RNA: RIBONUCLEIC ACID :

- how DNA communicates its message.

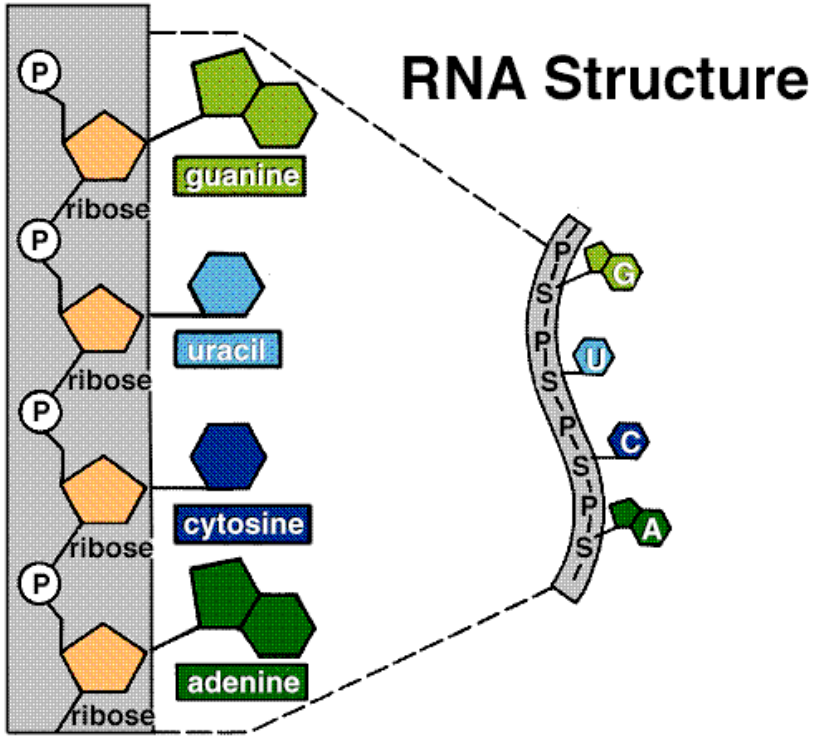
  - RNA is the genetic material of some **viruses** and is necessary in all organisms for **protein synthesis** to occur. RNA could have been the "original" nucleic acid when life first arose on Earth some 3.8 billion years ago.
  - Like DNA, all RNA molecules have a similar chemical organization, consisting of **nucleotides**.

Like DNA, each RNA nucleotide is also composed of three subunits:

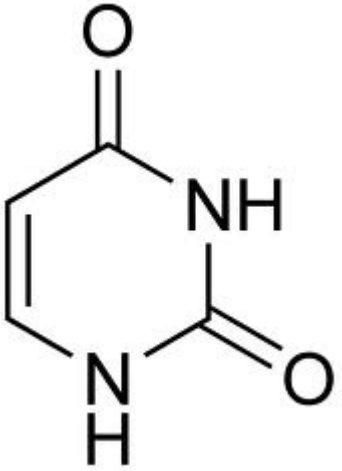
1.a 5-carbon sugar called **RIBOSE**.

2.a **PHOSPHATE group** that is attached to one end of the sugar molecule

3. one of several different **nitrogenous BASES** linked to the opposite end of the ribose.



There is **one base that is different** from DNA -- the base **URACIL** is used instead of **thymine**.(G, A, C, are otherwise the same as for DNA)

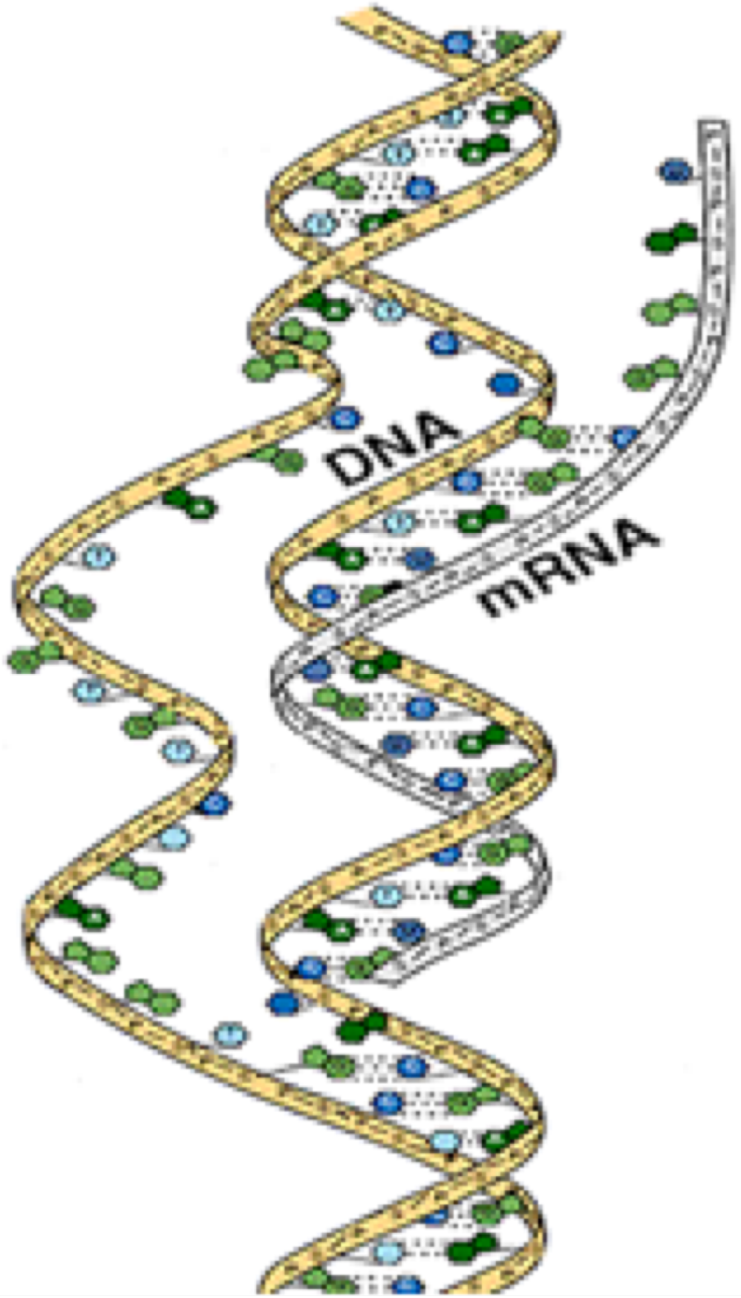


Uracil

UracilUracil

RNA is **SINGLE-STRANDED**, unlike DNA which is double stranded. RNA, therefore, is **not** a double helix.

- RNA is produced from DNA by a process called TRANSCRIPTION. The steps of transcription are as follows:
  1. A specific section of DNA unwinds, exposing a set of bases
  2. Along one strand of DNA (called the "sense" strand), complementary RNA bases are brought in. In RNA, Uracil binds to the Adenine on DNA. As in DNA, cytosine binds to guanine. The other strand of the DNA molecule (the "missense" strand), isn't read in eukaryotic cells.
  3. Adjacent RNA nucleotides form sugar-phosphate bonds.
  4. The RNA strand is released from DNA (RNA is a single-stranded nucleic acid).
  5. The DNA molecule rewinds, and returns to its normal double helix form. ANIMATION



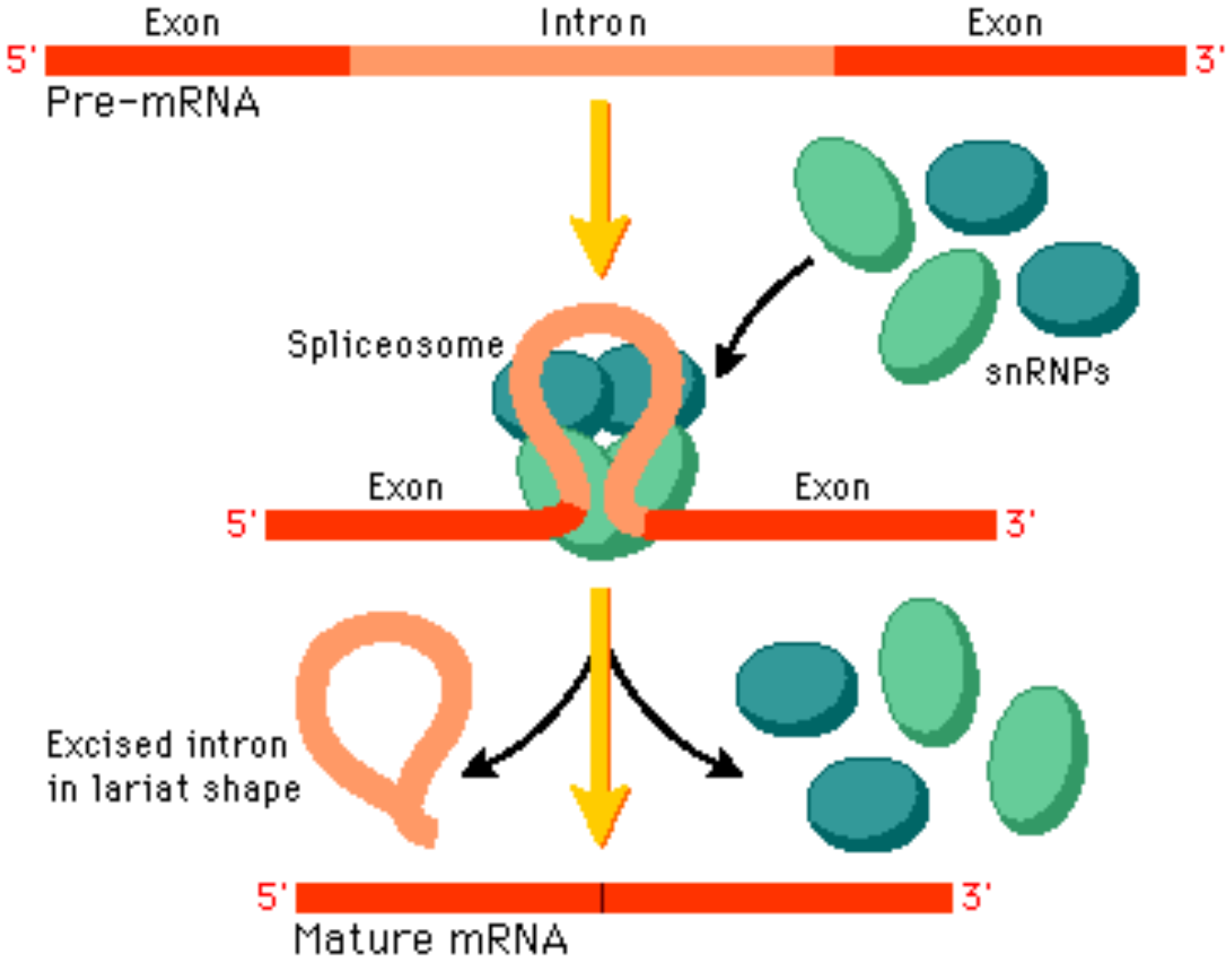
This mRNA transcript is ready to move into the cytoplasm.

Transcription is going on here—the nucleotides of mRNA are joined by the enzyme RNA polymerase in an order complementary to a strand of DNA.

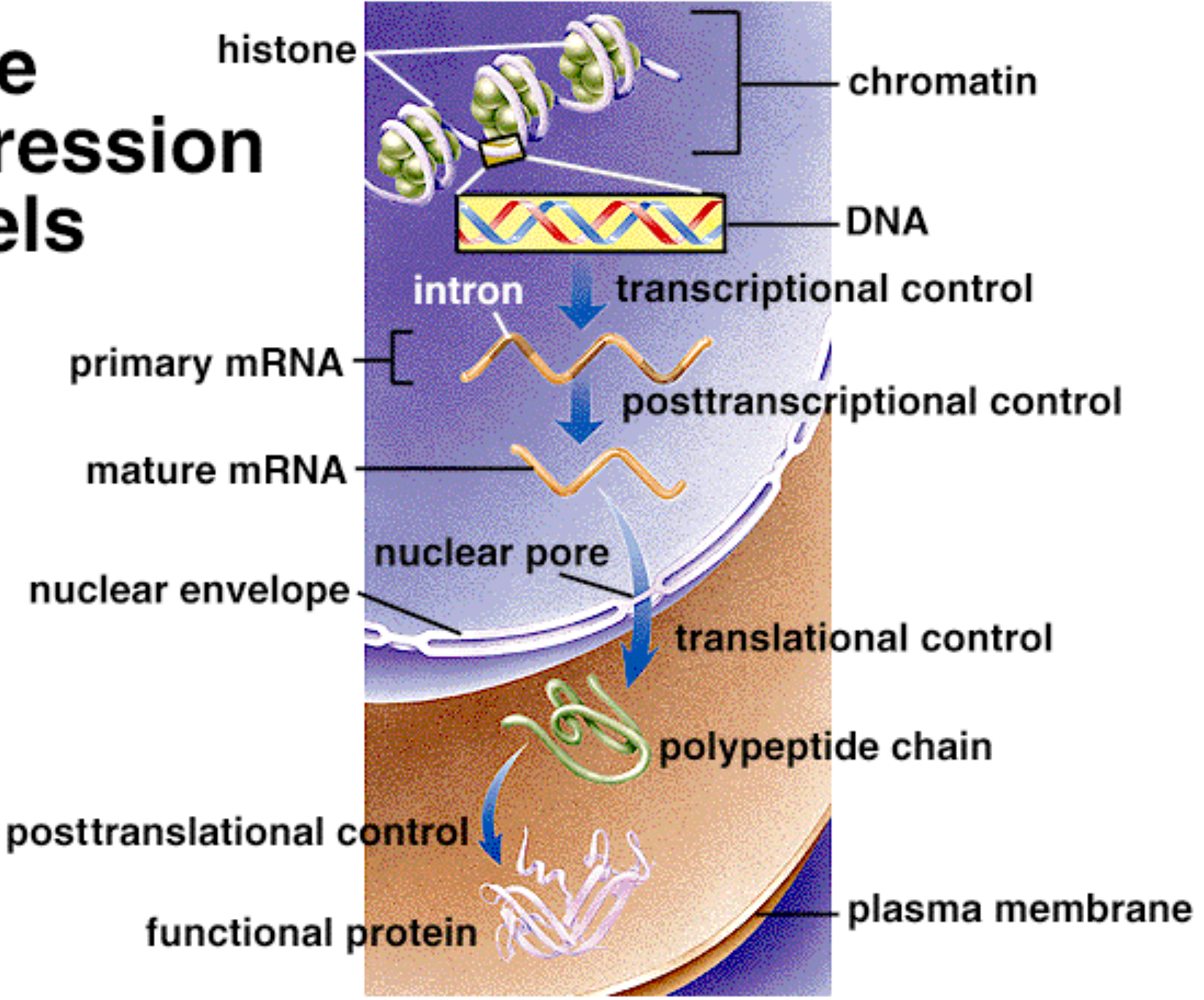
One portion of DNA—a particular gene or genes—is transcribed at one time.

- Once produced, the mRNA strand is often ***processed*** (certain sections called **introns** are cut out, a "Poly-A" tail is added to the 3' end, and a "cap" is added to the 5' end). Poly-A ANIMATION
  Splicing ANIMATION
- RNA can then **leave the nucleus** and go into the **cytoplasm**.

