Cellular & Molecular Biology
SQBS 1143

Chapter 4:
Translation

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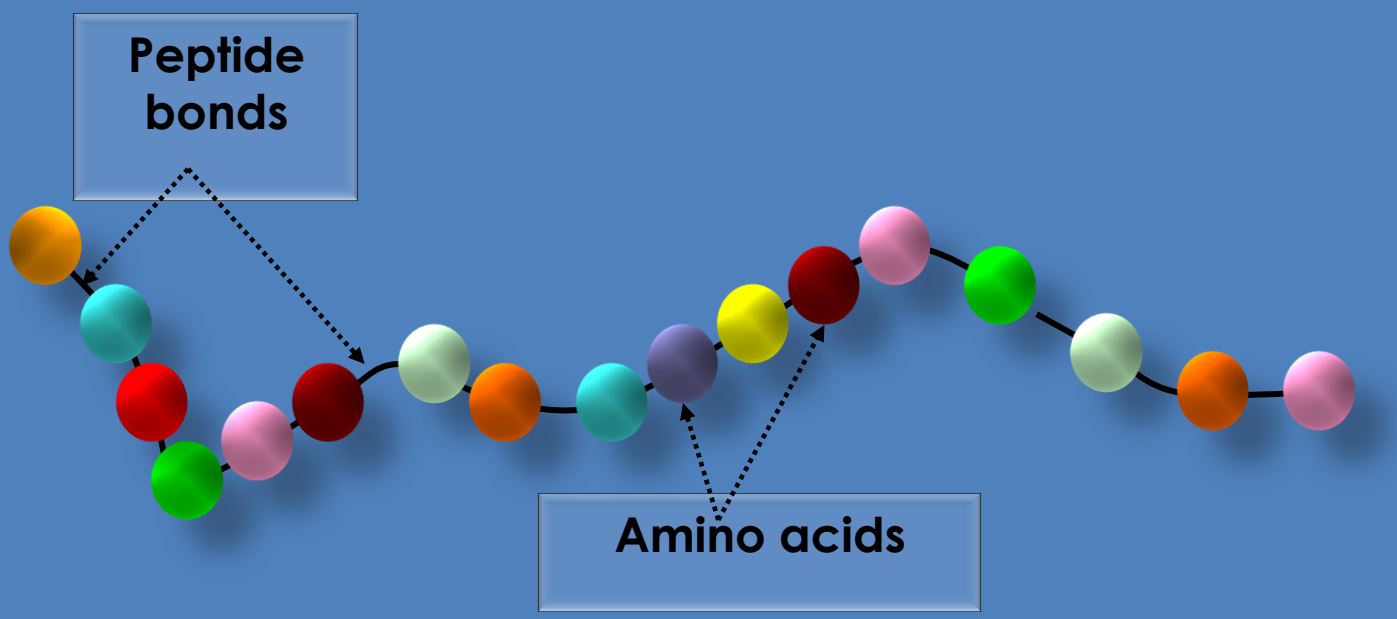
Introduction to Proteins

- When it comes to getting the real work of the cell done, the bureaucrats, DNA and RNA, are not much help. All the same, somebody has to get their hands dirty.

- **Proteins** are biological polymers that carry out most of the cell's day-to-day functions.

- Some proteins are merely **structural** or take part in cell movement, others help take up nutrients, others generate energy and yet others carry out biochemical reactions, including the synthesis of nucleotides and their assembly into nucleic acids.

POLYPEPTIDE CHAIN



- Molecules whose primary role is to carry information (nucleic acids like **DNA** and **messenger RNA**) are basically **linear molecules** with a regular repeating structure.

- Molecules that form cellular structures or have active roles carrying out reactions are normally folded into **three-dimensional (3-D)** structures. These include both **proteins** and certain **specialized RNA molecules** (**rRNA** and **tRNA**).

- Proteins are **made from a linear chain of monomers**, and are **folded** into a variety of **complex 3-D shapes**. A chain of amino acids is called a **polypeptide**. Some proteins consist of **more than one** polypeptide chain.

Role of Proteins in the Cell

We can subdivide proteins into four main categories:

- **Structural protein**
- **Enzyme**
- **Regulatory protein**
- **Transport protein**

} **Discussed in previous chapters**

i. Structural proteins

Structural proteins are found making up many **subcellular structures**. The flagella with which bacteria swim around, the microtubules used to control traffic inside cells of higher organisms, the fibers inside a muscle cell, and the outer coats of viruses are a few examples of structures built using proteins.

ii. Enzymes

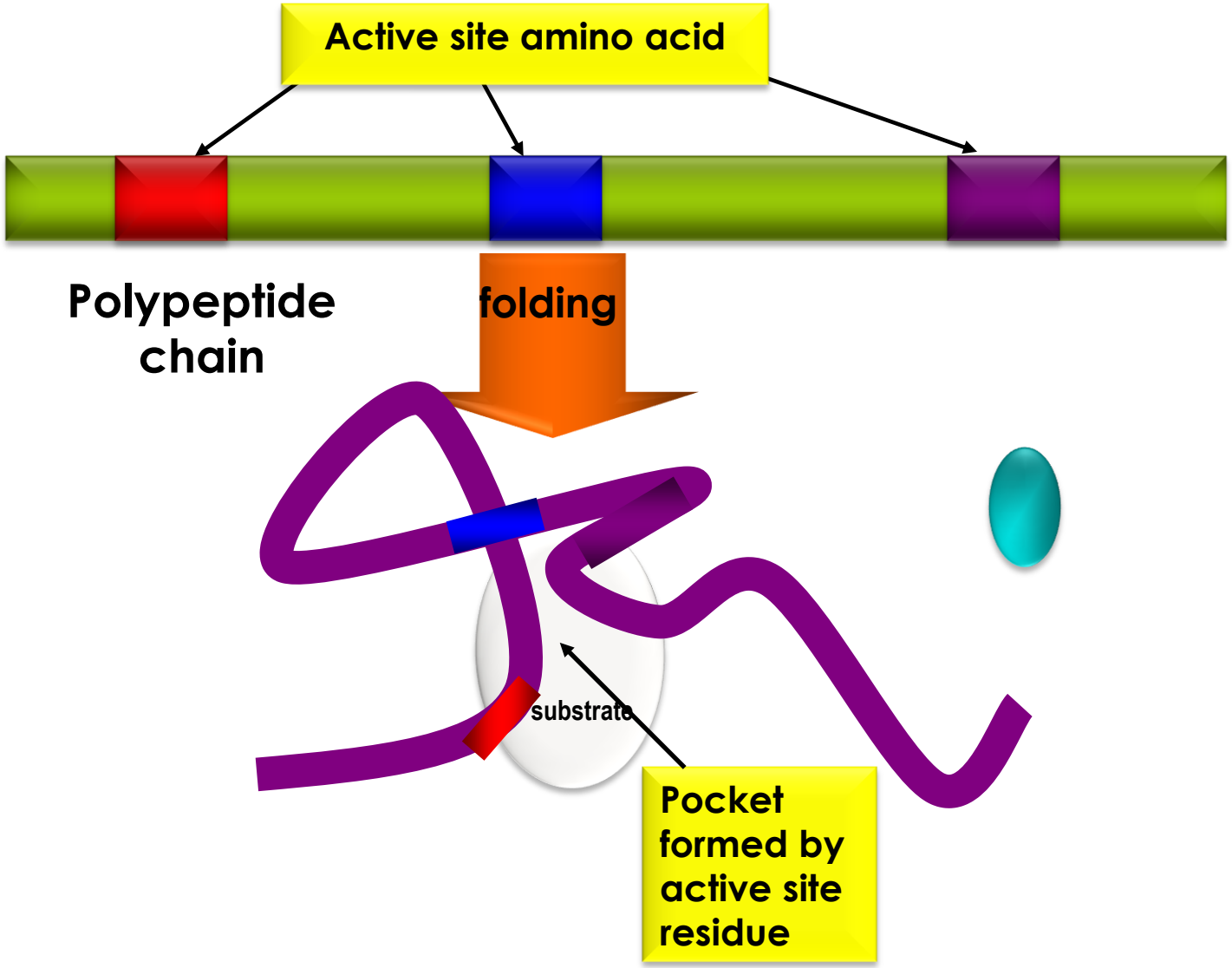
- **Enzymes** are proteins that carry out chemical reactions. An enzyme first binds another molecule, known as its **substrate**, and then performs some chemical operations with it.

- Some enzymes bind only **a single substrate** molecule; others may bind **two or more**, and react them together to make the final product.

- In any case, the enzyme needs an **active site**, a **pocket** or **cleft** in the protein, where the substrate binds and the reaction occurs.

- The active site is produced by **folding up the polypeptide chain correctly** so that amino acid residues that were spread out at great distances in the linear chain now come together and will co-operate in the enzyme reaction.

- The most famous enzyme in molecular biology is **β-galactosidase** encoded by the ***lac Z gene*** of the bacterium *Escherichia coli*.


Active site amino acid
Polypeptide chain
folding
substrate
Pocket formed by active site residue

Analog of lactose

- This is so easy to assay that it is widely used in genetic analysis. The natural substrate of β-galactosidase is the sugar **lactose**, made by linking together the two simple sugars, **glucose** and **galactose.**

- **Analogs** are molecules resembling natural substances well enough to fool the enzymes that use them. Some analogs **bind but not react** and simply **block the active site** and inhibit the enzyme. Such analogs are known as **competitive inhibitors** compete with the true substrate for the attention of the enzyme.

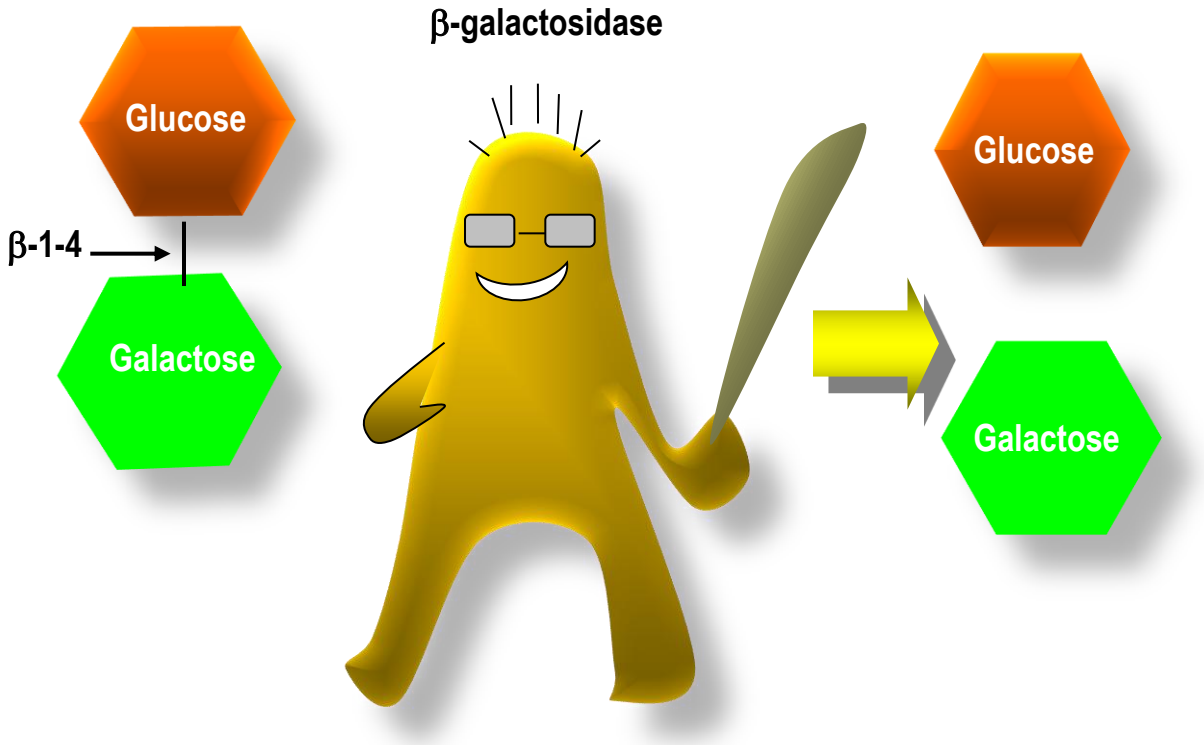
ONPG as lactose analog

- Other analogs do react. β-galactosidase splits many molecules in which galactose is linked to something else. We can take advantage of this by giving it **ONPG** which consists of **ortho-nitrophenol linked to galactose**.

