

Metabolism teaches us about genes

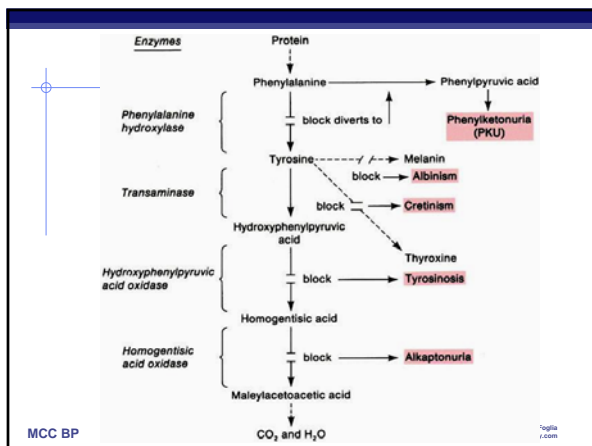
- Metabolic defects
 - studying metabolic diseases suggested that genes specified proteins
 - alkaptonuria (black urine from alkapton)
 - PKU (phenylketonuria)
 - each disease is caused by non-functional enzyme

Genes create phenotype

A → B → C → D → E

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Based on work by K. Foglia
www.kimunity.com



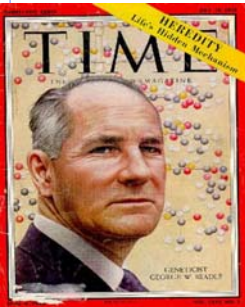
1 gene – 1 enzyme hypothesis


- **Beadle & Tatum**
 - ◆ Compared mutants of bread mold, *Neurospora* fungus
 - created mutations by X-ray treatments
 - ♦ X-rays break DNA
 - ♦ inactivate a gene
 - wild type grows on “minimal” media
 - ♦ sugars + required precursor nutrient to synthesize essential amino acids
 - mutants require added amino acids
 - ♦ each type of mutant lacks a certain enzyme needed to produce a certain amino acid
 - ♦ **non-functional enzyme = broken gene**

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Beadle & Tatum

1941 | 1958





George Beadle



Edward Tatum

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Beadle & Tatum's *Neurospora* experiment


| | Minimal medium (M ⁻) | M ⁻ + Ornithine | M ⁻ + Citrulline | M ⁻ + Arginine | |
|---|----------------------------------|----------------------------|-----------------------------|---------------------------|---|
| Wild type | | | | | Gene A → Precursor → Enzyme A → Ornithine → Gene B → Citrulline → Enzyme C → Arginine |
| Class I Mutants (mutation in gene A) | | | | | Precursor Enzyme A → Ornithine → Enzyme B → Citrulline → Enzyme C → Arginine |
| Class II Mutants (mutation in gene B) | | | | | Precursor → Enzyme A → Ornithine Enzyme B → Citrulline → Enzyme C → Arginine |
| Class III Mutants (mutation in gene C) | | | | | Precursor → Enzyme A → Ornithine → Enzyme B → Citrulline Enzyme C → Arginine |

(a) Experiment (b) Interpretation

So... What is a gene?

- One gene – one enzyme
 - ◆ but not all proteins are enzymes
 - ◆ but all proteins are coded by genes
- One gene – one protein
 - ◆ but many proteins are composed of several polypeptides
 - ◆ but each polypeptide has its own gene
- One gene – one polypeptide
 - ◆ but many genes only code for RNA
- One gene – one product
 - ◆ but many genes code for more than one product ...

Where does that leave us?!



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Defining a gene...

"Defining a gene is problematic because... one gene can code for several protein products, some genes code only for RNA, two genes can overlap, and there are many other complications."

gene → RNA


gene → polypeptide 1

gene → polypeptide 2

gene → polypeptide 3

— Elizabeth Pennings, Science 2003

It's hard to hunt for rabbits, if you don't know what a rabbit looks like.



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The "Central Dogma"

- How do we move information from DNA to proteins?


DNA → RNA → protein

transcription

translation

replication

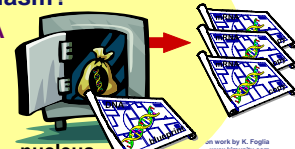
For simplicity sake, let's go back to genes that code for proteins...



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From nucleus to cytoplasm...