- The enzyme involved in transcription is known **as RNA polymerase**.
  - This process occurs in the **nucleus** (and, in particular, **dark coloured spots** in the nucleus called **nucleoli** (singular = **nucleolus**)


Exon
Intron
Exon
5'
3'
Pre-mRNA
Spliceosome
snRNPs
Exon
Exon
5'
3'
Excised intron
in lariat shape
5'
3'
Mature mRNA

Gene Expression Levels



Please transcribe the following DNA strand

G	A	C	A	A	C	T	G	G	A	T	C	G	A	C	DNA
III	II	III	II	II	III	II	III	III	II	II	III	III	II	III	
															mRNA

G	A	C	A	A	C	T	G	G	A	T	C	G	A	C	DNA
III	II	III	II	II	III	II	III	III	II	II	III	III	II	III	
C	U	G	U	U	G	A	C	C	U	A	G	C	U	G	mRNA

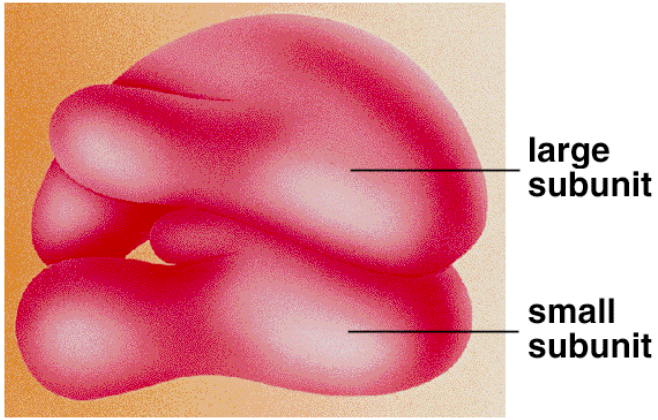
There are **3 types of RNA**, each with different functions.

- **rRNA**
- **tRNA**
- **mRNA**

– **The agents of Protein Synthesis**

RNA that is involved in protein synthesis belongs to one of three distinct types:

- **ribosomal RNA** (**rRNA**),
- **transfer RNA** (**tRNA**),
- **messenger RNA** (**mRNA**).

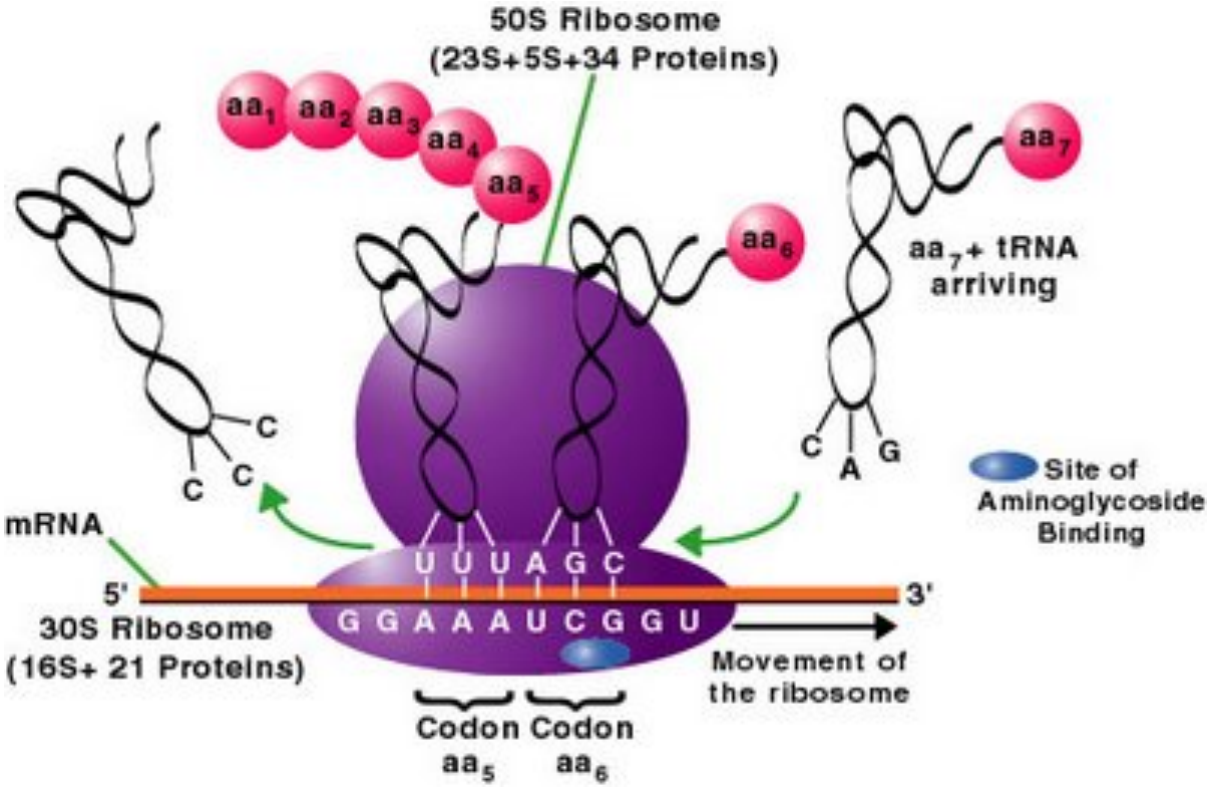


RIBOSOMAL RNA

(rRNA)

- **becomes a structural part of ribosomes** and serves as a genetic **link** between mRNA and tRNA. Ribosomal RNA is associated with protein, forming bodies called **ribosomes**.
- Ribosomes are the **sites** of **protein synthesis**.

•Ribosomal RNA varies in size and is the ***most plentiful*** RNA. It **constitutes 85% to 90%** of total cellular RNA.



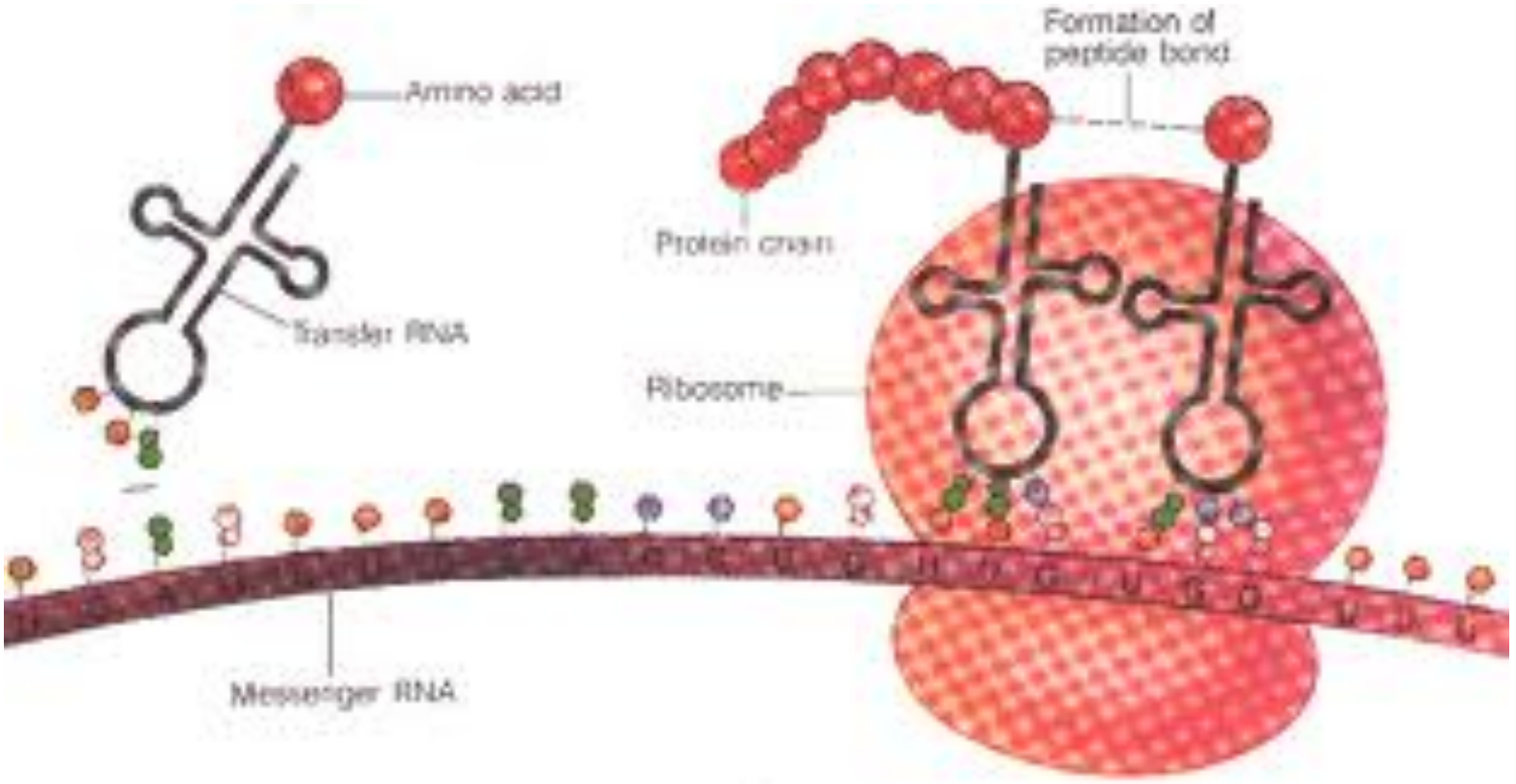
TRANSFER RNA (tRNA) - is used to **deliver amino acids from the cytoplasm to the ribosome**.

- There is a different tRNA for each amino acid. The function of each type of tRNA is to bring its specific amino acid to a ribosome.
- The tRNA molecules consist of about **80 nucleotides** and are structured in a **cloverleaf** pattern. They constitute about **5%** of the cell's total RNA.

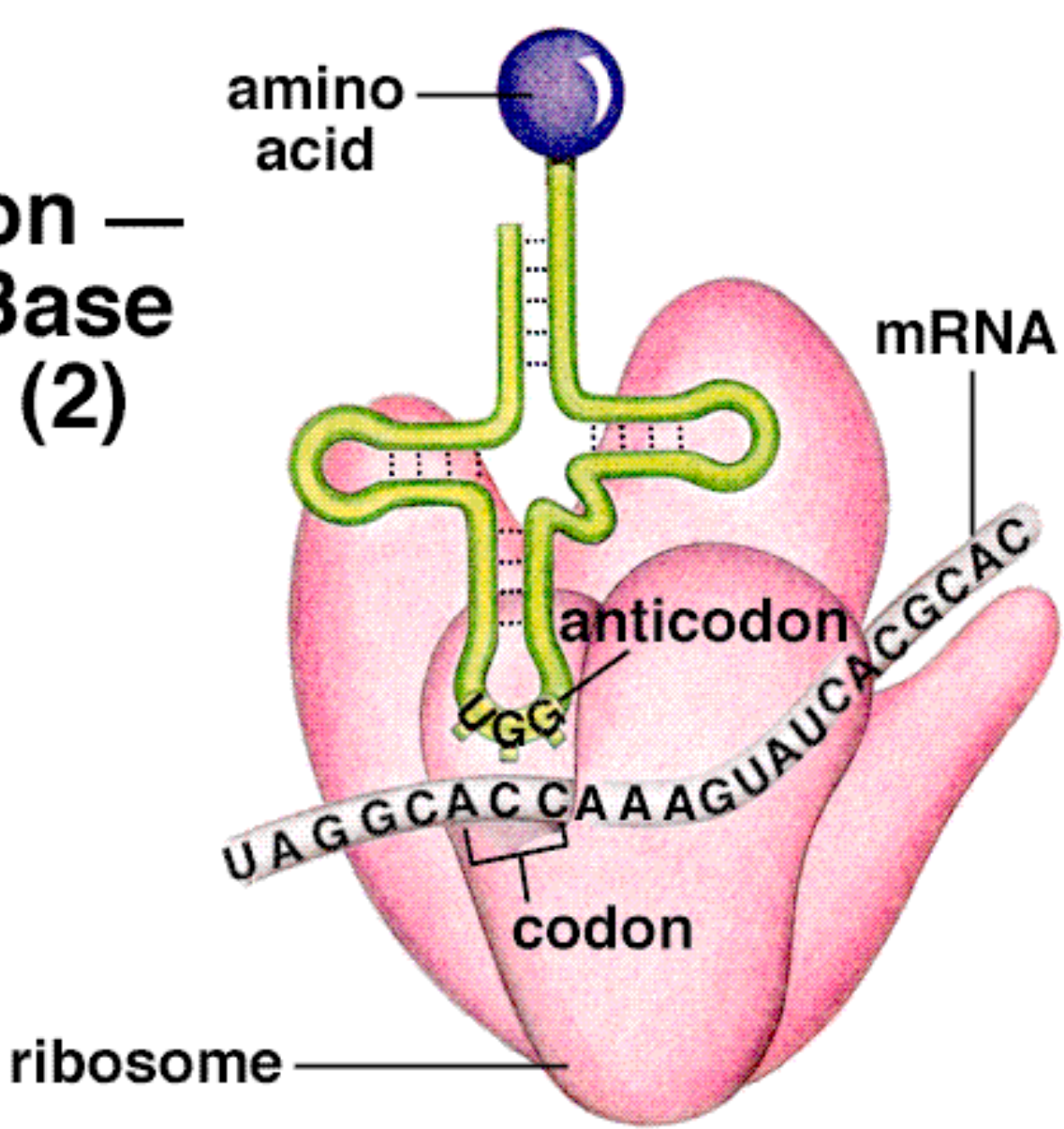
- **MESSENGER RNA** (mRNA) - carries **the genetic code** contained in the sequence of bases in the cell's DNA **from the nucleus to the Ribosome**.

- mRNA: acts as a "go-between" for DNA in the nucleus and the ribosomes in the cytoplasm.

- mRNA constitutes 5% to 10% of the cell's RNA.


