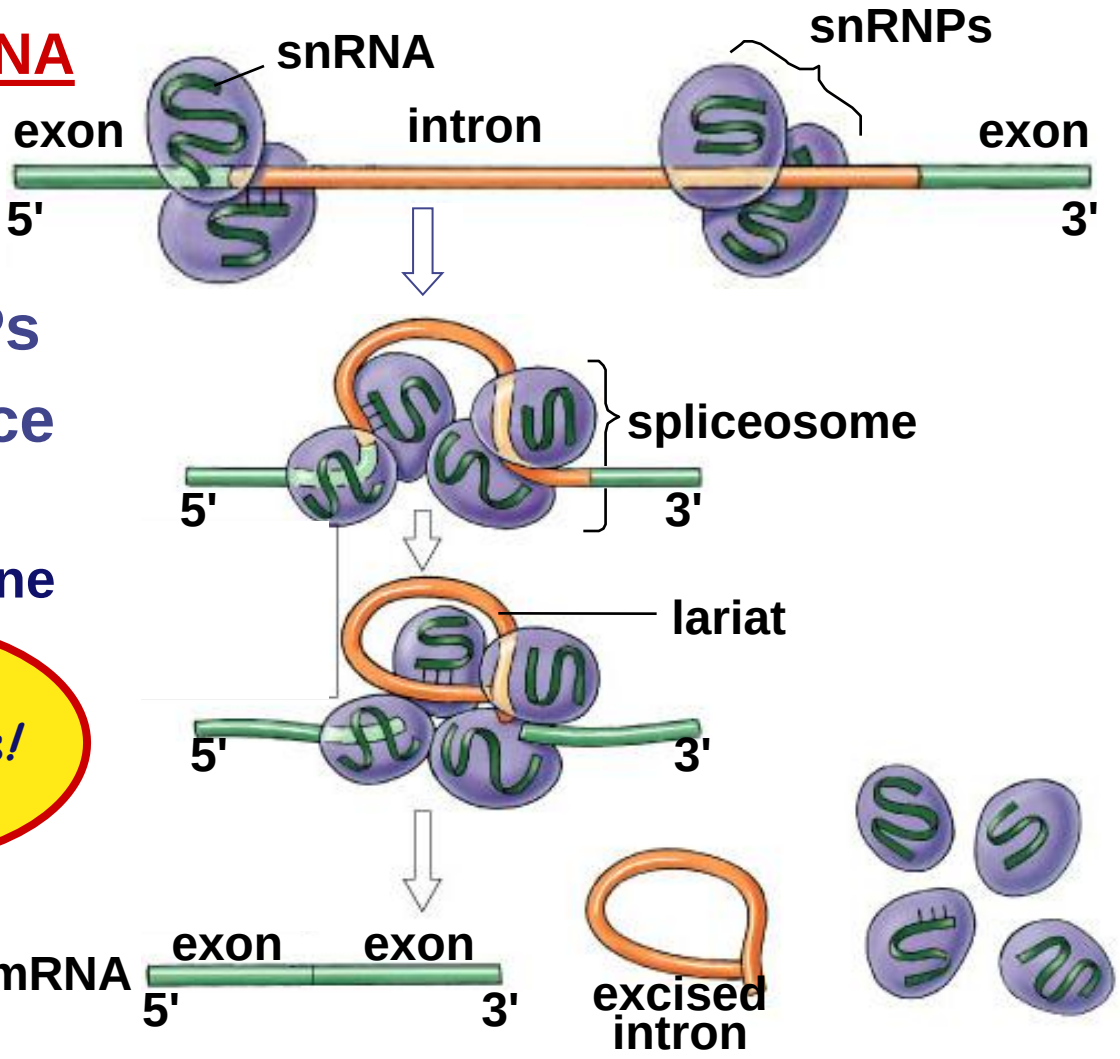
AUGCGGCTATGGGUCCGAUAAGGGCCAU
AUGCGGUCCGAUAAGGGCCAU
AUG|CGG|UCC|GAU|AAG|GGC|CAU
Met|Arg|Ser|Asp|Lys|Gly|His

AUGCGGCTATGGGUCCGAUAAGGGCCAU
AUGCGGGUCCGAUAAGGGCCAU
AUG|CGG|GUC|CGA|UAA|GGG|CCA|U
Met|Arg|Val|Arg|STOP|

RNA splicing enzym



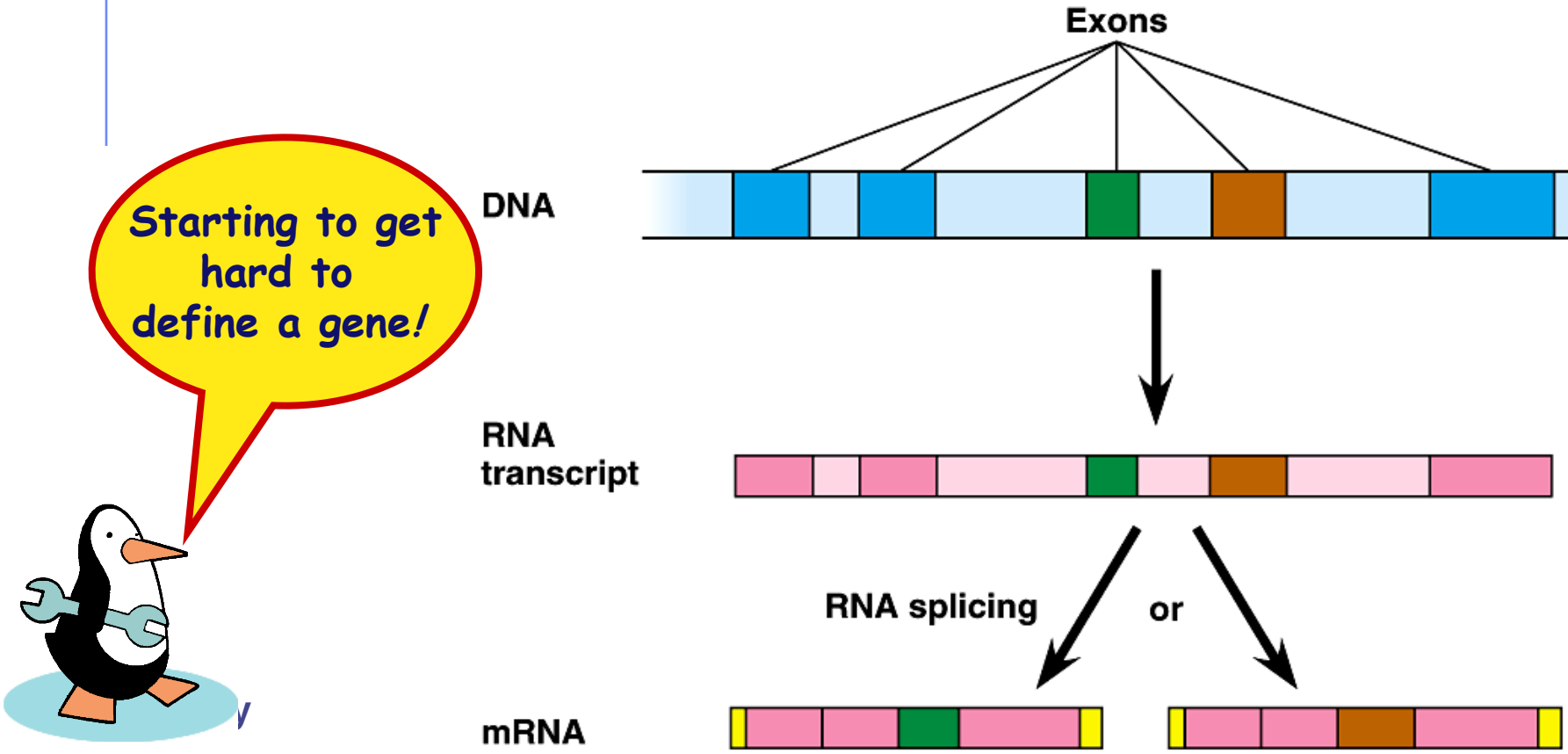
- **snRNPs**
  - small nuclear RNA
  - proteins
- **Spliceosome**
  - several snRNPs
  - recognize splice site sequence
    - cut & paste gene