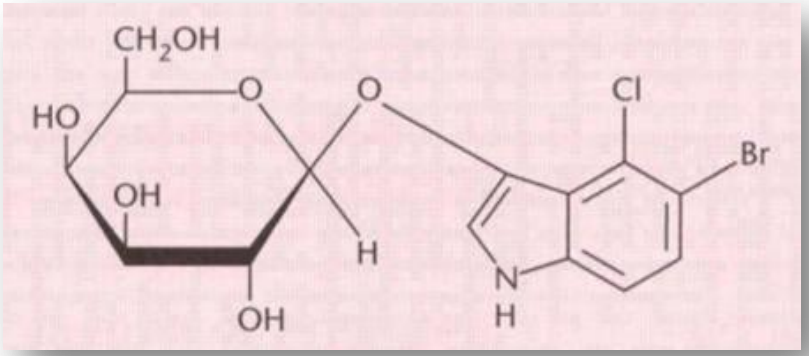
- When ONPG is split, we get galactose (colourless) and ortho-nitrophenol which is bright yellow. Using ONPG allows us to monitor the level of β-galactosidase by measuring the level of yellow colour.

X-gal is also split by β-galactosidase

- Similarly X-gal is split by β-galactosidase into a **blue dye** and **galactose**.



X-gal a lactose analog

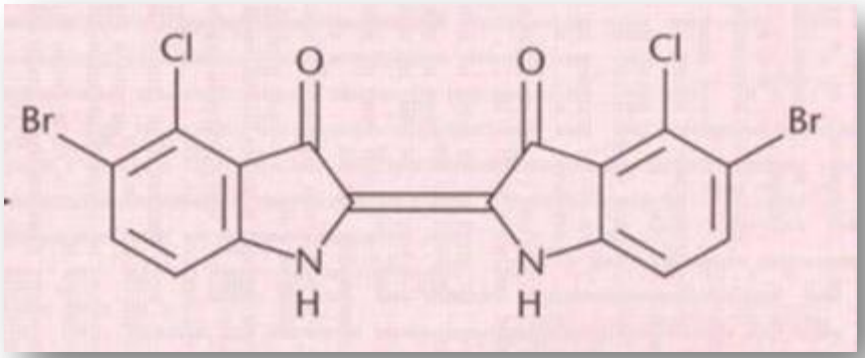


5-Bromo-4-chloro-3-indoxyl-ß-D-galactoside (X-gal)

ß-Galactosidase

5-Bromo-4-chloroindoxyl

Air oxidation



5,5’-Dibromo-4,4’-dichloroindigo

Regulatory Proteins and Transport Proteins

- Although regulatory proteins and transport proteins are not enzymes, they also bind other molecules and so they also need "active sites" to accommodate these.

iii. Regulatory proteins

- Regulatory proteins vary enormously. Many of them can bind both small signal molecules and DNA. The presence or absence of the signal molecule determines whether or not the gene is switched on.

iv. Transport proteins

- Transport proteins are found mostly in biological membranes, where they carry material from one side to the other.

- Strictly speaking, prosthetic groups are fixed to the protein, whereas cofactors are free to wander around from protein to protein. However, the terms are often used loosely. A protein without its prosthetic group is referred to as an **apoprotein**.

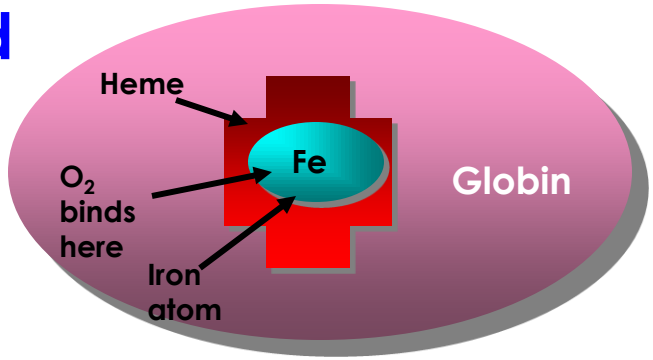
- Nutrients, such as sugars, must be transported into cells of all organisms, whereas waste products are deported. Multicellular organisms also have transport proteins to carry materials around the body, such as hemoglobin which carries oxygen in blood.

- To function properly many proteins need extra components, **cofactors** or **prosthetic groups**, which are not themselves proteins. Many proteins use single metal atoms as cofactors, others need more complex molecules.

- For example, oxygen carrier proteins such as **hemoglobin** have a ring-shaped cofactor with a **central iron atom**, called **heme**. The heme is bound in the active site of the apoprotein, in this case **globin**, and so we get hemoglobin.

- Oxygen binds to the iron atom at the center of the heme and the hemoglobin carries it around the body.

- Prosthetic groups are often **shared by more than one protein**, for example, heme is shared by hemoglobin and by myoglobin which receives oxygen and distributes it inside muscle cells.



How Are Proteins Constructed?

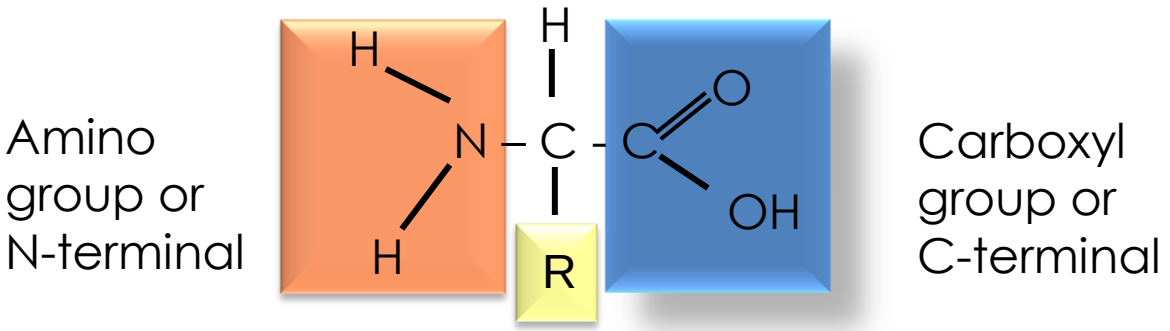
The monomer or subunit of a protein is known as an **amino acid**. There are **20 different amino acids** used in making proteins. They all have **central carbon atom**, **the alpha carbon**, surrounded by the four features.

Formation of Polypeptide Chains

- Amino acids are joined together by **peptide bonds** to give a linear polymer called a **polypeptide chain**.

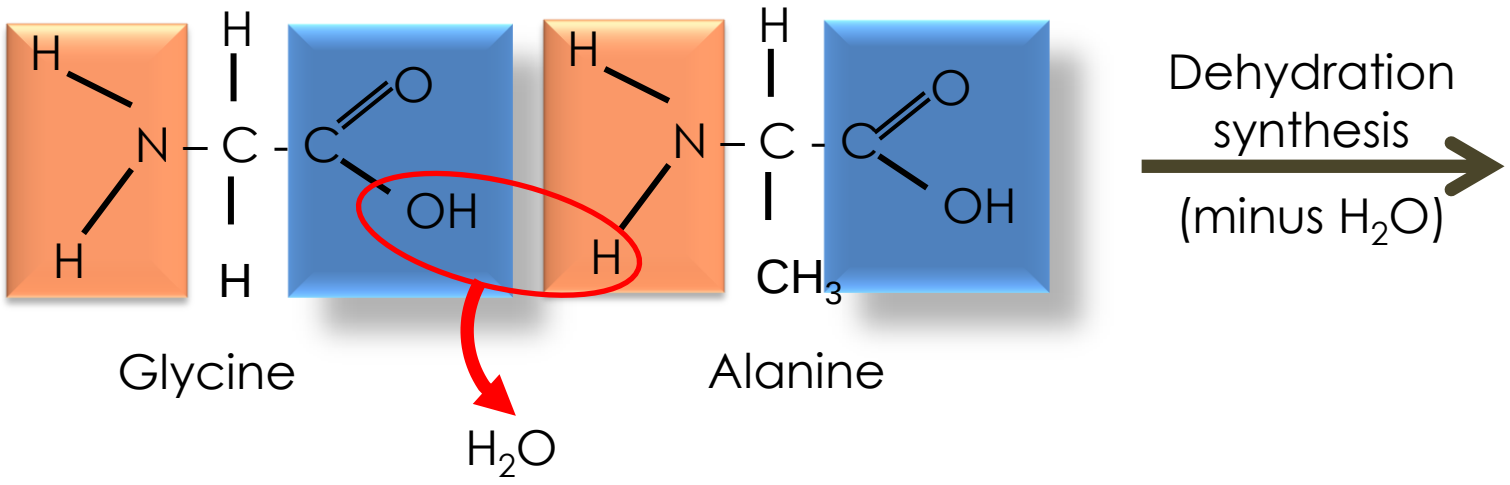
- The first amino acid in the chain retains its free **amino (NH_2) group** and this end is often called the **amino** or **N-terminus** of the polypeptide chain.

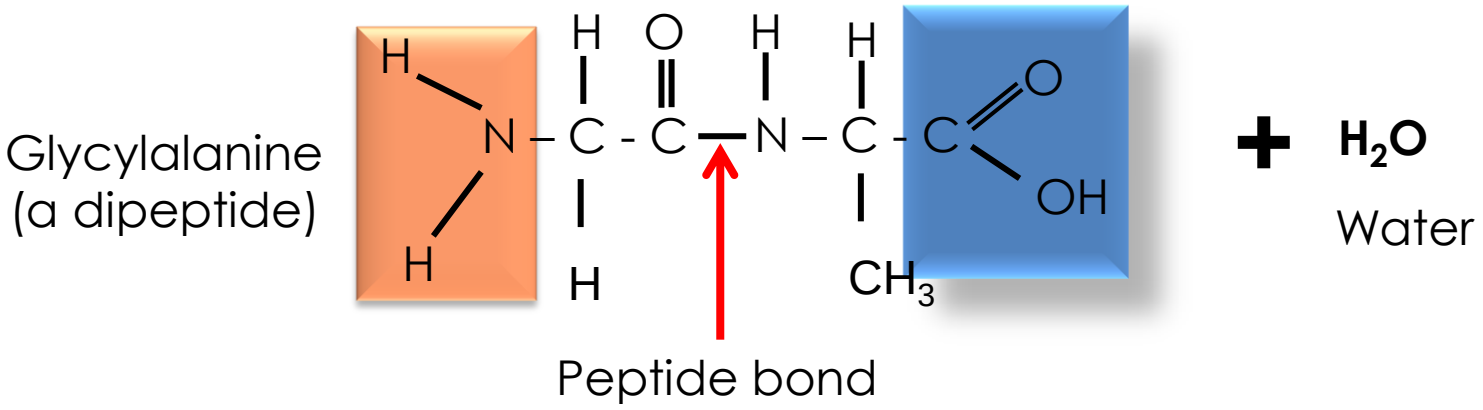
- The last amino acid to be added is left with a **free carboxyl (COOH) group** and this end is often called the carboxyl or **C-terminus**. The different side chains of the successive amino acids are labeled RI, R2, R3, etc.



R=side chain

- The following is an example of the **formation of peptide bond** between two peptides glycine and alanine. Notice how the bonds are constructed.





Three Dimensional Structures

- To make a complete protein we must next **fold** the polypeptide chain into the **correct 3-D structure**. Furthermore, a complete protein may have more than one polypeptide chain.

- Finally, many proteins have associated cofactors that are not made of amino acids. The structures of biological polymers, both protein and nucleic acid, are often divided into levels of organization.

- The first level or **primary structure** is the order of the monomers – i.e., the sequence of the amino acids for a protein, or of the nucleotides in the case of DNA or RNA.

Two Dimensional Structures

- **Secondary structure**: the folding or coiling of the original polymer chains by means of **hydrogen bonding**.

- In DNA, hydrogen bonding between base pairs forms Watson and Crick's double helix. In proteins there is instead **hydrogen bonding** between peptide groups.