Formation of peptide bond
Amino acid
Protein chain
Transfer RNA
Ribosome
Messenger RNA

Anticodon — Codon Base Pairing (2)



tRNA ~ amino acid at ribosome

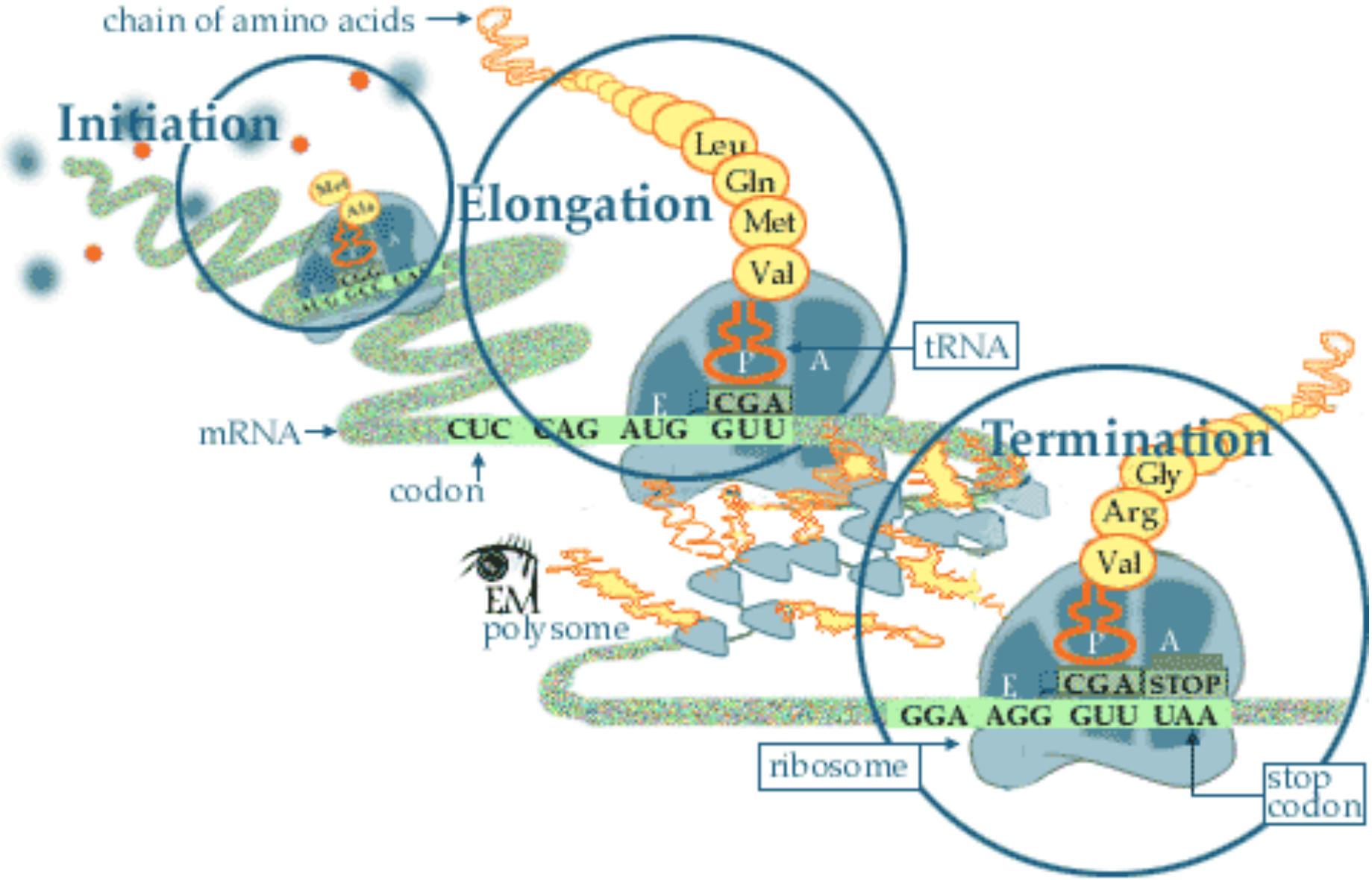
The Central Dogma of Molecular Biology

DNA → → mRNA → → Protein
ANIMATION

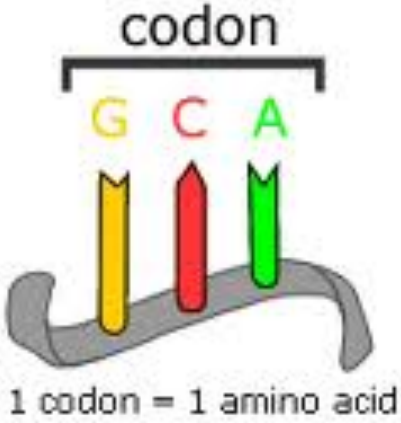
transcription translation Eng. version

- mRNA, once produced, leaves the nucleus through pores in the nuclear envelope, and enters the cytoplasm. This is where **TRANSLATION** occurs.

- **Translation is the process that changes the RNA message into the actual protein**. It occurs at the surface of the **RIBOSOME**.


chain of amino acids
Initiation
Elongation
Termination
Met
Ala
Leu
Gln
Met
Val
tRNA
P
A
E
CGA
mRNA
CUC GAG AUG GUU
codon
EM
polysome
Gly
Arg
Val
CGA
STOP
GGA AGG GUU UAA
ribosome
stop
codon

- The ***order of the bases in DNA***, and then subsequently ***mRNA***, ***determines the amino acid sequence*** of the protein being made.
- Each amino acids ***is coded for by 3 bases*** (this is known as a **TRIPLET CODE**)



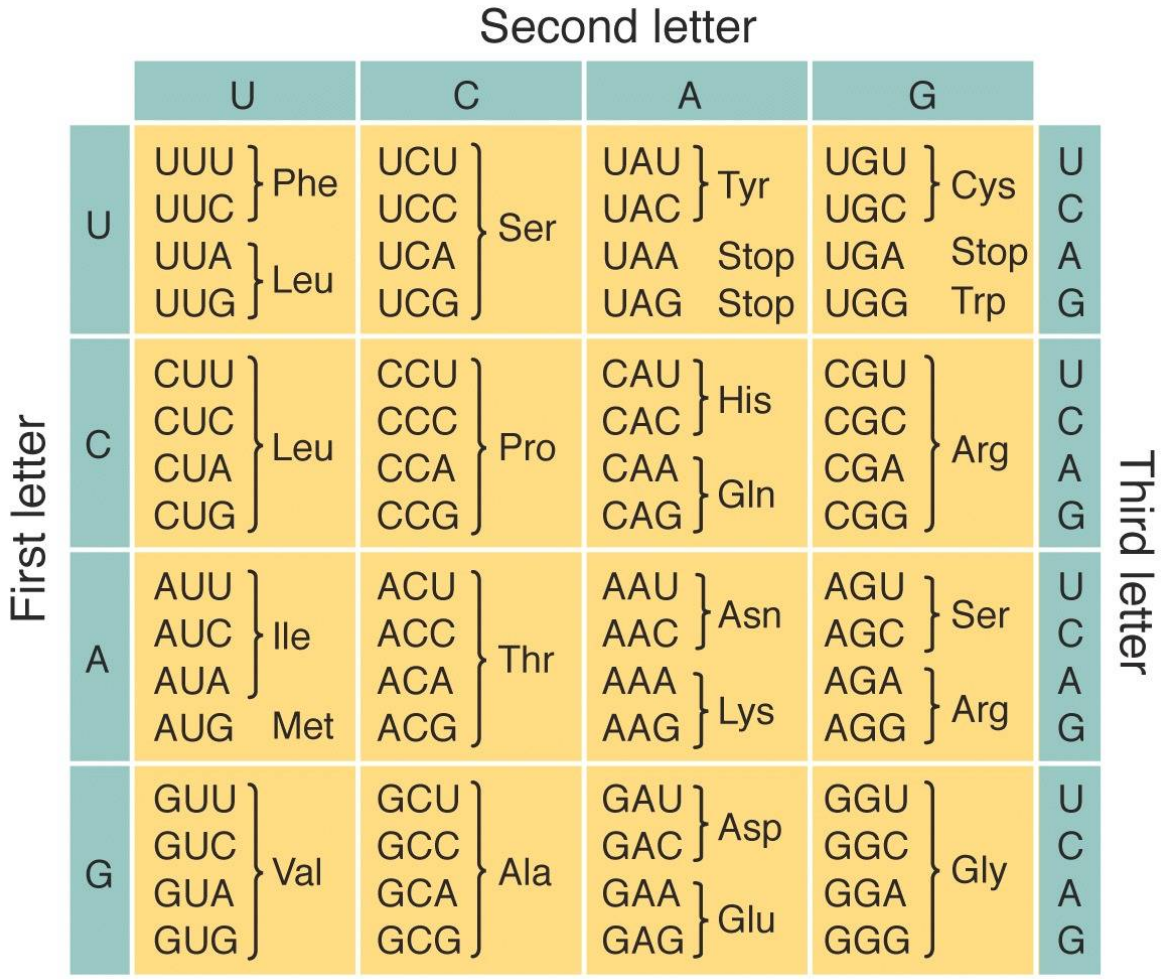
- There are **20 different amino acids** **ferent bases** in DNA/RNA.
- Each three-letter unit of mRNA is called a **CODON**.

- There are **4^3** (= **64**) codons possible --> therefore there are **easily enough codons to code for all the necessary amino acids**.

- In fact, the **same amino acid** is often **specified by more than one codon**. However (and this is **very important**), the reverse is **never** true: that is, any **one codon** only specifies **ONE amino acid** -- there is **no vagueness** in the code (e.g. CCU will *always* produce proline).

- The code also contains "**punctuation**." It tells when to **start** reading the gene for a particular protein and when to **stop**.

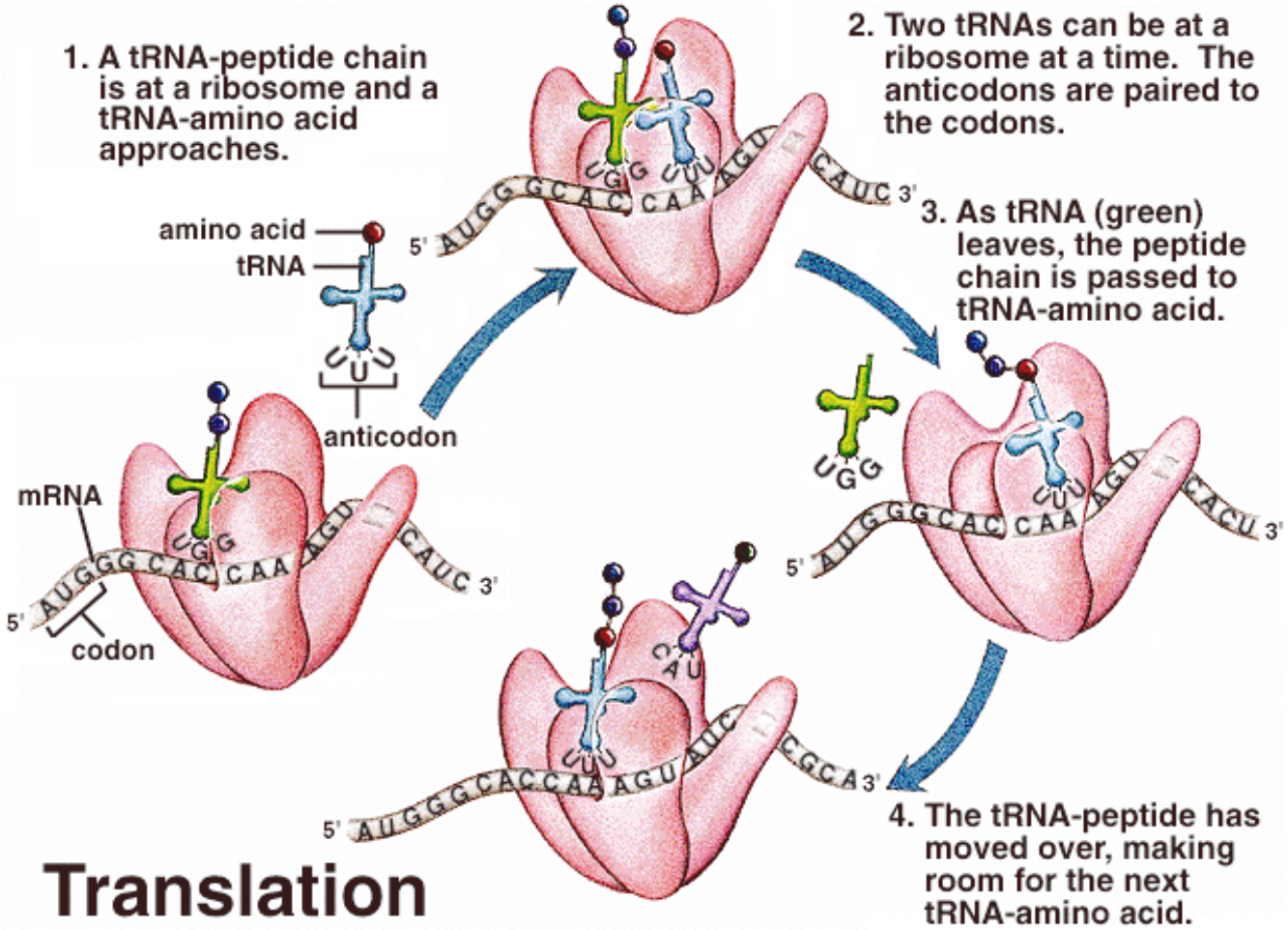
Each codon corresponds to an **amino acid**, or a "**start**" or "**stop**" synthesis signal. And *here it is*, the most important chart in all of Biology: the **GENETIC CODE**!



- The genetic code is **universal**: the **same codons** stand for the **same amino acids** in all living things (well, *almost* all living things).
- This "**Biochemical Unity**" suggests that all living things have a **common evolutionary ancestor**.