mRNA Splicing

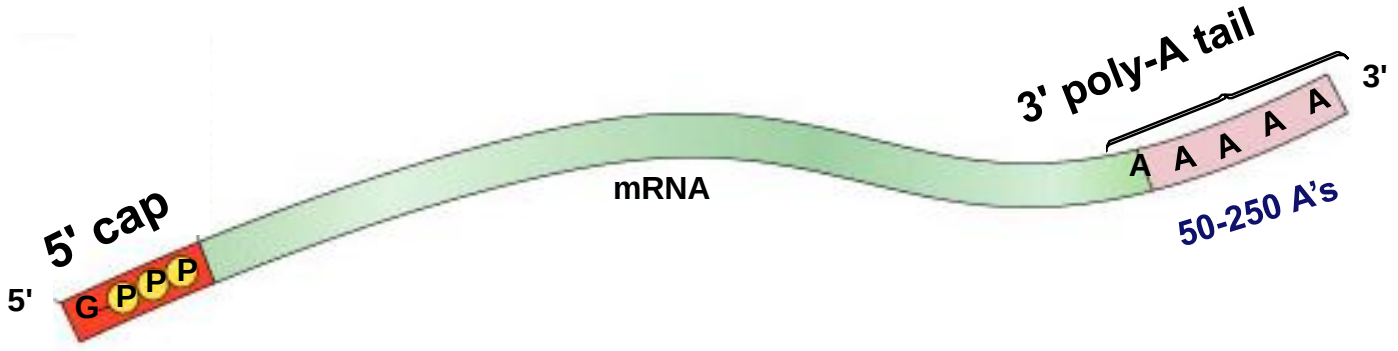
Alternative splicing

- Alternative mRNAs produced from same gene
  - when is an intron not an intron…
  - different segments treated as exons



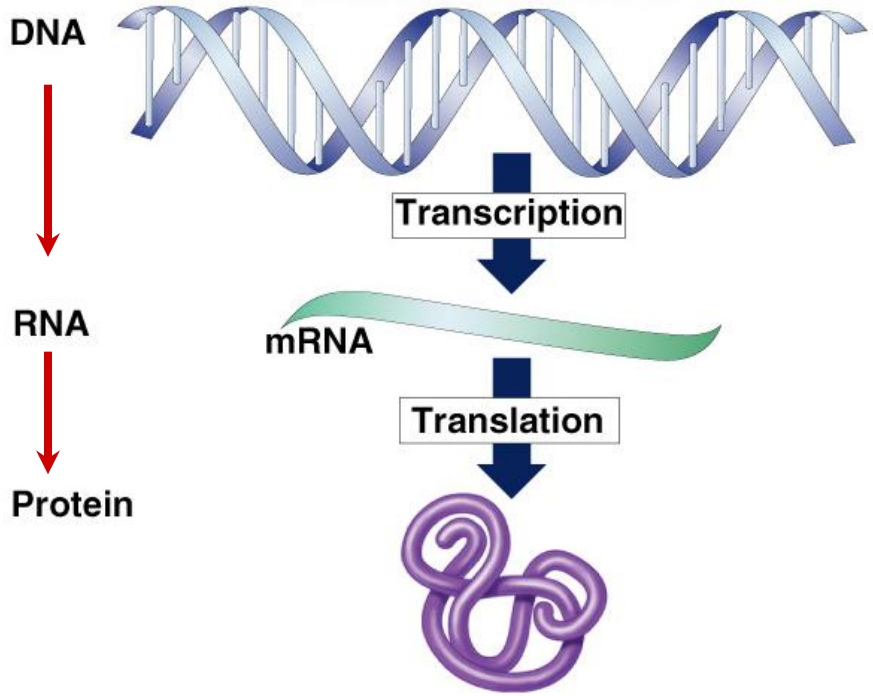
More post-transcriptional processing

- Need to protect mRNA on its trip from nucleus to cytoplasm
  - enzymes in cytoplasm attack mRNA
    - protect the ends of the molecule
    - add 5′ GTP cap
    - add poly-A tail
      - longer tail, mRNA lasts longer: produces more protein



Transcription Review

Translation

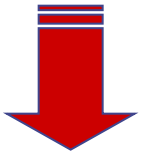


from
nucleic acid language
to
amino acid language

How does mRNA code for proteins?

DNA TACGCACATTTACGTACGCGG

4 ATCG



mRNA UGCGUGUAAAUGCAUGCGCC

4 AUCG

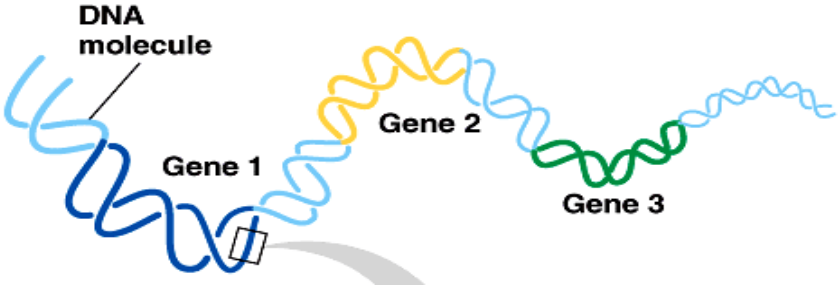
protein

20

Met Arg Val Asn Ala Cys Ala

How can you code for 20 amino acids with only 4 nucleotide bases (A,U,G,C)?

mRNA codes for proteins in triplets



DNA TACGCACATTTACGTACGCGG

codon

mRNA UGCGUGUAAAUGCAUGCGCC

protein Met Arg Val Asn Ala Cys Ala

Cracking the code

1960 | 1968
Nirenberg & Khorana

- **Crick**
  - **determined 3-letter (triplet) codon system**

WHYDIDTHEREDBATEATTHEFATRAT

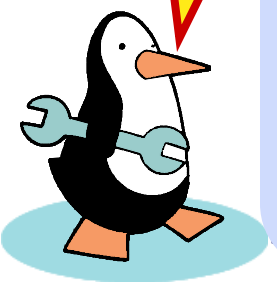
- **Nirenberg (47) & Khorana (17)**
  - **determined mRNA–amino acid match**
  - **added fabricated mRNA to test tube of ribosomes, tRNA & amino acids**
    - **created artificial UUUUU… mRNA**
    - **found that UUU coded for phenylalanine**

The code

- Code for ALL life!
  - strongest support for a common origin for all life
- Code is redundant
  - several codons for each amino acid
  - 3rd base "wobble"

