

SS

## Genetic code & Translation

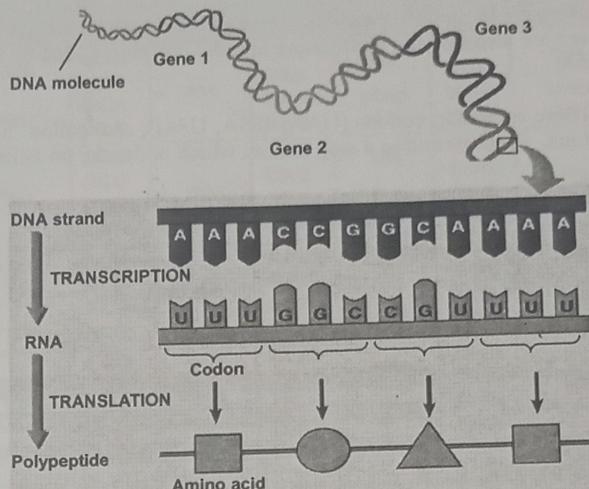


Figure 10.7

Transcription ke baad jo mRNA aata hai that is going to be translated into Protein, the sequence present in messenger RNA are going to be decoded.

## The Genetic Code

*The genetic code is the set of rules by which information encoded in genetic material (DNA or mRNA sequences) is translated into proteins (amino acid sequences) by living cells.*

**It is a set of 3 nucleotides on the mRNA known as CODON.**

(Since there are 4 bases, there are  $4^3 = 64$  possible codons, which must code for 20 different amino acids.)

Level - S →

### The Genetic Code

- is almost universal, used in both prokaryotes and eukaryotes. *in all org.*
- The code is written in the 5' → 3' direction.
- AUG is used as the start codon. All proteins are initially translated with methionine in the first position, although it is often removed after translation. There are also internal methionine in most proteins, coded by the same AUG codon.
- There are 3 stop codons (UAA, UGA, UAG), also called "nonsense" codons. Proteins end in a stop codon, which codes for no amino acid. *(Because it will not encode any amino acid, why we call it Stop codon)*
- The first codon establishes the Reading frame.
- Known to be nonoverlapping.

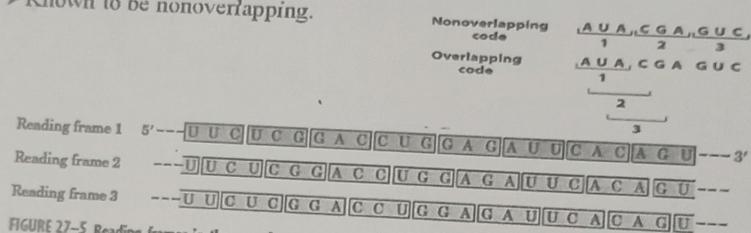


FIGURE 27-5 Reading frames in the genetic code. In a triplet, nonoverlapping code, all mRNAs have three potential reading frames, shaded here in different colors. The triplets, and hence the amino acids specified, are different in each reading frame.

### Breaking the Genetic Code

- One of the greatest scientific developments of the twentieth century
- First clue from Nirenberg and Matthaei:
  - poly(U) and 20 radiolabeled amino acids fed to *E.coli*  
→ only Phe produced Protein bni.  
bcz • Hence, UUU codes for Phe.
- Another step forward from Khorana:
  - used defined mRNAs in planned patterns
  - they used that is, (AC)<sub>n</sub> (alternating ACA and CAC codons → His and Thr)

multiple uridine residues  
chain

Virtually all organisms share the same genetic code  
“unity of life”

		Second Base					
		U	C	A	G		
First Base	U	UUU } phe UUC UUA UUG	UCU } ser UCC UCA UCG	UAU } tyr UAC UAA UAG	UGU } cys UGC UGA UGG	U C A G	
	C	CUU } leu CUC CUA CUG	CCU } pro CCC CCA CCG	CAU } his CAC CAA CAG	CGU } arg CGC CGA CGG	U C A G	
	A	AUU } ile AUC AUA AUG met (start)	ACU } thr ACC ACA ACG	AAU } asn AAC AAA AAG	AGU } ser AGC AGA AGG	U C A G	
	G	GUU } val GUC GUA GUG	GCU } ala GCC GCA GCG	GAU } asp GAC GAA GAG	GGU } gly GGC GGA GGG	U C A G	
Third Base							

There are 20 common, genetically encoded amino acids, by  
61/64 codons.

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## Most Amino Acids Have More Than One Codon

- Some codons are less subject to causing a mutation in an amino acid sequence because of degeneracy or because of the abundance of such tRNAs.
- There are 20 amino acids with 61 possible codons.
- Only Met and Trp have a **single** codon.

Mutate codon will encode single aa

Q How sequence will decode?

Sequence nucleotide pr hai mushtamil, & we have 4 nucleotide in RNA. In 4 nucleotides pe sequence ko aise read karegya k wo a.i.a one transform hogae.

Protein have special type of sequence on which all functions are depend on.

-tar Protein apne related gene se banta gene baki ha us me kya a.a ayegy & ek protein me a.a ke sequence leya ye us ke ek derive se differ karke hai or us ka function baki hai

→ usually the sequence present in RNA is going to be readed in triplet (codon)

Q Kio triplet me read kme ~~ki~~ <sup>one</sup> zaroori pair?

Because we have to fulfill 20 amino acids by 4 bases.

Reading frame:

jahan se ap read RNA shuru karegya wahan se reading frame set hogae ga, only 3 possibilities, but you read only one time.

If you read multiple time so it will called overlapping, or reading frame ~~non-overlapping~~ hta hai

Complementary present in the bottom of tRNA which is complementary to mRNA Sequence.

## Molecular Recognition:

Translation: A sequence which is present in mRNA, will be read in the form of Codon, & specific codon encodes specific amino acid, and amino acid was brought by tRNA so it will act as an adapter. Because no mRNA means se us sequence de patahaanta hai & kya Codon hai wahan ja bind kita hai, and waha specific amino acid le & ata hai jo se us codon se Related hai.

Eg

If AUG is present so tRNA will bring methionine because AUG encode Methionine.

- It has four arms, a-a arm, anticodon arm, tye arm, dihydro uridine arm.
- ✓ If arm a-a ke abne se bound ralchta h.
- ✓ Anticodon arm me anticodon hai, that is complementary to the Codon which is present in mRNA.
- ✓ Arrangement of tRNA & mRNA are antiparallel to each other (Means if tRNA is 3 → 3 to mRNA will 3 → 5)
- ✓ Anticodon ke 3 → 5, read Koenegy because us ko Codon k. Sath complementary kri hei.