Translation

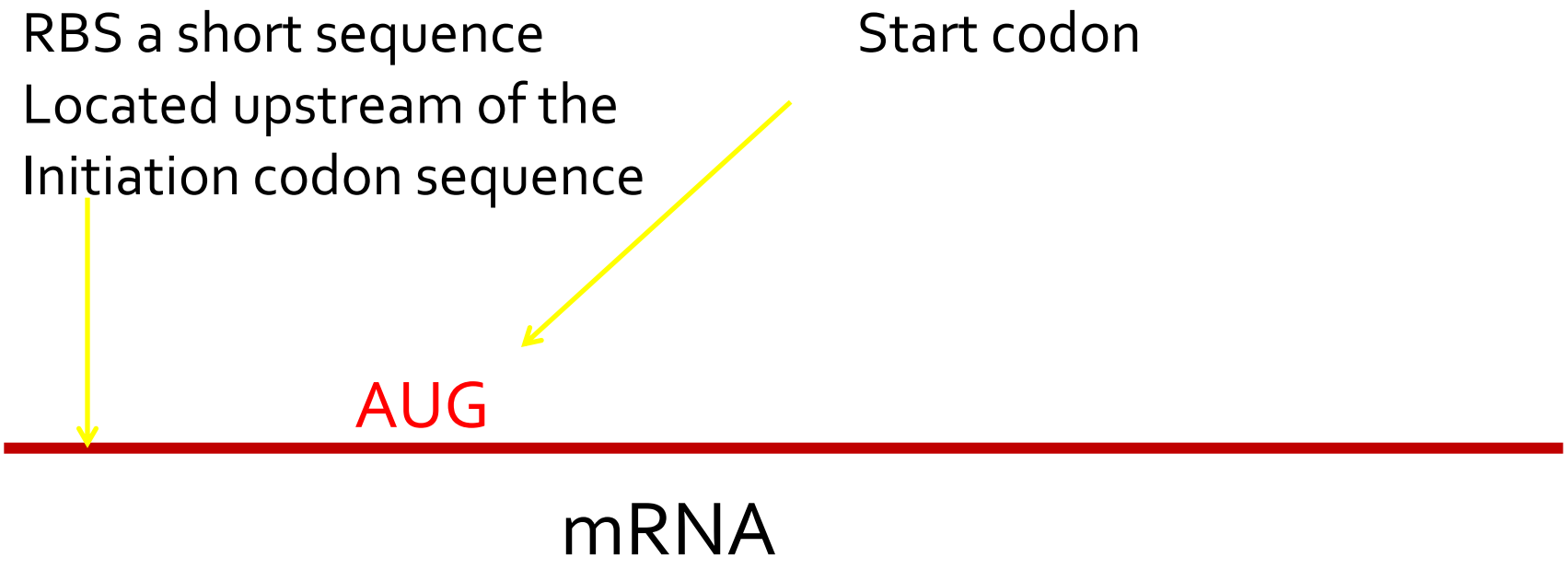
Human Genome Video



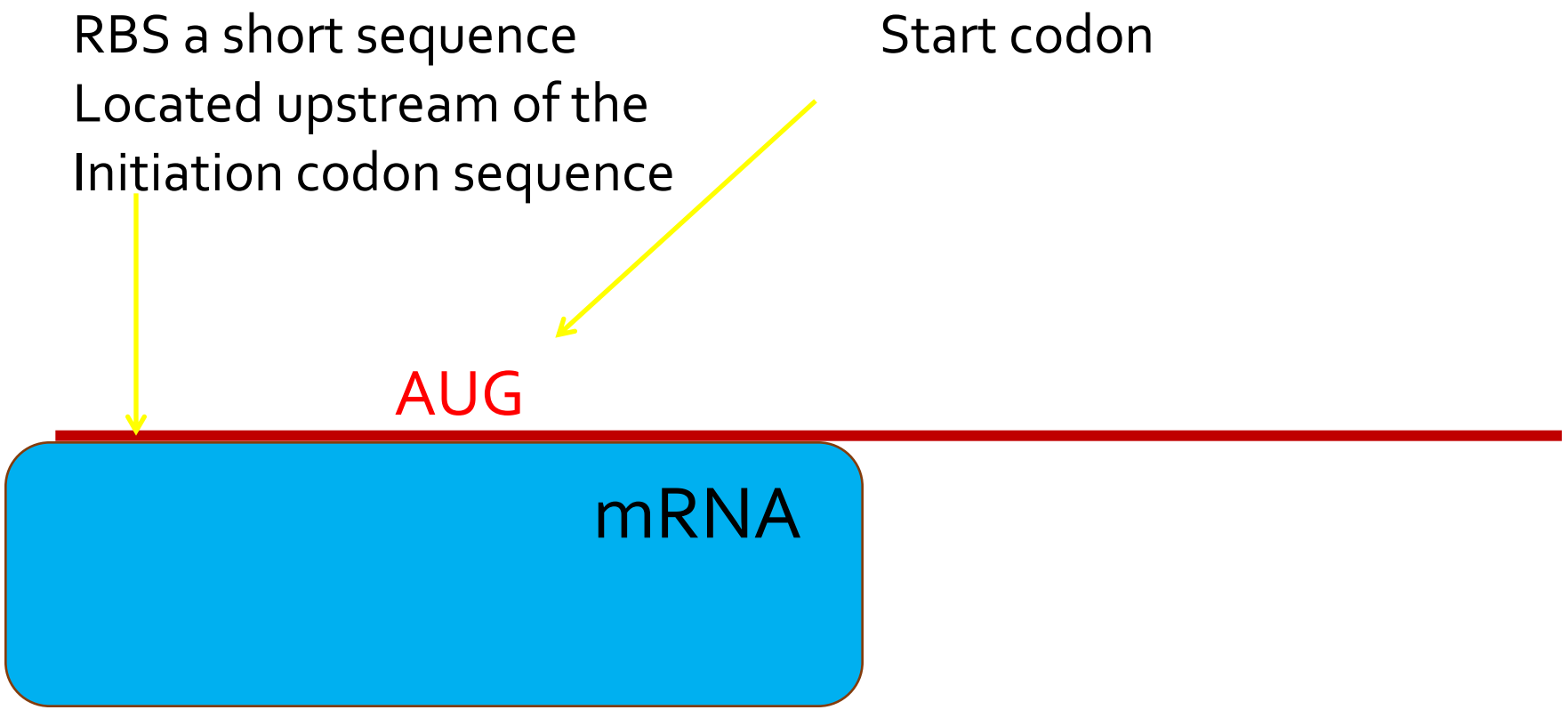
The steps in TRANSLATION: can be divided into 3 subprocesses:

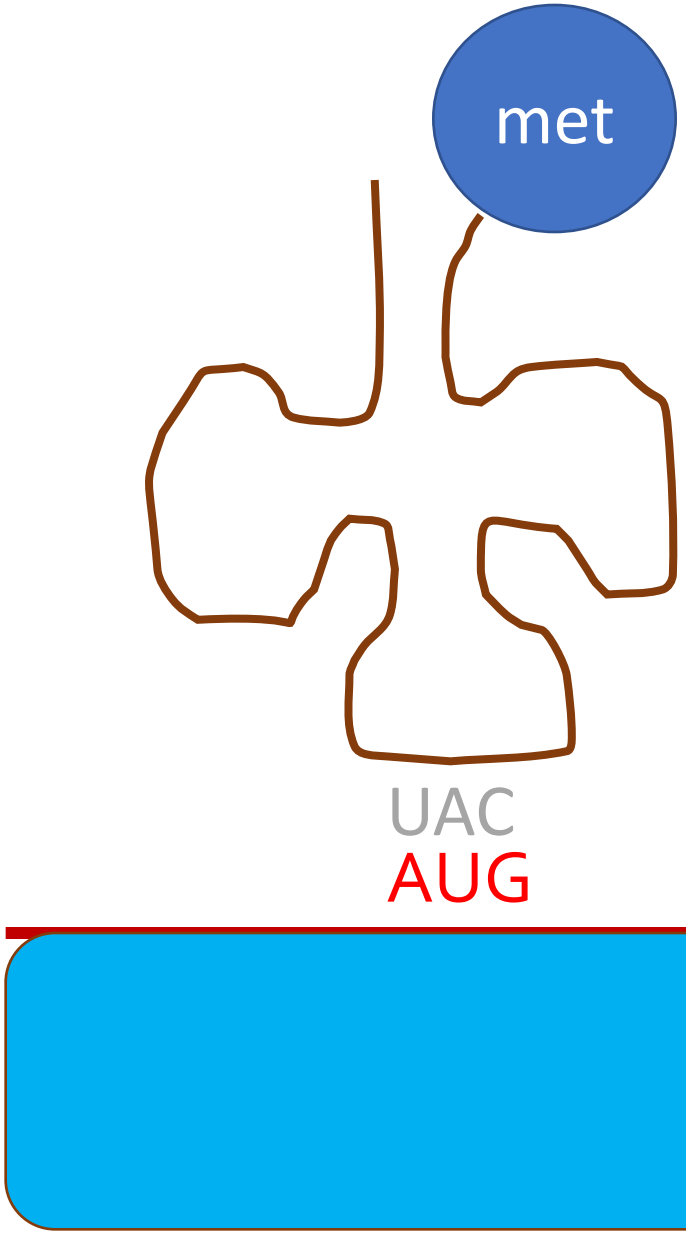
1. **Initiation**
2. **Elongation**
3. **Termination**

1. Initiation - : the mRNA, with its **START CODON (AUG)** attaches to the "R" site of the ribosome.



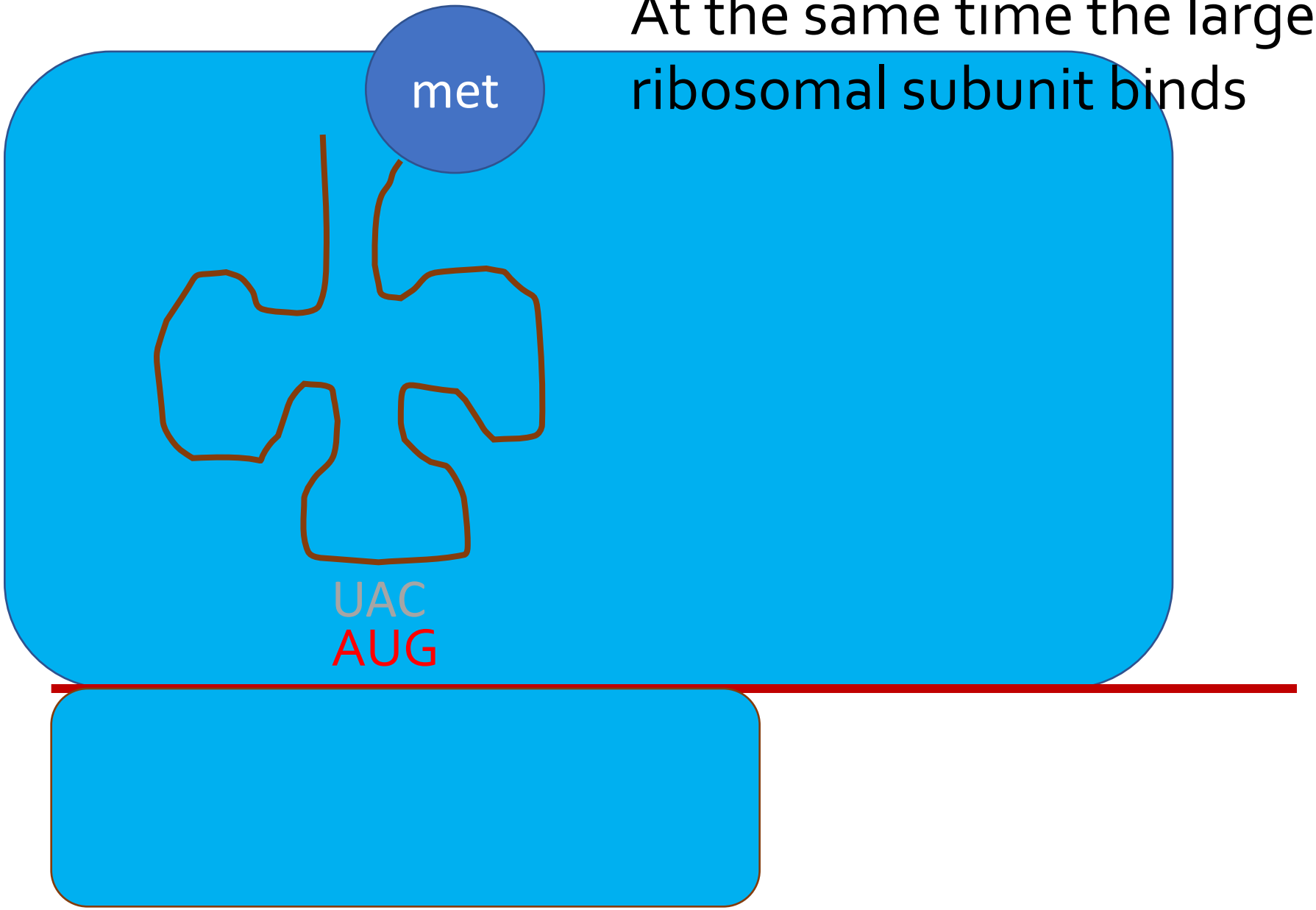
A small ribosomal subunit binds to mRNA.

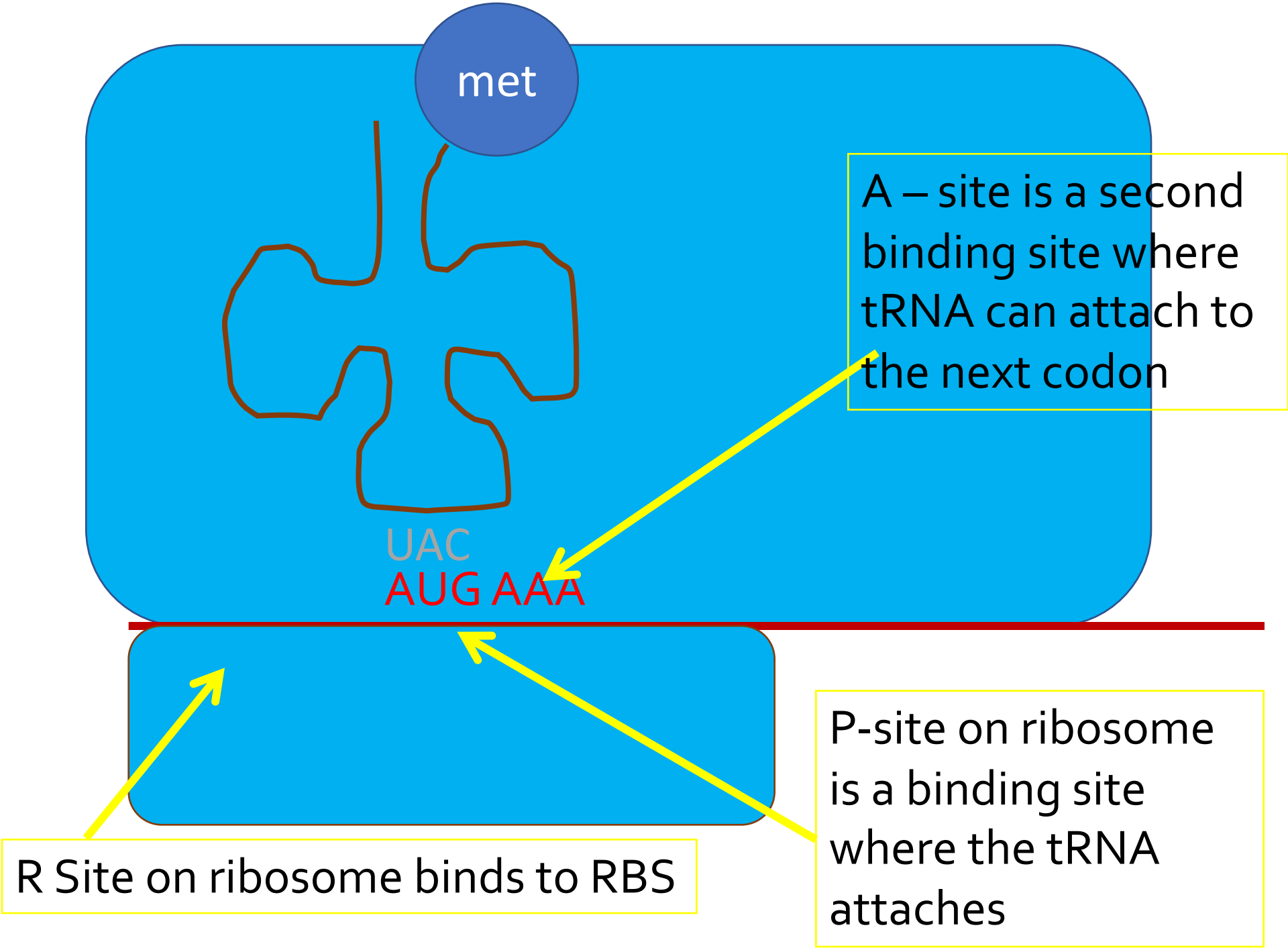


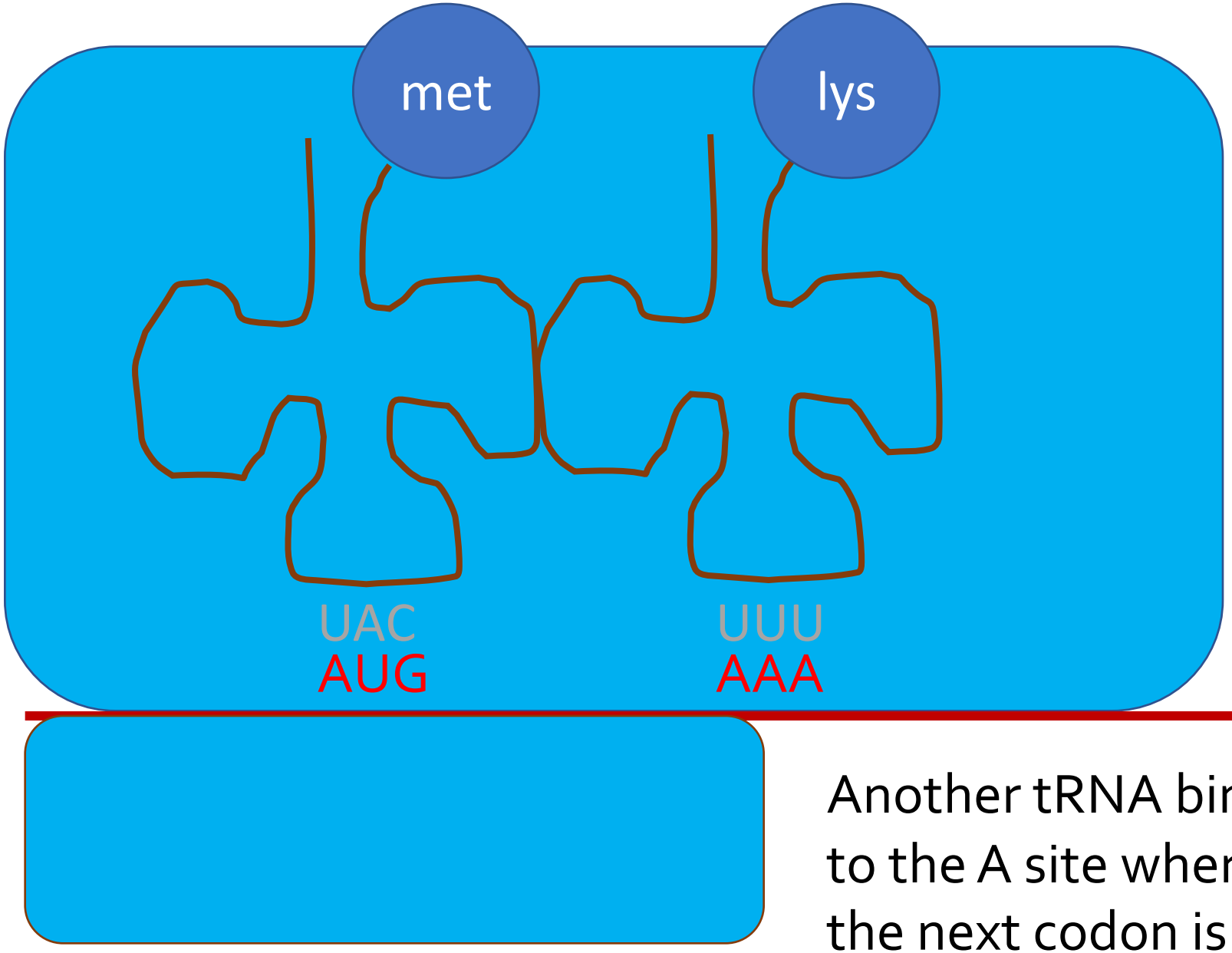


tRNA with a complementary anticodon (UAC)pairs with the initiation codon (AUG)
the amino acid methionine is bound to the tRNA

At the same time the large ribosomal subunit binds




met
UAC
AUG AAA
A – site is a second binding site where tRNA can attach to the next codon
R Site on ribosome binds to RBS
P-site on ribosome is a binding site where the tRNA attaches



Another tRNA binds to the A site where the next codon is located

1. **INITIATION**: the mRNA, with its **START CODON (AUG)** attaches to the "R" site of the ribosome.