First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

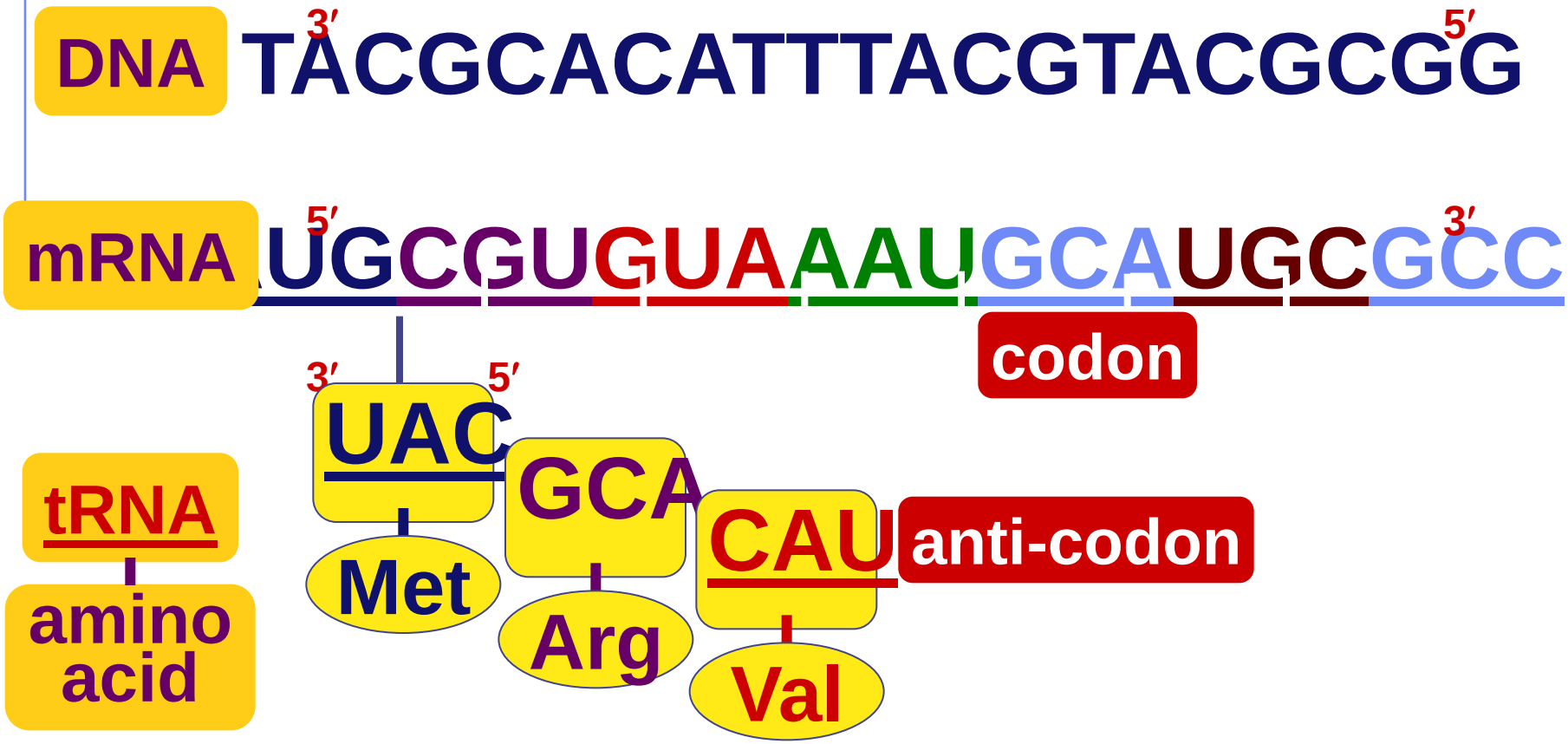
Why is the wobble good?



- Start codon
  - AUG
  - methionine
- Stop codons
  - UGA, UAA, UAG

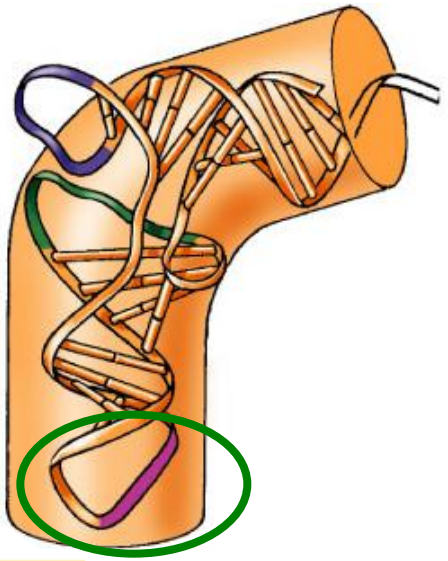
Triplet Code

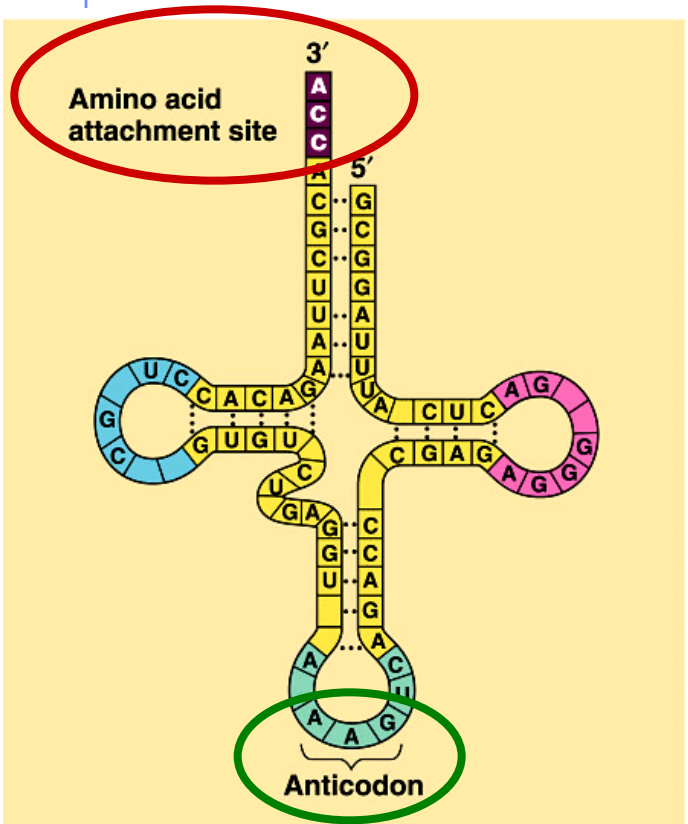
How are the codons matched to amino acids?

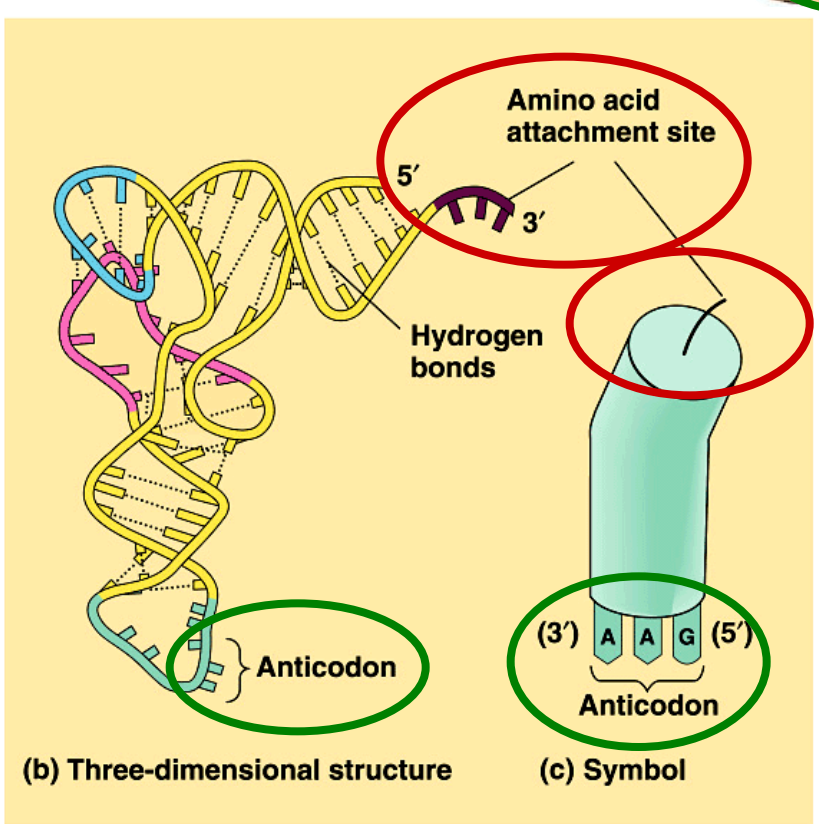


Transfer RNA structure

- "Clover leaf" structure
  - **anticodon** on "clover leaf" end
  - amino acid attached on 3′ end

