Lecture Note		
Subject: HAP, 201T	**SEM-2nd**	**UNIT-V**
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CHROMOSOME

Definition – Chromosome means: chroma - colour; some - body) Waldeyer coined the term chromosome first time in 1888.

A chromosome is a thread-like self-replicating genetic structure containing organized DNA molecule package found in the nucleus of the cell. E. Strasburger in 1875 discovered thread-like structures which appeared during cell division.

In all types of higher organisms (eukaryote), the well organized nucleus contains definite number of chromosomes of definite size, and shape.

The somatic chromosome number is the number of chromosomes found in somatic cell and is represented by 2n (Diploid). The genetic chromosome number is half of the somatic chromosome numbers and represented by n (Haploid).

The two copies of chromosome are ordinarily identical in morphology, gene content and gene order, they are known as homologus chromosomes.

Features of eukaryotic chromosome

- Chromosomes are best visible during metaphase
- Chromosomes bear genes in a linear fashion
- Chromosomes vary in shape, size and number in different species of plants and animals
- Chromosomes have property of self duplication and mutation
- Chromosomes are composed of DNA, RNA and protein

Chromosome size, shape and number

- Chromosome size is measured at mitotic metaphase generally measured in length and diameter *Plants usually have longer Chromosome than animals*
- Plant Chromosomes are generally 0.8-7μm in length where as animal chromosomes are 0.5-4μm in length. Chromosomes size varies from species to species

Chromosome shape

- The shape of chromosome is generally determined by the position of centromere.
- Chromosomes generally exits in three different shapes, ***rod shape, J shape and V shape***

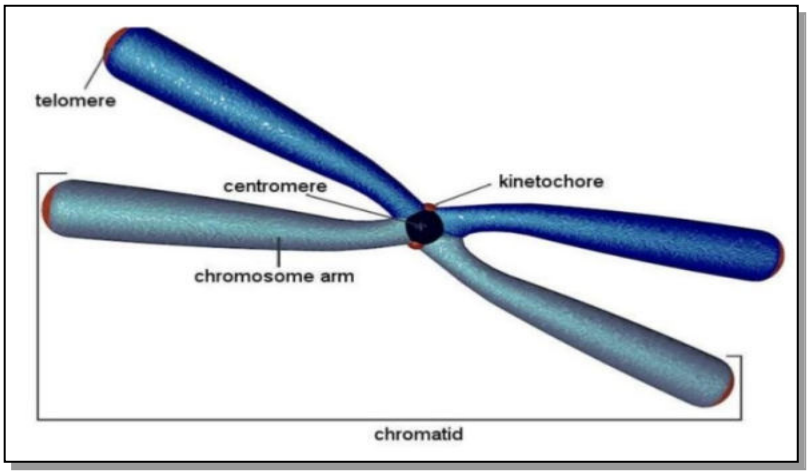
Chromosome number

- Each species has definite and constant somatic and genetic chromosome number

- Somatic chromosome number is the number of chromosome found in somatic cells while genetic chromosome number is the number of chromosome found in gametic cells.
- Somatic chromosome are always diploid and are denoted as 2n where as genetic chromosomes are always haploid and are denoted as n
- In human being somatic chromosomes are XX and XY where as genetic chromosome number is X and Y.

Chromosome structure • Structurally chromosomes consists of seven parts

1. Centromere 2. Chromatid 3. Secondary constriction and satellite 4. Telomere 5. Chromomere 6. Chromonema 7. Matrix



Centromere- It is a localized region of the chromosome with which spindle fibers attached is known as centromere or primary constriction or kinetochore

Chromatid- One of the two distinct longitudinal subunits of a chromosome is called as chromatid. Chromatids are of two types sister chromatids and non- sister chromatids.

Secondary constriction- Some chromosome exhibits secondary constriction in addition to primary constriction. The chromosomal region between telomere is called as satellite or trapant. The chromosome having satellite is called as satellite chromosome.

Telomere- The two ends of chromosome are called as telomeres. Telomere are highly stable and they do not fuse or unite with telomere of other chromosome.

Chromomere- The chromosomes of some of the species shows small bead like structures called as chromomeres. The structure of chromomeres in chromosome is constant.

Chromonema- Thread like coiled structures found in the chromosomes and chromatids are known as chromonema (plural chromonemata).

Matrix – It is a fluid part in which chromonemata are embedded is called as matrix.

Chemical composition of chromosome

- Chemically chromosomes are nucleoprotein in nature means are composed of RNA, DNA and protein.
- Generally chromosomes contains 30-40% DNA, 50-65% protein and 0.5- 10% RNA 1)

DNA- The amount of DNA present in somatic cell is constant. DNA content of gametic cell is half of that of somatic cell. DNA of chromosome is of two types

i) Unique DNA

ii) Repetitive DNA

i) Unique DNA- unique DNA consists of those DNA sequence which are present in a single copy per genome and are unique in nature, Unique DNA is also known as non repetitive DNA. Codes for protein which requires in large quantity for cell. eg- storage protein

ii) Repetitive DNA- Repetitive DNA consists of DNA nucleotides or base sequences, which are few to several hundred base pairs (bp) long and are present to several to a million copies per genome. Human genome contains 30% repetitive DNA.

Repetitive DNA is further divided into

i) Highly repetitive DNA and

ii) Moderately repetitive DNA

RNA- Purified chromatin contain 10-15% RNA. RNA associated with chromosome is messenger RNA, transfer RNA and ribosomal RNA.

Protein- Protein associated with chromosome is classified into two broad groups

i) Histone or basic protein

ii) Non histone protein

Non histone proteins are acidic in nature and histone proteins are basic in nature because of basic amino acids.

i) Histone protein- histones constitutes about 80% of the total chromosomal protein. They are present in an almost 1:1 ratio with DNA. Five fractions of histones are present like 1H1, 2H2a, 2H2b, 2H3 and 2H4

ii) **Non histone protein-** non histone proteins make up to 20% of the total protein mass. Content of non histone protein is different from species to species. Non histone protein includes many important enzymes like DNA and RNA polymerase.

Types of chromosome

• According to the relative position of centromere chromosomes

Middle: Metacentric

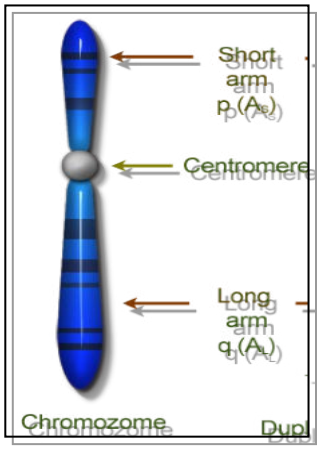
At one end : Acrocentric

Closer to one end: Submetacentric

Each chromosome has two arms,

labeled p (the shorter of the two) and q (the longer).

The p arm is named for "petite" meaning "small"; the q arm is named q simply because it follows p in the alphabet.



Metacentric chromosome

The centromere is located in the centre of chromosomes, i.e. the centromere is median. The centromere is localized approximately midway between each end and thereby two arms are roughly equal in length. Metacentric chromosome take V shape during anaphase.

Submetacentric Chromosome

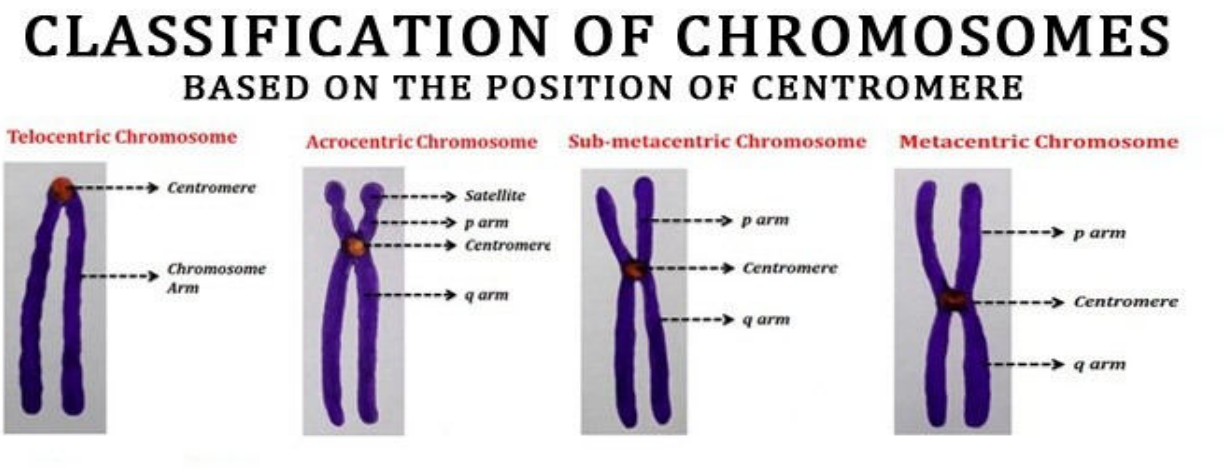
Centromere is located on one side of the central point of a chromosome. Centromere is submedian giving one longer and one shorter arms. Submetacentric chromosome may be J or L shaped during anaphase.

Acrocentric Chromosome

The centromere located close to one end of chromosomes. The centromere is more terminally placed and forms very unequal arm length (The "acro-" in acrocentric refers to the Greek word for "peak"). The p (short) arm is so short that is hard to observe, but still present. crocentric chromosome may be rod shape during anaphase.

Telocentric Chromosome

Centromere located at one end of chromosome (at terminal part of chromosome) lies at one end. Telocentic chromosome may be rod shape during anaphase.



Karyotype

• Complete set of chromosomes in a species, or in an individual organism is known as karyotype.

• The basic number of chromosomes in the somatic cells of an individual or a species is called the somatic number and is designated 2n. Thus, in humans 2n = 46.