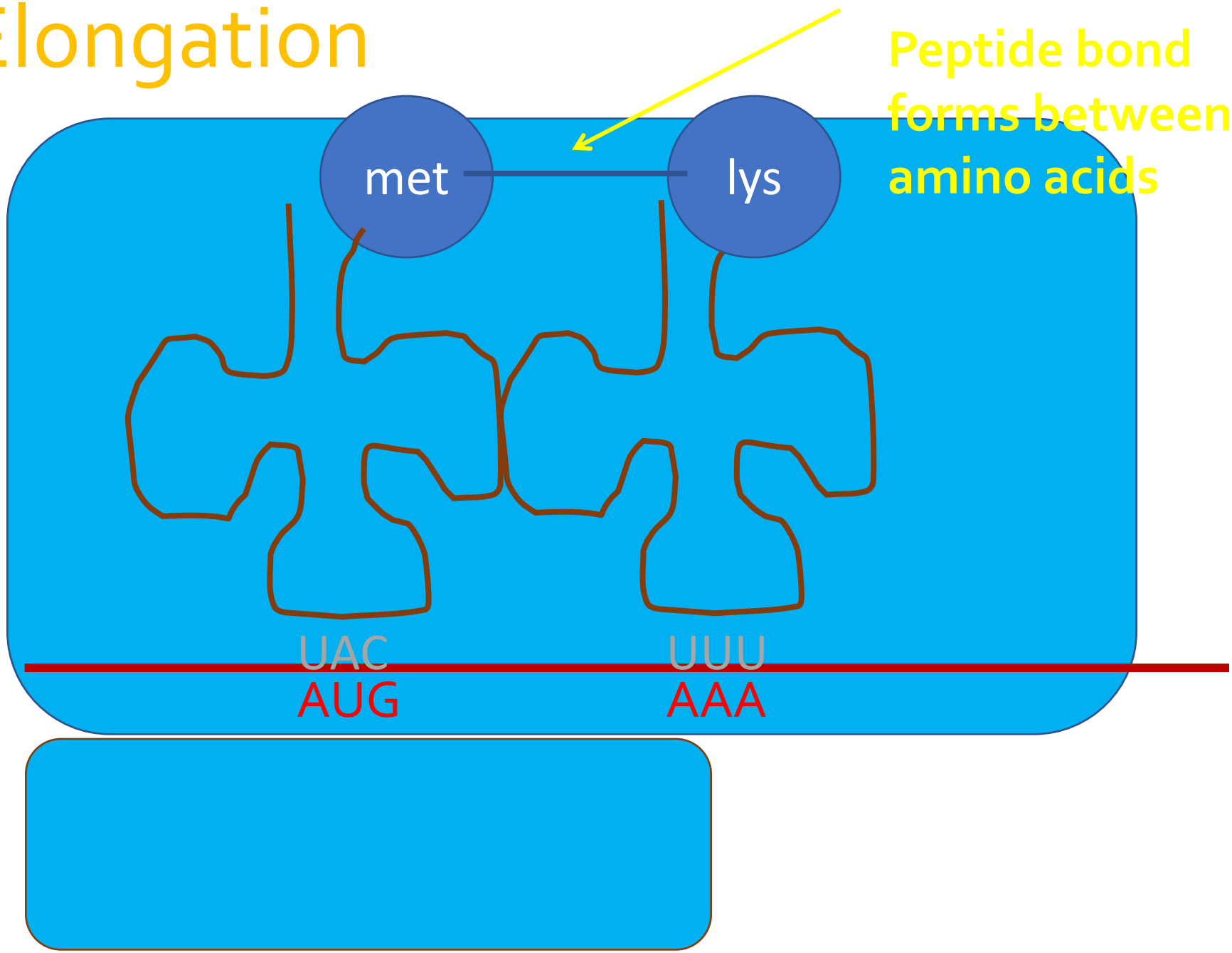
- The **AUG** codon **always initiates translation** and codes for the amino acid **methionine**.

- **tRNA** binds to the **start codon** of mRNA. The tRNA has a **binding site of 3 bases** called an **ANTICODON** that is **complementary** to the mRNA codon. Therefore, the codon of mRNA of AUG is "read" by a tRNA that has a **UAC anticodon**. The tRNA that has this anticodon carries, at it's tail, the amino acid **methionine**.

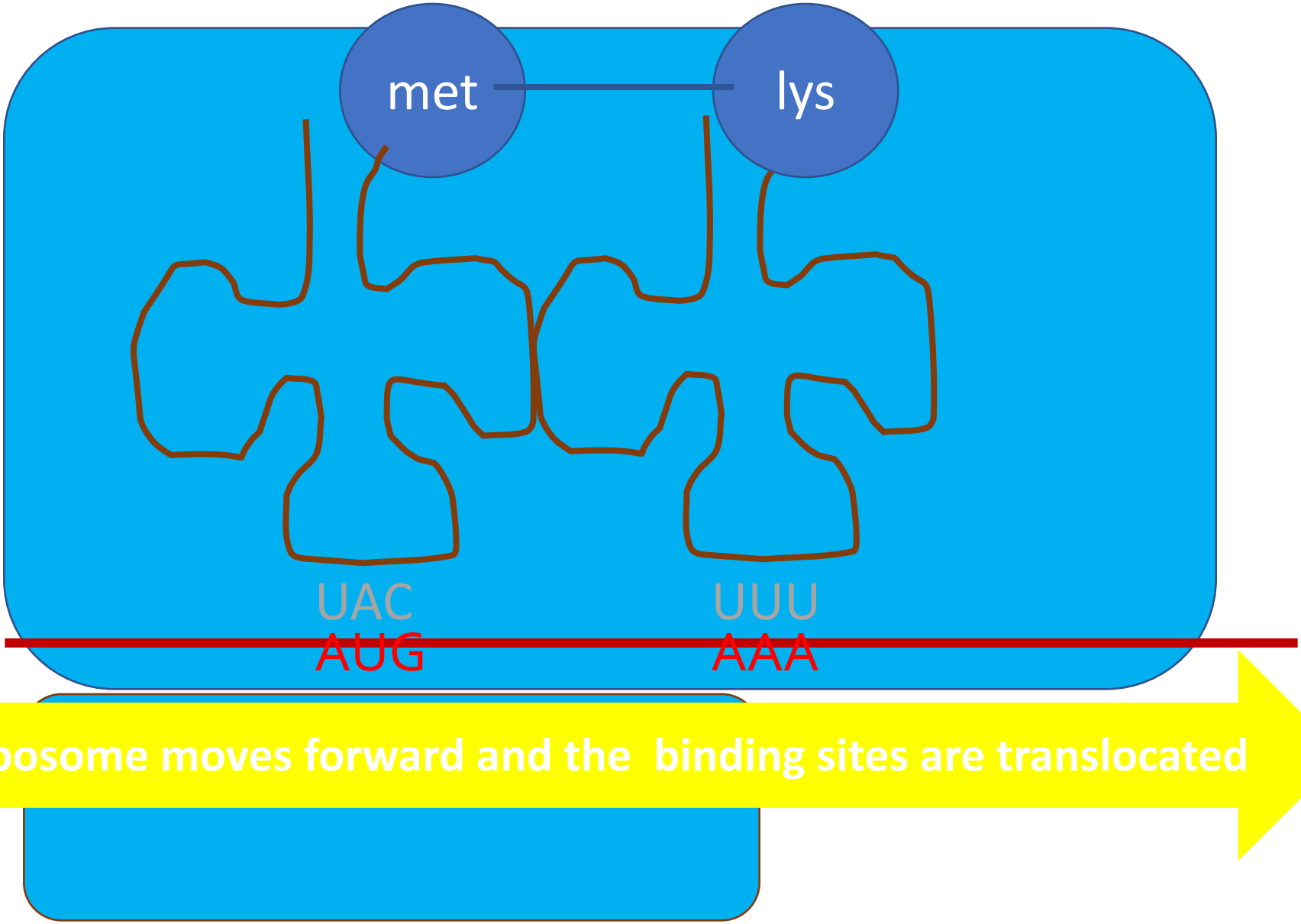
- This **methionyl-tRNA** is in the **P** site of the ribosome. The **A** site next to it is **available** to the tRNA bearing the next amino acid.

There is a specific tRNA for each mRNA codon that codes for an amino acid.

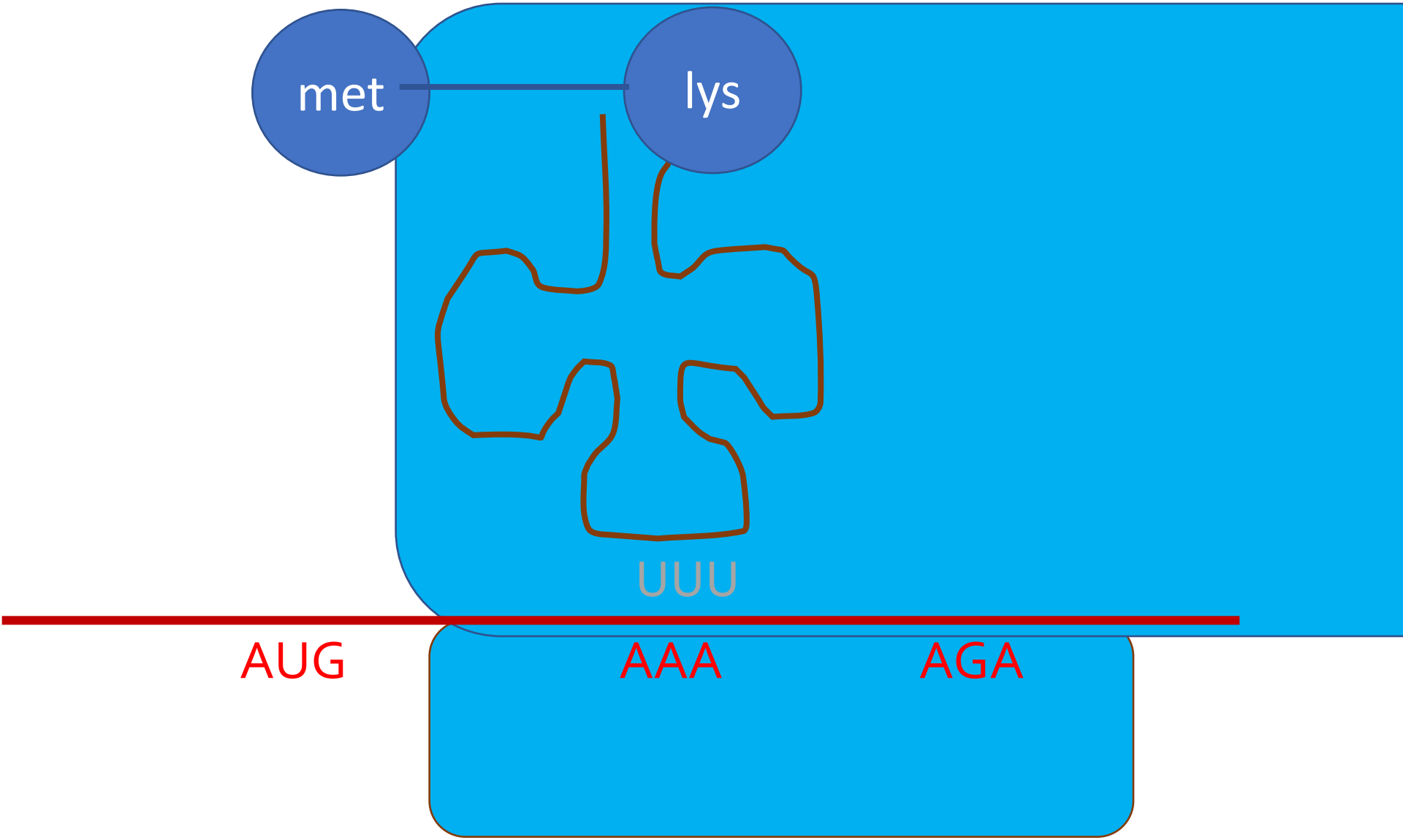
Elongation


Peptide bond forms between amino acids
met
lys
UAC
AUG
UUU
AAA

Elongation


met
lys
UAC
AUG
UUU
AAA
Ribosome moves forward and the binding sites are translocated

Elongation



- **more amino acids are added** and connected together to form a **polypeptide**, as specified by the mRNA sequence.

a. an incoming amino-acyl-tRNA (lets call this AA_2-$tRNA_2$) recognizes the codon in the A site and binds there.

b. a peptide bond is formed between the new amino acid and the growing polypeptide chain.