\* ek tRNA multiple code ko read krskta hai.

order

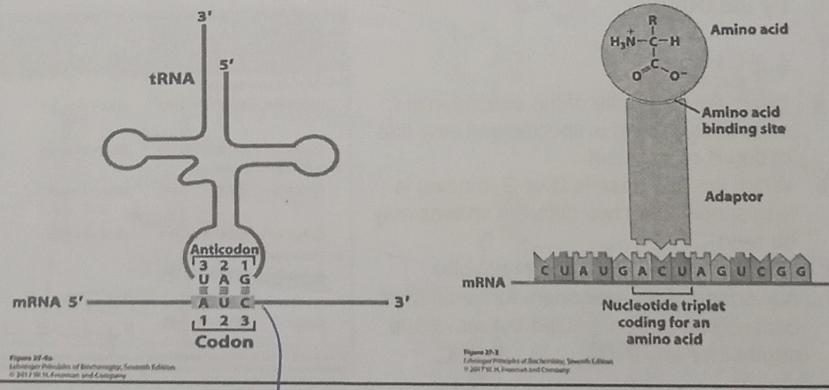
## The Genetic Code Is Universal, With a Few Exceptions

- It is used by prokaryotes and eukaryotes, across species.
- Mitochondria contain DNA and use a slightly different code. <sup>own</sup> Mitochondria have own protein factory.  
- UGA encodes Trp in vertebrate mtDNA (instead of STOP).  
- AGA/AGG encodes STOP in vertebrate mtDNA (instead of Arg).
- Mitochondria encode their own tRNAs, using 22 instead of 32. <sup>Rna</sup> for 61 codon who will encode 20 amino acids.

which se  
DNA transcribe  
to mRNA  
is made hai cu  
Protein Synthesis  
hii hai. Because  
its protein requirement is high,  
as there are  
many enzymatic  
reactions occurring

## Molecular Recognition of Codons in mRNA by tRNA

- The codon sequence is complementary with the anticodon sequence.
- The codon in mRNA base pairs with the anticodon in tRNA via hydrogen bonding.
- The alignment of two RNA segments is antiparallel.
- A reading frame without a termination codon among 50 or more codons is referred to as an **open reading frame (ORF)**.



This sequence will be read by anticodon or it will be mutatable w/o tRNA to base pair idr

Anticodon is present at the bottom of tRNA which is complementary to mRNA sequence.

## “Wobble” Pairings in tRNA with mRNA Can Occur in the Third Base

- The third base of a codon can form noncanonical base pairs with its complement (**anticodon**) in tRNA.
- Some tRNAs contain Inosinate (I), which can H-bond with U, C, and A.
  - These H-bonds are weaker and were named by Crick as “wobble” base pairs.
  - Example: In yeast, CGA, GGU, and CGC all bind to tRNA<sup>Arg</sup>, which has the anticodon 3'-GCI-5'.
    - Although sequences are usually written 5'→3', the anticodon here is written 3'→5' to illustrate its bonding to the mRNA codons.

Inosine will recognize U, A, C.

It will change

- Wobble Hypothesis** always Complementarity by
- The first two bases of codon always form strong base pairs with the corresponding bases anticodon and confer the coding specificity.
  - When an amino acid is specified by several different codons, the codons that differ in either of the first two bases require different tRNAs.
  - A minimum of 32 tRNAs are required to translate all 61 codons (31 to encode the amino acids and 1 for initiation).
  - The first base of the anticodon (reading in the 5'-3' direction; this pairs with the third base of the codon) determines the number of codons recognized by the tRNA.

wobble

- When the first base of the anticodon is C or A, base pairing is specific and only one codon is recognized.
- When the first base is U or G, binding is less specific and two different codons may be read.
- When inosine (I) is the first (wobble) nucleotide of an anticodon, three different codons can be recognized—the maximum number for any tRNA. (U, A, C)

C - G  
A - U  
U — A, G  
G — C, U  
I — A, U, C

agr first two  
change here  
to tRNA change  
higher.  
last base  
Pair is loose

TABLE 27-4 How the Wobble Base of the Anticodon Determines the Number of Codons a tRNA Can Recognize			
1. One codon recognized:	Anticodon (3') X-Y-C (5')	(3') X-Y-A (5')	
	— — —	— — —	
Codon	(5') X'-Y'-G (3')	(5') X'-Y'-U (3')	
2. Two codons recognized:	Anticodon (3') X-Y-U (5')	(3') X-Y-G (5')	
	— — —	— — —	
Codon	(5') X'-Y'-A (3')	(5') X'-Y'-C (3')	
3. Three codons recognized:	Anticodon (3') X-Y-I (5')		Purine
	— — —		
Codon	(5') X'-Y'-G (3')		Pyrimidine

Note: X and Y denote bases complementary to and capable of strong Watson-Crick base pairing with Z' and Y', respectively. Wobble bases—in the 3' position of codons and at positions of anticodons—are shaded in white.

Concept of wobble is because of U, I.

Chloroplast  
20 aminoacyl-tRNA, mRNA

Ug it  
Points

→ Euk tRNA multiple codon ko read krskta  
hau because of wobble base pairing.  
*"Five stages of Protein Synthesis"*

Activation

- Activation me a-a are attach to tRNA &  
it will require ATP, so it <sup>wo</sup> will attach  
karega activated a-a ko

Omitation

smaller Subunit, mRNA, first tRNA with methionine  
Sub assemble hinge jese hi large  
Subunit attach hoga ye elongation me  
transfer hoga ga, or read krta rathyga  
codon ko, peptide bond bnti rathyga or  
Protein synthesize hti rathy g.

After, in the last jese hi Stop Codon  
Process terminate hoga ga  
Protein disassemble hoga g, donc  
Subunit separate hogaengy tRNA drag  
hoga ga

After Protein Synthesis it is going for  
further modification. functional line se  
Phiele, jis me Protein fold hoga, 3  
dimensional Structure bnaengi, additional  
mol add hinge if needed, After that  
it will further go toward Post modification spe

Kr k rachta hau mRNA, tRNA & many many it

et

- Ribosomes Structure is not simple because it contains lots of Protein.

Q Why we need these Proteins? Because it holds mRNA, it forms peptide bond, it also have tRNA, that's why it is complicated

Bacterial Ribosome } Eukaryotic ribosome  
larger              Size      smaller

No. of Proteins

57	" "	a "	80	types of Protein is
"	"	Bacterial	Present in Eukaryotic	
ribosome (36 in large)			Ribosome (47 in larger	
(21 in smaller subunit)			(33 in smaller	
			Subunit)	Subunit

Chloroplast & mitochondrial ribosomes are simplest, because they synthesize their own ribosome from mitochondria. Perform many functions, no. of pathways are going to place inside it, so it synthesizes its own Protein, apart DNA me specific RNA. DNA is apron Protein kud synthesise karta hai;

Generalization Starts when protein come under  
 → 20 amino acids + 20 aminoacyl-tRNA hinge, or  
 Yahan Pata lagya ga + tRNA pe kitne aakar  
 jise rata or Kaise jise rata, so basically it  
 will recognize tRNA by some recognition points.