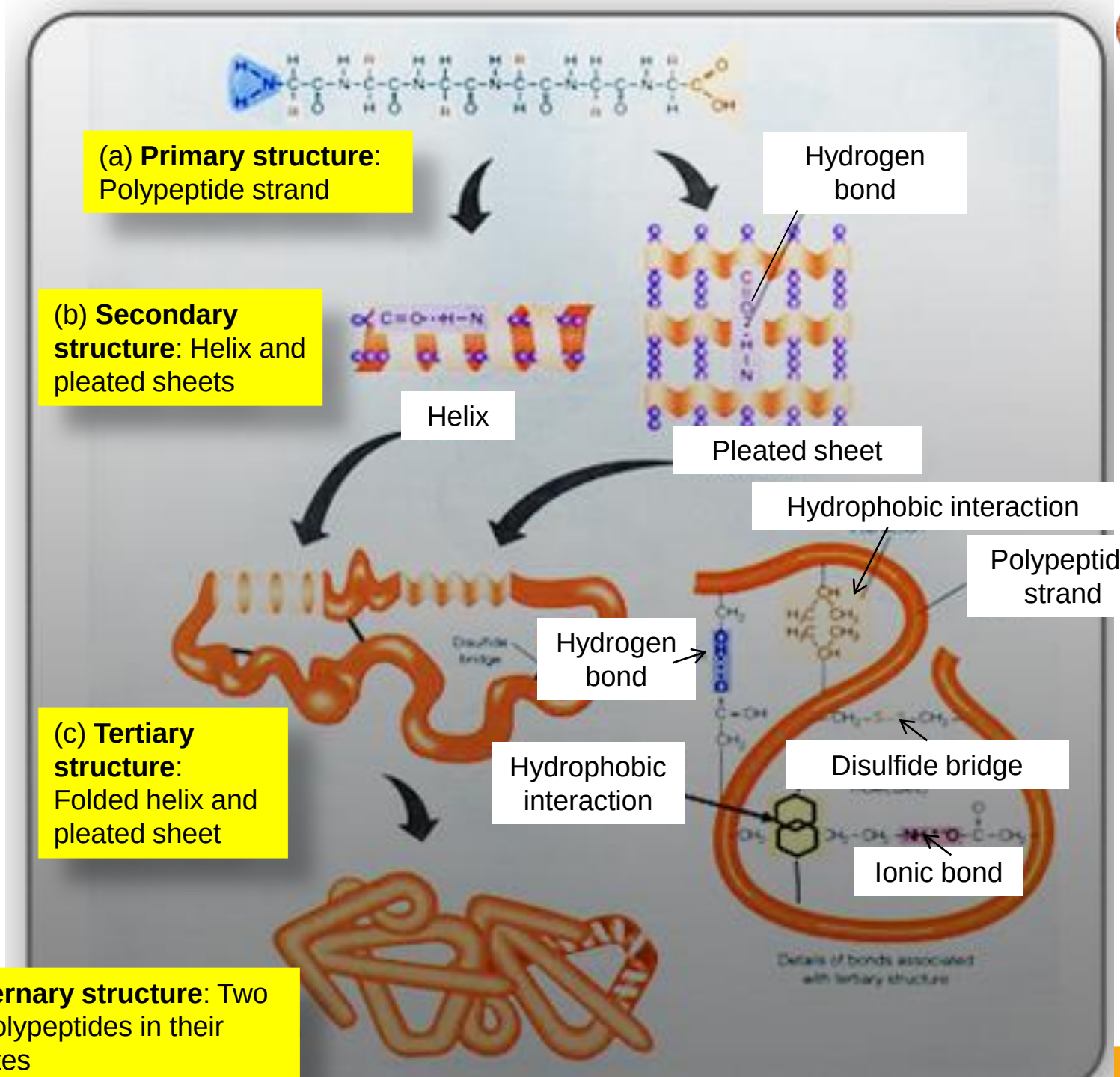
- In protein here are **two alternative secondary structures**: the **α-(alpha) helix** and the **β-(beta) sheet**.

- In the α-helix, a single polypeptide chain is coiled to make the α-helix and the **hydrogen bonds run vertically up and down** the helix-axis, **not sideways** across the helix.

- Actually, the hydrogen bonds in an α-helix are not quite vertical. They are slightly tilted relative to the helix axis because there are about **5.6 amino acids per turn** rather than a whole number.

- The **β-sheet** also has **hydrogen bonding between peptide groups** but in this case the **polypeptide chain is folded back on itself** to give a flattish structure.
- The **hydrogen bonds do go sideways** in the β-sheet. From each peptide group, **one hydrogen bond goes to one side** and **a second to the other side**.
- The next level is the **tertiary** structure. In a nucleic acid this would be **supercoiling**. In a protein we fold the polypeptide chain, with its preformed regions of α-helix and β-sheet, to give the final 3-D structure.

- This **level of folding** depends on the **side chains** of the **individual amino acids**. Since there are **20 different amino acids**, **a whole variety** of final 3-D conformations are possible.
- Nonetheless, most proteins are roughly **spherical**. This 3-D folding is largely the result of two factors acting in concert.
- Many of the amino acids have **side chains** (R-groups) which are very **water soluble** (**hydrophilic**).
- These side chains prefer to be on the **surface of the protein** so they can dissolve in the water surrounding the protein.
- In contrast, another set of side chains are **water repellent** (**hydrophobic**) and huddle together inside the protein **away from the water**. Since **hydrophobic** molecules are **greasy and insoluble**, this arrangement is known as the **oil drop model** of protein structure.


(a) **Primary structure**: Polypeptide strand
Hydrogen bond
(b) **Secondary structure**: Helix and pleated sheets
Helix
Pleated sheet
Hydrophobic interaction
Polypeptide strand
Hydrogen bond
(c) **Tertiary structure**: Folded helix and pleated sheet
Hydrophobic interaction
Disulfide bridge
Ionic bond
(d) **Quarternary structure**: Two or more polypeptides in their folded states

Quaternary Structure of Proteins

- This is the assembly of several individual polypeptide chains to give the final structure.
- Not all proteins have more than one polypeptide chain some just have one so they have **no quarternary structure** .
- In those having **more than one polypeptide chain**, the same hydrophilic and hydrophobic forces responsible for tertiary structure are involved.
- **To stick two polypeptide chains together the original chains are designed slightly different**.

- Some of the **hydrophobic side chains** are left as **a cluster exposed to the water** at the **protein surface**.

- This is an **unstable** arrangement and then **two polypeptide** chains **with exposed hydrophobic patches come into contact with each other**, they stick together, rather like **velcro**.

Twenty Different Amino Acids

- Unlike nucleic acids that have only **four different bases**, there are **20 different amino acids** in proteins. This allows for a great variety of 3-D structure and of chemical reactivity.

- The 20 amino acids may be represented by both **three letter** and **one letter** abbreviations.

- The latter are used when writing out protein sequences.

- Most are obvious, but since **some letters of the alphabet** have **several amino acids**, the others need a little imagination.

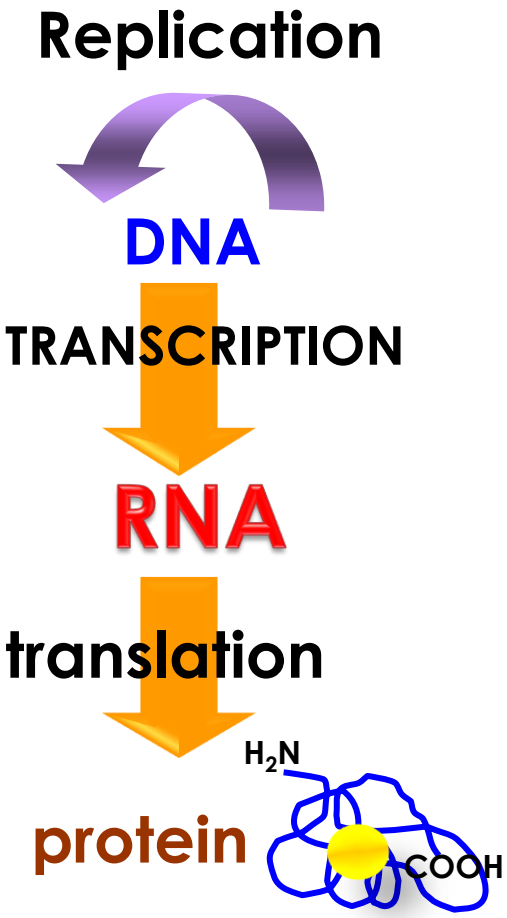
How are Proteins Made?

- Each protein is made using the genetic information stored on the chromosomes. The genetic information is transmitted in two stages.
- First the information in the **DNA is transcribed** into messenger RNA.
- The next step uses the information carried by the mRNA to give the sequence of amino acids making up a polypeptide chain.

- This involves converting the **nucleic acid "language"** the **genetic code**, to **protein "language**" and is therefore known as **TRANSLATION**.

- An early rule of molecular biology stated that there is only one gene for each protein.
  Although exceptions have been found, it is still usually true that each gene in the DNA gives rise to a single protein.

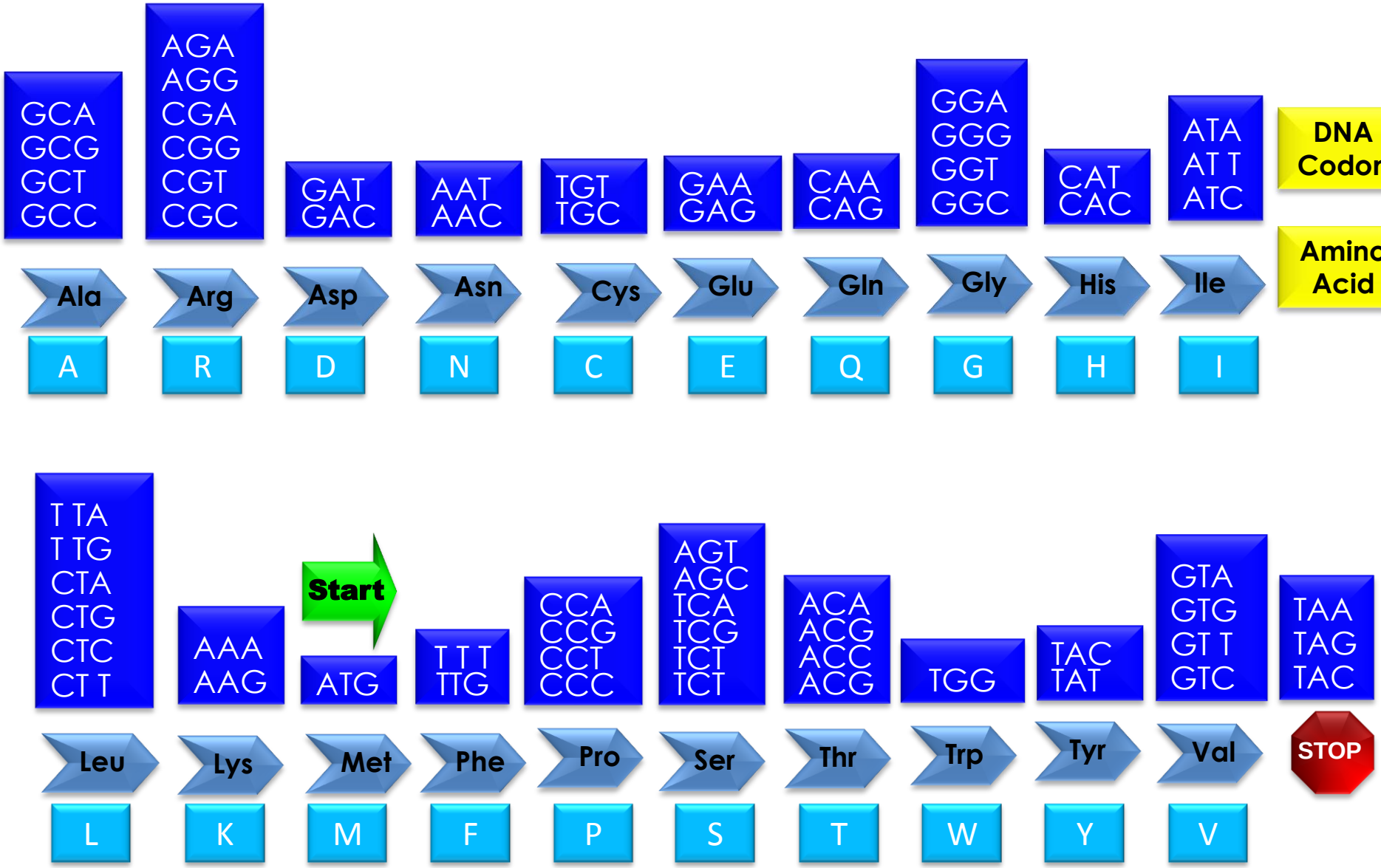
The Central Dogma



- The overall flow of information in biological cells is known as **CENTRAL DOGMA** of molecular biology was first formulated by Sir Francis Crick.