- Where are the genes?
 - ◆ genes are on chromosomes in nucleus
- Where are proteins synthesized?
 - ◆ proteins made in cytoplasm by ribosomes
- How does the information get from nucleus to cytoplasm?
 - ◆ messenger RNA




The diagram shows a yellow circle representing the nucleus. Inside, a DNA double helix is shown with a red arrow pointing to a messenger RNA molecule. The word 'nucleus' is written below the circle. To the right, several messenger RNA molecules are shown moving away from the nucleus.

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RNA

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

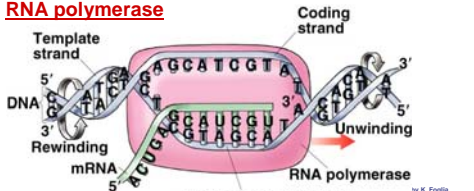


The diagram shows two DNA double helices on the right, labeled 'DNA'. On the left, a single RNA strand is shown, labeled 'RNA'. Labels include 'Nitrogenous Bases', 'Base pair', and 'Sugar-phosphate backbone'. A red arrow labeled 'transcription' points from a yellow box 'DNA' to another yellow box 'RNA'.

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Transcription

- Transcribed DNA strand = **template strand**
 - ◆ untranscribed DNA strand = **coding strand**
- Synthesis of complementary RNA strand
 - ◆ transcription bubble
- Enzyme
 - ◆ **RNA polymerase**



The diagram shows a DNA double helix being unwound. The top strand is labeled 'Template strand' with sequence 5'-ATCA-3' and the bottom strand is 'Coding strand' with sequence 3'-TAGT-5'. An 'RNA polymerase' enzyme is shown moving along the template strand, synthesizing an 'mRNA' strand with sequence 5'-AUGCAUCGU-3'. The region where the strands are separated is labeled 'Unwinding' and 'RNA-DNA hybrid helix'. The process is also labeled 'Rewinding'.

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Transcription in Prokaryotes

- **Initiation**
 - ◆ RNA polymerase binds to **promoter sequence** on DNA

Role of promoter

1. Where to start reading
= starting point
2. Which strand to read
= template strand
3. Direction on DNA
= always reads DNA 3'→5'

Transcription in Prokaryotes

- **Promoter sequences**

RNA polymerase molecules bound to bacterial DNA

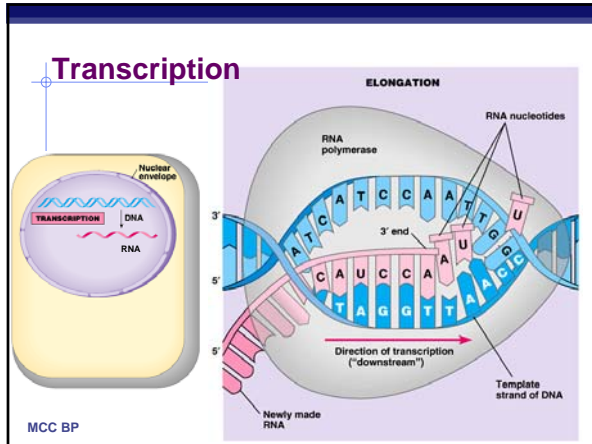
Transcription in Prokaryotes

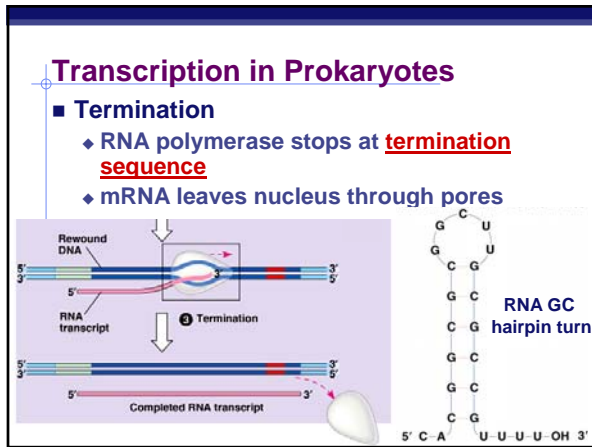
- **Elongation**
 - ◆ RNA polymerase unwinds DNA
~20 base pairs at a time
 - ◆ reads DNA 3'→5'
 - ◆ builds RNA 5'→3' (the energy governs the synthesis!)