### Requirements

TABLE 27-5 Components Required for the Five Major Stages of Protein Synthesis in <i>E. coli</i>	
Stage	Essential components
1. Activation of amino acids	20 amino acids 20 aminoacyl-tRNA synthases 32 or more tRNAs ATP $Mg^{2+}$ a-a attachment to tRNA
2. Initiation	mRNA N-Formylmethionyl-tRNAfMet Initiation codon in mRNA (AUG) 30S ribosomal subunit 50S ribosomal subunit Initiation factors (IF1, IF2, IF3) GTP $Mg^{2+}$
3. Elongation	Functional 70S ribosomes (initiation complex) Aminoacyl-tRNAs specified by codons Elongation factors (EF-Tu, EF-Ts, EF-G) GTP $Mg^{2+}$
4. Termination and ribosome recycling	Termination codon in mRNA Release factors (RF1, RF2, RF3, RRF) EF-G IF3
5. Folding and posttranslational processing	Chaperones and folding enzymes (PPI, PDI); specific enzymes, cofactors, and other components for removal of initiating residues and signal sequences, additional proteolytic processing, modification of terminal residues, and attachment of acetyl, phosphoryl, methyl, carboxyl, carbohydrate, or prosthetic groups

tRNA aakar aakar aakar aakar aakar some ke liye jio bind  
 ribosoma ribosoma ribosoma ribosoma thoda kya it is imp

### The Ribosome Is a Key Player in Protein Synthesis

- Make up ~25% of dry weight of bacteria
- ~65% rRNA, 35% protein
  - rRNA forms the core.
  - RNA does the catalysis of peptide bond formation.
- Made of two subunits bound together (30S and 50S) in bacteria, with mRNA running through them
- Holds mRNA

TABLE 27-6 RNA and Protein Components of the *E. coli* Ribosome

Subunit	Number of different proteins	Total number of proteins	Protein designations	Number and type of rRNAs
30S	21	21	S1-S21	1 (16S rRNA)
50S	33	36	L1-L36*	2 (5S and 23S rRNAs)

\*The L1 to L36 protein designations do not correspond to 36 different proteins. The protein originally designated L7 is a modified form of L12, and L8 is a complex of three other proteins. Also, L26 proved to be the same protein as S20 (and not part of the 50S subunit). This gives 33 different proteins in the large subunit. There are four copies of the L7/L12 protein, with the three extra copies bringing the total protein count to 36.

Q) Why Ribosome Play Important Role in Protein Synthesis: Because it holds mRNA, helps in peptide bond formation. Basically jo leg haan mRNA wo jaa ke attack hoji ribosome me, or proper complex peptide bond bana raha hogya, tRNA ate rabege, ye assumption kr ke rakhta hai mRNA, tRNA ay itself that why it is imp.

## Ribosomes in Bacteria and Eukaryotes

- Overall, very similar
- Still two subunits with mRNA running between
- In eukaryotes, larger (80S), more complex, contain > 80 proteins
- Chloroplasts and mitochondria have ribosomes simpler than those in bacteria.

*because it makes their own protein*

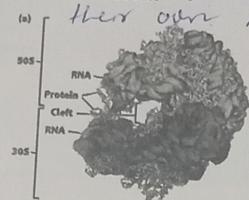


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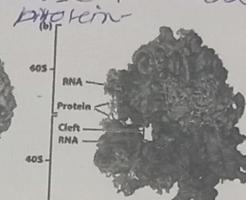
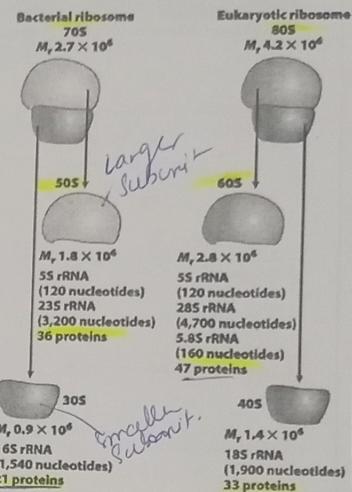
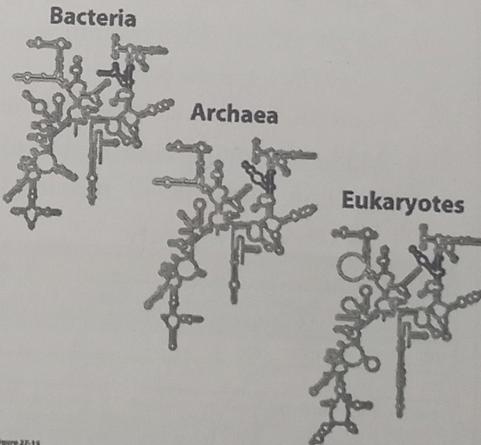


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## rRNAs Have Complex Secondary Structures

- The three ss rRNAs having specific 3-D structure with extensive intrachain base pairs.
- Shape of rRNAs are highly conserved.
- The red, yellow, and purple indicate areas where the structures of the rRNAs from bacteria, archaea, and eukaryotes have diverged.
- Conserved regions are shown in green.



They are associated with each other.

## Characteristics of tRNAs

*After folding by  
self complementary  
Base pair  
it will form*

- ssRNA of 73–93 nucleotides in both bacteria and eukaryotes
- Cloverleaf structure in 2-D, “Twisted L” shape in 3-D
- Serve as interpreters during translation
- Most have G at 5'-end; all have CAA at 3'-end
- Have modified bases
  - methylated bases, and so on

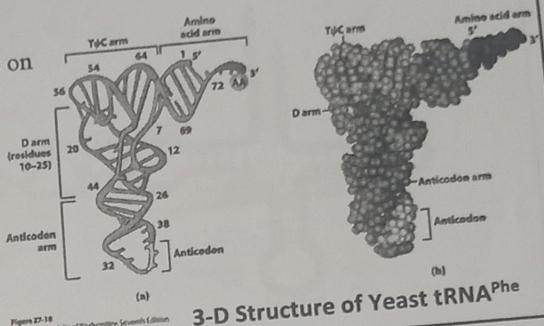


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3-D Structure of Yeast tRNA<sup>Phe</sup>

## Characteristics of tRNAs

- Of have 4 terms.