According to the number of the centromere the eukaryotic chromosomes may be

- Acentric :without any centromere
- Mono centric: with one centromere
- Dicentric : with two centromeres
- Polycentric: with more than two centromeres

GENES

SUMMARY OF EVOLUTION OF GENE CONCEPT

YEAR	SCIENTIST	GENE CONCEPT
1866	G.J. MENDEL	A UNIT FACTOR THAT CONTROLS SPECIFIC PHENOTYPIC TRAIT
1902	SIR A.E.GARROD	ONE GENE –ONE METABOLIC BLOCK THEORY
1940	BEADLE & TATUM	ONE GENE-ONE ENZYME THEORY
1957	U.M.INGRAM	ONE GENE-ONE POLYPEPTIDE THEORY
1960s	C.YANOFSKY & CO-WORKERS	GENE IS A UNIT OF RECOMBINATION
Early 1970s	E.B.LEWIS	COMPLEMENTATION TEST IN *DROSOPHILA*

General concept:

- The gene is to genetics what the atom is to chemistry.
- The gene is the unit of genetic information that controls a specific aspect of the phenotype.
- The gene is the unit of genetic information that specifies the synthesis of one polypeptide.

Classical definition:-Gene is the Unit of Function (one gene specifies one character), Recombination, and Mutation.

Classical concept:

- Genes are discrete particles inherited in mendelian fashion that occupies a definite locus in the chromosome and responsible for expression of specific phenotypic character.
- Number of genes in each organism is more than the number of chromosomes; hence several genes are located on each chromosome.
- The genes are arranged in a single linear order like beads on a string.
- Each gene occupies specific position called locus.
- If the position of gene changes, character changes. Genes can be transmitted from parent to off springs.
- Genes may exist in several alternate formed called alleles.
- Genes are capable of combined together or can be replicated during a cell division.
- Genes may under for sudden changes in position and composition called mutation.
- Genes are capable of self duplication producing their own exact copies.

Mordern definition:

Includes coding as well as non-coding regulatory sequences. Gene is the Unit of Genetic Information, i.e., the sequence of DNA that specifies one polypeptide.

Concept:

S. Benzer (1957) coined different terms for different nature of gene and genetic material in relation to the chromosome on the basis of genetic phenomena to which they involve.

i) Genes as unit of transmission or cistron.

ii) Genes as unit of recombination or recon.

iii) Gene as unit of mutation or muton.

The part of DNA specifying a single polypeptide chain is termed as cistron. A cistron can have 100 nucleotide pairs in length to 30,000nucleotide pairs. It transmits characters from one generation to other as unit of transmission. The smallest segment of DNA capable of being separated and exchange with other chromosome is called recon. A recon consists of not more than two pairs of nucleotides.

Muton is the smallest unit of genetic material which when changed or mutated produce a phenotypic trait. Muton is delimited to a singlenucleotide.

Type:

On the basis of their behavior the genes may be categorized into the following types:

I. **Basic genes:** These are the fundamental genes that bring about expression of particular character.
II. **Lethal genes:** These bring about the death their possessor.
III. **Multiple gene:** When two or more pairs of independent genes act together to produce a single phenotypic trait.
IV. **Cumulative gene:** Some genes have additive effects on the action of other genes. These are called cumulative genes.
V. **Pleiotropic genes:** The genes which produce changes in more than one character is called pleiotropic gene.
VI. **Modifying gene:** The gene which cannot produce a character by itself but interacts with other to produce a modified effect is called modifier gene.
VII. **Inhibitory gene:** The gene which suppresses or inhibits the expression of another gene is called inhibitory gene.

Gene Action: The influence of gene resulting in the expression of a genetic character is called gene action.

The genes are generally associated with the production of enzymes, which they synthesize from chemical substance available in the body cells through a process of autocatalysis.

As a rule one gene affects one enzyme. The various actions of genes are expressed in their development of

- pigments
- Colours
- hormones
- size and form
- production of proteins
- antigen and antibody

Production decisive effect on human disease like

- albinism,
- Tyrosinosis etc.

Structural genes:

- It regulates to produce specific mRNA
- determine the kind of protein to be synthesized. Operator genes:
- These genes act as switches to turn on or turn off the activities of structural genes
- regulating the elongation and termination of polypeptide chain. Regulator genes:
- These genes produce certain proteinacous substance called repressures, which prevent the operator genes from their action.

Essential feature:

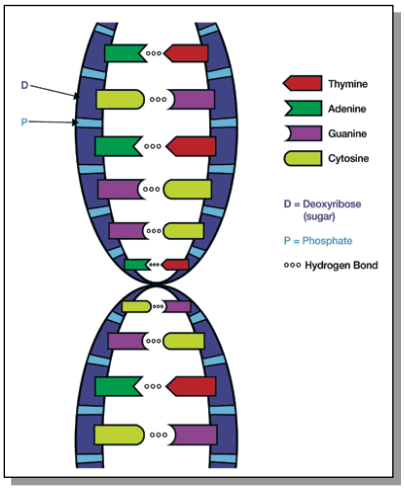
- Determines the physical as well as physiological characters.
- Situated in the chromosome.
- Occupies a specific position known as Locus.
- Arranged in single linear order.
- Occur in functional states called Alleles.
- Some have more than 2 alleles known as Multiple Alleles.
- May be transferred to its homologous (Cross- over) or non-homologous counterpart (Translocation).
- Can duplicate themselves very accurately (Replication).
- Synthesizes a particular Protein. o Determines the sequence of amino acid in the polypeptide chain (The Genetic Code).

DNA

DNA or deoxyribonucleic acid is a genetic material that transfer the genetic information from one organism to their off spring.

Located in nucleus and mitochondria .The information in DNA is stored as code (made up of A,G,C,T).

99% of base are same. The order of bases determines the individuality.



STRUCTURE

DNA is a long chain polymer of nucleotides

Consist of:

Deoxyribose=5 pentose sugar

Phosphate group

Organic bases Adenine, Guanine (purines) Cytosine, thymine (pyrimidines)

DNA is a double helix with 2 strands which gives ladder like shape with base pairs

BASE PAIRING PRINCIPLE

- Base pairing is an application of hydrogen bonding principle
- Adenine= Thymine pair interacts through 2 hydrogen bonds
- Guanine= Cytosine pair interacts through 3 hydrogen bonds
- The diameter of the double helix is 20 Angstroms

BONDING PATTERNS OF DNA

- The nitrogenous base are single or double ring structure that are attached to the 1st carbon atom
- The base is attached to the sugar by N-glycosidic bond
- Nucleoside is converted into nucleotide by attachment of a phosphate group.
- The linkage between the nucleotides in a polynucleotide is a phosphodiester bond
- The 5' carbon atoms has not participated in phosphodiester bond and called 5' end
- The molecule which are un reacted called as 3' hydroxyl group or 3' end
- Hydrogen bonds gives stability
- It occurs between neucleobases& is internal to DNA
- HB also influence on replication

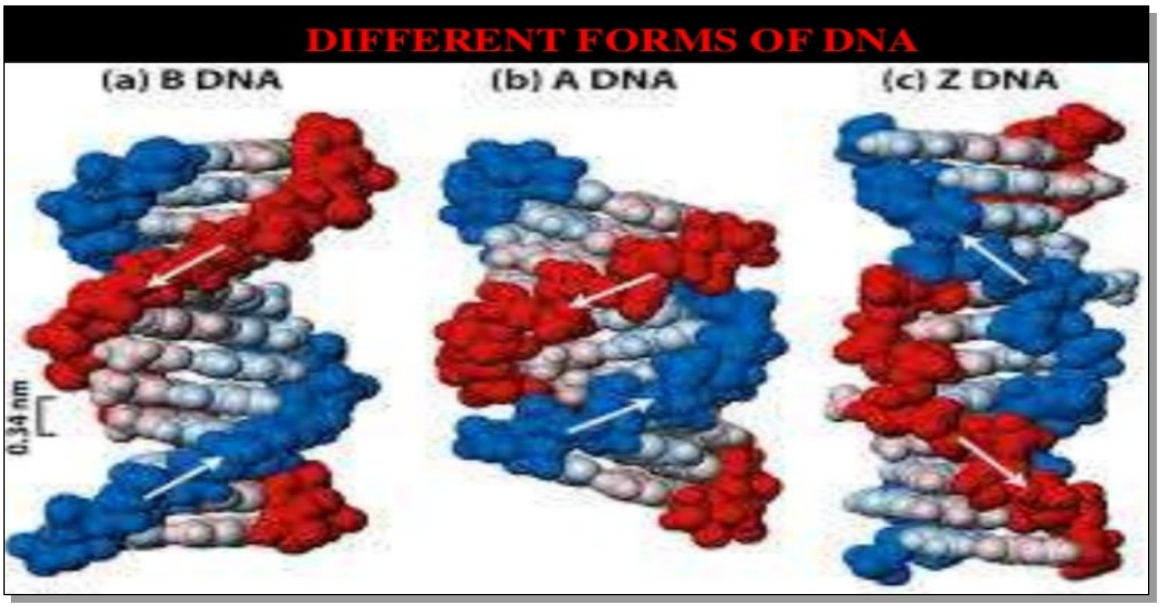
BASE STACKING:

- It is a non-covalent interaction
- Depend on vanderwaal's dispersive forces
- Electrostatic effects influence stability
- Purines stacks strongly than pyrimidines
- It influence replication

DIFFERENT CONFORMATION OF DNA

DIFFERENT CONFORMATION OF DNA			
FEATURE	**B DNA**	**A DNA**	**Z DNA**
TYPE OF HELIX	RIGHT-HANDED	RIGHT-HANDED	LEFT-HANDED
NO.OF. BP PER TURN	10	10	12
DISTANCE BETWEEN BP(nm)	0.34	0.29	0.37
DISTANCE COMPLETE PER TURN(nm)	3.4	3.2	4.5
DIAMETER(nm)	2.37	2.55	1.84
MAJOR GROOVE	WIDE,DEEP	NARROW,DEEP	FLAT
MINOR GROOVE	NARROW,SHALLOW	WIDE,SHALLOW	NARROW,DEEP

DIFFERENT FORMS OF DNA



FUNCTION OF DNA

Replication

Double helix unwinds and act as a template and forms double helix with the aid of DNA polymerase

Encoding Information

A codon specifies a particular amino acid that which produce a particular protein

Mutation/Recombination DNA plays a role in evolution of a species

- DNA can repair itself through recombination and mutation occurs due to illegal base pairing
- Both mutation and recombination either beneficial or create genetic diseases

Gene Expression

- Cells from different tissues & organ, look & behave differently
- DNA can respond to produce a particular protein by express a particular protein through transcription and translation Transcription-making RNA
- Translation-making protein

APPLICATION

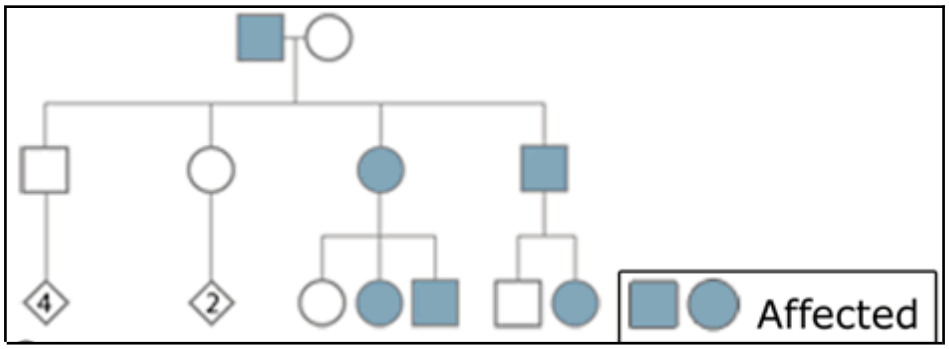
- DNA FINGER PRINTING
- DNA SEGUENCING
- DNA VACCINES
- RECOMBINANT TECHNOLOGY.