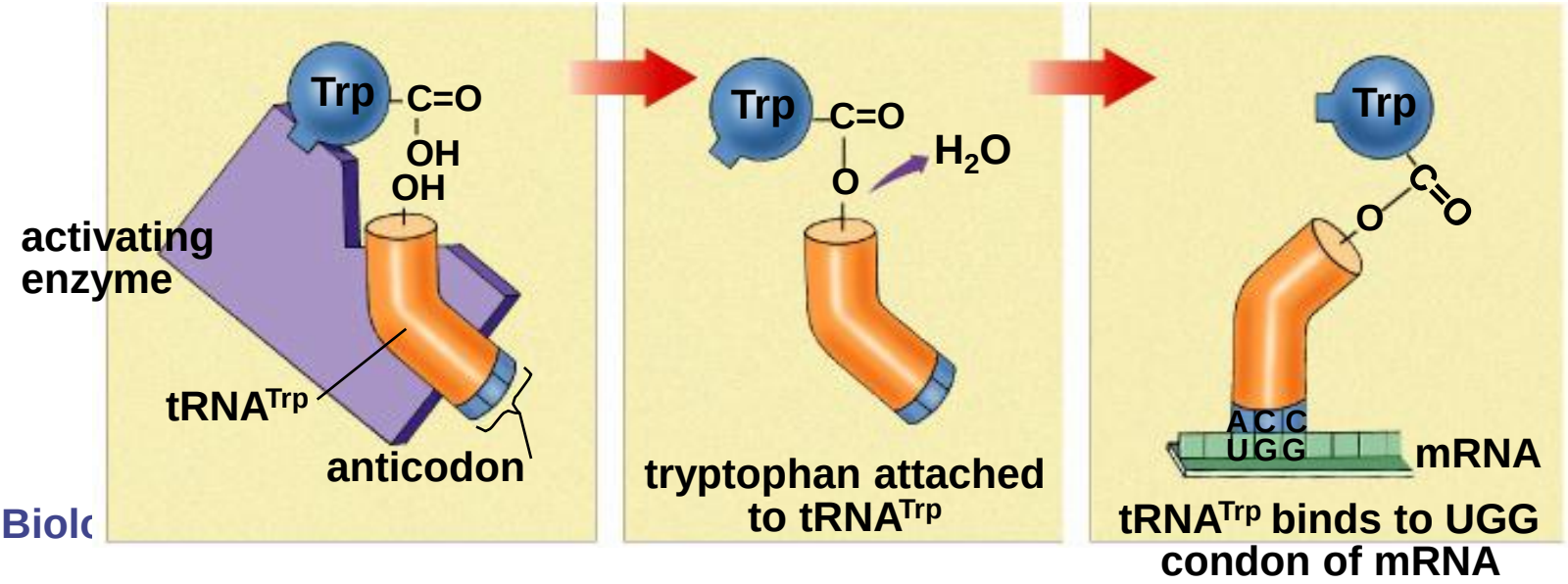




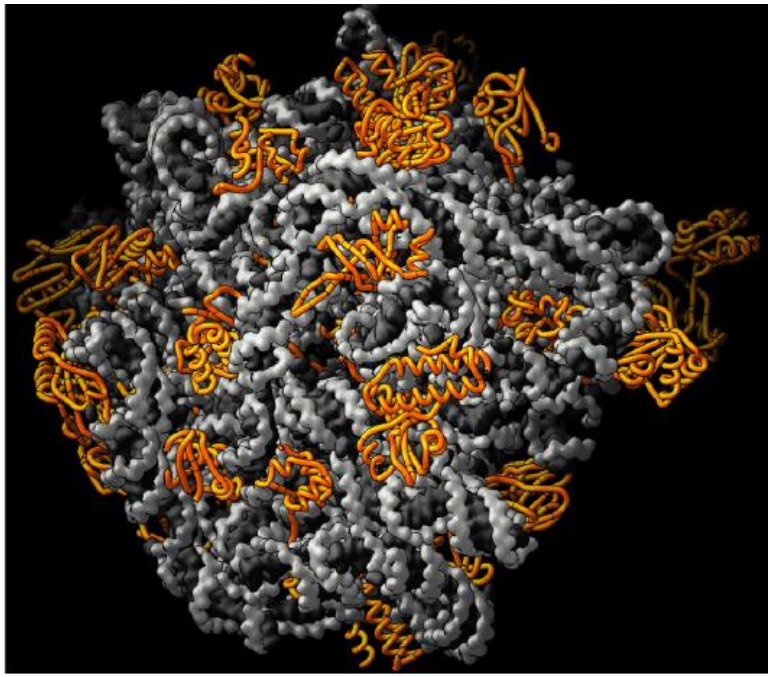


Loading tRNA

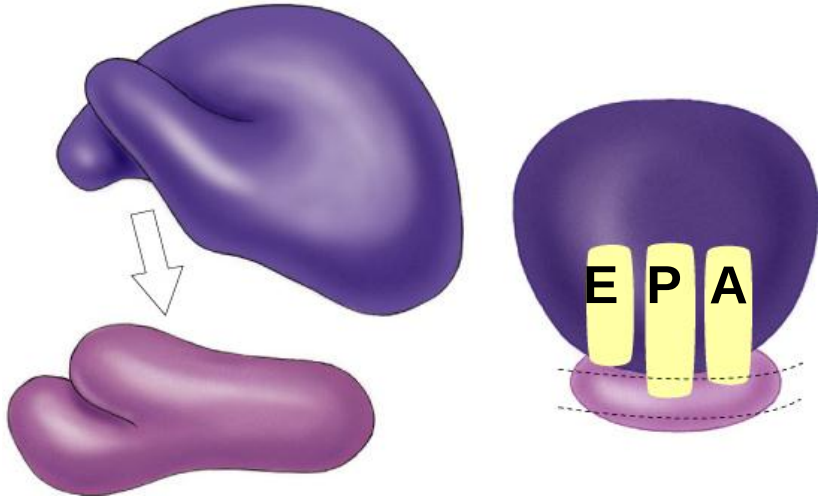
- **Aminoacyl tRNA synthetase**
  - enzyme which bonds amino acid to tRNA
  - bond requires energy
    - ATP → AMP
    - bond is unstable
    - so it can release amino acid at ribosome easily