- **Amino acid arm**

- has amino acid esterified via carboxyl group to the 2'-OH or 3'-OH of the A of the terminal CAA codon

- **Anticodon arm**

- **D arm**

- contains dihydrouridine (D)
- contributes to folding

- **T<sub>ψ</sub>C arm**

- contains pseudouridine ( $\psi$ )—has bonding between base and ribose
- helps in folding

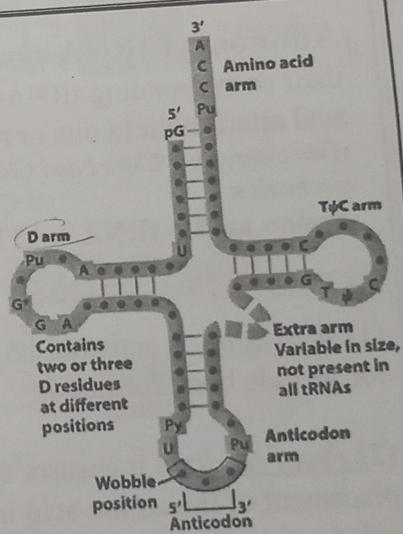
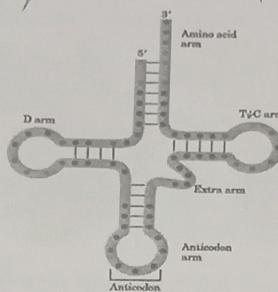


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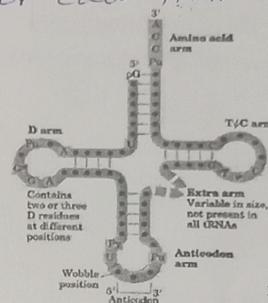
### Recognition points of aminoacyl-tRNA synthetases

- Each tRNA molecule has a triplet anticodon on one end and an amino acid attachment site on the other
- Black dots are same in all tRNAs
- Green & Blue Dots are the Recognition points (orange for one) or (green for more) aminoacyl-tRNA synthetases.

Orange dot are specific for each tRNA



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Each synthetase enzyme is specific for a particular amino acid and can recognize by specific recognizing points. On the basis of which amino acid is attached it will recognize the tRNA.

### Stage 1: Activation of Amino Acids

(Aminoacyl-tRNA synthetases esterify the 20 amino acids to their corresponding tRNAs. Each enzyme is specific for one amino acid attachment to one or more corresponding tRNAs.)

• Two classes i.e. Class I and Class II: Based on substantial differences in reaction mechanism



- Synthetases have two properties?

(1) Activation: it activates an amino acid for peptide bond formation

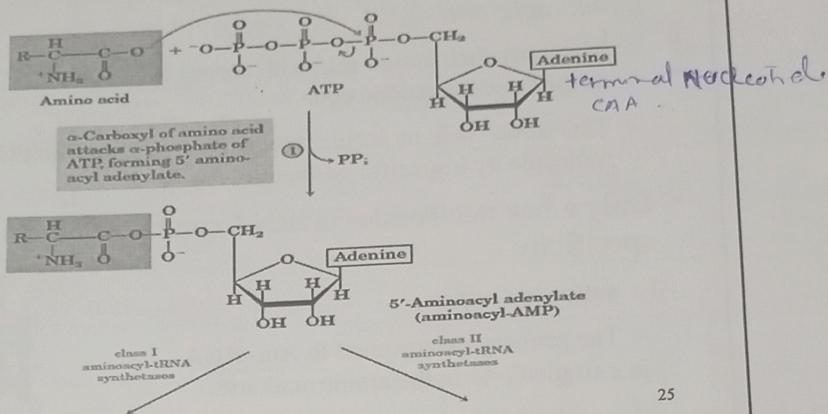
(2) Proof reading: it ensures appropriate placement of the amino acid in a growing polypeptide.

TABLE 27-7		The Two Classes of Aminoacyl-tRNA Synthetases			
		Class I a.o.		Class II a.g.	
Arg	Leu			Ala	Lys
Cys	Met			Asn	Phe
Gln	Trp			Asp	Pro
Glu	Tyr			Gly	Ser
Ile	Val			His	Thr

Note: Here, Arg represents argylyl-tRNA synthetase, and so forth. This classification applies to all organisms for which tRNA synthetases have been analyzed and is based on protein structural distinctions and on the mechanistic distinction outlined in Figure 27-19.

AA don't release & salt attach hoje,  
aa k carbonylic groups & salt attach  
hoja so aagy ja k shift krawaejj tRNA.

### Activation of Amino Acids

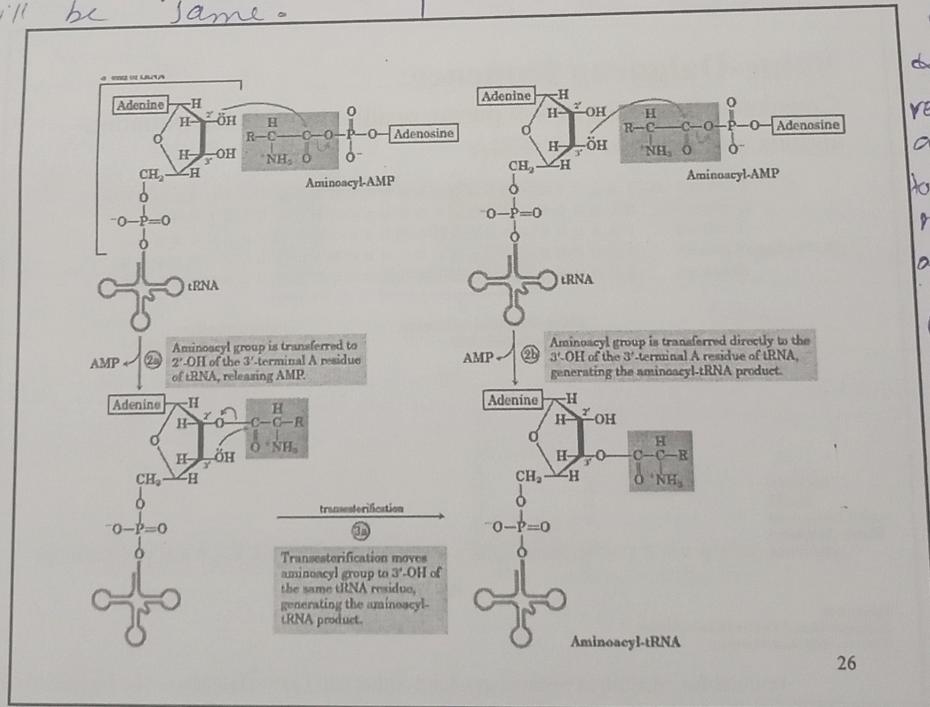


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will same mechanism. Aa first come to carbon no 2 , then come to carbon no 3 , Resultant molecule will be same.

A-A along with amb in activated form is k carbonylic group detach ho k AMP ko release karega , and attach karega carbon no 3 k salt , or Lone electron pair krawaejj attack resultant your aa is attach to adenine residue (terminal adenine) of tRNA)

(Indirect attachment)



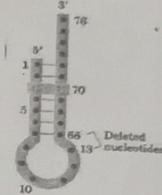
26

(direct attachment)

After study 13-16 delete last 4 nucleotides. So the G-U triplets diya it is a recognition point for tRNA who carries alanine, alanine wala synthetase, just G & U ko penetaanta hai only. So synthetase ne tRNA reaction perform diya or adenine ka sahi alanine ko attach karne ka. Synthetase alone recognition point ko pehchaan koi le. Sahe attachment thoda hai.

## The Second Genetic Code

- Aminoacyl-tRNA synthetases must be specific for both amino acid and tRNA.
- Matching each amino acid with correct tRNA can be viewed as the "second genetic code."
  - The "code" is in molecular recognition of a specific tRNA molecule by a specific synthetase.
- Only a few nucleotides in tRNA confer the binding specificity.
  - anticodon region
  - The primary determinant in Ala-tRNA is a single G=U in the amino acid arm.