GENETIC PATTERN OF INHERITANCE

How traits and characteristics are passed on from one generation to another

The different patterns of inheritance

Genomic or parental imprinting



Observations of the way traits, or characteristics, are passed from one generation to the next in the form of identifiable phenotypes probably represent the oldest form of genetics. However, the scientific study of patterns of inheritance is conventionally said to have started with the work of the Austrian monk Gregor Mendel in the second half of the nineteenth century.

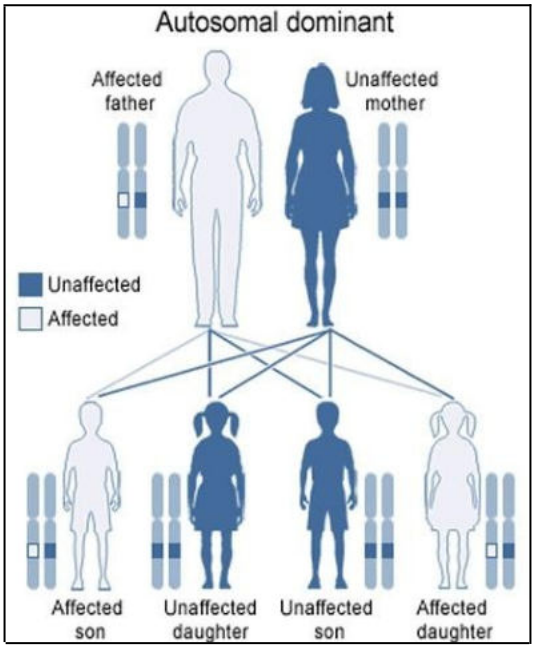
In diploid organisms each body cell (or 'somatic cell') contains two copies of the genome. So each somatic cell contains two copies of each chromosome, and two copies of each gene. The exceptions to this rule are the **sex chromosomes** that determine sex in a given species. For example, in the XY system that is found in most mammals - including human beings - males have one X chromosome and one Y chromosome (XY) and females have two X chromosomes (XX). The paired chromosomes that are not involved in sex determination are called **autosomes**, to distinguish them from the sex chromosomes. Human beings have 46 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes (X and Y).

The different forms of a gene that are found at a specific point (or locus) along a given chromosome are known as **alleles**. Diploid organisms have two alleles for each autosomal gene - one inherited from the mother, one inherited from the father.

Mendelian inheritance patterns

Within a population, there may be a number of alleles for a given gene. Individuals that have two copies of the same allele are referred to as **homozygous** for that allele; individuals that have copies of different alleles are known as **heterozygous** for that allele. The inheritance patterns observed will depend on whether the allele is found on an autosomal chromosome or a sex chromosome, and on whether the allele is **dominant** or **recessive**.

Autosomal dominant If the phenotype associated with a given version of a gene is observed when an individual has only one copy, the allele is said to be autosomal dominant. The phenotype will be observed whether the individual has one copy of the allele (is heterozygous) or has two copies of the allele (is homozygous).



Autosomal recessive

If the phenotype associated with a given version of a gene is observed only when an individual has two copies, the allele is said to be autosomal recessive. The phenotype will be observed only when the individual is homozygous for the allele concerned. An individual with only one copy of the allele will not show the phenotype, but will be able to pass the allele on to subsequent generations. As a result, an individual heterozygous for an autosomal recessive allele is known as a **carrier**.

Sex-linked or X-linked inheritance

In many organisms, the determination of sex involves a pair of chromosomes that differ in length and genetic content - for example, the XY system used in human beings and other mammals.

The X chromosome carries hundreds of genes, and many of these are not connected with the determination of sex. The smaller Y chromosome contains a number of genes responsible for the initiation and maintenance of maleness, but it lacks copies of most of the genes that are found on the X chromosome. As a result, the genes located on the X chromosome display a characteristic pattern of inheritance referred to as **sex-linkage** or **X-linkage**. Females (XX) have two copies of each gene on the X chromosome, so they can be heterozygous or homozygous for a given allele. However, males (XY) will express all alleles present on the single X chromosome that they receive from their mother, and concepts such as 'dominant' or 'recessive' are irrelevant.

A number of medical conditions in humans are associated with genes on the X chromosome, including haemophilia, muscular dystrophy and some forms of colour blindness.

Non-Mendelian Inheritance Patterns

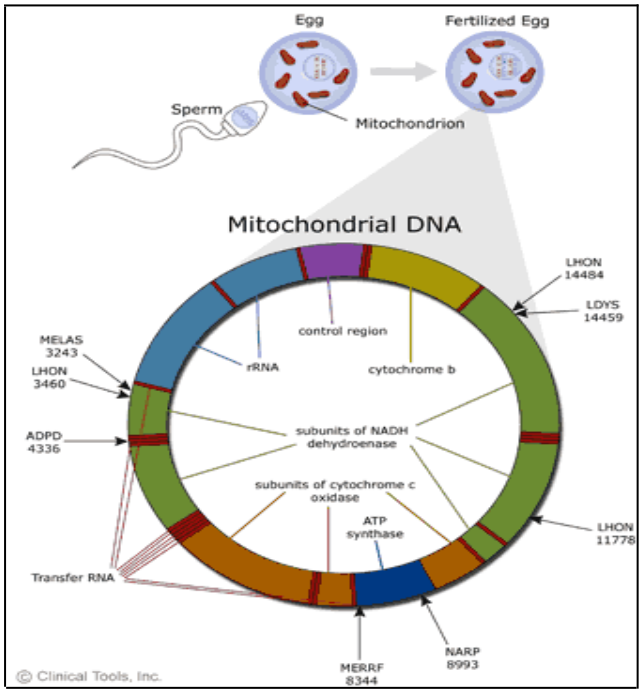
Complex and multifactorial inheritance

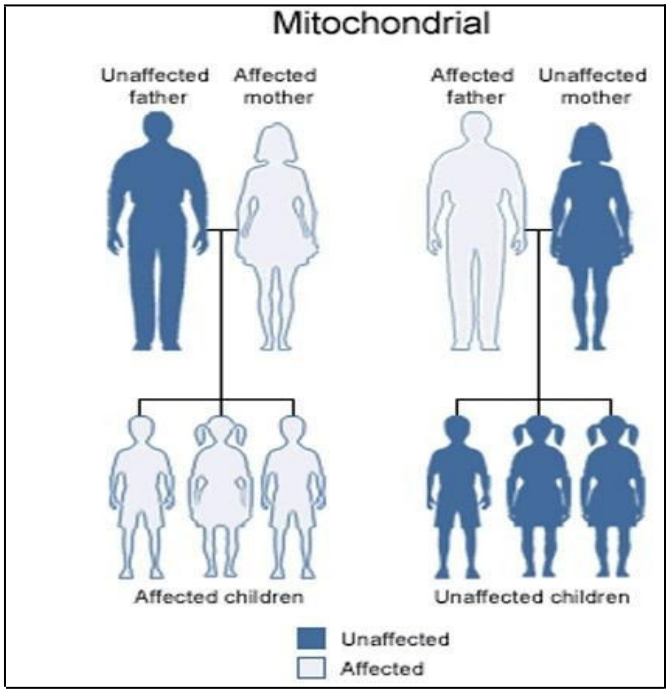
Some traits or characteristics display **continuous variation**, a range of phenotypes that cannot be easily divided into clear categories. In many of these cases, the final phenotype is the result of an interaction between genetic factors and environmental influences.

An example is human height and weight. A number of genetic factors within the individual may predispose them to fall within a certain height or weight range, but the observed height or weight will depend on interactions between genes, and between genes and environmental factors (for example, nutrition). Traits in which a range of phenotypes can be produced by gene interactions and gene-environment interactions are known as **complex** or **multifactorial.**

Mitochondrial inheritance

Animal and plant cells contain mitochondria that have their evolutionary origins in protobacteria that entered into a symbiotic relationship with the cells billions of years ago. The chloroplasts in plant cells are also the descendants of symbiotic protobacteria. As a result, mitochondria and chloroplasts contain their own DNA.





Mitochondria are scattered throughout the cytoplasm of animal and plant cells, and their DNA is replicated as part of the process of mitochondrial division. A newly formed embryo receives all its mitochondria from the mother through the egg cell, so mitochondrial inheritance is through the maternal line.