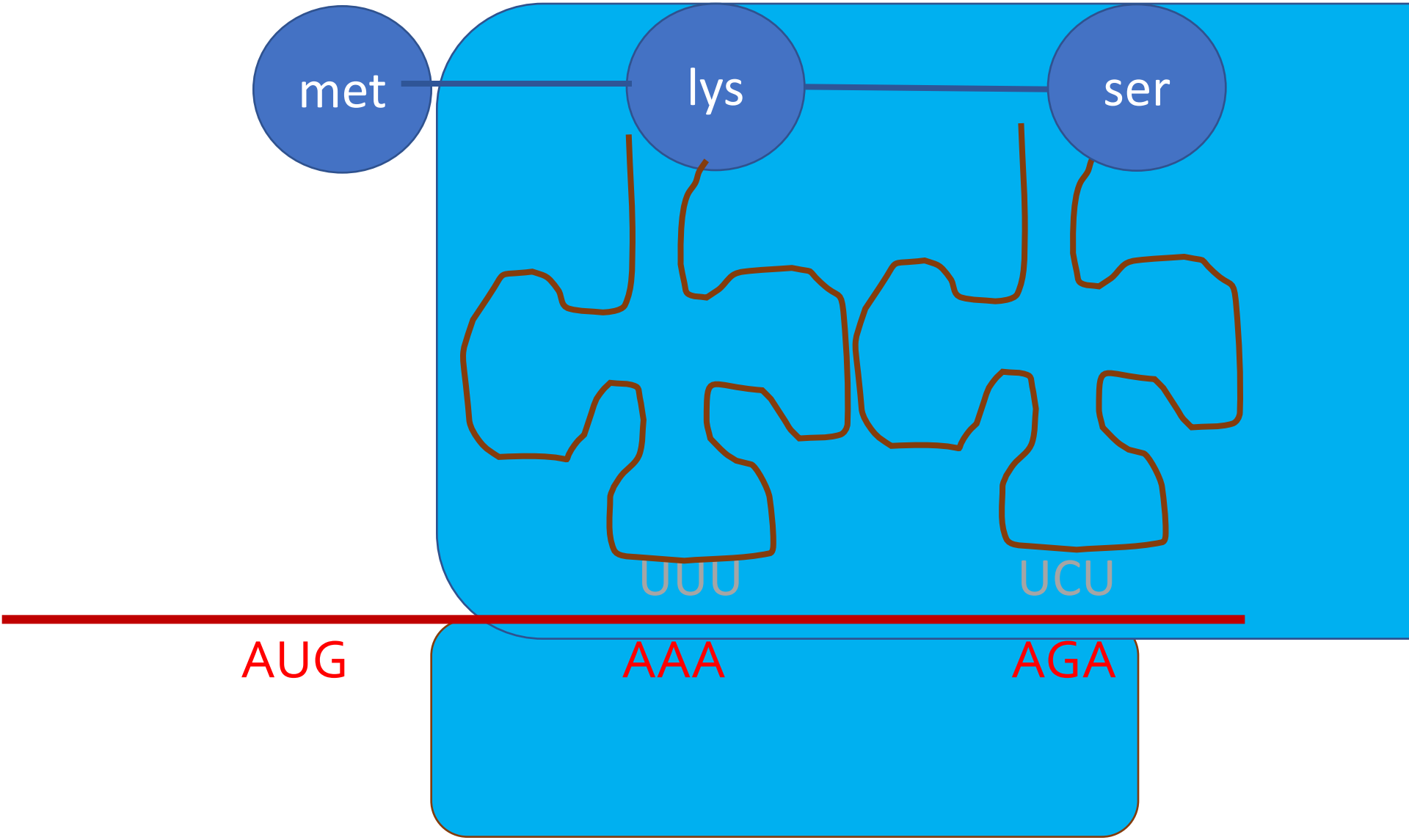
c. the amino acid is removed from $tRNA_1$ (bond breaks between aa_1 and $tRNA_1$)

d. the $tRNA_1$ that was in the P site is released, and the tRNA in the A site is translocated to the P site.

e. the **ribosome moves over one codon** along the mRNA (to the right in our diagram, or more specifically in the 5' ----> 3' direction.)

f. This movement shifts the $tRNA_2$ (which is attached to the growing amino acid chain) to the P site.

Elongation



g. $tRNA_3$ with aa_3 can now move into A site and bind with the next codon on mRNA.

h. **THIS PROCESS <u>REPEATS</u>**, and the CHAIN **<u>ELONGATES</u>** as long as there are new codons to read on the mRNA.

3. **TERMINATION**: The process above repeats until a special codon, called a **STOP CODON**, is reached. There are 3 Stop codons: **UAA, UAG, UGA**.

   a. the stop codons **do not code for amino acids** but instead act as **signals to stop translation**.

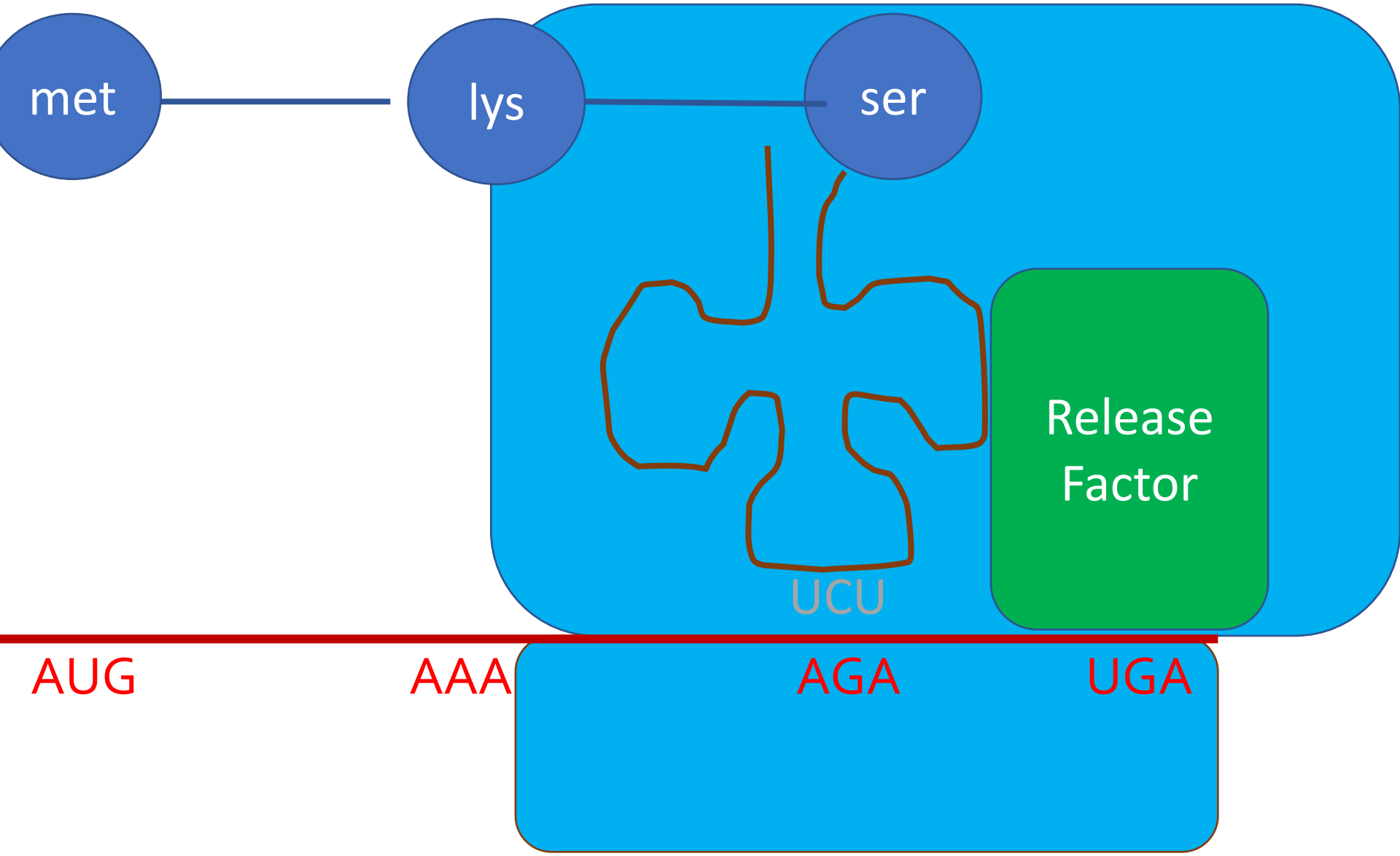
   b. a protein called ***release factor*** **binds directly to the stop codon** in the A site. The release factor causes a **water molecule** to be added to the end of the polypeptide chain, and the **chain then separates from the last tRNA**.

   .

c. **the protein is now complete**. The **mRNA is now usually broken down**, and the ribosome splits into its large and small subunits.

d. the new protein is sent for **final processing** into the **endoplasmic reticulum** and **golgi apparatus**

Termination

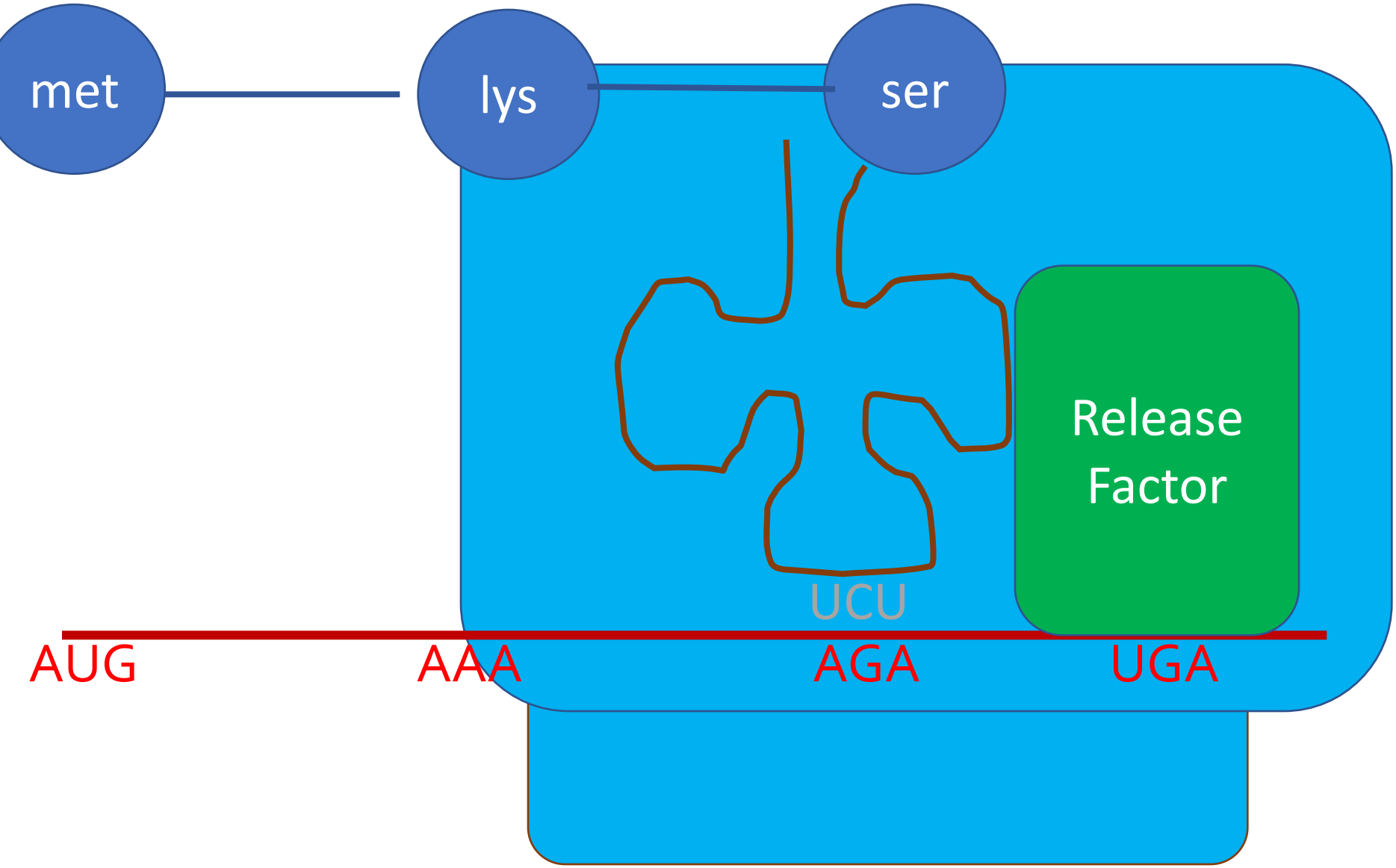


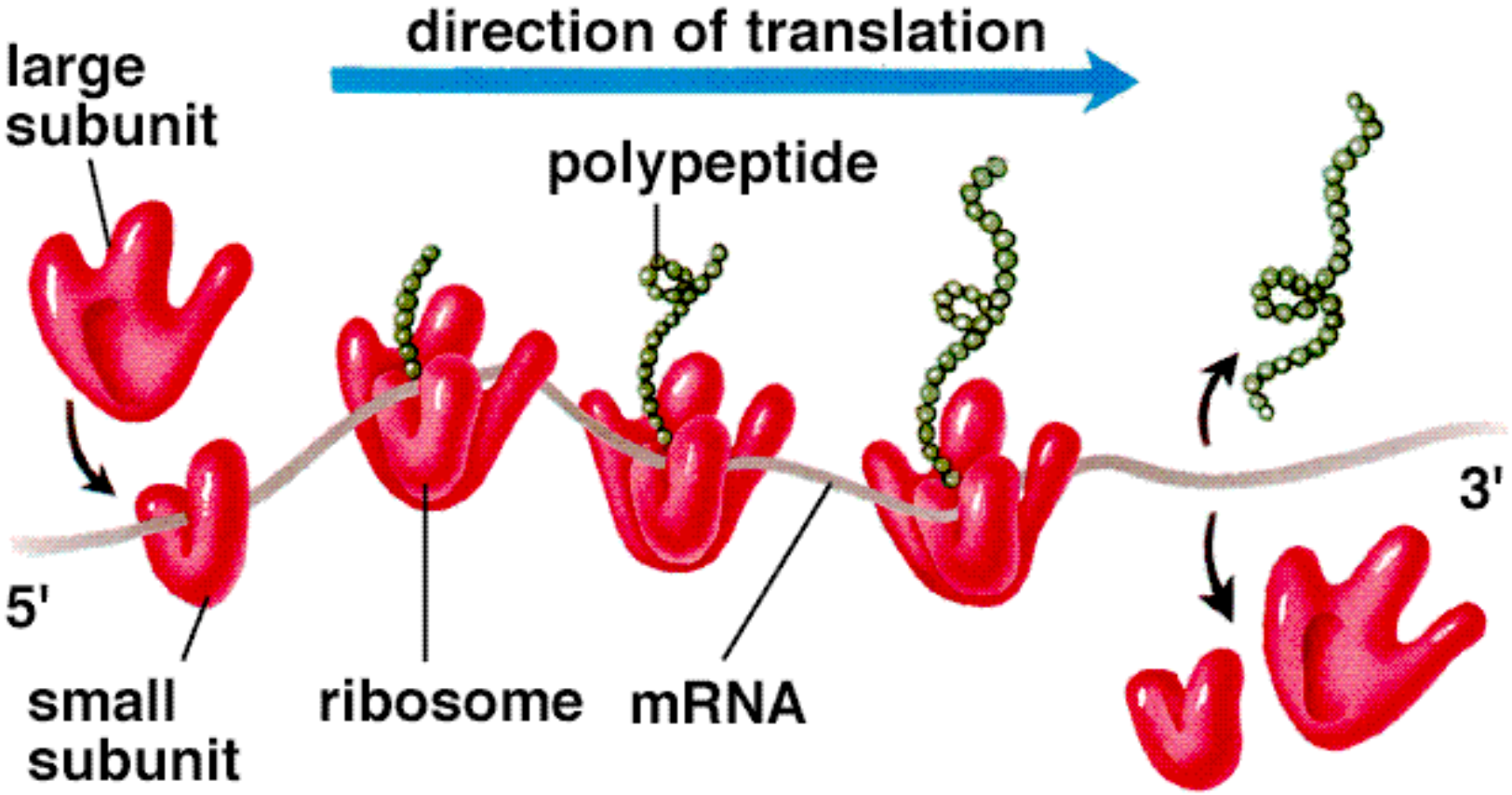
The release factor

- Comes to a stop codon on the mRNA and binds to the site

- Hydrolyzes the bond between the last tRNA at the P site and the polypeptide. This releases them
- The ribosomal subunits dissociate

Termination




direction of translation
large subunit
polypeptide
5'
3'
small subunit
ribosome
mRNA

Often, **many ribosomes** will **simultaneously transcribe** the **same mRNA**. In this way, **many copies** of the same protein can be made quickly. These clusters of ribosomes are called **polysomes**.

Working Example!

- Translating DNA into proteins

- Given the following DNA nucleotide sequence:
- **TGTCAACGTACTG**

1. Give the mRNA sequence that would be transcribed from it!
-

Working Example!