Synthetase alanine ke tRNA ke sahi attachment karne ka so far this is need only G-U triplet ka ho ya wo us se kar tag ni pata, so wo delete karne diya jata hai.

### Shine-Dalgarno sequence:

Consensus Sequence of 4-9 purine residue provide the initiation signals and binds to 16S rRNA

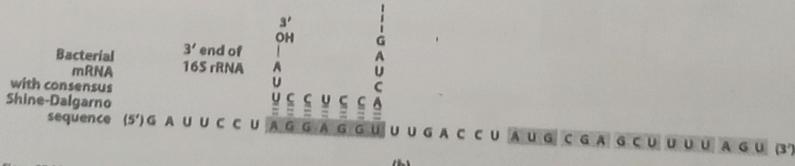
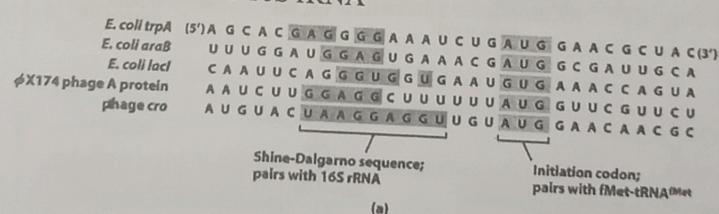


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(named for Australian researchers John Shine and Lyrne Dalgarno, who identified it) 28

Shine dalgarno sequence Shakespearian se phle kinaa, sequence hai is k sahi smaller subunit attachment

Q) Why we call it d-arm? Because it poses d-hydroxyl groups.

Anticodon arms of tRNA have anticodon sequence, so it is specific for mRNA.

A-A arm which has a-a attach hoga, the attachment of a-a to the specific tRNA is unique. No other a-a amino tRNA can attach hoga.

Activation of A-A: tRNA has a-a ki attachment & it is specific & it is carried out by Enzyme Amino-acyl tRNA synthetase - 20 amino acids & 4 types 20 synthetases.

Q) What synthetase does? It helps in attachment of a-a to tRNA. Through ATP activation.

First it will activate a-a through ATP then attachment karna ja.

B) Km Properties ki waja se ye recognize kita hai ta a-a attachment karna ske?

Activation: Sy

ATP se attach karna & mol ko aloe kr k tRNA se attach karega, then peptide bond baa dega.

Proof reading:

mRNA ki sequence niche hai so tRNA is going to work as an adapter to link this mRNA to peptide bond, corrections are taken up by synthetase enzyme because after attachment koi correction bhi nahi karega & sali attach hua k rhi.

**Handouts Pg # 12**

Basic Reaction  $a\text{-a} + \text{tRNA} + \text{ATP} \rightarrow \text{Aminoacyl-tRNA} + \text{AMP}$

Amino acid attach with tRNA with the release of  $\text{PP}_i$

off one ATP, then ATP amp convert hoga, Pyrophospho-phate release. Energy is utilized for the attachment of a-a to tRNA.

The attachment pattern of a-a is different so on the bases of attachment it will divide into two classes.

Q) What is the diff b/w class 1 & 2?  
A) tRNA ka ribose sugar hai, us ke sathe a-a attach kruana hai. ek carbon 2, ek carbon 3 kuch directly b pe attach hoga, kuch phle 2 pe ja then 3 pe,  
Date: \_\_\_\_\_

AA Amino residue ke sathe attach hogta, a-a ke carbonylic group ke sathe X attach hogta jo aayi ja ke shift krua gaya tRNA.

Shine-Dalgarno Sequence :-  
Expensive in terms of energy, ek or recognizing Point - hr stage pe energy ja abhi hai, to error na aye, corrected Protein bne, hr stage pe checking hogi, jb initiation hogi, mRNA attach hogta to ribosome (attachment is specific)

Q) Kya specific hai? there is a particular sequence just before the start Codon jisay smaller subunit Pehchaane ge, then it will assemble on first AUG or tRNA ke attach krua ga. The seq present in red is called Shine Dalgarno Seq. for  
1 Shine Dalgarno was a scientist who observed this sequence which was recognized by ribosome and made a complex, and it is specific to the sequence present on ribosome. It is called Shine Dalgarno Sequence"

### Initiation (Stage 2) Pg # 15

- The smaller subunit is going to bind with mRNA, initially it needs two factors, IF1 which is required for the attachment of A side of ribosome, IF3 which binds with ribosome

IF2: tRNA ki attachment kruana to mRNA

IF3: Ye mukarrak larger subunit ko bind on one deta jb tk complex na bne

## Stage 2: Initiation

Bacterial Ribosomes have three sites that bind tRNAs, aminoacyl (A) site, the peptidyl (P) site, and exit (E) site.

**Step 1:** The 30S ribosomal subunit binds two initiation factors, IF-1 and IF-3.

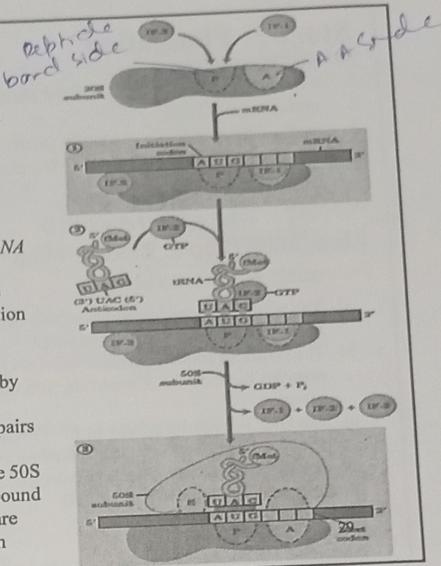
*Factor IF-3 prevents the 30S and 50S subunits from combining prematurely.*

*Factor IF-1 binds at the A site and prevents tRNA binding at this site during initiation.*

The mRNA then binds to the 30S subunit. The initiating (5')AUG is guided to its correct position by the Shine-Dalgarno sequence.

**Step 2:** The complex consisting of the 30S ribosomal subunit, IF-3, and mRNA is joined by both GTP-bound IF-2 and the initiating fMet-tRNA<sup>fMet</sup>. The anticodon of this tRNA now pairs correctly with the mRNA's initiation codon.

**Step 3:** This large complex combines with the 50S ribosomal subunit; simultaneously, the GTP bound to IF-2 is hydrolyzed to GDP and Pi, which are released from the complex. All three initiation factors depart from the ribosome at this point.



## Initiation in Eukaryotes

Eukaryotic mRNAs are bound to the ribosome as a complex with a number of specific binding proteins.

- At the 3' end, the mRNA is bound by the poly(A) binding Proteins (PAB).
- The protein eIF4A has an RNA helicase activity removes secondary structure within the mRNA seq.
- eIF4F complex (eIF4A, E, & G) and eIF4B scan mRNA from the 5' end until the first AUG is encountered.