Decoding the Genetic Code

- There are 20 amino acids in proteins but only four different bases in the messenger RNA. So we cannot simply use one base of a nucleic acid code for a single amino acid when making a protein.
- During translation, the bases of mRNA are read off in **groups of three**, which are known as **codon** represents a particular amino acid.
- Since there are four different bases, there are **64** possible **groups of three bases** (4^3), that is, 64 different codons in the genetic code.
- However, there are only 20 different amino acids making up proteins, so **some amino acids** are **encoded by more than one codon**.


DNA Codon
Amino Acid
GCA GCG GCT GCC
AGA AGG CGA CGG CGT CGC
GAT GAC
AAT AAC
TGT TGC
GAA GAG
CAA CAG
GGA GGG GGT GGC
CAT CAC
ATA ATT ATC
Ala
Arg
Asp
Asn
Cys
Glu
Gln
Gly
His
Ile
A
R
D
N
C
E
Q
G
H
I
TTA TTG CTA CTG CTC CTT
AAA AAG
Start
ATG
TTT TTG
CCA CCG CCT CCC
AGT AGC TCA TCG TCT TCT
ACA ACG ACC ACG
TGG
TAC TAT
GTA GTG GTT GTC
TAA TAG TAC
Leu
Lys
Met
Phe
Pro
Ser
Thr
Trp
Tyr
Val
STOP
L
K
M
F
P
S
T
W
Y
V

CODON TABLE (Genetic Code)

2nd base

1st base	U	C	A	G	3rd base
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA STOP UAG STOP	UGU Cys UGC Cys UGA STOP UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG MET	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Ribosome - The Cell's Decoding Machine

- The decoding process is carried out by a submicroscopic machine called a **ribosome** that binds messenger RNA and translates it, so synthesizing a polypeptide chain. Here we'll talk about protein synthesis in bacteria. (The details of protein synthesis differ between bacteria and higher organisms).

- The ribosome consists of two subunits, **large (50S)** and **small (30S)** can be pictured as a snowman.

- After binding to the mRNA, the ribosome moves along it, adding a few amino acids to the growing polypeptide chain each time it reads a codon from the message.

How Many tRNA Molecules are There?

- To read the codon, we need a set of **adapter molecules** that recognize the codon on the mRNA at one end and carry the corresponding amino acid attached to their other end. These adapters are a third type of RNA, **transfer RNA** or **tRNA**.

- **Each transfer RNA** carries only **a single amino acid** so we need **at least 20 different** tRNAs because there are 20 different amino acids. On the other and, there are **4 codons to be recognized** as some amino acids have **more than one codon**.

- In practice, we have a sloppy compromise and the **number of different tRNA** molecules is somewhere **between 20 and 64**. Some tRNAs can read **more than one codon**, though, of course, these **must** all **code for** the **same amino acid**.

- At one end, the tRNA has an **anticodon** consisting of three bases that are **complementary** to the three bases of the **codon on the messenger RNA**.

- The **codon and anticodon recognize each other** by base pairing and are held together by **hydrogen bonds**. At its other end, each tRNA carries the amino acid corresponding to the codon it recognizes.

- Since only complementary bases can pair, how does a **tRNA with one anticodon** read **more than one codon**? Easy - it cheats! The rules or dishonesty in base pairing are known as the **wobble rules**. The first base of the tRNA anticodon can wobble around a little because it is not squeezed between other bases in a helix structure.

- For example, if the first anticodon base is **G** it can pair with **C**, as usual, or, in wobble mode, with **U**. Therefore tRNA for **histidine**, with **GUG** as anticodon, can **recognize both** the **CAC** and **CAU** codons.

- Whenever an amino acid is encoded by a pair of codons, the third codon bases are **U** and **C** (e.g., **histidine**, **tyrosine**) or **A** and **G** (e.g., **lysine**, **glutamic acid**), but never other combinations.

- Similarly, those privileged **amino acids with four or six codons** may be regarded as having **two or three such pairs**. As originally **transcribed** RNA contains only the four bases A, U, G and C.

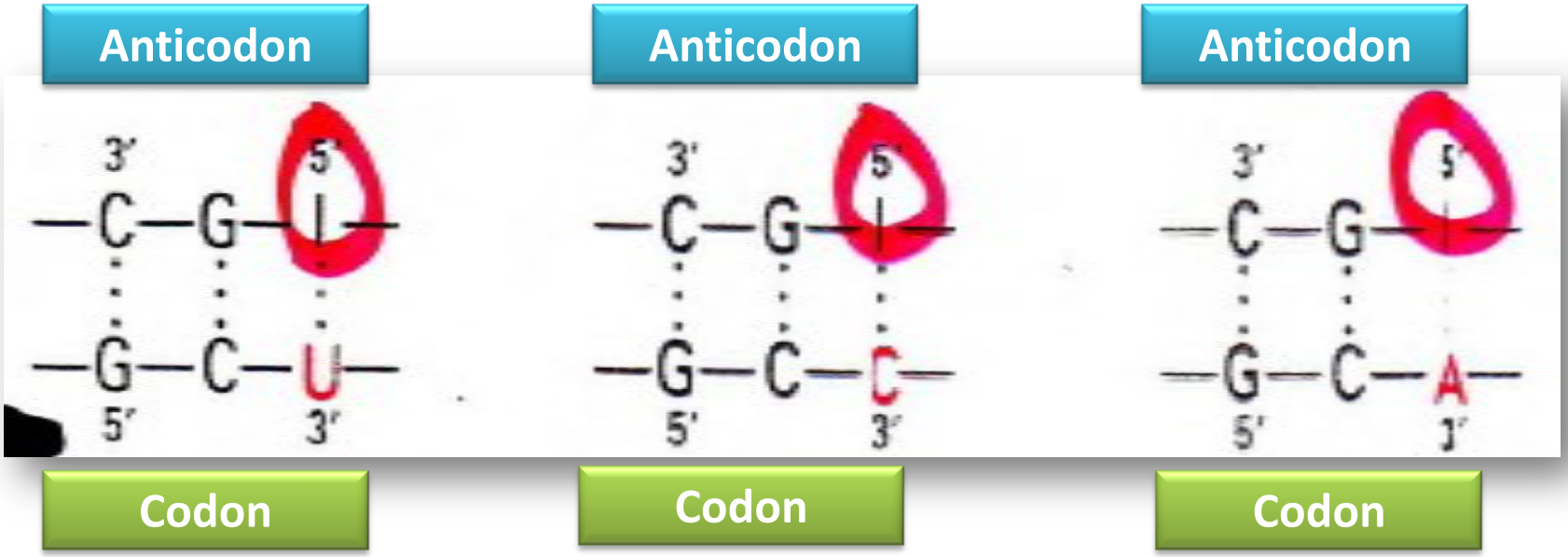
- However, some RNA molecules contain bases that are **altered chemically** after the RNA has been made.

- This is especially true for tRNA. In fact the anticodon itself may contain the weird base **I** for **Inosine** , which is occasionally used as the first anticodon base because it can pair with any of U, C or A.

The Wobble Theory

First Base of Anticodon	Pairs with: Third Base of Codon	
	normal	by wobble
G	C	Or U
U	A	Or G
I	-	C or A or U
C	G only	no wobble
A	U only	no wobble

“Wobble” codon base (3’) pairs with (5`) - I of anticodon

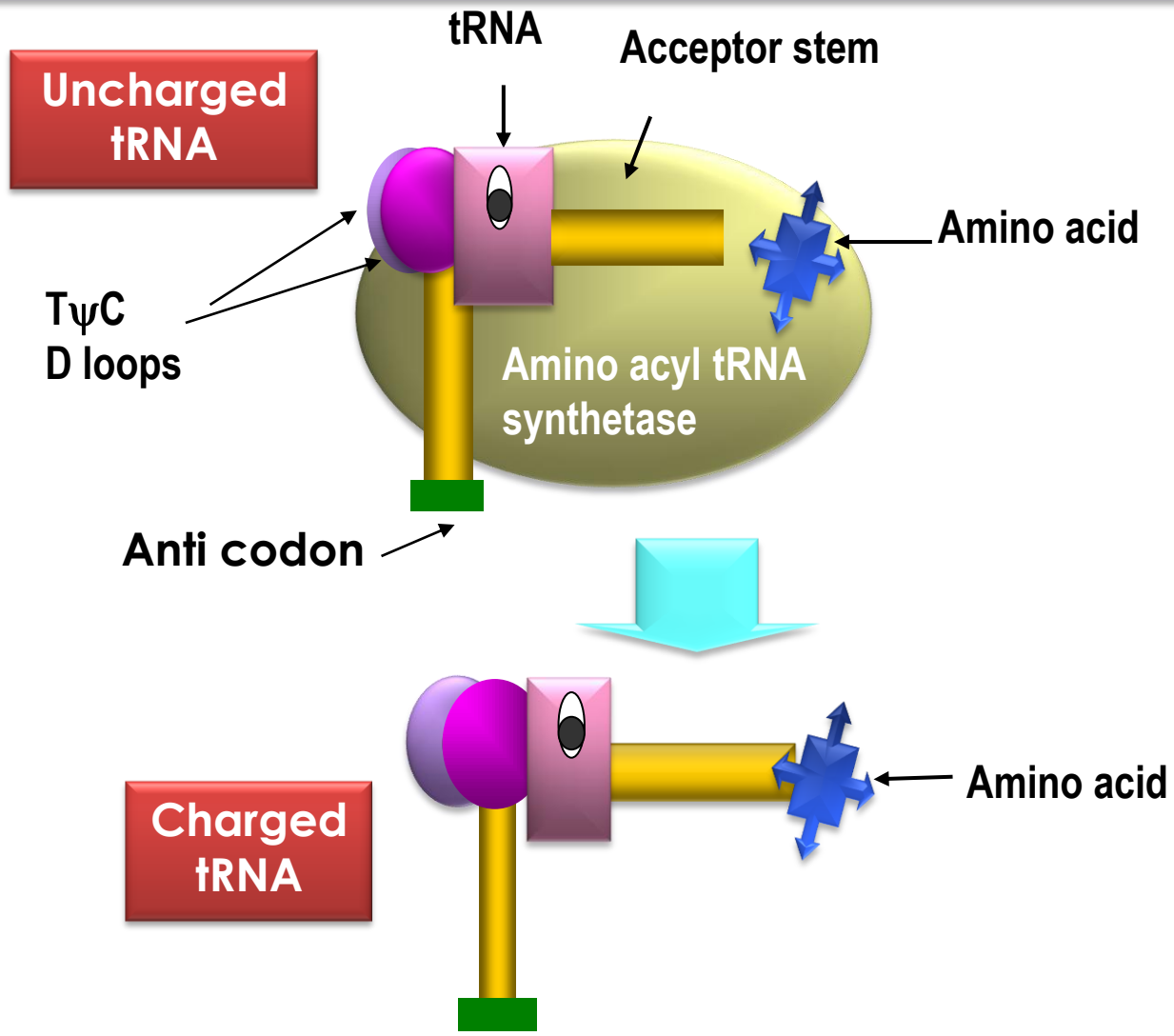


[3 Ala codons read by only one tRNA anticodon]

How Does the tRNA get its Amino Acid?

- For each tRNA, there is a special enzyme that recognizes both the tRNA and the corresponding amino acid.
- The enzymes, known as **aminoacyl tRNA synthetase** attach the amino acid to the tRNA.
- This is called **charging the tRNA**. Empty tRNA is known as uncharged tRNA, while tRNA with its amino acid is charged tRNA.