Ribosomes

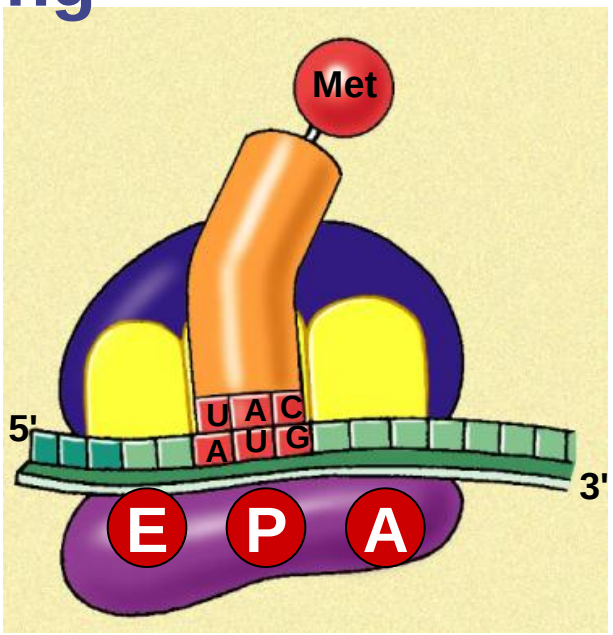


- Facilitate coupling of tRNA anticodon to mRNA codon
  - organelle or enzyme?
- Structure
  - ribosomal RNA (rRNA) & proteins
  - 2 subunits
    - large
    - small



Ribosomes

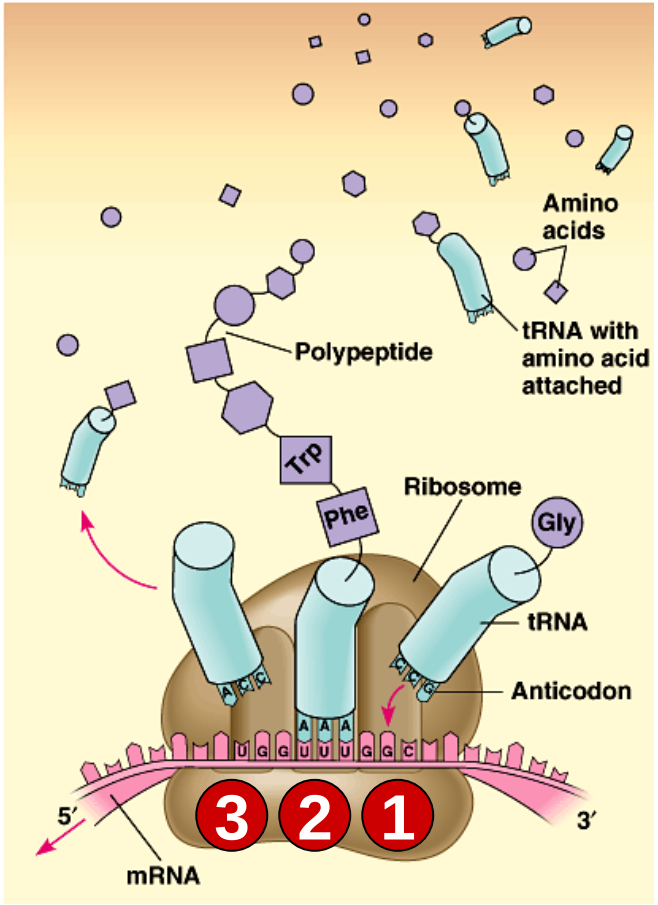
- A site (aminoacyl-tRNA site)
  - holds tRNA carrying next amino acid to be added to chain
- P site (peptidyl-tRNA site)
  - holds tRNA carrying growing polypeptide chain
- E site (exit site)
  - empty tRNA leaves ribosome from exit site

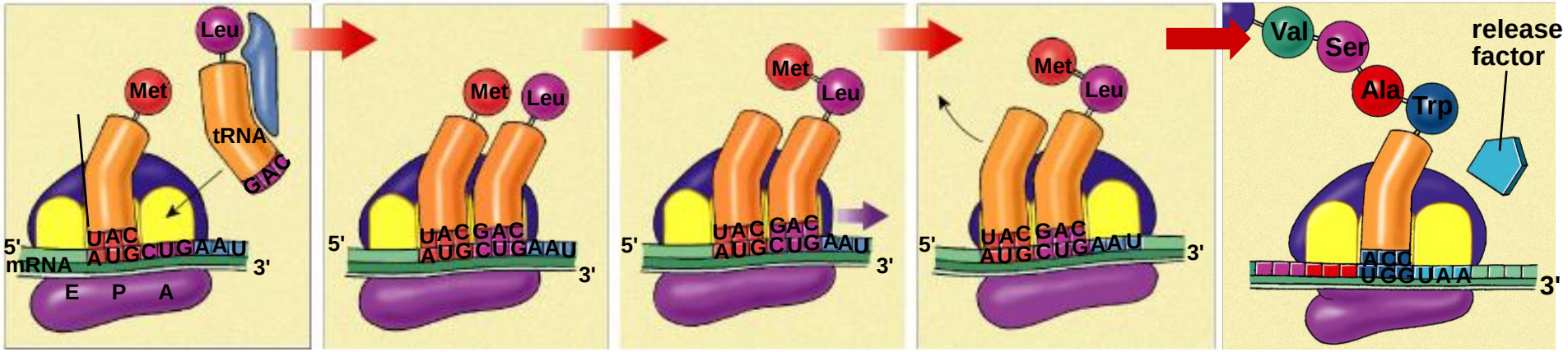


Transcription video

Building a polypeptide

- **Initiation**
  - brings together mRNA, ribosome subunits, initiator tRNA
- **Elongation**
  - adding amino acids based on codon sequence
- **Termination**
  - end codon