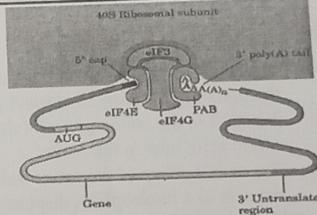


FIGURE 27-22 Protein complexes in the formation of a eukaryotic initiation complex. The 3' and 5' ends of eukaryotic mRNAs are linked by a complex of proteins that includes several initiation factors and the poly(A) binding protein (PAB). The factors eIF4E and eIF4G are part of a larger complex called eIF4F. This complex binds to the 40S ribosomal subunit.

TABLE 27-8 Protein Factors Required for Initiation of Translation in Bacterial and Eukaryotic Cells

Factor	Function
<b>Bacterial</b>	
IF-1	Prevents premature binding of tRNAs to A site
IF-2	Facilitates binding of fMet-tRNA <sup>fMet</sup> to 30S ribosomal subunit
IF-3	Binds to 30S subunit; prevents premature association of 50S subunit; enhances specificity of P site for fMet-tRNA <sup>fMet</sup>
<b>Eukaryotic*</b>	
elf2	Facilitates binding of initiating Met-tRNA <sup>fMet</sup> to 40S ribosomal subunit
elf2B, elf3	First factors to bind 40S subunit; facilitate subsequent steps
elf4A	RNA helicase activity removes secondary structure in the mRNA to permit binding to 40S subunit; part of the elf4F complex
elf4B	Binds to mRNA; facilitates scanning of mRNA to locate the first AUG
elf4E	Binds to the 5' cap of mRNA; part of the elf4F complex
elf4G	Binds to elf4E and to poly(A) binding protein (PAB); part of the elf4F complex
elf5	Promotes dissociation of several other initiation factors from 40S subunit as a prelude to association of 60S subunit to form 80S initiation complex
elf6	Facilitates dissociation of inactive 80S ribosome into 40S and 60S subunits

### Stage 3: Elongation

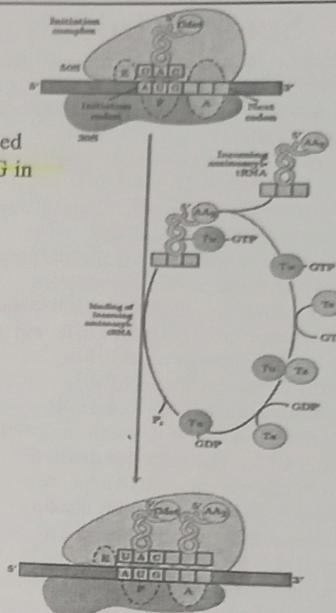
Elongation requires

1. the initiation complex described above,
2. aminoacyl-tRNAs,
3. a set of three soluble cytosolic proteins called elongation factors (EF-Tu, EF-Ts, and EF-G in bacteria).
4. GTP.

*category in  
3 parts/ steps?*

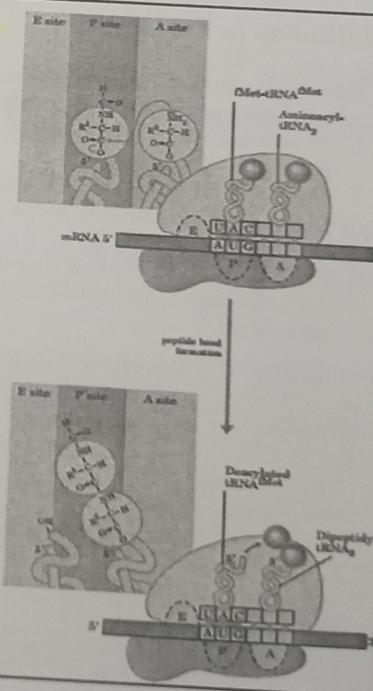
#### Elongation Step 1: Binding of an Incoming Aminoacyl-tRNA

- a) The appropriate incoming aminoacyl-tRNA binds to a complex of GTP-bound EF-Tu.
- b) The resulting aminoacyl-tRNA-EF-Tu-GTP complex binds to the A site of the 70S initiation complex.
- c) The GTP is hydrolyzed and an EF-Tu-GDP complex is released from the 70S ribosome.
- d) The EF-Tu-GTP complex is regenerated in a process involving EF-Ts and GTP.



#### Elongation Step 2: Peptide Bond Formation

- a) A peptide bond is formed by the transfer of the initiating N-formylmethionyl group from its tRNA to the amino group of the second amino acid, now in the A site.
- b) The amino group of the amino acid in the A site acts as a nucleophile, displacing the tRNA in the P site to form the peptide bond.
- c) This reaction produces a dipeptidyl tRNA in the A site, and the now "uncharged" (deacylated) tRNAfMet remains bound to the P site.
- d) The enzymatic activity that catalyzes peptide bond formation has historically been referred to as *peptidyl transferase* (assumed to be intrinsic to one or more of the proteins in the large ribosomal subunit). We now know that this reaction is catalyzed by the 23S rRNA adding to the known catalytic property of ribozymes.



Q) why IF1 binds? Because first tRNA with methionine is directly attach to P-side not on A-side. After that all tRNA is going to attach to A-side.

Q) why first tRNA will bind to P-side? Because IF1 will inhibit / block A-side. as it is required for initiation

- ↗ First tRNA will bind to P
- ↗ After that all tRNA bind to A then it will slide backward to P
- ↗ First tRNA need IF2 which is with GTP (ATP), GTP helps in attachment of tRNA, after attachment GTP will hydrolyse
- ↗ After GTP hydrolysis (IF1, IF2, IF3 will release)
- | Then larger subunit will bind
- IF3 : IF3 is binding in the delta subunit initiation complex made on site after complex will formed it will release a larger subunit will bind tRNA with methionine mRNA
- Smaller Subunit,

## Initiation In Eukaryotes:

Eukaryotic transcript come with cap on tail.

Capping is required when complex, it have many EIF (factors) which is attach with smaller subunit.

- It will hold cap on tail is bind by Polyadenylated Protein

- eIF have Scanning Process, jis me cap wala Sirha Peeche ki taraf milta hai, tail aagya ki Side karta hai.

- After scanning it will stop at first codon, then First RNA will assemble here, and other subunit will attach here.

Elongation = Stage 3 Pg # 16

Step 1 :- First methionine will bind,

> Next a-a will bind to A side, for its binding we need Tu factor along with GTP

> Tu factor will release, GTP will hydrolyse after binding

> For performing function again its GTP will need to replace by GDP which was replaced by Ts

> Later GDP will replace Ts, Tu is activated; it will bring tRNA

Step 2 :- Ribosomes combine on aya with the mRNA, tRNA attaches with methionine, initiation complete hogi, later another tRNA is going to attach to A side

In the next stage Peptide bond is formed by ribosome - Ribosomes have Peptidyl transferase activity which will catalyze the peptide bond,

Bond bnegi carbonylic in Amne group & break me 2 different Amne acids are there with two tRNA attaches on it -

Amne group have lone electron pair will disperse bond carbonylic jo synthetare ne DNA thi -

Break kr ke apne isek rejoin karyga

Step #3 Translocation is carried out by Translocase activity - Translocase activity is facilitated by another elongation factor "G" EFG, which will come along with GTP - It will further hydrolyze GTP for its energy. Ribosomes will move forward - The Codon come on inner side or occupies A side. Then A side will convert into P side, and a new side "E" is also formed. When only tRNA leaves go later on release tRNA goes.