Charging of tRNA



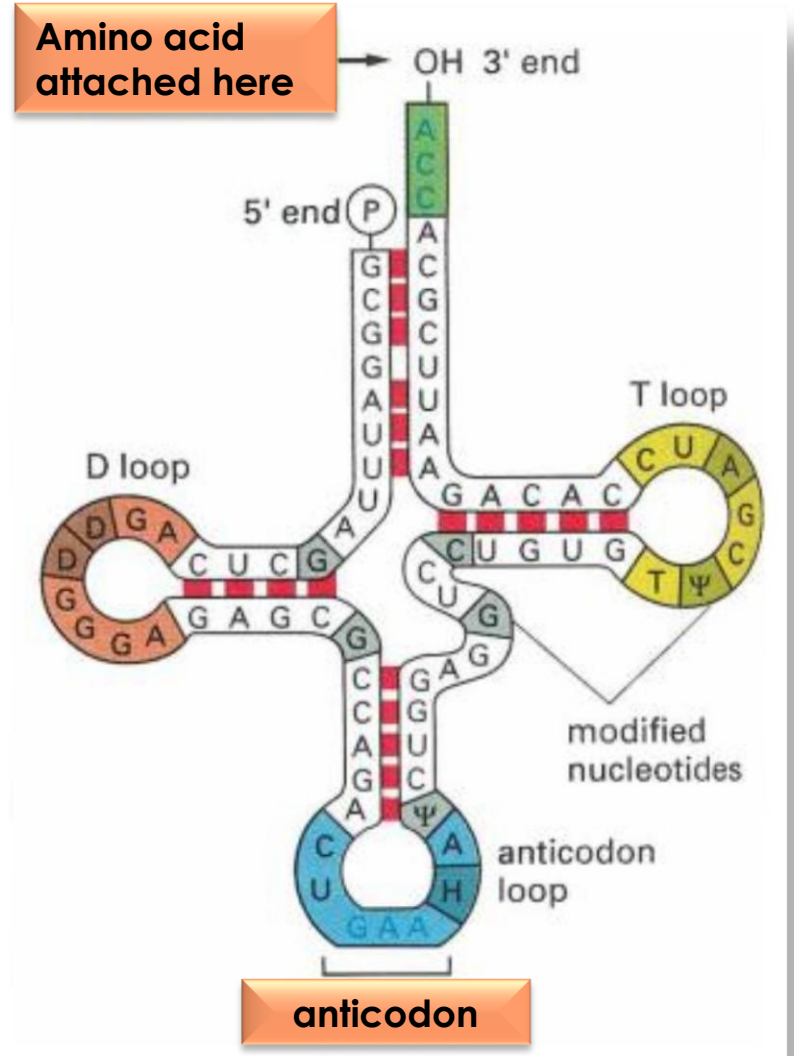
Structure of Transfer RNA

- A typical RNA has **four short base-paired stems** and **three loops**. This is the cloverleaf structure intended to reveal details of base-pairing, and shows the tRNA spreads out. The **amino acid** is bound at the **free end of the acceptor stem**.

- The **anticodon** is at the **opposite end** in the **anticodon loop**. The other two loops of tRNA are named after modified bases. The TψC loop contains **"ψ"** (spelt "psi" it pronounced "sigh") which stands for **pseudouracil** and the D-loop has "D" for **dihydrouracil**.

Structure of Transfer RNA

- These weird bases are required for proper folding and operation of the tRNA. The TψC loop and the D-loop are needed for binding to the ribosome and for recognizing the enzyme which sticks the amino acids onto the tRNA (amino acyl tRNA synthetase).
- We still haven't told you the true 3-D structure of tRNA.
- In real life the tRNA cloverleaf is folded up further. The TψC loop and D-loops are pushed together and the molecule is bent into an L-shape.


Amino acid attached here
OH 3' end
5' end
P
D loop
T loop
modified nucleotides
anticodon loop
anticodon

Reading Frames

- The bases of mRNA are read in groups of three, starting at the 5' end. We always begin with the **start codon**, **AUG**. However, consider the following message:

1 2 3

5'GAAAUGUAUGCAUGCCAAAGGAGGCAUCUAAGGA3'

- We have **three** possible **start codons** (underlined). Each of these starts at a slightly different point. Each of the three leads us to take quite different groups of three bases

- The three alternatives are illustrated below where bases considered to be part of the same codon have been given the same numbers. These three possibilities are known as "**reading frames**".

- As there are three bases in a codon, changing the reading frame by three or (a multiple of three) gets you back to where you started.

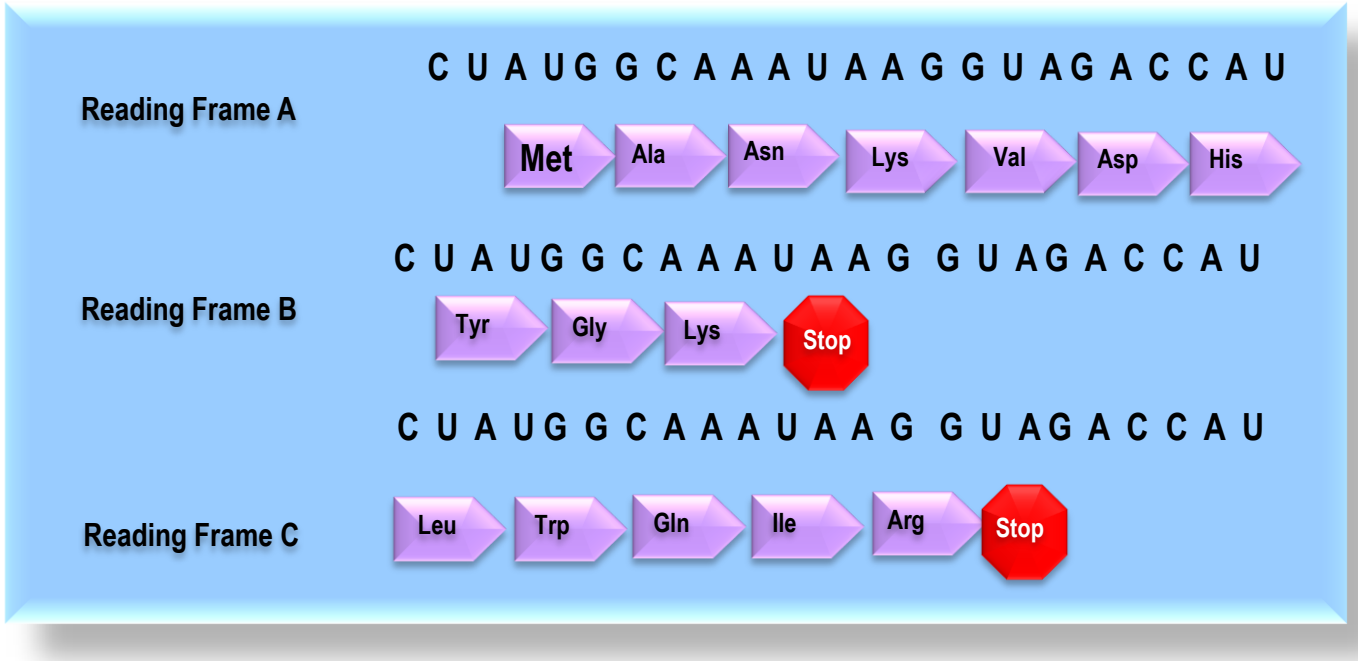
- A stretch of RNA, beginning with a start codon, and which can therefore be translated into a protein, is known as an **Open Reading Frame** often abbreviated to **ORF**. Any messenger RNA will **have several possible ORFs** and we have to find the correct one.

G A A A U G U A U G C A U G C C A A A G G A G G C A U C U A A G G A

1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8

1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8

1 1 1 2 2 2 3 3 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8



Messenger RNA can be Translated in more than one "Reading Frame". An overlapping triplet code that is read in three different frames. The mRNA is the same sequence in both lines but is read in a different "frame" and would lead to the synthesis of different proteins.

Getting Protein Synthesis Started

- The first codon is always **AUG**, which stands for the amino acid **methionine**. The special tRNA, the initiator tRNA will be charged with **chemically tagged methionine** (**formyl-methionine** or **fmet**) and will **bind to the start codon**.

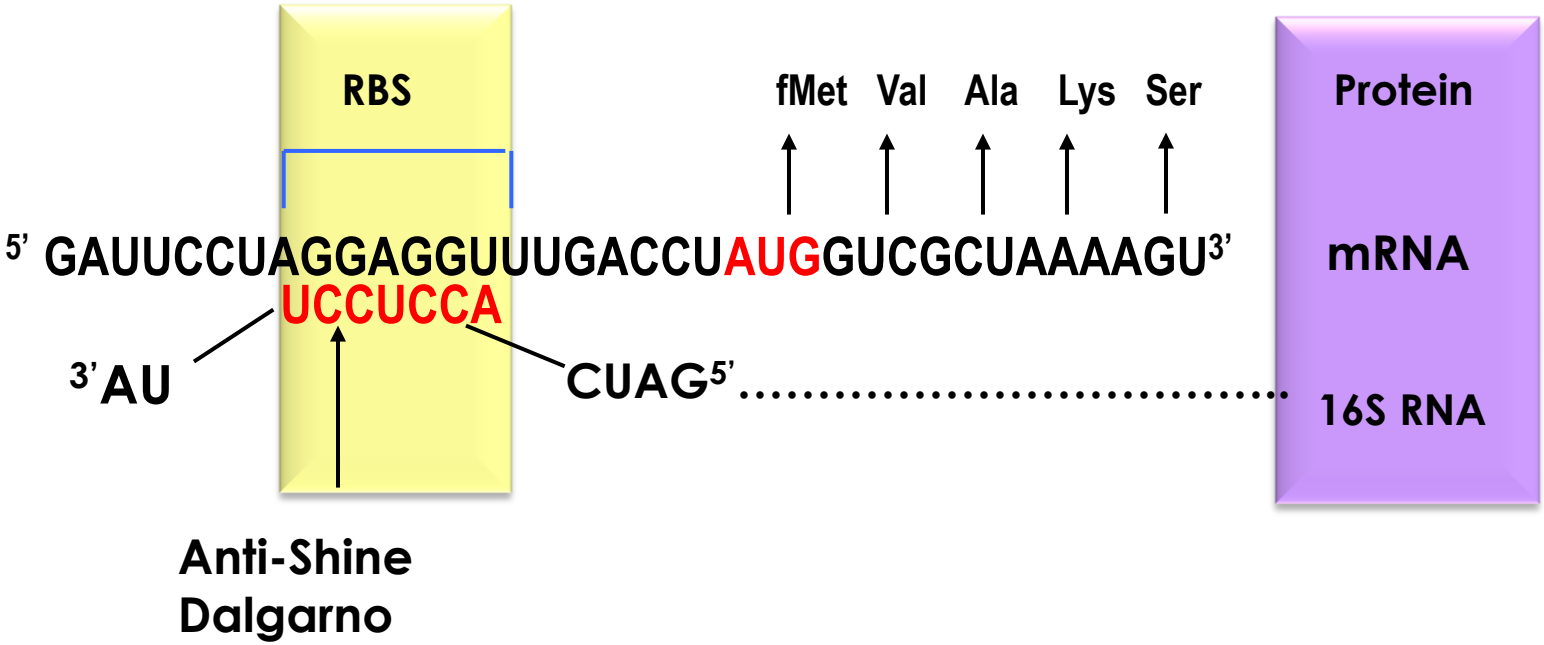
- So all polypeptide chains begin with methionine. Sometimes the initial methionine is snipped off later, so **mature proteins** do not always begin with methionine.

- There are also AUG codons in the middle of a message and, consequently, methionines in the middle of proteins.

So how does the ribosome know which AUG codon to start with?

- Near the front (the 5' end) of the messenger RNA is a special sequence, the **ribosome binding site** is usually called **Shine Dalgarno sequence** or S-D sequence, after its two discoverers.
- The sequence complementary to this, the **anti Shine-Dalgarno** sequence is found close to the 3' end of the 16S ribosomal RNA and this causes the mRNA to bind to this mRNA.
- In some cases the S-D is an exact match to the anti-S-D sequence and these mRNAs are translated efficiently. In other cases the match is poorer and translation is less efficient. The **start codon** is the next **AUG** codon **after the ribosome binding site**. It's that simple!