Genomic imprinting

The expression of a small number of human genes is influenced by whether the gene has been inherited from the mother or father. This process - called **genomic (or parental) imprinting**

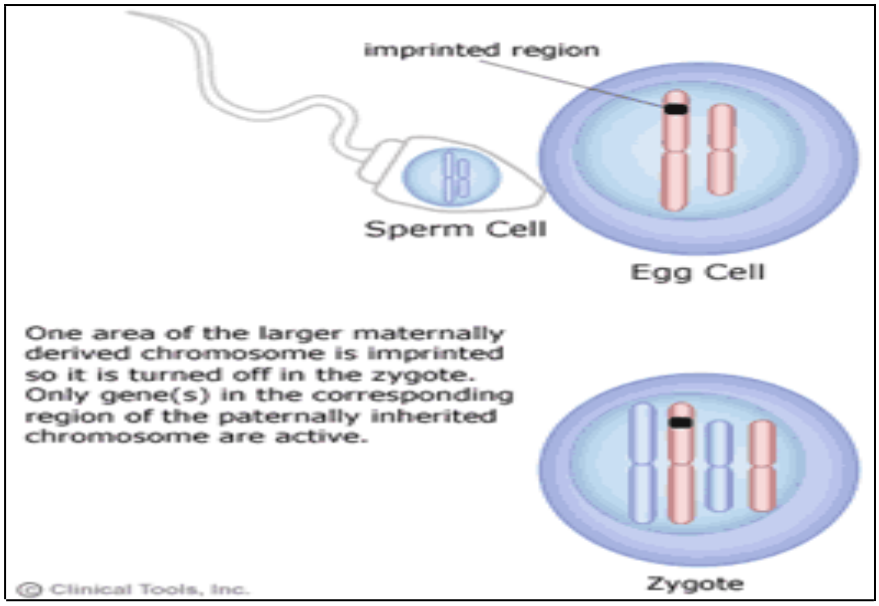
- usually means that the organism expresses one its alleles but not both. In many cases the non-expressed allele is inactivated - for example, by DNA methylation. (High levels of DNA methylation are known to inhibit gene activity.)

Imprinting involves three stages:

- the inactivation of an allele in the ovaries or testes before or during the formation of egg cells or sperm

- the maintenance of that inactivation in the somatic cells of the offspring organism the removal, then re-establishment, of the inactivation during the formation of egg cells or sperm in the offspring organism

- The pattern of imprinting is maintained in the somatic cells of the organism but can alter from generation to generation.



Protein synthesis

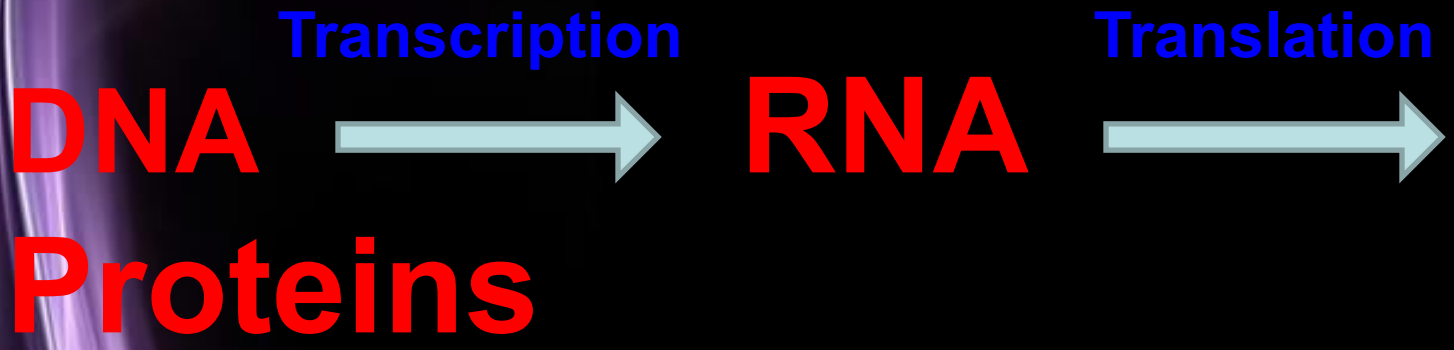
Lecture Note

- Transcription
- Genetic code
- tRNA
- Ribosomes
- Translation
- Polyribosomes

Transcription

- **Transcription**, or RNA synthesis, is the process of creating an equivalent RNA copy of a sequence of DNA.

- Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language that can be converted back and forth from DNA to RNA in the presence of the correct enzymes.



Central dogma

- During transcription, a DNA sequence is read by RNA polymerase, which produces a complementary, antiparallel RNA strand.

- As opposed to DNA replication, transcription results in an RNA compliment that includes uracil (U) in all instances where thymine (T) would have occurred in a DNA compliment.

- **Transcription is the first step leading to gene expression.**

- **The stretch of DNA transcribed into an RNA molecule is called a *transcription unit* and encodes at least one gene.**

- **If the gene transcribed encodes for a protein, the result of transcription is messenger RNA (mRNA), which will then be used to create that protein via the process of translation.**

- Alternatively, the transcribed gene may encode for either ribosomal RNA (rRNA) or transfer RNA (tRNA), other components of the protein-assembly process, or other ribozymes.

A DNA transcription unit encoding for a protein contains not only the sequence that will eventually be directly translated into the protein (the *coding sequence*) but also *regulatory sequences* that direct and regulate the synthesis of that protein.

- As in DNA replication, DNA is read from 3' → 5' during transcription. Meanwhile, the complementary RNA is created from the 5' → 3' direction.

- Only one of the two DNA strands, called the template strand, is used for transcription.

- The other DNA strand is called the coding strand, because its sequence is the same as the newly created RNA transcript (except for the substitution of uracil for thymine).

- **Transcription is divided into 3 stages:**

1. ***initiation***
2. ***elongation***
3. ***termination*.**

Initiation

- In bacteria, transcription begins with the binding of RNA polymerase to the promoter in DNA.
- RNA polymerase is a core enzyme consisting of five subunits: 2 α subunits, 1 β subunit, 1 β' subunit, and 1 ω subunit.
- At the start of initiation, the core enzyme is associated with a sigma factor that aids in finding the appropriate -35 and -10 base pairs downstream of promoter sequences.

Elongation

- One strand of DNA, the ***template strand*** (or noncoding strand), is used as a template for RNA synthesis.
- As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy.
- Although RNA polymerase traverses the template strand from 3' → 5', the coding (non-template) strand and newly-formed RNA can also be used as reference points, so transcription can be described as occurring 5' → 3'.

- This produces an RNA molecule from 5' → 3', an exact copy of the coding strand (except that thymines are replaced with uracils, and the nucleotides are composed of a ribose (5-carbon) sugar where DNA has deoxyribose (one less oxygen atom) in its sugar-phosphate backbone).

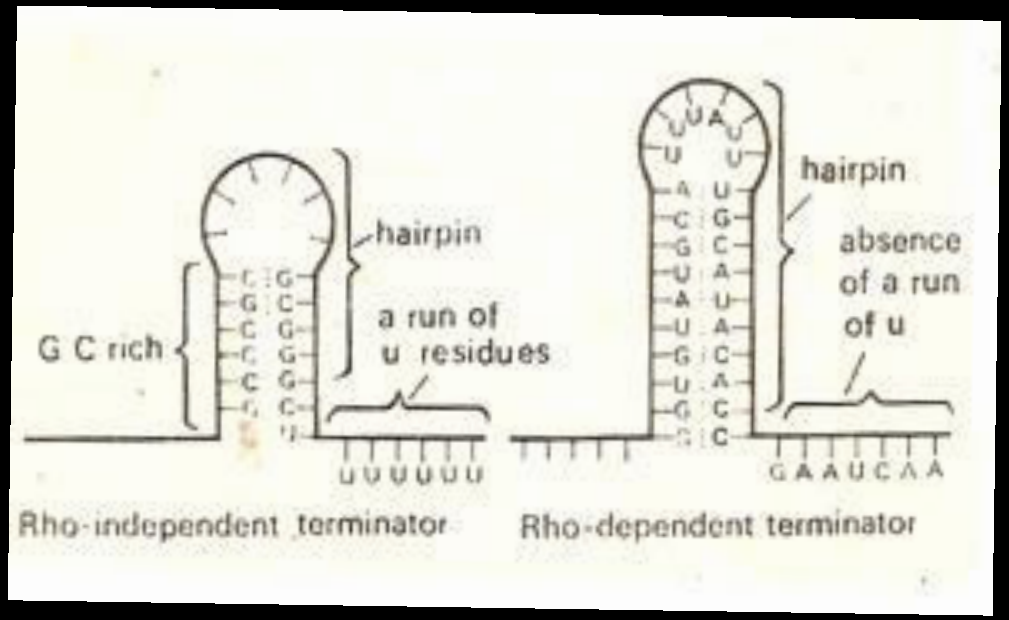
- Unlike DNA replication, mRNA transcription can involve multiple RNA polymerases on a single DNA template and multiple rounds of transcription (amplification of particular mRNA), so many mRNA molecules can be rapidly produced from a single copy of a gene.

- Elongation also involves a **proofreading mechanism** that can replace incorrectly incorporated bases.

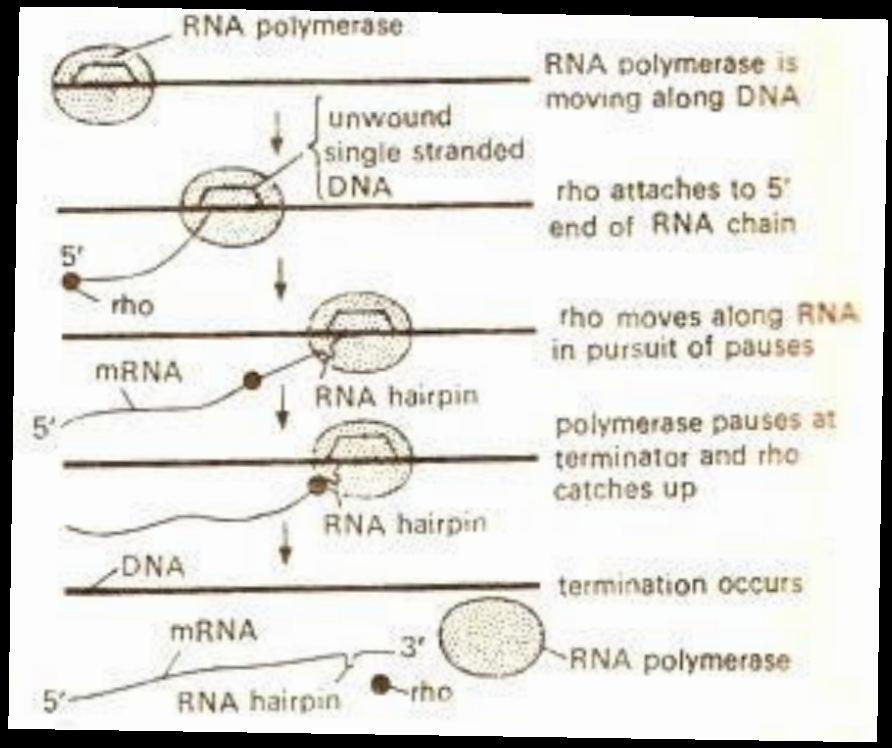
Termination

- Bacteria use two different strategies for transcription termination: Rho-independent and Rho-dependent

In **Rho-independent transcription** termination, RNA transcription stops when the newly synthesized RNA molecule forms a G-C rich hairpin loop followed by a run of U's, which makes it detach from the DNA template.