- Translating DNA into proteins

- Given the following DNA nucleotide sequence:
- **TGTCAACGTACTG**

1. Give the mRNA sequence that would be transcribed from it!
- ACAGUUGCAUGAC

2. **Give the mRNA codons**

3. Give the tRNA anticodons

2. **Give the mRNA codons**

ACA GUU GCA UGA C

3. Give the tRNA anticodons

2. **Give the mRNA codons**

ACA GUU GCA UGA C

3. Give the tRNA anticodons

UGU CAA CGU ACU G

•4. Give the amino acid sequence that would be translated from it.

- **a. Take your mRNA codons**
- **ACA GUU GCA UGA C**

- **b. Refer to Fig. 24.8 p 492**

•**4. Give the amino acid sequence that would be translated from it.**

- **a. Take your mRNA codons**
- **ACA GUU GCA UGA C**

- **b. Refer to Fig. 24.8 p 492**

•**Threonine – Valine – Alanine - Stop**

II. Determining DNA sequences from Proteins

Given the following amino acid sequence, give a possible DNA sequence that could code for the sequence:

Lysine, Asparanine, Methionine, Glutamic Acid, Alanine, Stop.

Lysine, Asparagine, Methionine, Glutamic Acid, Alanine, Stop.

- Step 1
- Look up codons on fig. 24.8

- Lys – Asp - Met - Glu - Ala – Stop
- AAA – AAU – AUG – GAA – GCU - UAA
- AAG AAC GAG GCC - UAG
- GCA UGA
- GCG

There are multiple codons for Asparagine, Glutamate , Alanine and stop

Find the complementary base pairs and remember that Uracil is not a base in DNA.

AAA-AAU-AUG-GAA-GCU-UAA - mRNA

TTT-TTA-TAC-CTT-CGA-ATT - DNA

- For Protein Synthesis Quiz:
- Stated Clearly Video

E3-E4: Mutations

Amoeba sisters: Mutations Animation

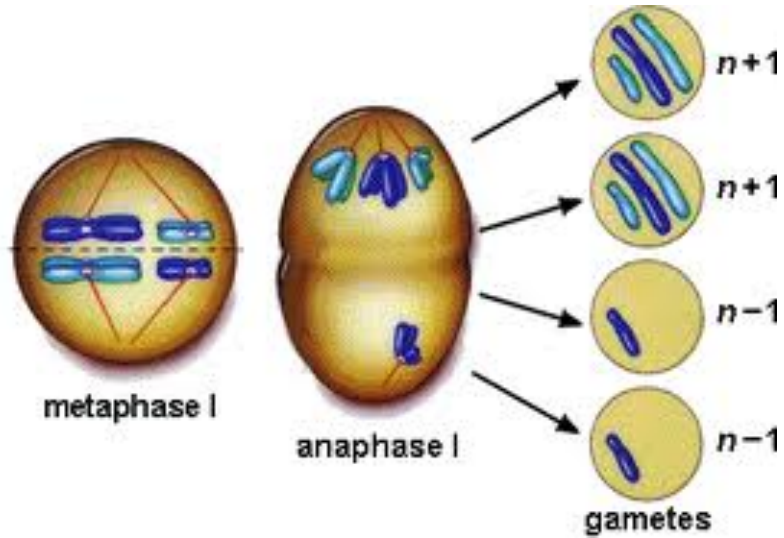
- **I. MUTATIONS & DEFECTS**

A. Chromosomal abnormalities

- **1. Recall: during meiosis, homologous chromosomes (doubled, earlier in the process) line up at the center of the cell and engage in "crossing over" (increases variety of gene combinations)…**
-

•2. Problems occasionally arise, causing changes in the physical structure of a chromosome

- **i) Usually involves thousands of genes**
- **i) homologues get stuck together, and don't separate (called "non-disjunction")**
- **a) in sex chromosomes …**
- **b) in autosomes**
-



Non disjunction in sex chromosomes

Sex chromosome of defective sperm	Sex chromosome of normal egg	Sex chromosome of fetus (genotype)	Phenotype of offspring
O	X	XO	F-Turner Syndrome
XX	X	XXX	F – Trisomy X
YY	X	XYY	M – XYY males
XY	X	XXY	M – Klinefelter Syndrome

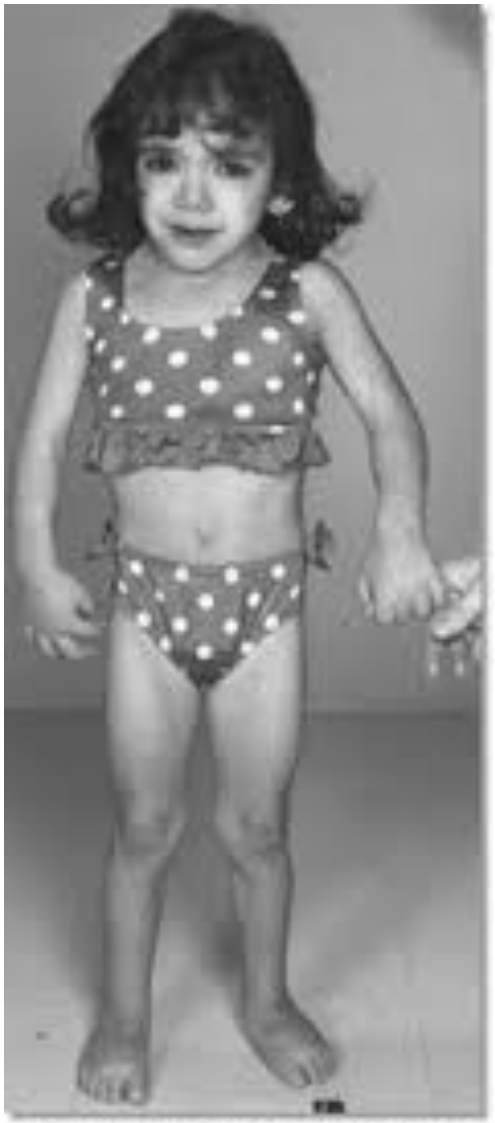
Sex chromosome of defective egg	Sex chromosome Of normal sperm	Sex chromosomes of fetus (genotype)	Phenotype of offspring
O	X	XO	F – Turner Syndrome
O	Y	YO	fatal
XX	X	XXX	F – trisomy X
XX	Y	XXY	M – Klinefelter Syndrome

Turner Syndrome (XO)

Characteristics:

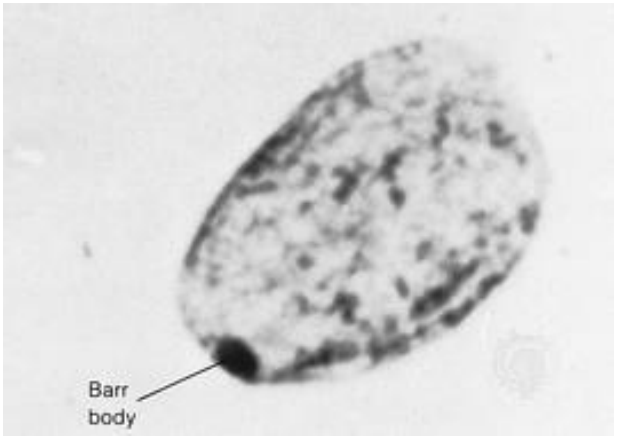
- Swollen hands and feet (child)
- Webbed neck (child)

- Incomplete puberty
- Broad shield-like chest
- Drooping eyelids
- Dry eyes
- Infertility
- Short
- Absence of menstruation



Trisomy X

Due to the deactivation of X-chromosomes and the formation of Barr bodies, only one X-chromosome is active in any given cell.



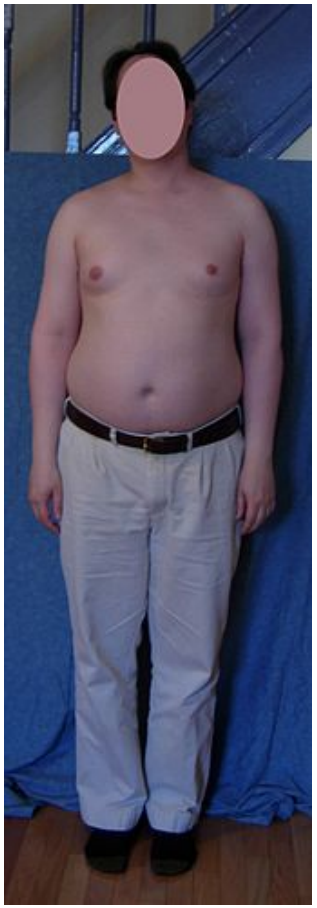
Therefore, most girls with trisomy X are unaffected by the extra X chromosome.

XYY (Jacobs Syndrome)

- Taller
- May have persistant acne
- Tend to have speech and reading problems
- Once thought to be aggressive – since disproven

XXY – Klinefelter Syndrome

- Abnormal body proportions (long legs, short trunk, shoulder equal to hip size)
- Abnormally large breasts
- Infertility
- Sexual problems
- Less than normal amount of pubic, armpit, and facial hair
- Small testicles
- Tall height



b) in autosomes:

1) results in gametes that are:

- **missing the chromosome in question**
- **have 2 copies of chrom. in question**

2) when fused w/ normal gamete, zygote has either 1 or 3 copies of chromosome in question

3) 1 copy = fatal; embryo aborts so early that woman never knows she was pregnant

4) 3 copies = fatal in most cases; miscarriage later in pregnancy: exceptions: trisomy 13, 18 may make the full 40 weeks and be born; trisomy 21 (Down Syndrome)

c) non-disjunction influenced by age of parents, esp. mother …

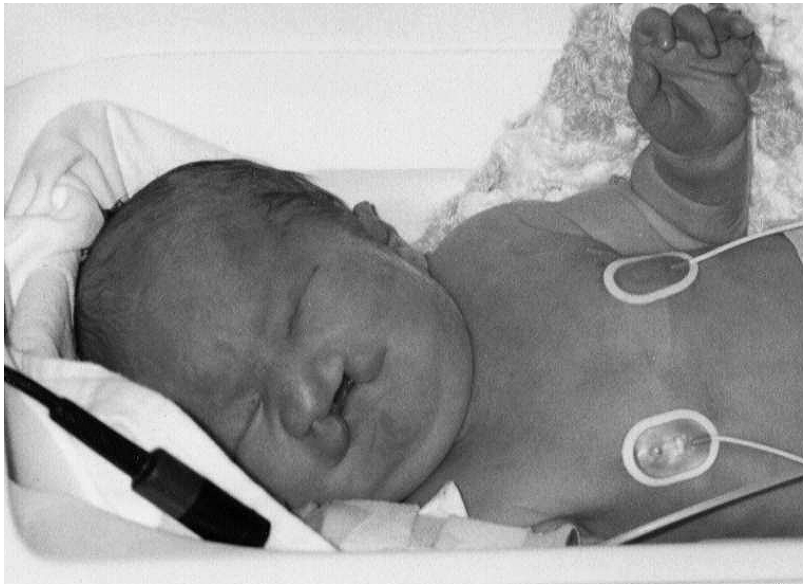
1) a woman's eggs begin to develop in her ovaries when she is still a fetus in her mother's uterus!

Over 35, non-disjunction risk rises exponentially

2) male "age effect" is smaller (meiosis cycle about 2 weeks) (about 25% of Down Syn. Cases)

Trisomy 13 - Patau Syndrome

- Cleft lip or palate
- Clenched hands (with outer fingers on top of the inner fingers)
- Close-set eyes -- eyes may actually fuse together into one
- Decreased muscle tone
- Extra fingers or toes
- Hernias:
- Hole, split, or cleft in the iris
- Low-set ears
- Intellectual Disability, Severe
- Scalp defects (missing skin)
- Seizures
- Single palmar crease
- Skeletal (limb) abnormalities
- Small eyes, head and lower jaw
- Unusual to survive past 1st birthday



Trisomy 18 – Edwards Syndrome

Clenched hands

Crossed legs (preferred position)

Feet with a rounded bottom (rocker-bottom feet)

Low birth weight

Low-set ears

Intellectual disability

Small head

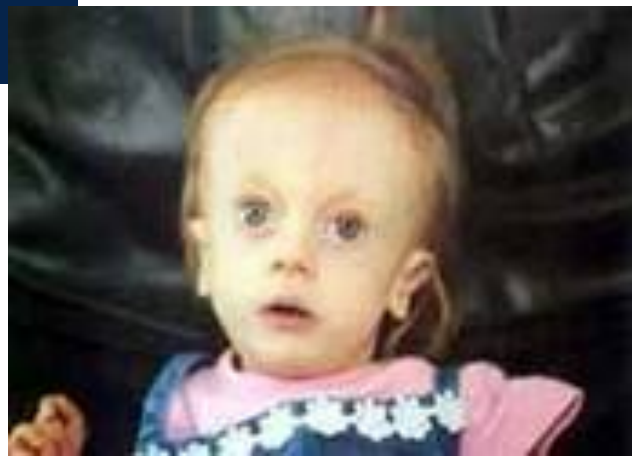
Small jaw

Underdeveloped fingernails

Undescended testicle

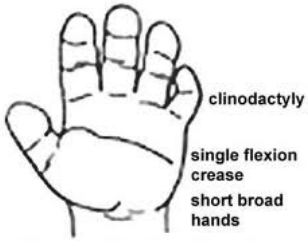
Unusual shaped chest

50% of infants do not survive past 1 week. Some make it into teenage years with serious developmental and medical problems



Trisomy 21 – Down Syndrome

- Decreased muscle tone at birth
- Excess skin at the nape of the neck
- Flattened nose
- Separated joints between the bones of the skull (sutures)
- Single crease in the palm of the hand
- Small ears
- Small mouth
- Upward slanting eyes
- Wide, short hands with short fingers
- White spots on the colored part of the eye
- Impulsive behavior
- Poor judgment
- Short attention span



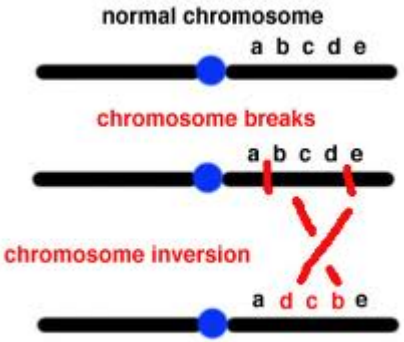
Trisomy 21 - Hand Features

B. Chromosomal Mutations

- **Def’n: change in chromosome structure that can be detected microscopically**

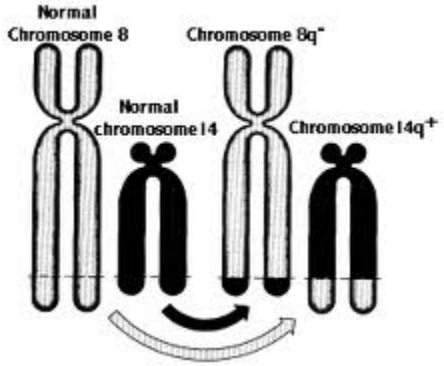
- **1. Segments of chromosomes can be affected**
  - **(in crossing over, or break due to radiation, chemicals, viruses…) and change the gene sequence of that chromosome**
- **2. Results: missing genes, extra copies of genes,or “garbling” of base-pair sequences**

•i)Flipped-over pieces (“inversion”)