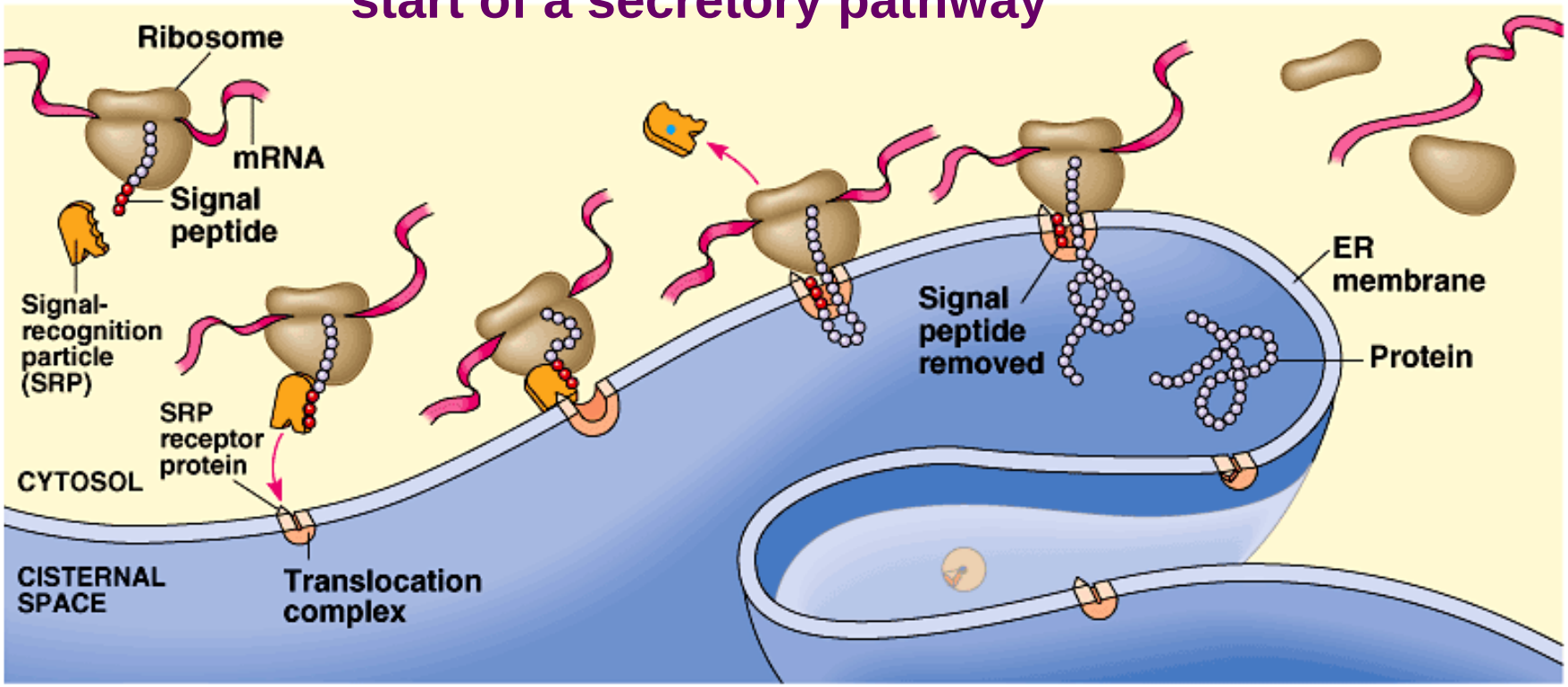
Protein targeting

- **Signal peptide**
  - **address label**

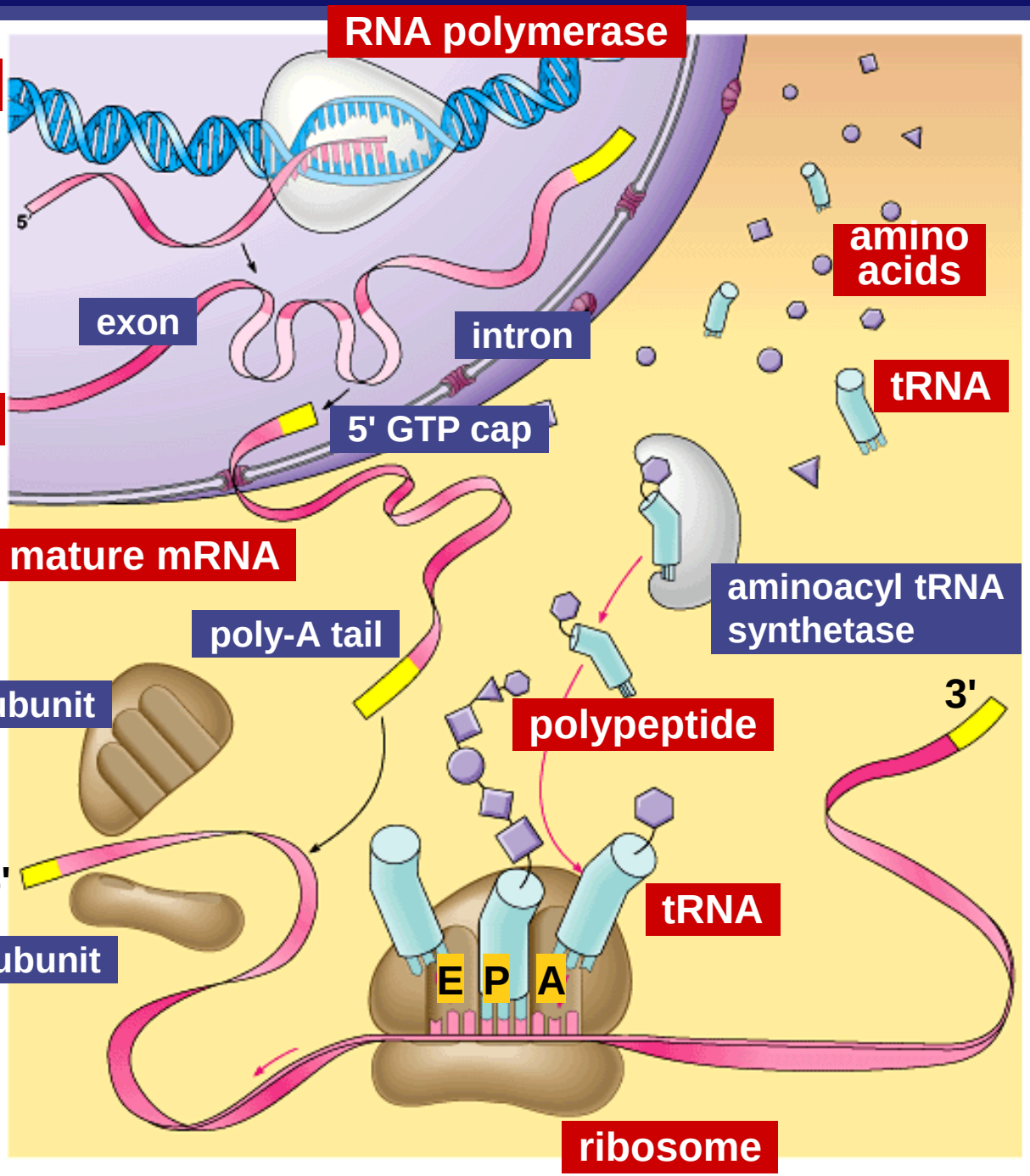
Destinations:

- secretion
- nucleus
- mitochondria
- chloroplasts
- cell membrane
- cytoplasm
- etc…