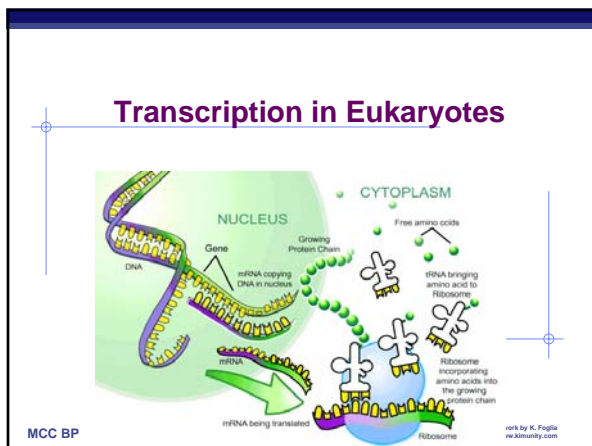
No proofreading

- 1 error/10⁵ bases
- many copies
- short life
- not worth it!

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Prokaryote vs. Eukaryote genes

| | |
|---|--|
| <ul style="list-style-type: none"> ■ Prokaryotes ◆ DNA in cytoplasm ◆ circular chromosome ◆ naked DNA ◆ no introns | <ul style="list-style-type: none"> ■ Eukaryotes ◆ DNA in nucleus ◆ linear chromosomes ◆ DNA wound on histone proteins ◆ introns vs. exons |
|---|--|

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Transcription in Eukaryotes

- 3 RNA polymerase enzymes
 - ◆ RNA polymerase I
 - only transcribes rRNA genes
 - ◆ **RNA polymerase I I**
 - transcribes genes into mRNA
 - ◆ RNA polymerase I I I
 - only transcribes rRNA genes
- ◆ each has a specific promoter sequence it recognizes

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Transcription in Eukaryotes

- Initiation complex
 - ◆ **transcription factors** bind to **promoter region** upstream of gene
 - proteins which bind to DNA & turn on or off transcription
 - **TATA** box binding site
 - ◆ only then does RNA polymerase bind to DNA

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Post-transcriptional processing

- **Primary transcript**
 - ◆ eukaryotic mRNA needs work after transcription
- **Protect mRNA**
 - ◆ from RNase enzymes in cytoplasm
 - add 5' cap
 - add polyA tail
- **Edit out introns**

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Transcription to translation

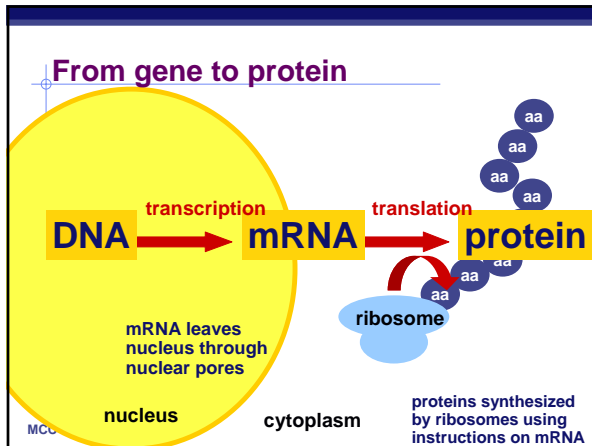
- **Differences between prokaryotes & eukaryotes**
 - ◆ time & physical separation between processes
 - ◆ RNA processing