In the "Rho-dependent" type of termination, a protein factor called "Rho" destabilizes the interaction between the template and the mRNA, thus releasing the newly synthesized mRNA from the elongation complex.



Genetic code

Properties of genetic code

- **The code is universal.** All prokaryotic and eukaryotic organisms use the same codon to specify each amino acid.

- **The code is triplet.** Three nucleotides make one codon. 61 of them code for amino acids and 3 viz., UAA, UAG and UGA are nonsense codons or chain termination codons.

- **The code is degenerate.** For a particular amino acid more than one word can be used

- **The code is non overlapping.** A base in mRNA is not used for two different codons

- **The code is commaless.** There is no special signal or commas between codons.

- **The code is non ambiguous.** A particular codon will always code for the same amino acid, wherever it is found.

The Genetic Code

	U	C	A	G	
U	UUU Phenyl alanine UUC UUG Leucine UUA	UCU UCC Serine UCA UCG	UAU Tyrosine UAC UAA Stop UAG	UGU Cysteine UGC UGA Stop UGG Tryptophan	U C A G
C	CUU CUC Leucine CUA CUG	CCU CCC Proline CCA CCG	CAU Histidine CAC CAA Glutamine CAG	CGU CGC Arginine CGA CGG	U C A G
A	AUU AUC Isoleucine AUA AUG Methionine	ACU ACC Threonine ACA ACG	AAU Asparagine AAC AAA Lysine AAG	AGU Serine AGC AGA Arginine AGG	U C A G
G	GUU GUC Valine GUA GUG	GCU GCC Alanine GCA GCG	GAU Aspartic acid GAC GAA Glutamic acid GAG	GGU GGC Glycine GGA GGG	U C A G

tRNA

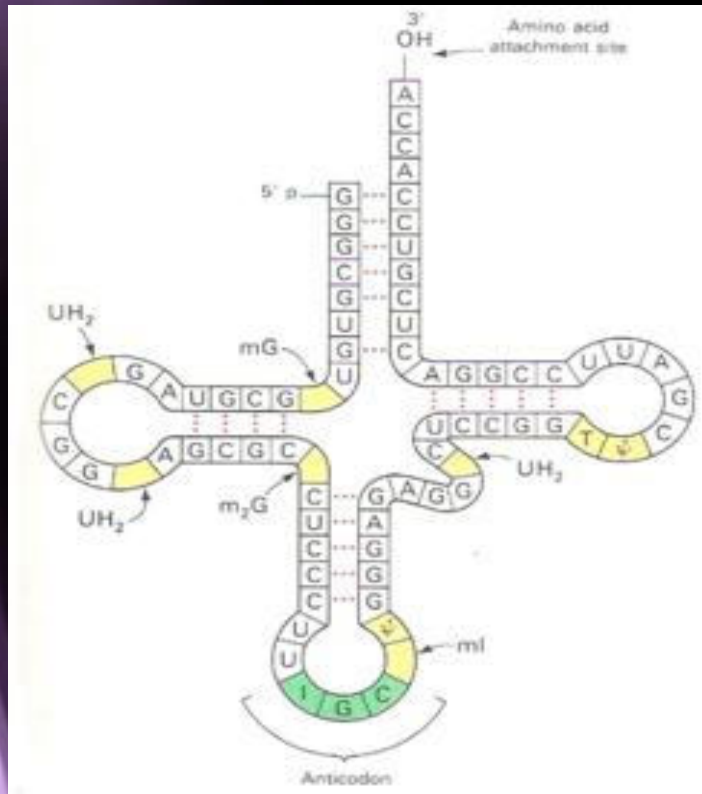


Fig. Base sequence of yeast alanyl tRNA

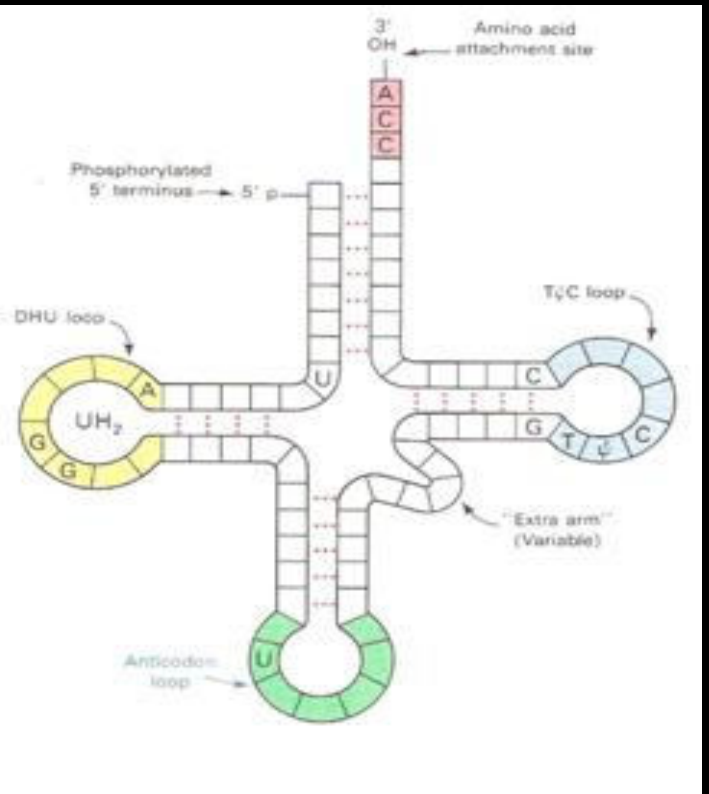


Fig. Common features of tRNA molecule

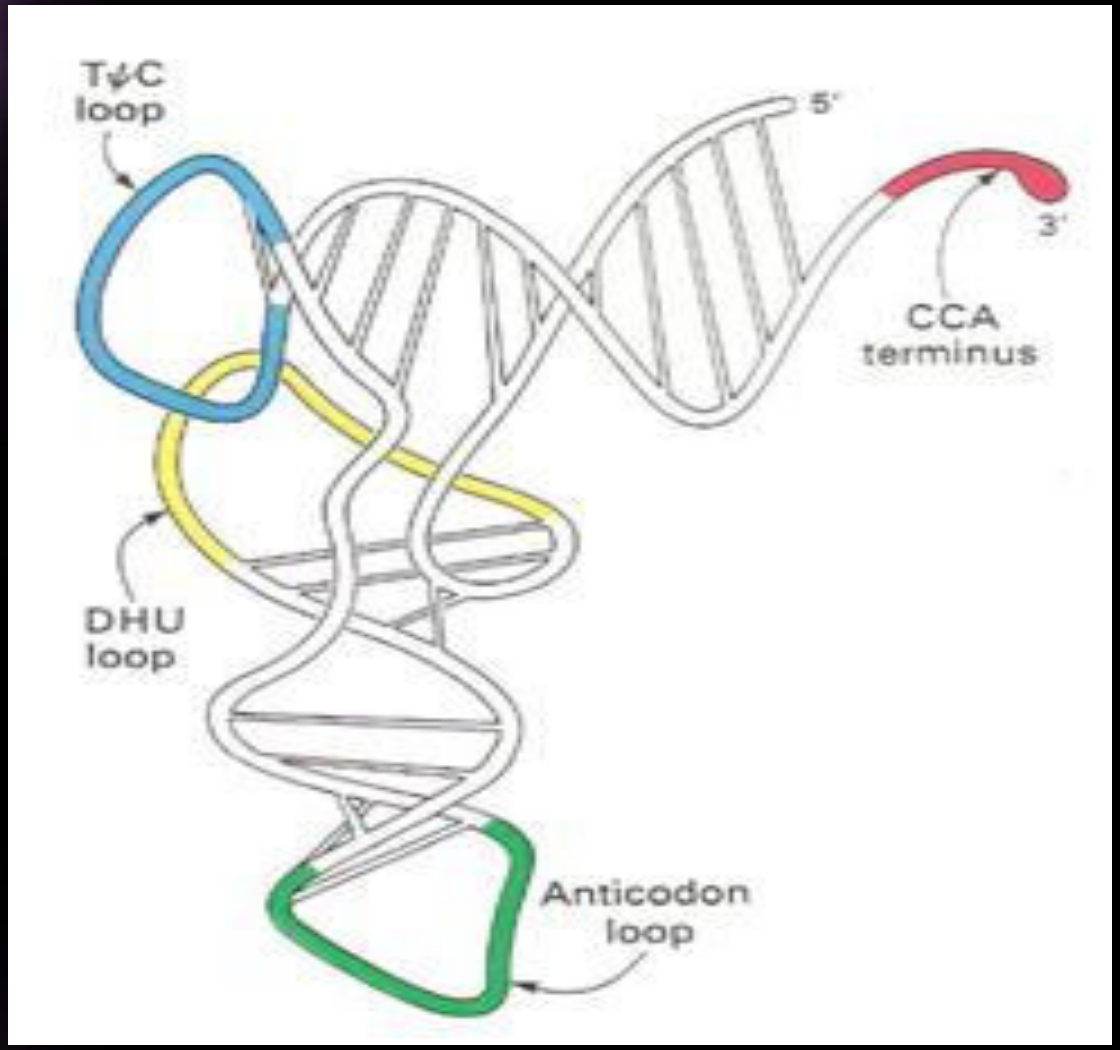


Fig. 3D structure of tRNA

Ribosomes

- Bacterial ribosomes consists of two subunits of unequal size, the larger having a sedimentation coefficient of 50S and the smaller of 30S.

- The two ribosomal subunits have irregular shapes which fit together in such a way that a cleft is formed through which mRNA passes as the ribosome moves along it during the translation process and from which the newly formed polypeptide chain emerges.