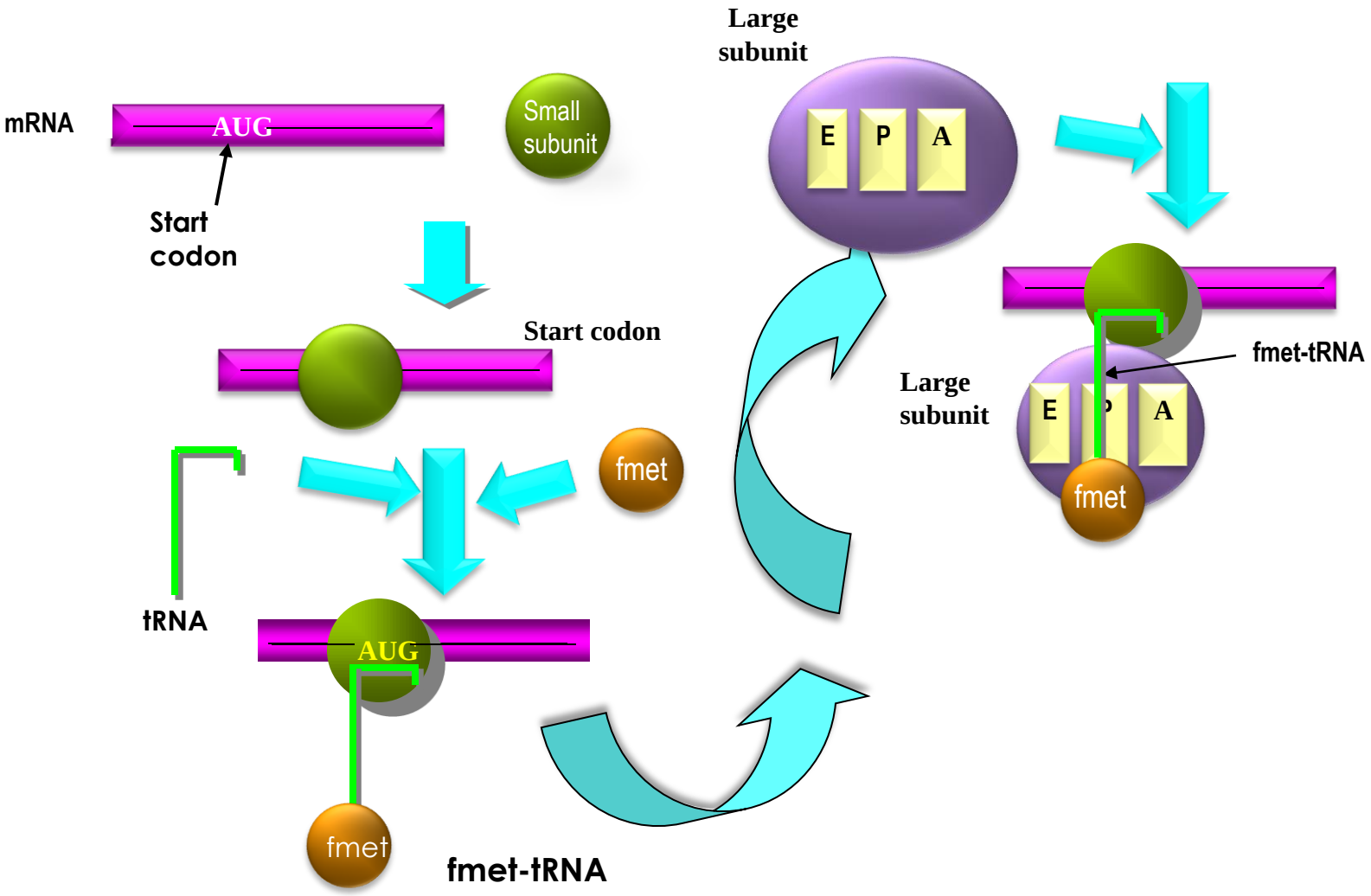
Ribosome binding site (RBS)



Initiation of Translation

- Before protein synthesis starts, the two subunits of the ribosome are floating around separately. Because the **16S rRNA**, with the complementary, anti-Shine Dalgarno sequence, is in the **small subunit of the ribosome**, the messenger RNA binds to a free small subunit.
- Next the initiator tRNA, **carrying fMet,** recognizes the AUG start codon. You also need three proteins known as **initiation factors**, that help arrange all the components correctly.
- Finally, the large subunit arrives, and joins its smaller partner as the initiation factors drop off. This sequence events in the **initiation** is shown below.

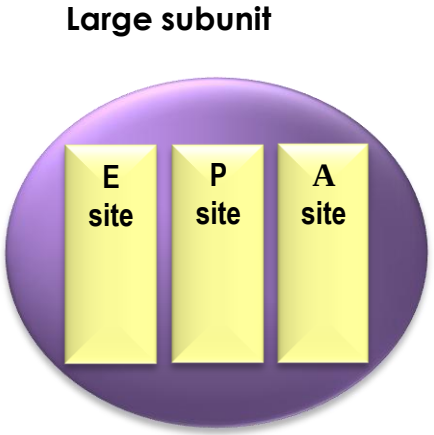
Formation of the Initiation Complex



Elongation of a Growing protein

- After the large subunit of the ribosome has arrived, the polypeptide chain is made. The ribosome has three sites for tRNA: the **E-site** (*Exit site*), **A-site** (*Acceptor site*) and **P-site** (*Peptide site*) start with the **fMet initiator tRNA** in the **P-site**.

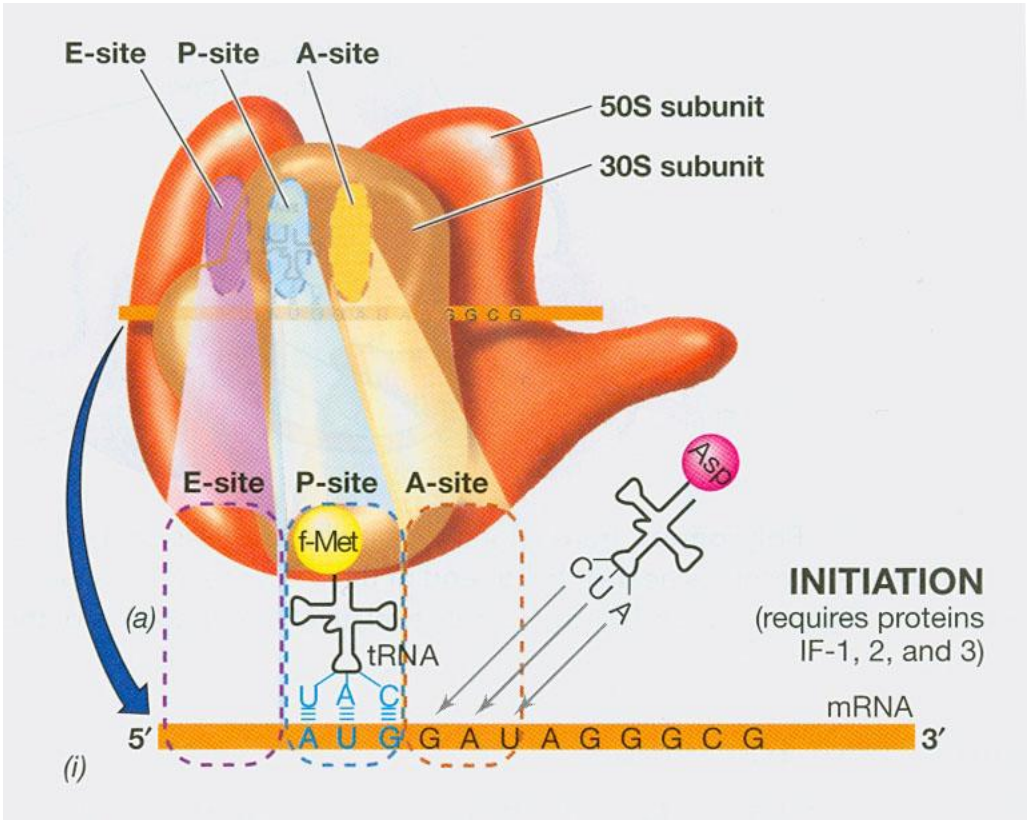
- Another tRNA, carrying the exact amino acid, arrives and enters the A-site. The fMet is cut loose from its tRNA and bonded to amino acid No. 2 instead. So tRNA No. 2 now carries two linked amino acids, the beginnings of our growing protein chain.

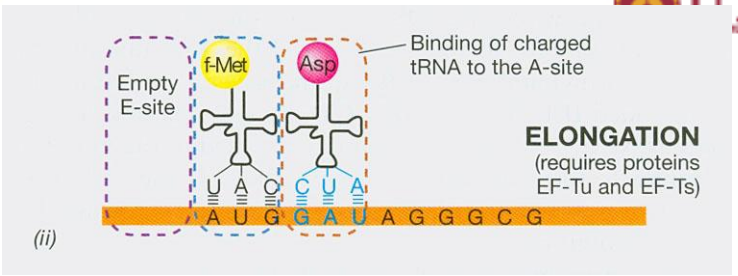


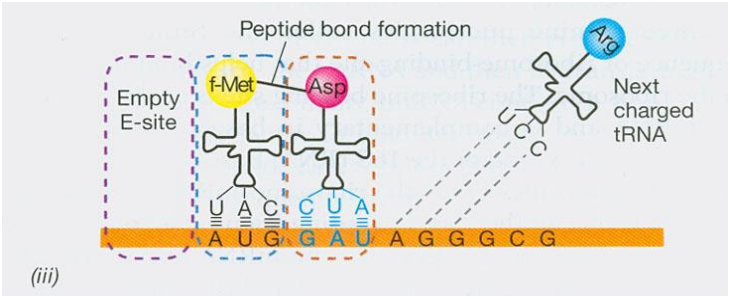
- Next, another charged tRNA arrives carrying the third amino acid. In order to fit the newcomer into the A-site we must push tRNA No. 2 sideways into the P-site.

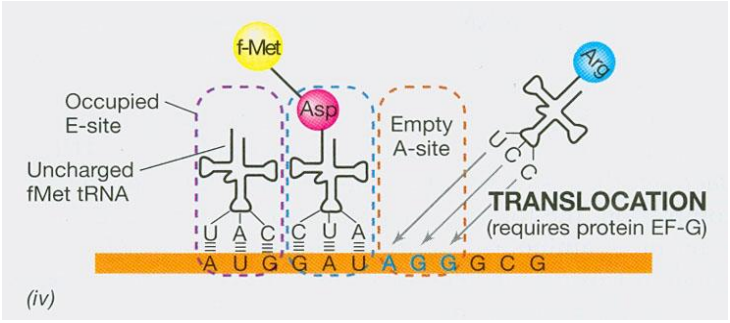
- This in turn pushes the free tRNA in the P-site to the E-site. (It's rather like trying to fit three members of the **circumferentially-challenged**?? into the back seat of a small car!!) As the third gets in, the first one is pushed close to the opposite door.

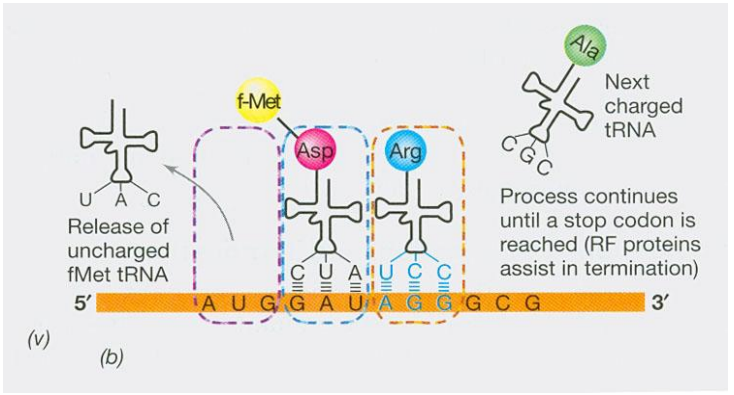
- As the peptide chain continues to grow, it is constantly cut off from the tRNA holding it (which occupies the P-peptide site) and joined instead to the newest amino acid to be brought by its tRNA into the A-site, hence the name "acceptor" site.