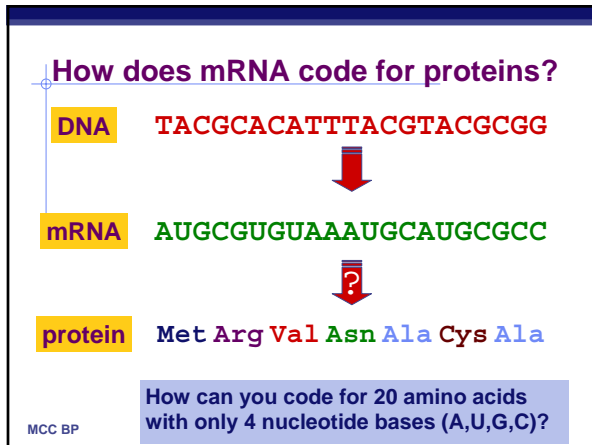
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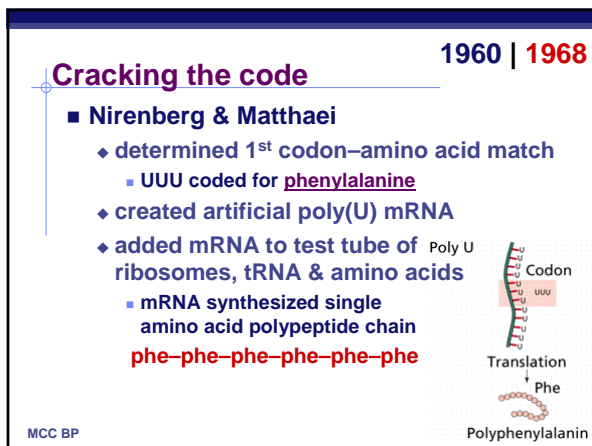
Translation in Prokaryotes

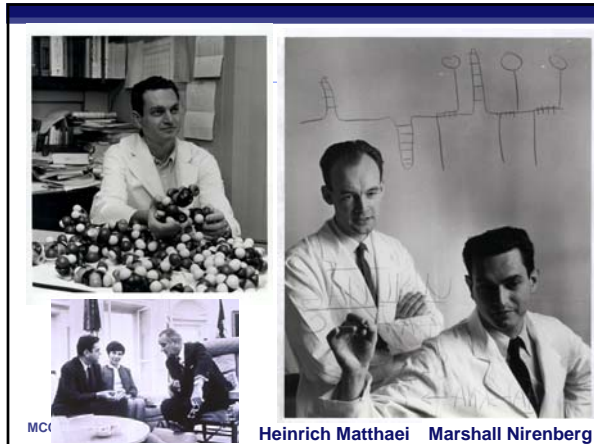
- **Transcription & translation are simultaneous in bacteria**
 - ◆ DNA is in cytoplasm
 - ◆ no mRNA editing needed

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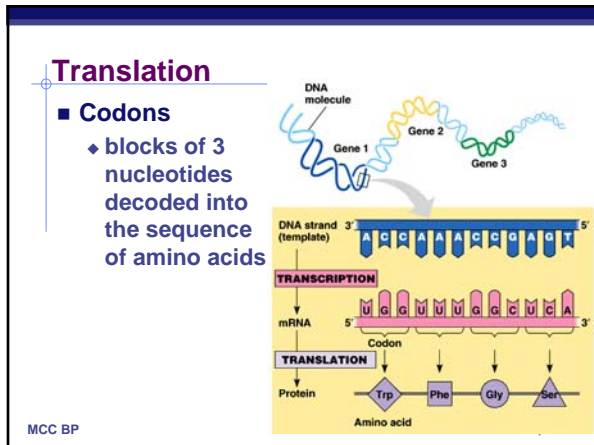




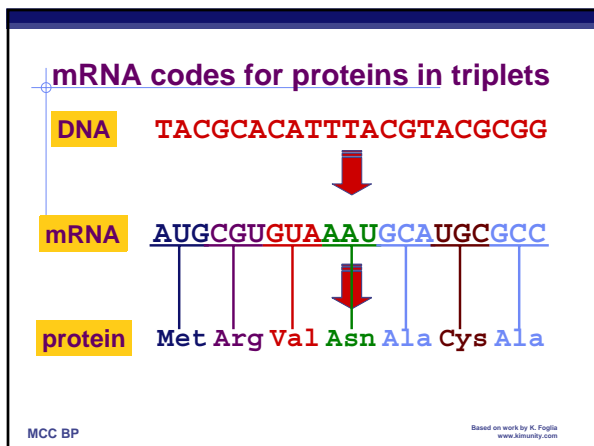




Heinrich Matthaei Marshall Nirenberg



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The code

- For **ALL** life!
 - strongest support for a common origin for all life
- Code is **redundant**
 - several codons for each amino acid

Why is this a good thing?

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

MC

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

How are the codons matched to amino acids?