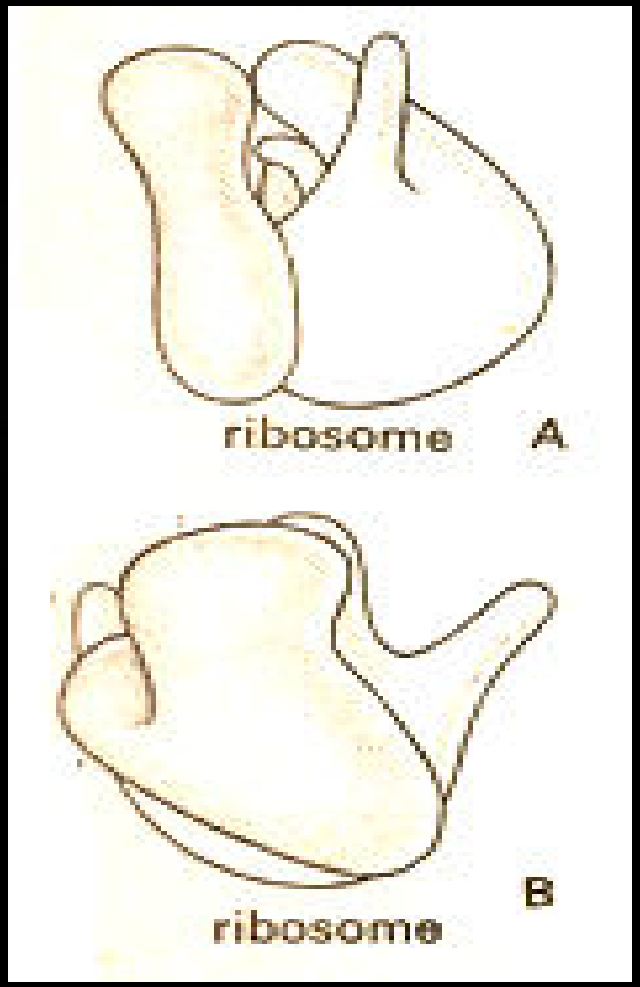


Fig. Ribosomes

Translation

- **Translation** is the first stage of protein biosynthesis (part of the overall process of gene expression).

- Translation is the production of proteins by decoding mRNA produced in transcription.

- It occurs in the cytoplasm where the ribosomes are located.

- Ribosomes are made of a small and large subunit which surrounds the mRNA.

- In translation, messenger RNA (mRNA) is decoded to produce a specific polypeptide according to the rules specified by the genetic code.

- This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acid that form a protein.

- Many types of transcribed RNA, such as transfer RNA, ribosomal RNA, and small nuclear RNA are not necessarily translated into an amino acid sequence.

- **Translation** proceeds in four phases:
  - activation,
  - initiation,
  - elongation and
  - termination

  (all describing the growth of the amino acid chain, or polypeptide that is the product of translation).

  Amino acids are brought to ribosomes and assembled into proteins.

Activation

- In activation, the correct amino acid is covalently bonded to the correct transfer RNA (tRNA).
- While this is not technically a step in translation, it is required for translation to proceed.
- The amino acid is joined by its carboxyl group to the 3' OH of the tRNA by an ester bond.
- When the tRNA has an amino acid linked to it, it is termed "charged".

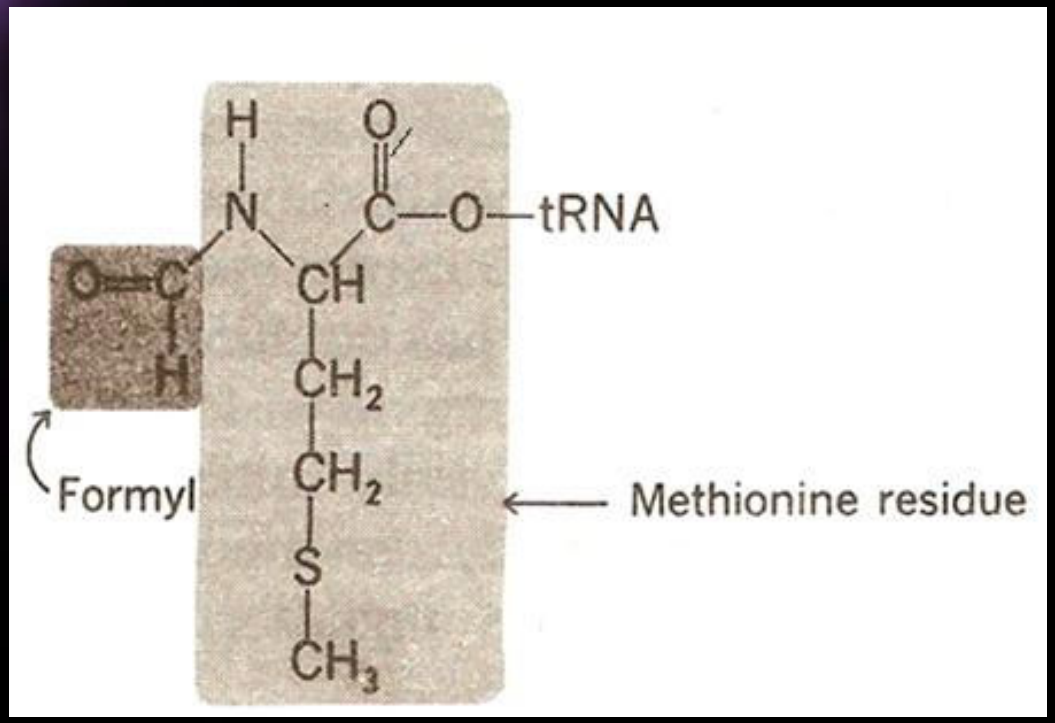


Fig. Charged tRNA (N-formyl methionyl tRNA $_f^{met}$)

In Prokaryotes initiation requires the large and small ribosome subunits, the mRNA, the initiator tRNA and three initiation factors (IF1, IF2, IF3) and GTP. The overall sequence of the event is as follows

- IF3 bind to the free 30S subunit, this helps to prevent the large subunit binding to it without an mRNA molecule and forming an inactive ribosome

- IF2 complexed with GTP and IF1 then binds to the small subunit. It will assist the charged initiator tRNA to bind.

- The 30S subunit attached to an mRNA molecule making use of the ribosome binding site (RBS) on the mRNA

- The initiator tRNA can then bind to the complex by base pairing of its anticodon with the AUG codon on mRNA.

- At this point, IF3 can be released, as its role in keeping the subunits apart and helping the mRNA to bind are complete.

- This complex is called 30S initiation complex

• The 50S subunit can now bind, which displace IF1 and IF2, and the GTP is hydrolysed in this energy consuming step.

•This complex is called as 70S initiation complex.

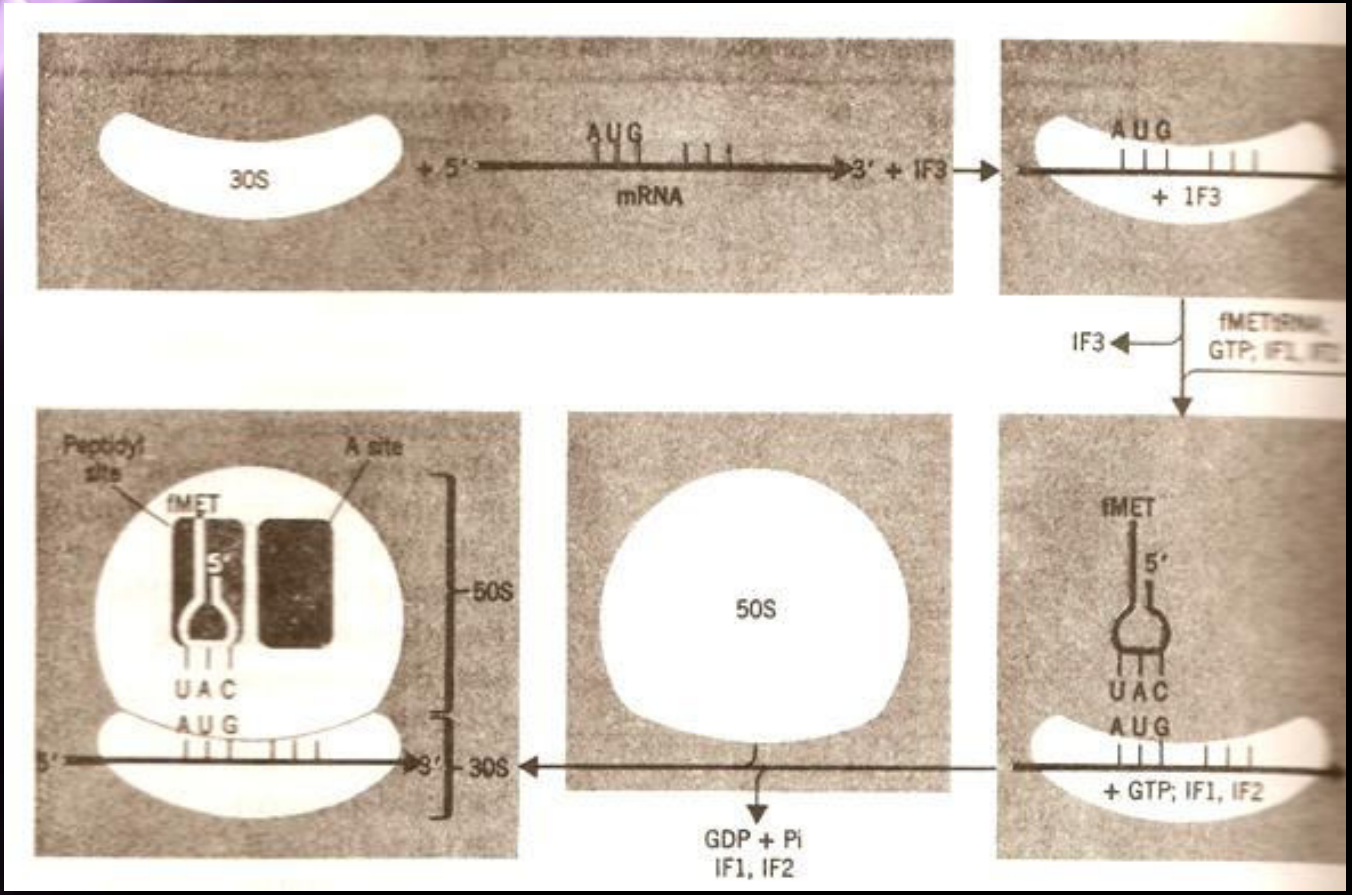


Fig. Formation of the 70S initiation complex

- The assembled ribosome has two tRNA binding sites.
- These are called the A and P sites, for amino acyl and peptidyl sites.
- The A site is where incoming amino acyl tRNA molecules bind, and the P site is where the growing polypeptide chain is usually found.
- These sites are in the cleft of small subunit and contain adjacent codons that are being translated.