E-site
P-site
A-site
50S subunit
30S subunit
E-site
P-site
A-site
f-Met
Asp
tRNA
(a)
INITIATION
(requires proteins
IF-1, 2, and 3)
mRNA
5′
A U G G A U A G G G C G
3′
(i)


Empty
E-site
f-Met
Asp
Binding of charged
tRNA to the A-site
ELONGATION
(requires proteins
EF-Tu and EF-Ts)
A U G G A U A G G G C G
(ii)


Peptide bond formation
Empty
E-site
f-Met
Asp
Arg
Next
charged
tRNA
A U G G A U A G G G C G
(iii)

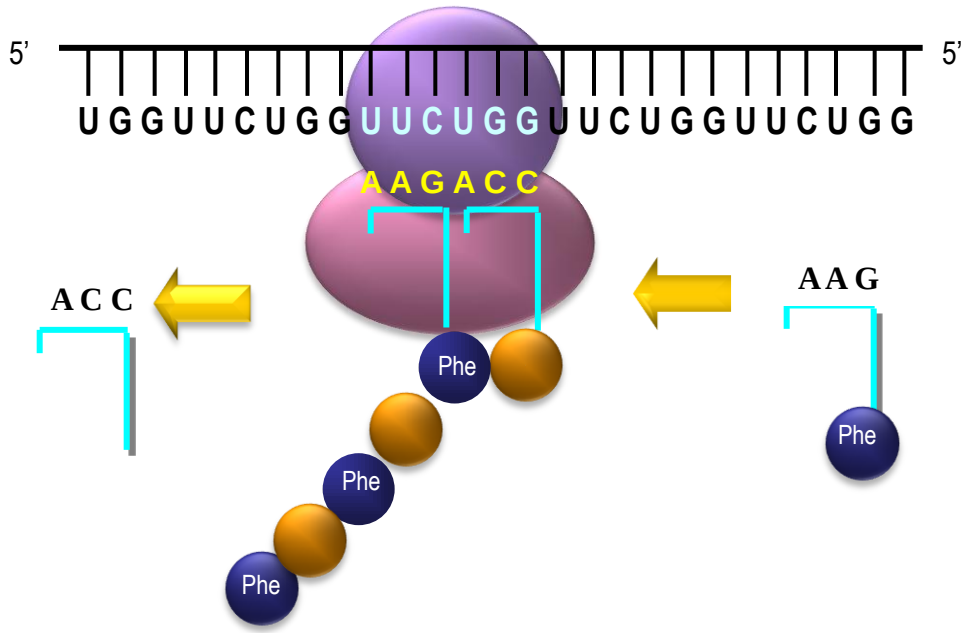

f-Met
Occupied
E-site
Asp
Empty
A-site
Arg
Uncharged
fMet tRNA
TRANSLOCATION
(requires protein EF-G)
A U G G A U A G G G C G
(iv)


Ala
Next
charged
tRNA
f-Met
Asp
Arg
Release of
uncharged
fMet tRNA
Process continues
until a stop codon is
reached (RF proteins
assist in termination)
5′
A U G G A U A G G G C G
3′
(v)
(b)

The Elongation Factors

- The arrival and sideways shuffling of the tRNAs on the ribosome is supervised by protein known as **elongation factors**.
- Both elongation factors require a supply of energy in order to move the tRNA molecule around.
- The tRNA is delivered to the ribosome and installed into the A-site by elongation factor **EF-T** (which is actually a pair of proteins, **EF-Tu** and **EF-Ts**).
- The second elongation factor, **EF-G**, oversees moving everybody sideways at the correct time.

Elongation of Polypeptide Chain



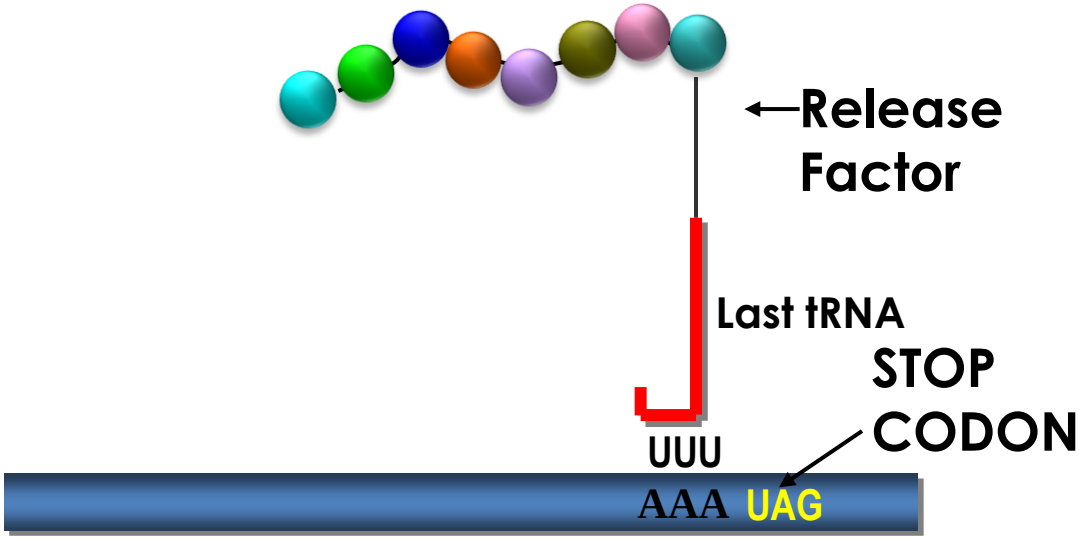
Termination of Protein Synthesis

- Eventually we reach the end of the message. This is marked by a **stop codon**. There are three of these, **UGA**, **UAG**, and **UAA**.

- As **no tRNA exists** to read these three codons, the chain can no longer grow. Instead, proteins known as **release factors** read the stop signal and chop the completed polypeptide chain of the final tRNA.

- This event is so unnerving to the ribosome that it goes all to pieces and falls apart into its separate subunits. This the **termination** of polypeptide.

Termination of polypeptide synthesis

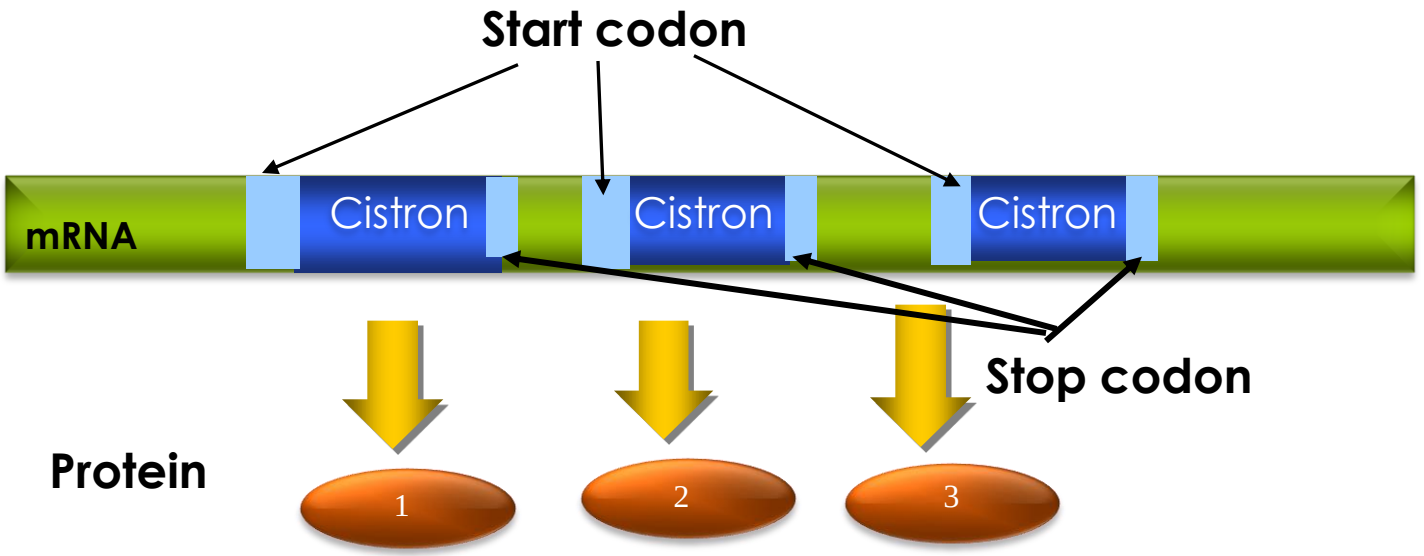


One Messenger RNA Can Code for Several Proteins

- In bacteria, several proteins may be encoded by the same messenger RNA. As long as each open reading frame has its own Shine-Dalgarno sequence in front of it, the ribosome will bind and start translating.

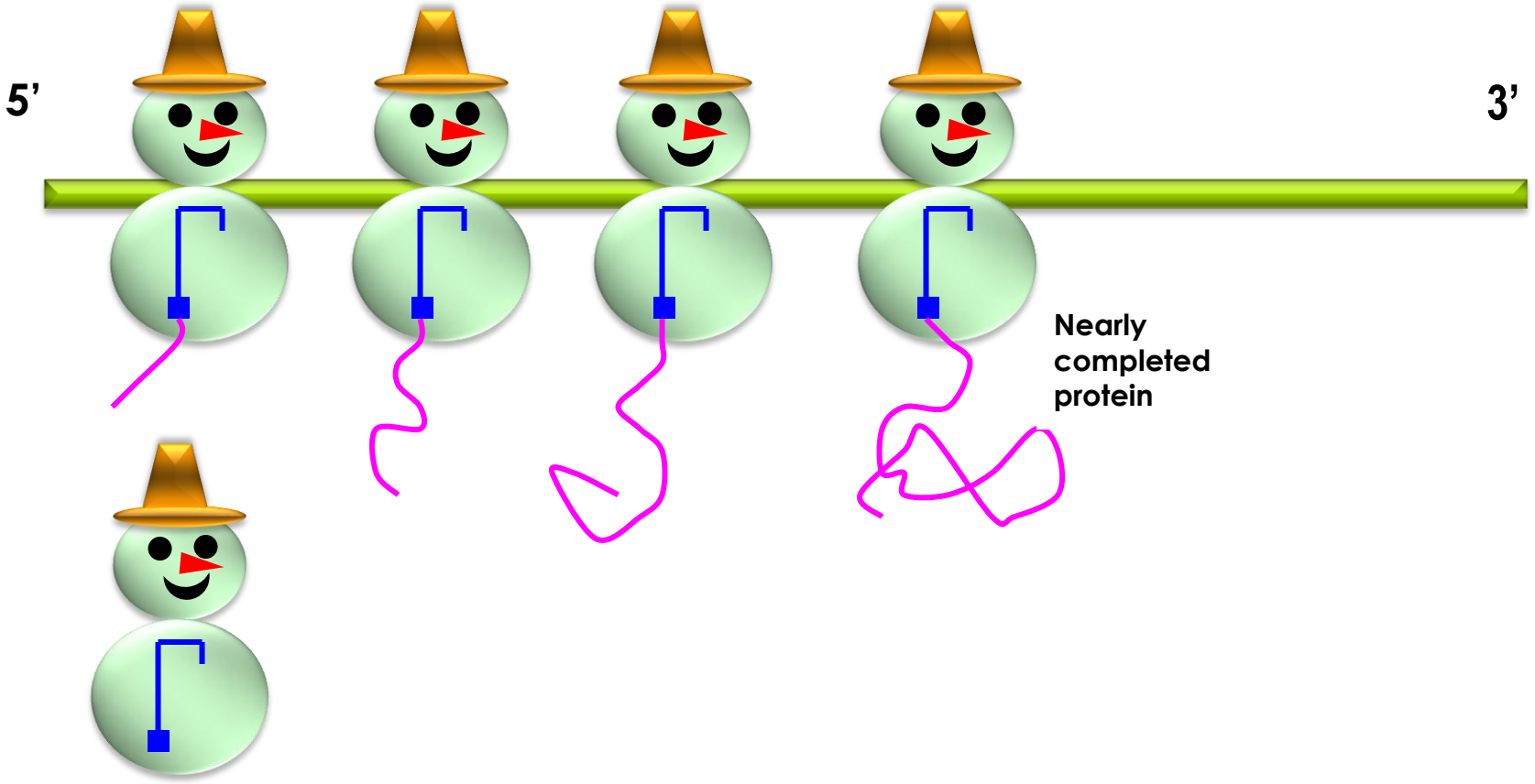
- **Open reading frames that are translated into proteins** are sometimes known as **cistrons**. mRNA which carries several of these is therefore called **polycistronic mRNA**.