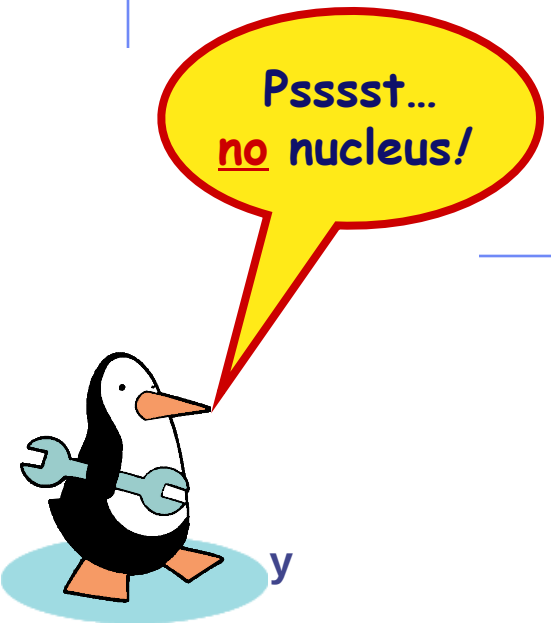
start of a secretory pathway

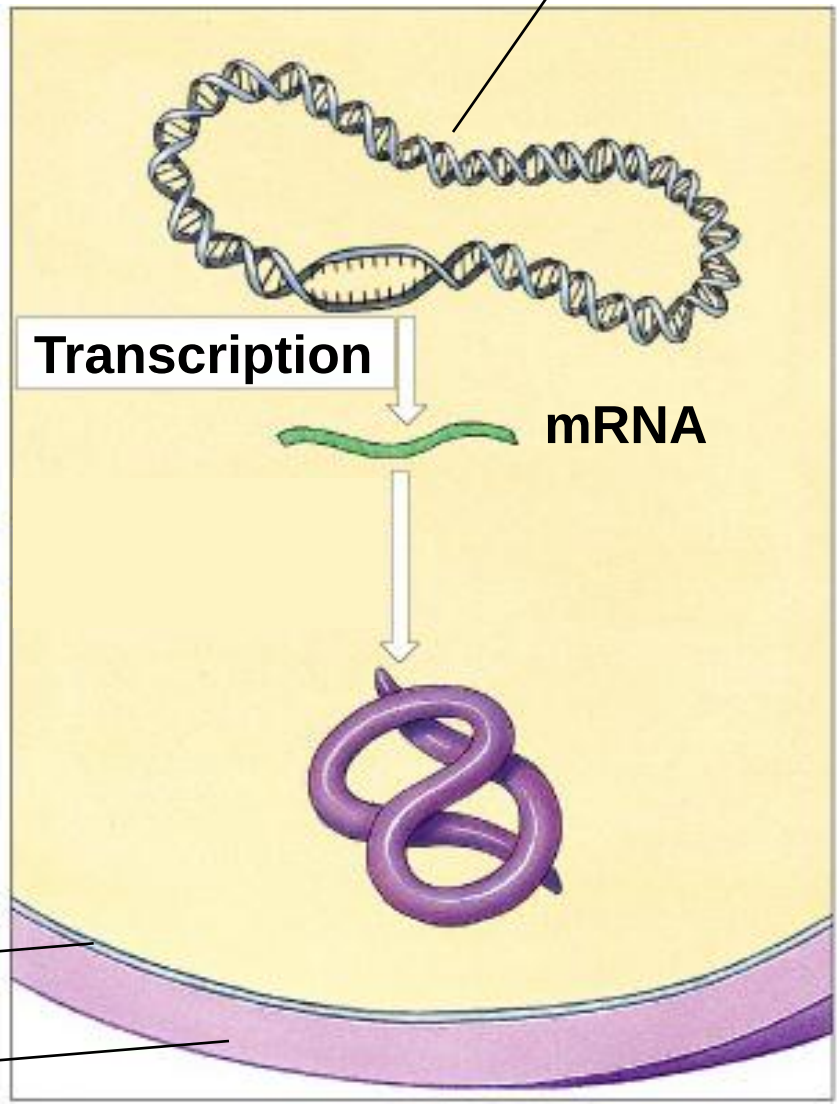


Can you tell the story?



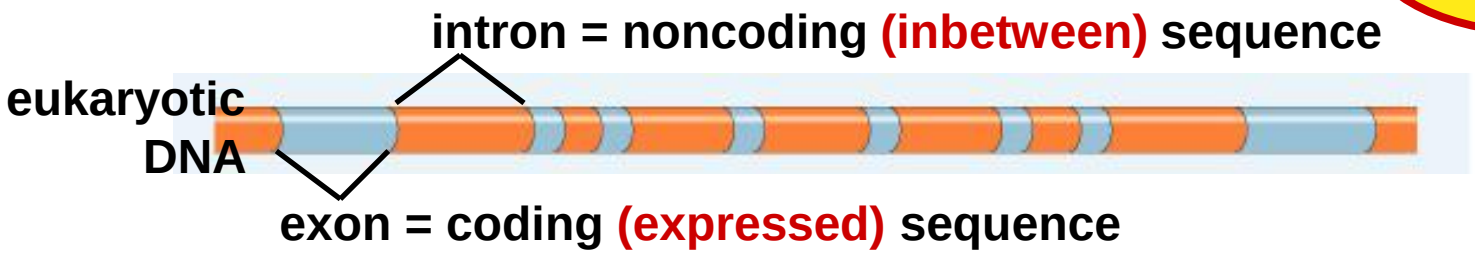
Protein Synthesis in Prokaryotes

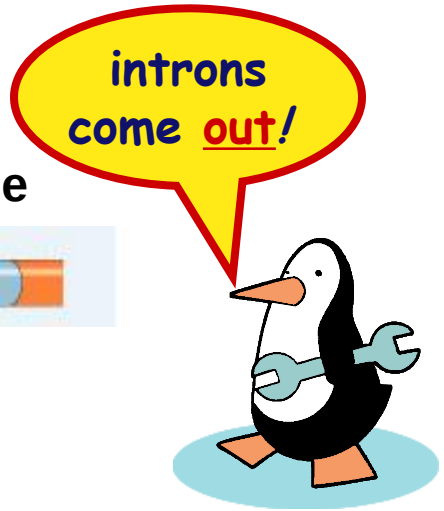




Prokaryote vs. Eukaryote genes

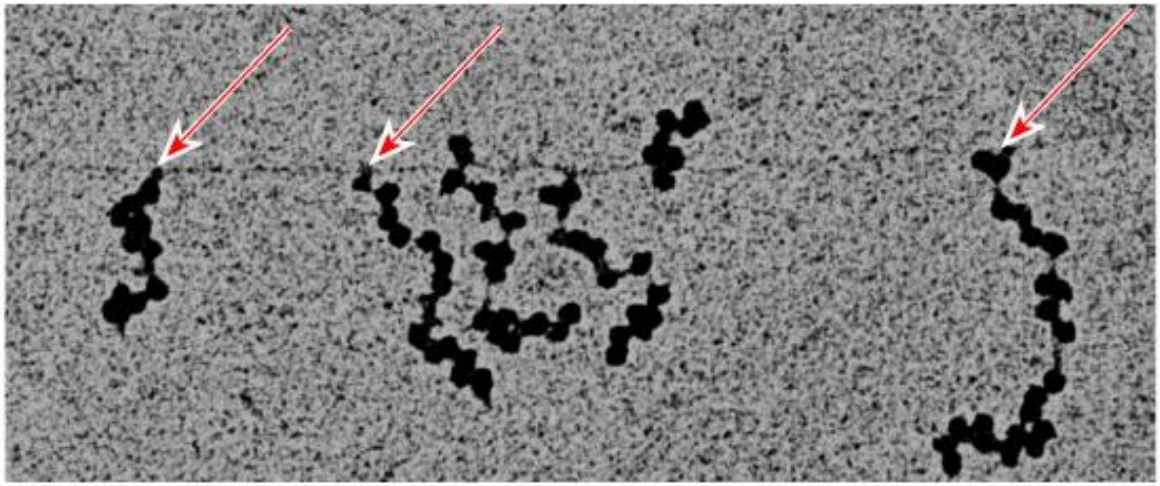
- **Prokaryotes**
  - DNA in cytoplasm
  - circular chromosome
  - naked DNA
  - no introns
- **Eukaryotes**
  - DNA in nucleus
  - linear chromosomes
  - DNA wound on histone proteins
  - introns vs. exons