- One major outcome of initiation is the placement of the initiator tRNA in the P site.
- It is the only tRNA that does this, as all other must enter the A site.

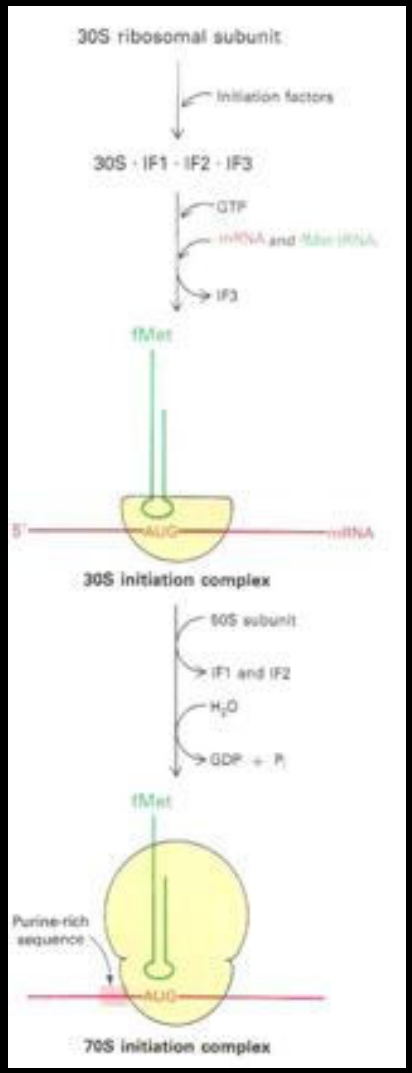


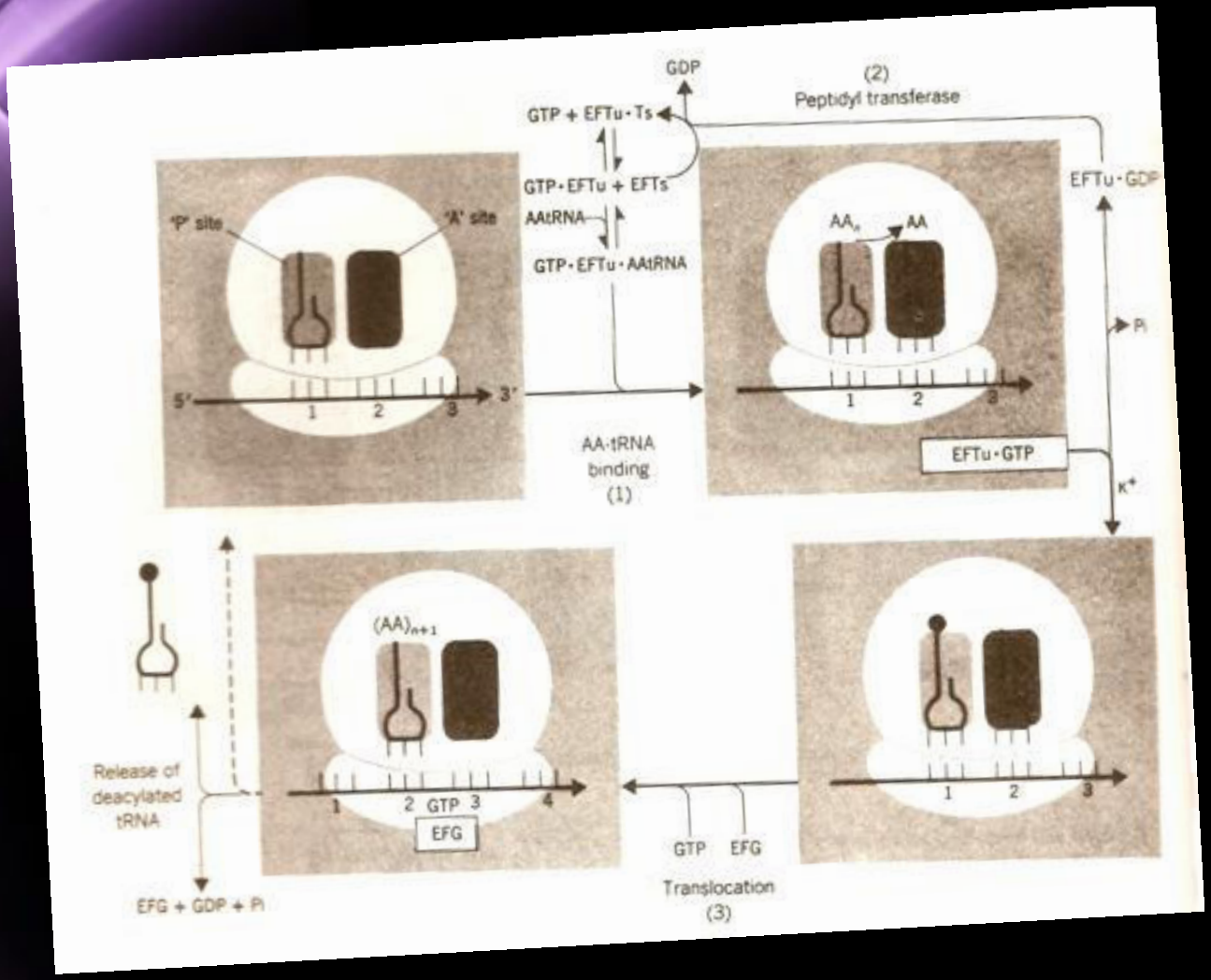
Fig. Initiation phase of protein synthesis

- With the formation of 70S initiation complex the elongation cycle can begin.
- In involves three elongation factors EF-Tu, EF-Ts and EF-G, GTP, charged tRNA and the 70S initiation complex.

Elongation is divided into three steps:

1. Amino acyl tRNA delivery.

- EF-Tu is required to deliver the amino acyl tRNA to the A site and energy is consumed in this step by the hydrolysis of GTP.
- The released EF-Tu. GDP complex is regenerated with the help of EF-Ts.
- In the EF-Tu EF-Ts exchange cycle EF-Ts displaces the GDP and subsequently is displaced itself by GTP.
- The resultant EF-Tu.GTP complex is now able to bind another amino acyl tRNA and deliver it to the ribosome.
- All amino acyl tRNAs can form this complex with EF-Tu except the initiator tRNA.

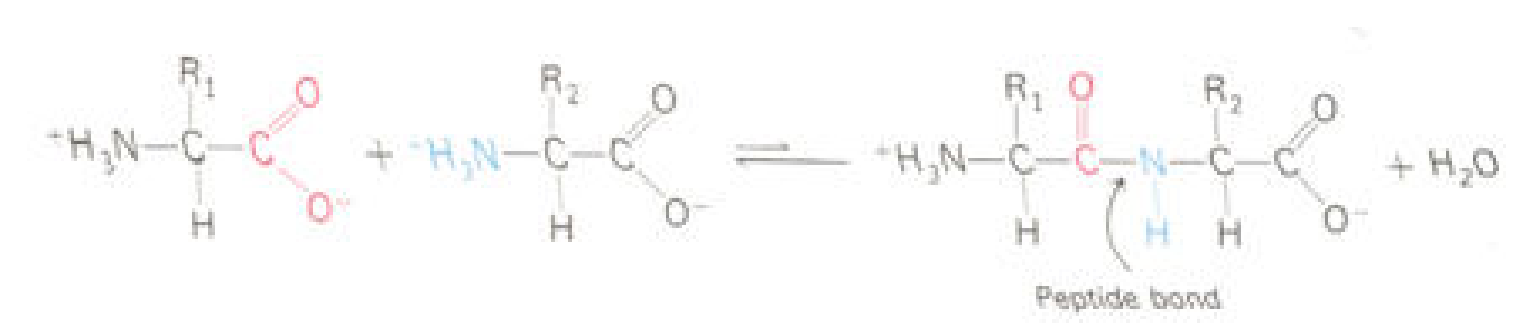


- **Fig. Elongation of polypeptide chain**

2. Peptide bond formation.

- After aminoacyl-tRNA delivery, the A- and P- sites are both occupied and the two amino acids that are to be joined are in close proximity.

- The peptidyl transferase activity of the 50S subunit can now form a peptide bond between these two amino acids without the input of any more energy, since energy in the form of ATP was used to charge the tRNA.



- **Fig. Peptide bond formation**

3. Translocation.

- A complex of EF-G (translocase) and GTP binds to the ribosome and, in an energy consuming step, the discharged tRNA is ejected from the P-site, the peptidyl-tRNA is moved from the A-site to the P-site and the mRNA moves by one codon relative to one codon to the ribosome.

- GDP and EF-G are released, the latter being reusable. A new codon is now present in the vacant A-site.

The cycle is repeated until one of the termination codons (UAA, UAG and UGA) appear in the A-site.