DNA 3' TACGCACATTTACGTACGCGG 5'

mRNA 5' AUGCGUGUA **AAUGCAUG** GCC 3'

codon

tRNA 3' UAC 5'

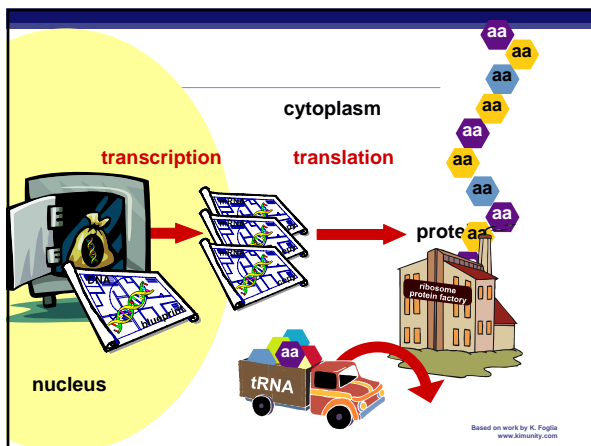
amino acid Met

GCA Arg

CAU anti-codon Val

MC

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tRNA structure

- “Clover leaf” structure
 - ◆ anticodon on “clover leaf” end
 - ◆ amino acid attached on 3' end

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Loading tRNA

- Aminoacyl tRNA synthetase
 - ◆ enzyme which bonds amino acid to tRNA
 - ◆ endergonic reaction
 - ATP → AMP
 - ◆ energy stored in tRNA-amino acid bond
 - unstable
 - so it can release amino acid at ribosome

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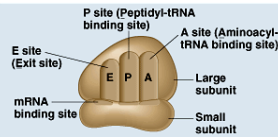
Ribosomes

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

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Ribosomes

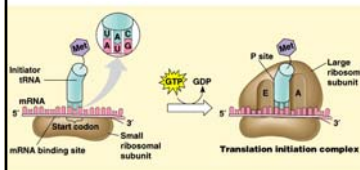
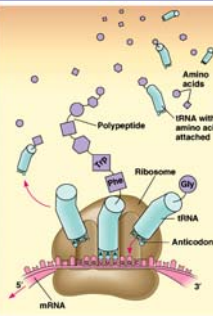
- **P site (peptidyl-tRNA site)**
 - ◆ holds tRNA carrying growing **polypeptide** chain
- **A site (aminoacyl-tRNA site)**
 - ◆ holds tRNA carrying next **amino acid** to be added to chain
- **E site (exit site)**
 - ◆ empty tRNA leaves ribosome from exit site



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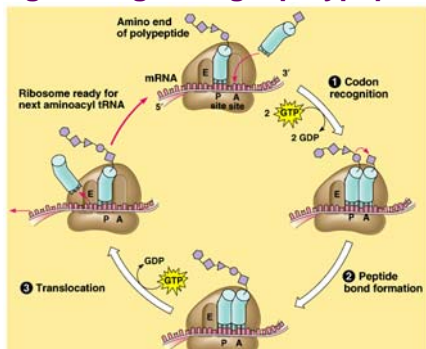
Building a polypeptide

- **Initiation**
 - ◆ brings together mRNA, ribosome subunits, proteins & initiator tRNA
- **Elongation**
- **Termination**

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Elongation: growing a polypeptide



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Termination: release polypeptide

- Release factor
 - ◆ “release protein” bonds to A site
 - ◆ bonds water molecule to polypeptide chain

Now what happens to the polypeptide?

Protein targeting

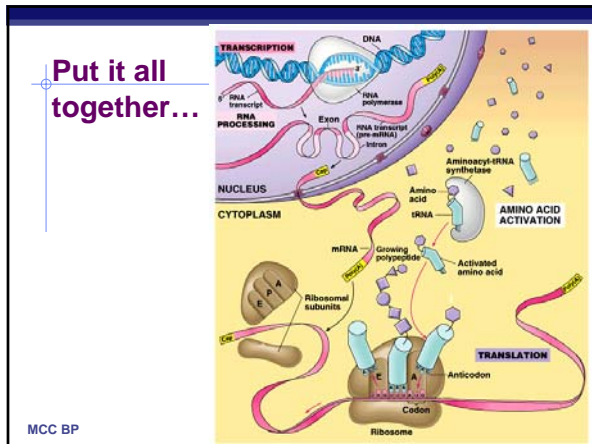
- Signal peptide
 - ◆ address label

Destinations:

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

start of a secretory pathway

Can you tell the story?



Any Questions??

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