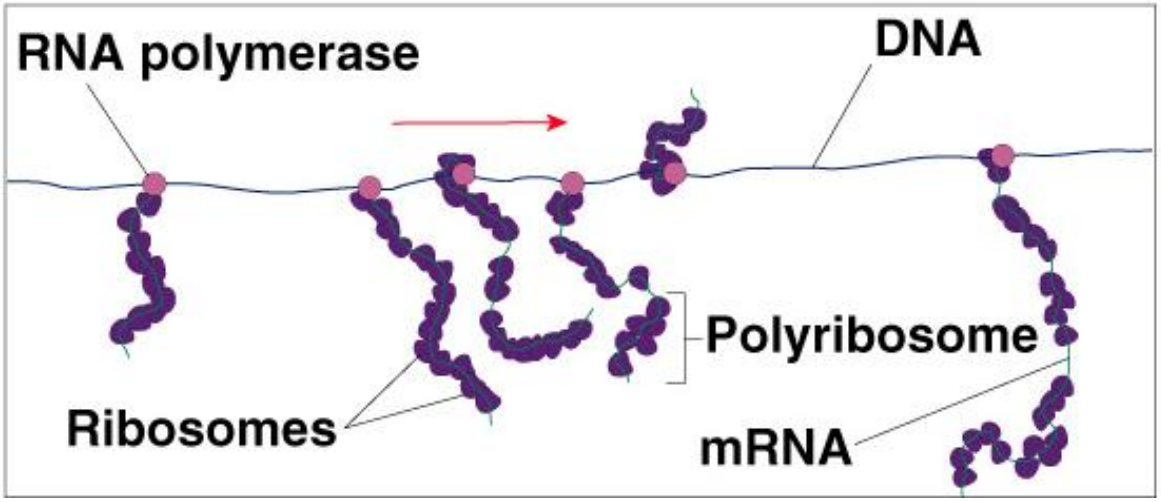




Translation in Prokaryotes

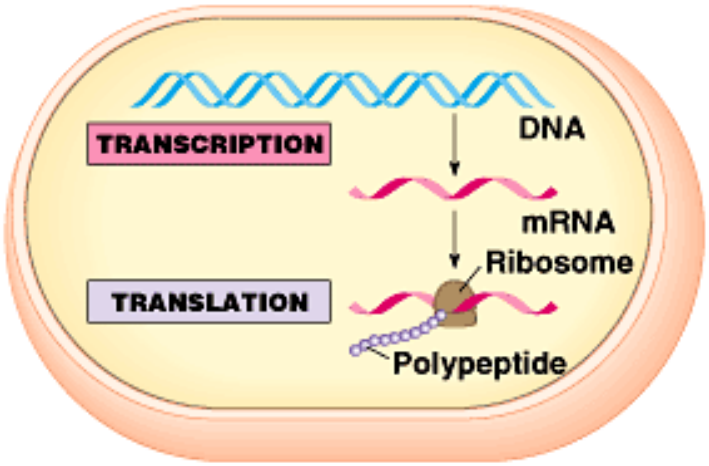
- **Transcription & translation are simultaneous in bacteria**
  - DNA is in cytoplasm
  - no mRNA editing
  - **ribosomes** read mRNA as it is being transcribed



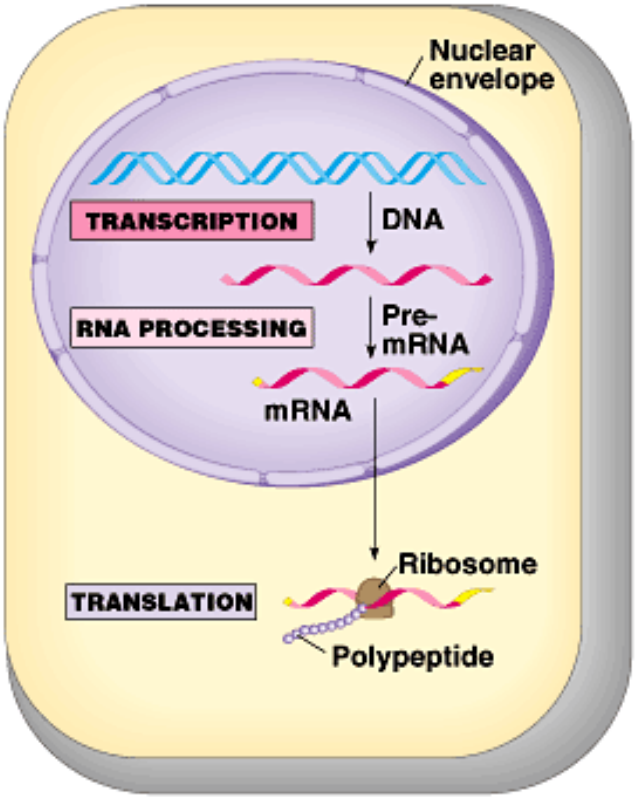


Translation: prokaryotes vs. eukaryotes

- **Differences between prokaryotes & eukaryotes**
  - time & physical separation between processes
    - takes eukaryote ~1 hour from DNA to protein
  - no RNA processing



(a) Prokaryotic cell



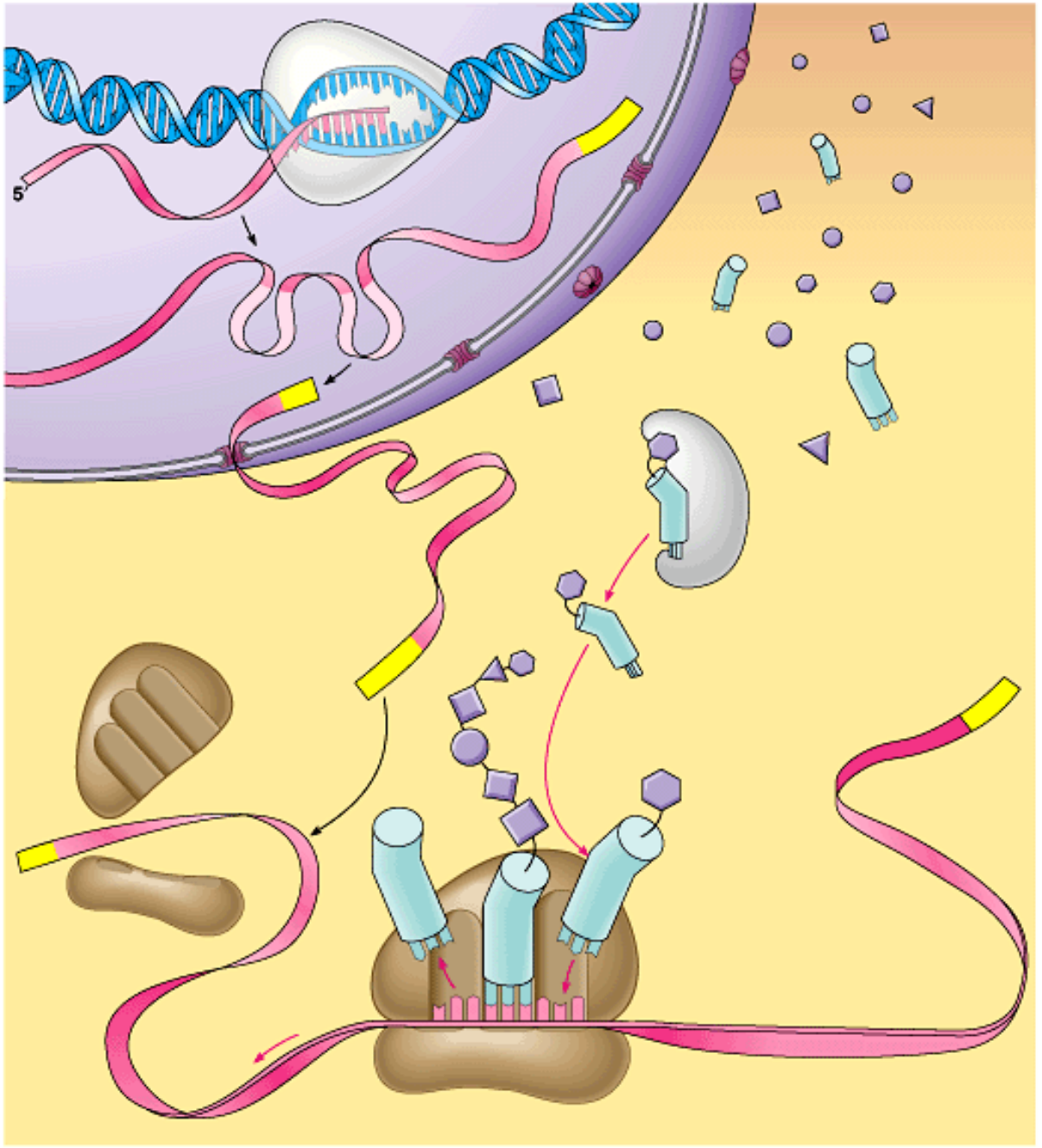
(b) Eukaryotic cell

Any Questions??

What color would a smurf turn if he held his breath?

Substitute Slides for Student Print version

Can you tell the story?



The Transcriptional unit