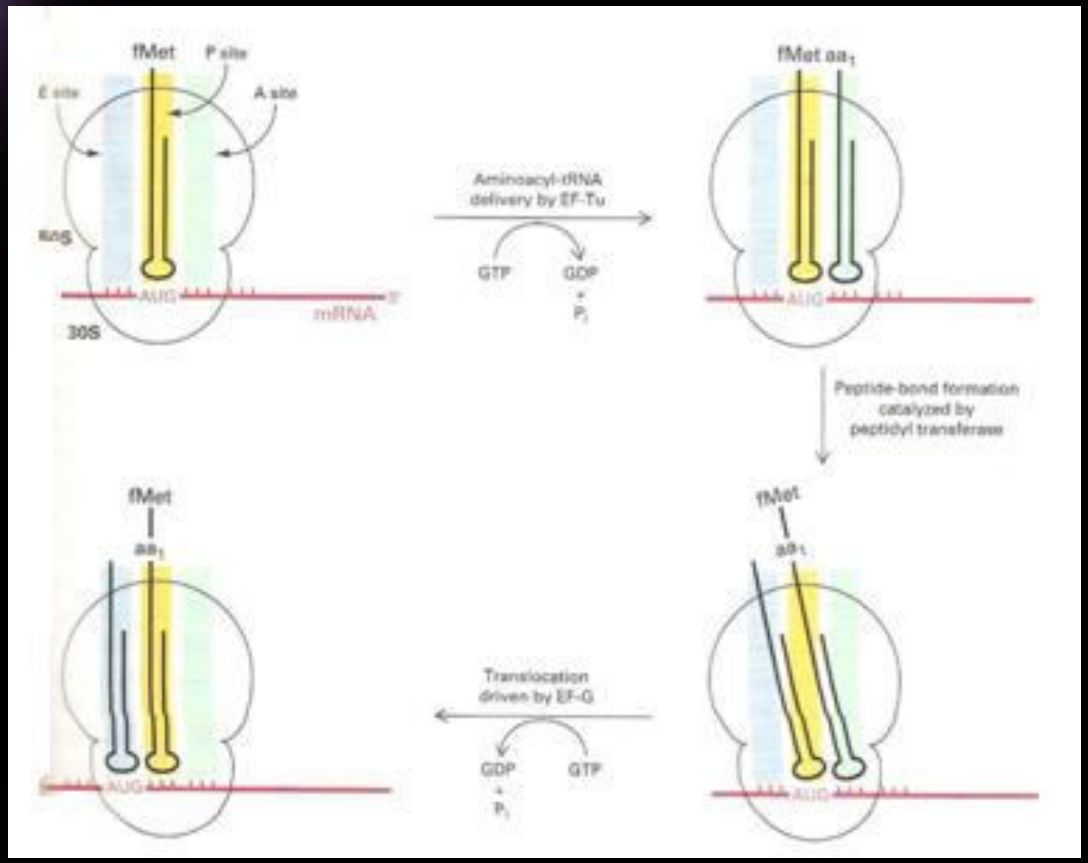


Fig. Translocation

- **Termination** of the polypeptide happens when the A site of the ribosome faces a stop codon (UAA, UAG, or UGA).

- When this happens, no tRNA can recognize it, but a **releasing factor** can recognize nonsense codons and causes the release of the polypeptide chain.

- The 5' end of the mRNA gives rise to the protein's N-terminus, and the direction of translation can therefore be stated as N->C.

- Termination of polypeptide synthesis is signalled by one of the three termination codons in the mRNA (UAA, UAG and UGA) immediately following the last amino acid codon.
- In prokaryotes, once a termination codon occupies the ribosomal A-site, three termination or release factors, viz., the protein RF1, RF2 and RF3 contribute to-

➢ The hydrolysis of the terminal peptidyl-tRNA bond.

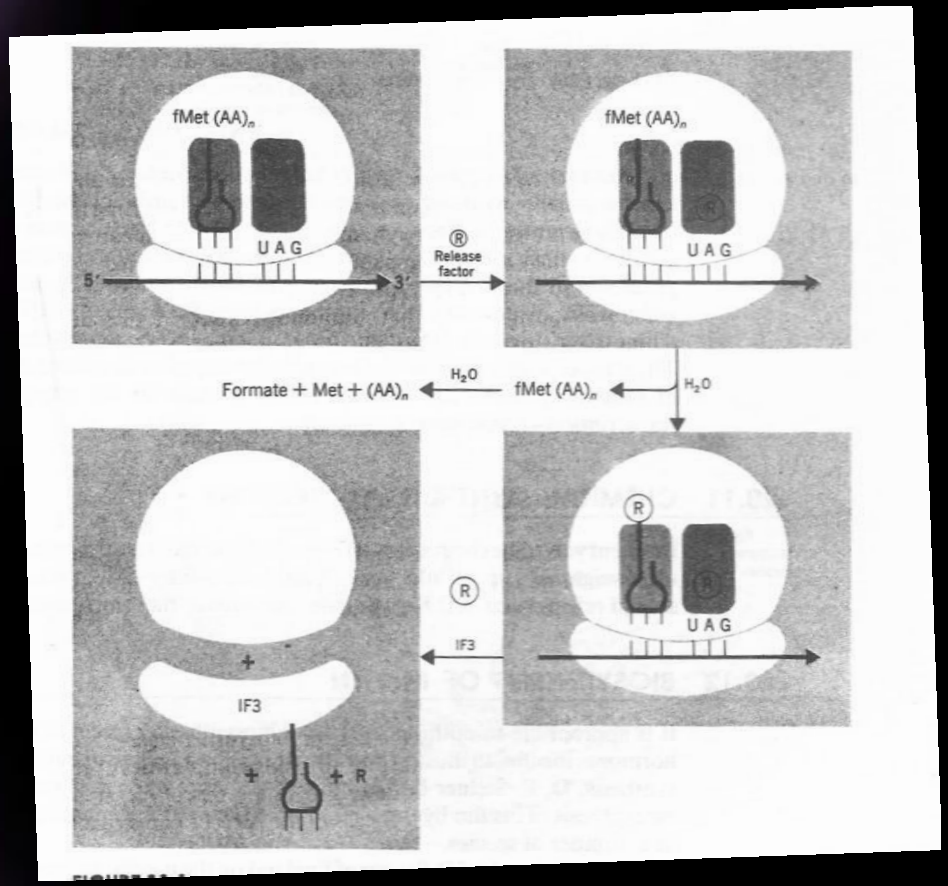
➢ Release of the free polypeptide and the last uncharged tRNA from the P-site

➢ The dissociation of the 70S ribosome into its 30S and 50S subunits

- RF1 recognizes the termination codon UAG and UAA and RF2 recognize UGA and UAA.

- Either RF1 or RF2 binds at the termination codon and induces peptidyl transferase to transfer the growing peptide chain to a water molecule rather than to another amino acid.

- Function of RF3 is not known.


fMet (AA)n
U A G
5′
3′
® Release factor
fMet (AA)n
U A G
H2O
Formate + Met + (AA)n
fMet (AA)n
H2O
R
U A G
® IF3
IF3
+
-
+
+ R

Polyribosomes

- A single strand of mRNA is translated simultaneously by many ribosomes, spaced closely together.
- Such clusters of ribosomes are called polysomes or polyribosomes.
- The simultaneous translation of a single mRNA by many ribosomes allow highly efficient use of the mRNA

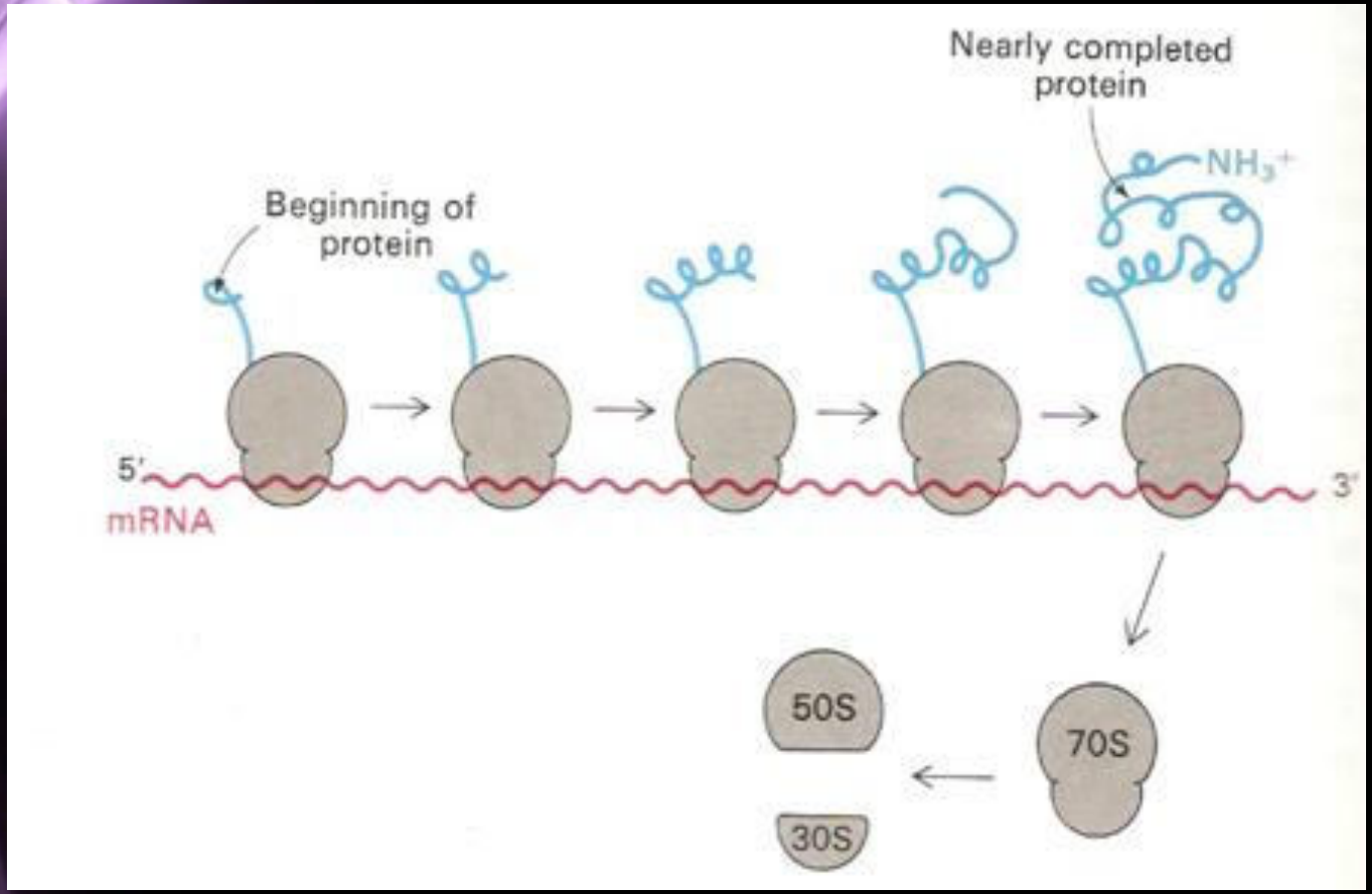


Fig. Polyribosomes

THANK YOU