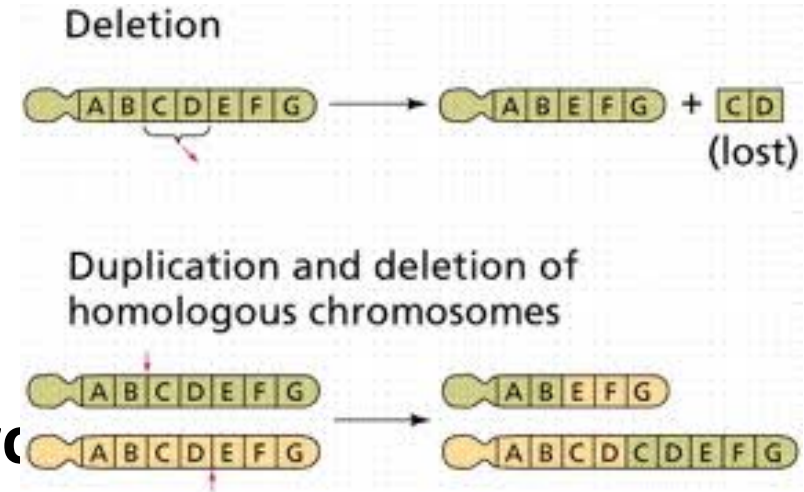


•ii) Exchange of pieces with a non-homologue (“translocation”)

•



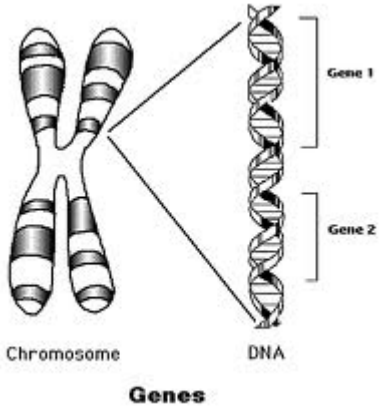
•iii) Exchange of pieces with a homologue (“duplication”)



•iv) Missing pieces from two
closes up, leaving a missing piece behind (called “deletion”)

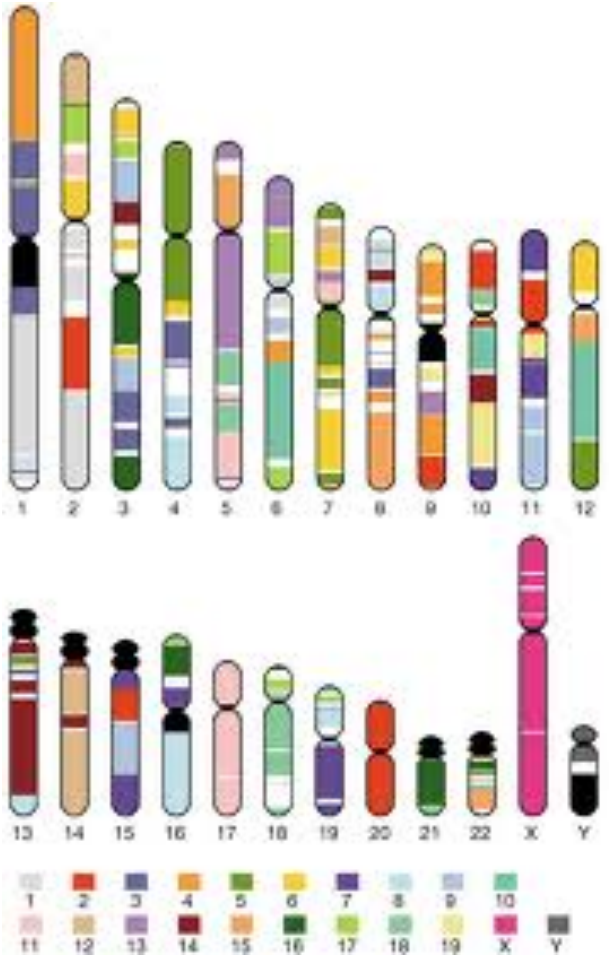
C. Gene Mutations

1. GENE (def' n) the segment of DNA on a chromosome that codes for ONE protein



2. Gene Mutation (def' n) change in the nucleotide sequence of a gene

3. The human genome (all the DNA in all 46 chromosomes in one human cell) is approximately 3 billion base pairs -- only 10 - 15 % of this DNA is actual genes



4. Types of gene mutation:

a) FRAMESHIFT mutations: caused by insertions or deletions in the base-pair sequence

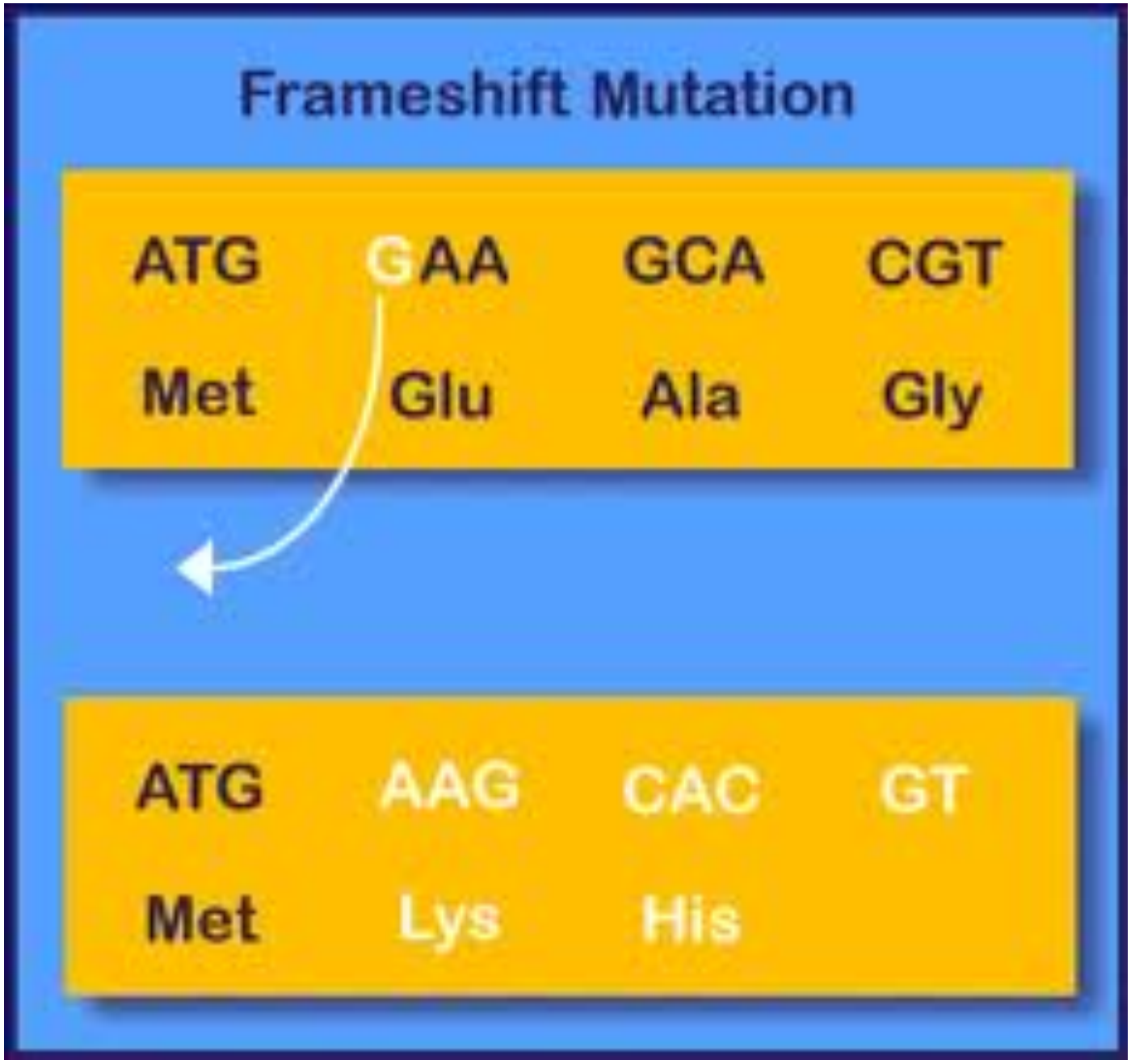
i) effect how the codons are read:

e.g.: THE MAN BIT THE DOG

delete the first H, and this becomes:

TEM ANB ITT HED OG_

ii) result: a completely non-functioning protein; all amino acids are wrong


Frameshift Mutation
ATG GAA GCA CGT
Met Glu Ala Gly
ATG AAG CAC GT
Met Lys His

b) POINT mutations: a change in a single nucleotide, resulting in a change of one codon

i) results vary…

1) SILENT mutation: no effect on protein

e.g.: ACU → ACG or ACC or ACA

- Because all 4 code for amino acid Threonine

2) NONSENSE mutation: shortens protein

e.g.: UCG → UCA
[cysteine] [stop codon!]

- very serious; makes protein non-functional!

3. MISSENSE mutation: substitution of one amino acid for another

e.g.: GUA → GAA
[valine] [glutamate]

effect: variable … if the amino acids have similar chemical properties, or are located in a noncritical area of the protein, there will be little to no effect.

TED-ED Gene Mutations through time

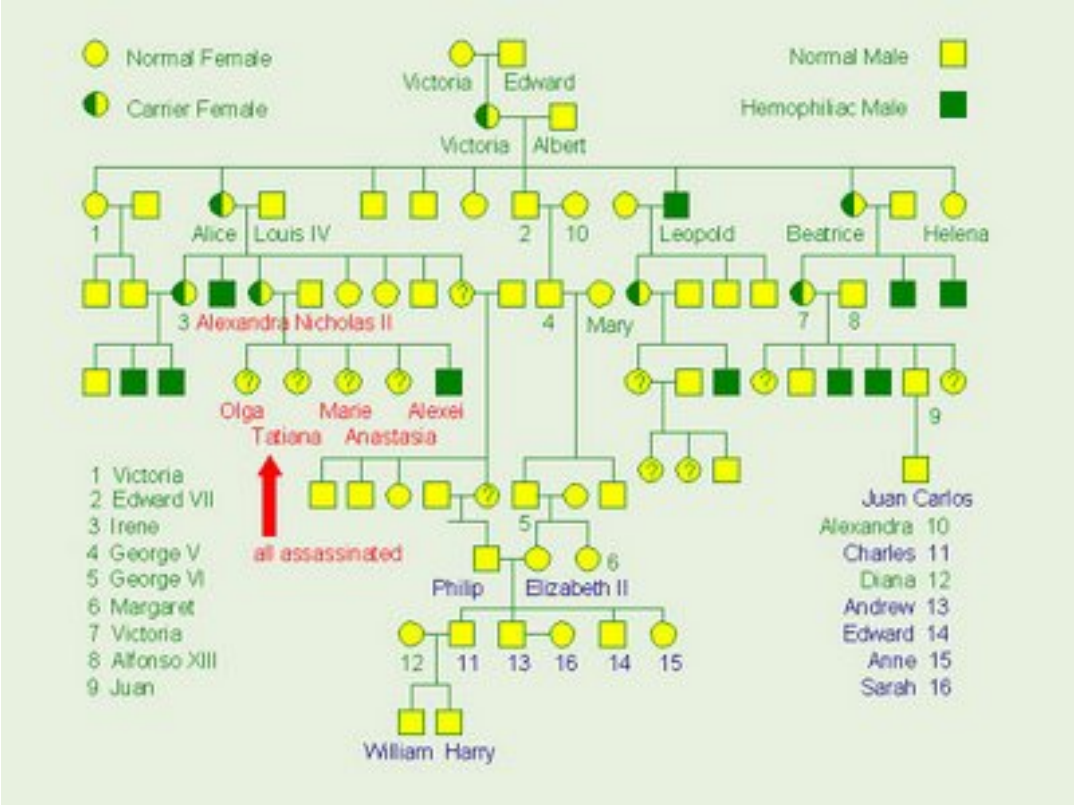
If they are quite different and/or in critical area, can cause disease (the above substitution causes sickle-cell anaemia!) TED –ED Sickle Cell

II. MUTATIONS: LOCATIONS/CAUSES

A. Germinal (in tissue that gives rise to sperm or eggs)

1. Can be passed on to offspring

e.g.: Queen Victoria & haemophilia



B. Somatic Mutation (in body tissues)

1. Not inheritable
2. Responsible for many cancers

C. Replication errors

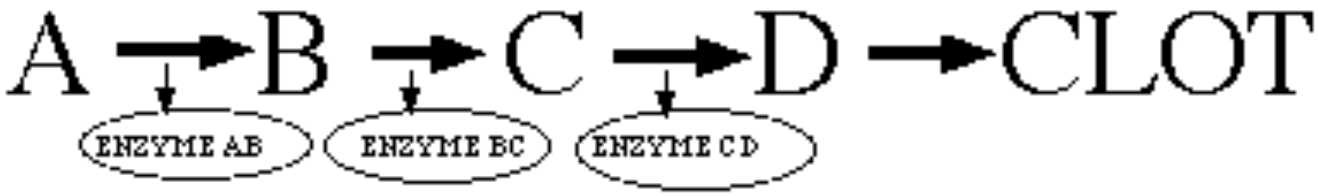
1. Pretty rare; enzyme (DNA polymerase) that carries out replication also "proofreads" and makes corrections; only about 1 per 109 base pairs replicated!

D. MUTAGENS (def'n): environmental substances that cause mutations

1. Radiation: X-rays, UV rays, exposure to radioactive elements
2. Chemicals: generally organic chemicals; pesticides, cigarette smoke, etc.

III. HOW MUTATIONS CAUSE GENETIC DISORDERS

A. Normally, chemical reactions occur in "pathways"…



B. If Enzyme BC is mutated (nonfunctional) then compounds C and D would not be made, and clot doesn't form -- Haemophilia

Working Example, cont'd

Given the following DNA nucleotide sequence:

TGTCAACGTACTG

For each mutation below:

a) work out the NEW amino acid sequence
b) identify the type of mutation
c) predict the consequences for protein function

1. a G is inserted in between the double As

TGTCAACGTACTG

1.mRNA:

- ACAGUUGCAUGAC

2.Separate into codons

- ACA GUU GCA UGA C
- 3. Find A.A.s to match code
- Thr – Val – Ala- Stop

TGTCAGACGTACTG

1.mRNA:

- ACAGUCUGCAUGAC
- 2. Separate into codons
- ACA GUC UGC AUG AU
- 3. Find A.A.s to match code
- Thr – Val- Cys- Met…..

This is a frame shift mutation that results in the protein not terminating. The result is likely a non functional protein.

The second T from the left is deleted

TGTCAACGTACTG

1.mRNA:

- ACAGUUGCAUGAC

2.Separate into codons

- ACA GUU GCA UGA C
- 3. Find A.A.s to match code
- Thr – Val – Ala- Stop

TGCAACGTACTG

1.mRNA:

- ACGUUGCAUGAC

2.Separate into codons

- ACG UUG CAU GAC
- 3. Find A.A.s to match code
- Thr –Leu – His- Asp

This is a deletion mutation that results in the protein not terminating. The result is likely a non functional protein.

The third T from the left is changed to a C

TGTCAACGTACTG

1.mRNA:

- ACAGUUGCAUGAC

2.Separate into codons

- ACA GUU GCA UGA C
- 3. Find A.A.s to match code
- Thr – Val – Ala- Stop

TGTCAACGCACTG

1.mRNA:

- ACAGUUGCGUGAC

2.Separate into codons

- ACA GUU GCG UGA C
- 3. Find A.A.s to match code
- Thr – Val – Ala- Stop

This is a silent missense mutation that results in the protein terminating as originally because although the DNA sequence changed, the amino acids encoded remained identical.

Protein Synthesis Quiz

Stated Clearly Video

CRSPR Video- In a Nutshell

Ethics Lesson

Show some of these videos:
Questioning Bioengineering
You are NOT your genes
ELSI Ethics
ELSI ANIMATION
Biology Ethics
Case Study: Vaccines
Poll Everywhere Activity