- In higher organisms, it does not happened. Instead of a Shine-Dalgarno sequence, the front (5' end) of the messenger RNA molecule is recognized, the first open reading frame is translated.


Start codon
mRNA
Cistron
Cistron
Cistron
Stop codon
Protein
1
2
3

Several Ribosomes Can Read the Same Message at Once

- Once the first ribosome has got moving, another can jump onto the same messenger RNA and travel along behind.
- In practice, several ribosomes will move along the same mRNA about a hundred bases apart.
- This structure is called a **polysome** (short for **polyribosome**).

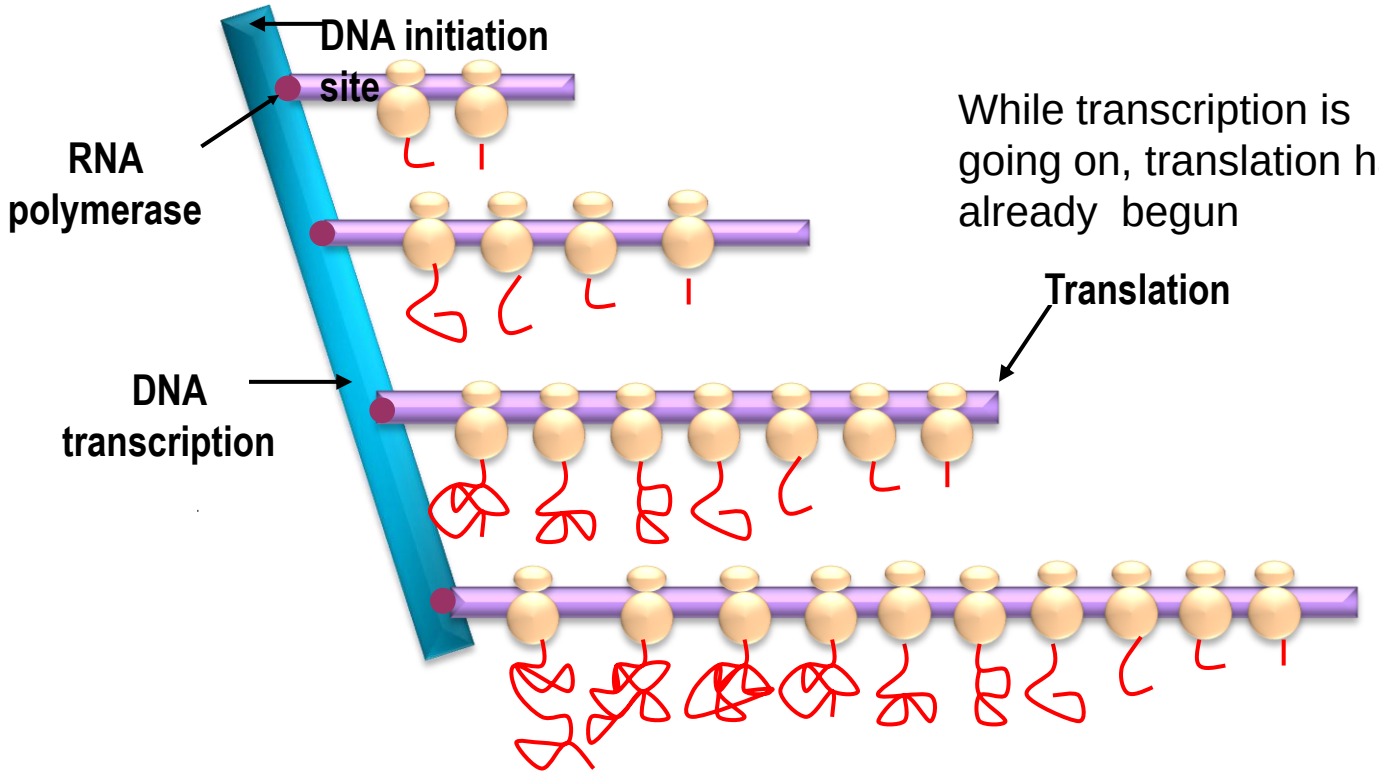

5'
3'
Nearly completed protein

Coupled Translation and Transcription in Bacteria

- When mRNA is transcribed from the original DNA template, its synthesis starts at the 5' end. The mRNA is also read by the ribosome starting at the 5' end.

- What this means is that the ribosome can start translating the message before the synthesis of the messenger RNA molecule has actually been finished.

Coupled Translation and Transcription



While transcription is going on, translation has already begun

Polyribosomes:A polyribosome is formed when several ribosomes simultaneously translate a single mRNA

The result is that you find partly finished mRNA, still attached to the bacterial chromosome via RNA polymerase, with a group of enthusiastic ribosomes already jumping aboard to get started.

Some Proteins Come to a Bad End

- Murphy 's Law tells us that if something can go wrong, it will. Ribosomes have their problems, too. One snag they sometimes come across is when they receive a **defective messenger RNA**.

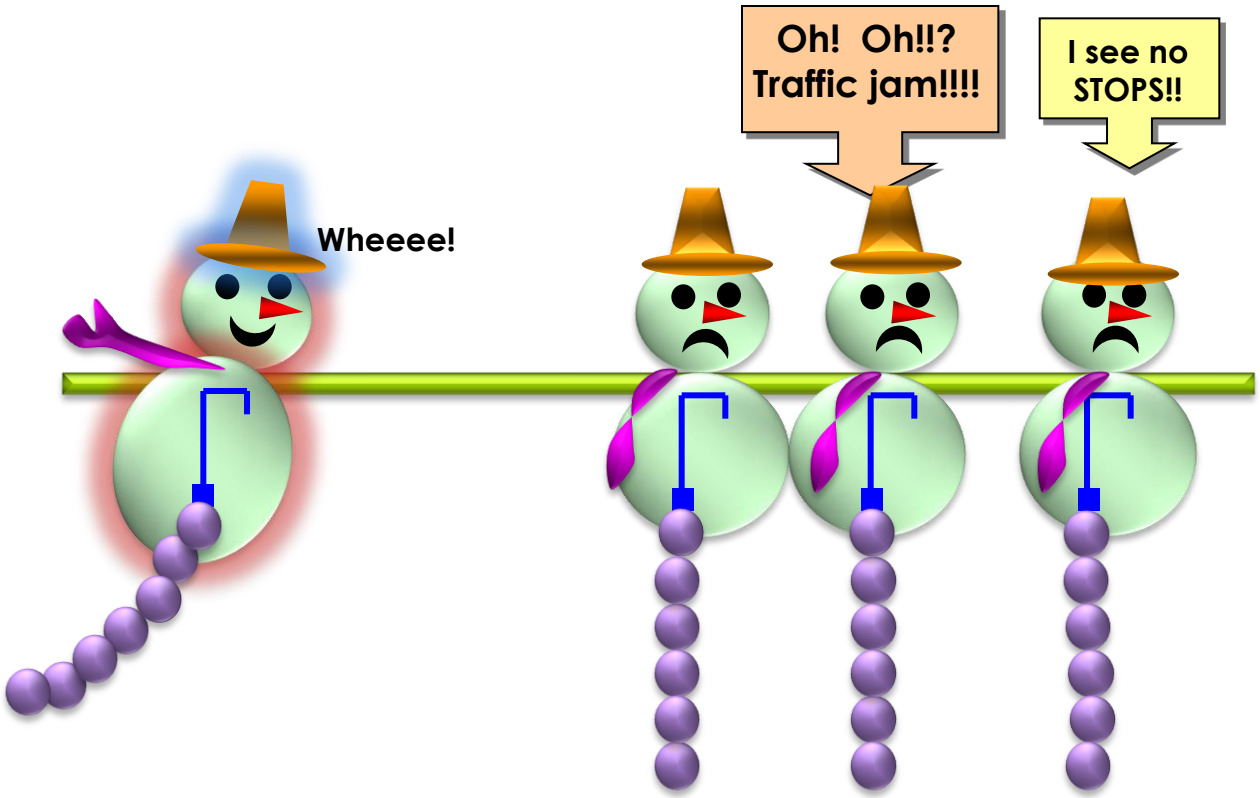
- Whether the mRNA was **never properly finished** or whether it was **mistakenly snipped short** by an over-enthusiastic RNA cutting enzyme, it can cause havoc.

- A ribosome that is translating a message into protein expects, sooner or later, to **come across a stop codon**.

- Even if an mRNA molecule comes to an abrupt end, ribosomes may only be released by release factor and this in turn **needs a stop codon**.

- If the mRNA is defective and there is **no stop codon**, a ribosome that reaches the end will just sit there forever and the ribosomes behind it will all be stalled too (**ribosome pile up**).

- So what do we do to free these trapped ribosomes? It turns out that bacterial cells contain a small but heroic RNA molecule that **rescues stalled ribosomes**.



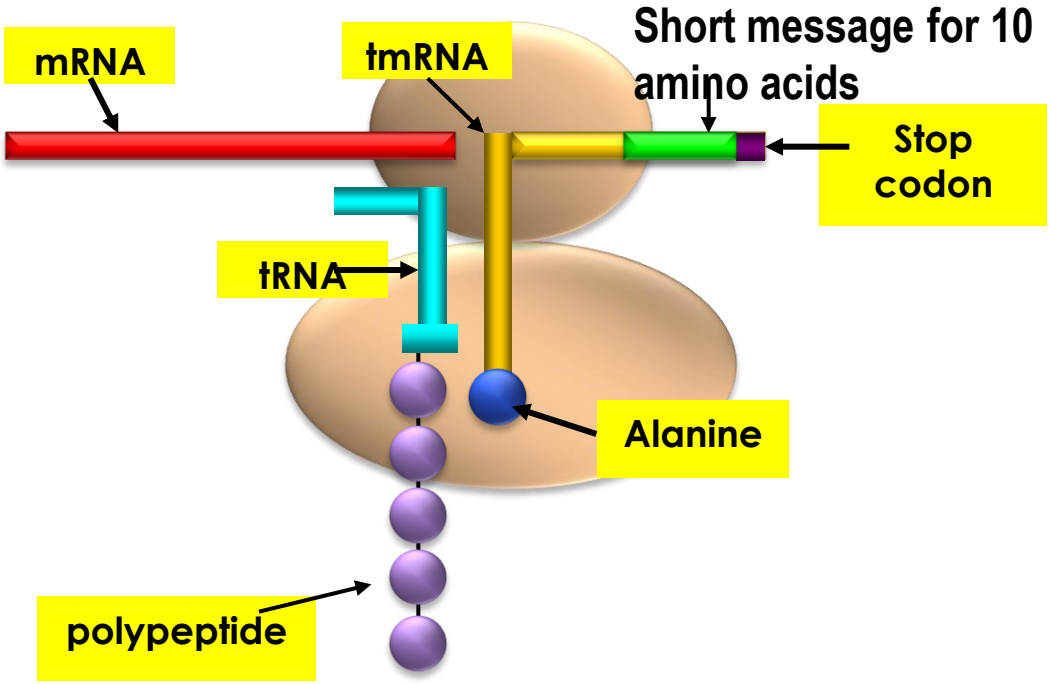
Defective mRNA

- This was named **tmRNA** because it acts partly like transfer RNA and partly like messenger RNA. Like a tRNA, the tmRNA carries an amino acid, actually **alanine**. When it sees a stalled ribosome it binds beside the defective mRNA.

- Protein synthesis now continues, first using the alanine carried by tmRNA, and then continuing on to translate the **short stretch of message** (of about 10 amino acids) that is also part of the tmRNA.

- Finally, the tmRNA provides **apropriate stop codon** so that release factor can disassemble the ribosome and free it for its next assignment.

tmRNA



Ribosome Rescued by tmRNA

- But what about the protein we just made? Clearly, it too is defective.
- Shouldn't it be destroyed? The tmRNA is one step ahead of you!
- The short stretch amino acids specified by the message part of mRNA and added to the end of the defective protein acts as a signal.
- This is recognized by **tail-specific protease** which munches unnecessary proteins. Perhaps we should think of tmRNA as meaning "terminate me" RNA!

References:

- Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2009). Brock Biology of Microorganisms: Pearson Education, USA.

- Clark, D.P. and Russel, L.D. (2000). Molecular Biology Made Simple and Fun: Cache River Press, USA.