#### Step #4

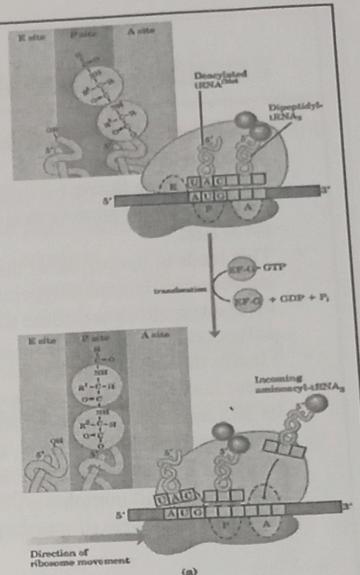
For termination chain will grow, tRNA will exchange, a.a. will exchange by the same process as elongation - Later on Stop Codon will come, which will not be encoded by any tRNA so nothing will bind to it. Only releasing factor will bind to it.

Releasing factor: It is a Protein which help to release peptide by occupying stop codon.

After attachment of RF Peptide bond will release as a complex will assemble all the things will separate out - For protein synthesis so it will recycle.

### Elongation Step 3: Translocation

- The ribosome moves one codon toward the 3' end of the mRNA. This movement shifts the anticodon of the dipeptidyl tRNA, which is still attached to the second codon of the mRNA, from the A site to the P site, and shifts the deacylated tRNA from the P site to the E site, from where the tRNA is released into the cytosol.
- The third codon of the mRNA now lies in the A site and the second codon in the P site.
- Movement of the ribosome along the mRNA requires EF-G (also known as translocase) and the energy provided by hydrolysis of another molecule of GTP.
- A change in the three-dimensional conformation of the entire ribosome results in its movement along the mRNA. Because the structure of EF-G (bind to A site and displace the peptidyl-tRNA) mimics the structure of the EF-Tu-tRNA complex.

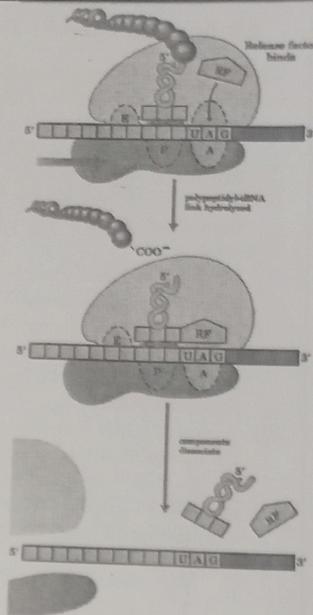


### Stage 3: Elongation (Eukaryotic)

- Steps are similar to bacteria
- Elongation factors – eEF $1\alpha$  (EF-Tu), eEF $1\beta\gamma$  (EF-Ts), eEF $2$  (EF-G)
- Difference: Eukaryotic ribosomes do NOT have an E site; the uncharged tRNAs are released from the P site.

## Stage 4: Termination

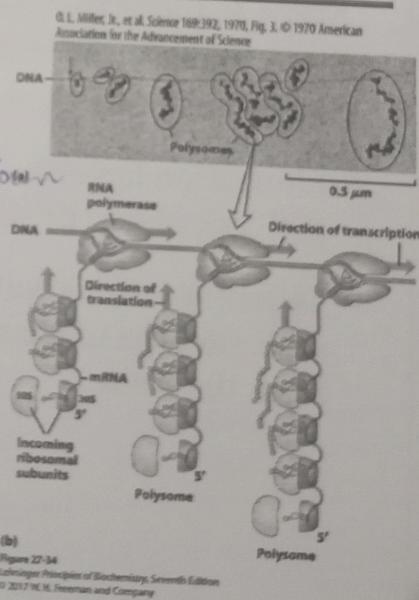
- Termination occurs in response to a termination codon in the A site.
- First, a release factor, (RF-1 or RF-2, depending on which termination codon is present) binds to the A site.
- This leads to hydrolysis of the ester linkage between the nascent polypeptide and the tRNA in the P site and release of the completed polypeptide.
- Finally the mRNA, deacylated tRNA, and release factor leave the ribosome which dissociates into its 30S and 50S subunits aided by ribosome recycling factor (RRF), IF-3, and energy provided by EF-G-mediated GTP hydrolysis.
- The 30S subunit complex with IF-3 is ready to begin another cycle of translation.



Polyosome : A single mRNA can contain multiple ribosome attachemnt sites and it can form a beaded structure.

## Features of Protein Synthesis

- Large energy cost (122 kJ/mol of phosphodiester bond energy, to generate a peptide bond, which has a standard free energy of hydrolysis of about -21 kJ/mol)
- Can be rapid when accomplished on clusters of ribosomes called a polysome on breakdown def
- In bacteria, tightly coupled to transcription
  - Translation can begin before transcription is finished.



(b)  
Figure 27-34  
Lodriguez Principles of Biochemistry, Seventh Edition  
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Post-translational  
It is performed after translation by it will  
activate Proteins.

## Posttranslational Modifications

Newly Synthesize polypeptide chains Undergo Folding and Processing

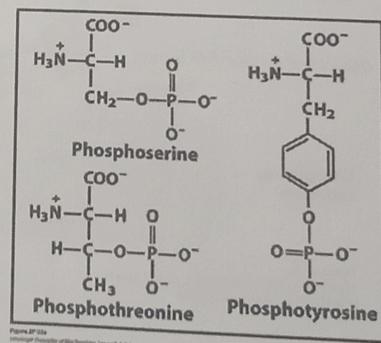
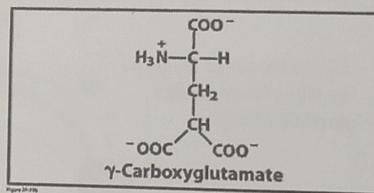
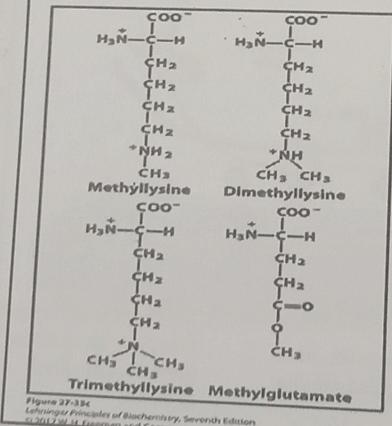
1. Amino-Terminal and Carboxy-Terminal Modifications
  - Removal of Formyl-Methionine and Methionine
  - Removal of Additional Amine Terminal
  - Removal of Additional Carboxy Terminal *if eukaryote*
  - Acetylation at N-terminal (50% of eukaryotic proteins) *& acetylated*
2. Removal of Signal Sequence (Direct the movement of proteins in ER)
3. Phosphorylation: ex: Casien
4. Attachment of Carbohydrate Side Chains (Glycosylation)
5. Addition of Isoprenyl Groups (*5c carbonyls req for Fatty acid chain of protein*)
6. Addition of Prosthetic Groups (Biotin in carboxylases, heme in hemoglobin and cytochromes)
7. Proteolytic Processing *Koch Protein bina k baad methylation hti kwaya jata hai.*
8. Formation of Disulfide Cross-Links *hai os k baad <sup>37</sup> very active*

*addition of phosphate group*

*Trypsinogen — Trypsin.*

## Posttranslational Modifications

- Modifying amino acids with additional phosphates, carboxylic acid groups, and so on

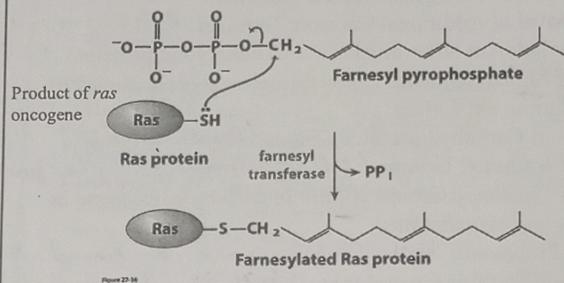


Ras protein is in inactive form, when Isoprenyl group attaches to it, it becomes active, it is an oncogene if can convert into cancerous. If Isoprenyl group attaches to it, if Isoprenyl does not attach to this protein is non-functional.

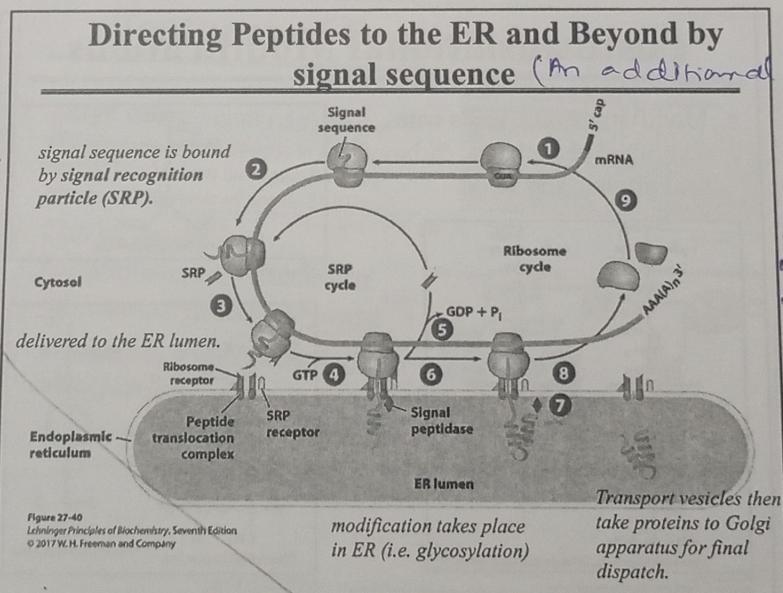
## More Modified Amino Acids

Addition of isoprenyl groups (such as farnesyl pyrophosphate) from intermediates of cholesterol synthesis pathway

Isoprene group helps anchor proteins in membranes for carcinogenic activity.



As we go on  
Problem synthesis  
for Schrödinger  
will start.  
Later on after  
detachment,  
Problem synthesis  
will start.



Sequence  
which is going  
to be  
recognize by  
the receptor  
which is present  
Dr ER-

In ER, Protein processing loops, it have receptor SRP to track its destruction, SRP is for signal recognition particle K type. It is a receptor signal sequence who recognize it, and help it to attach at inner side after attachment SRP, <sup>28</sup>RP molecule & tail bond break -

### Post Translational Modification

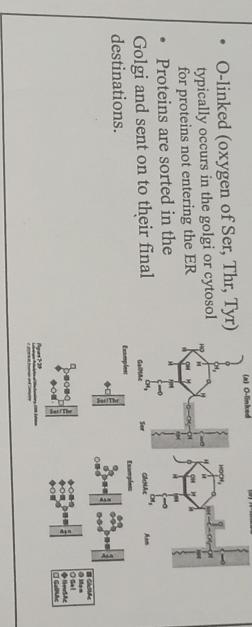
Post translation modifications have methylation  
(attachment of methyl group). Any group, phosphate  
group, methyl group attaches to R-chain on  
outer side.

- In A.A., amine or carboxy group formed bond  
on R groups is their for attachment on Protein
- Methylation, carbonylation, phosphorylation isobenzyl  
Protein be attach with ha on Proteins

Glycosylation starts when protein come under the molecule.

## Glycosylation of Proteins

- Glycoproteins form by linking an oligosaccharide to a side group on the peptide. (two types)
  - N-linked (nitrogen of Asn or Arg)
    - begins in the RER
  - O-linked (oxygen of Ser, Thr, Tyr)
    - typically occurs in the Golgi or cytosol
    - for proteins not entering the ER
- Proteins are sorted in the Golgi and sent on to their final destinations.



## Glycosylation of Proteins

(proteins) oligosaccharide to the protein attach the main functional groups on it will transform to all over the body were it's needed.

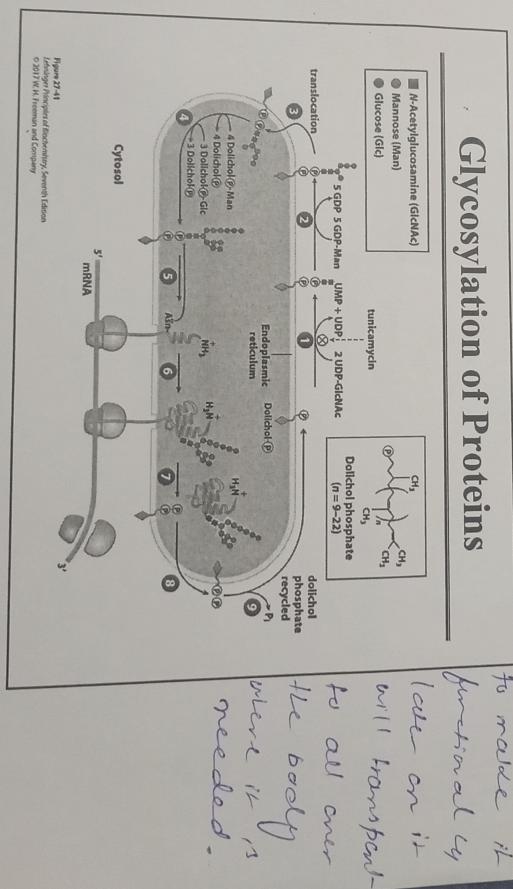


Figure 27-41  
Lodish et al., Principles of Molecular Cell Biology, Seventh Edition  
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