AS-2185

M.Sc (III Semester) Examination, 2013

BOTANY

Paper : LBC 901/LBT-301

(Cell Biology and Biochemistry)

Answer 1.

(a) Fill in the blanks:

(i) Protein Modification

(ii) Cytosol

(iii) Prokaryotes

(b) Tick the most appropriate answer:

(iv) (b)

(v) (b)

(vi) (d)

(vii) (c)

(c) Define the following:

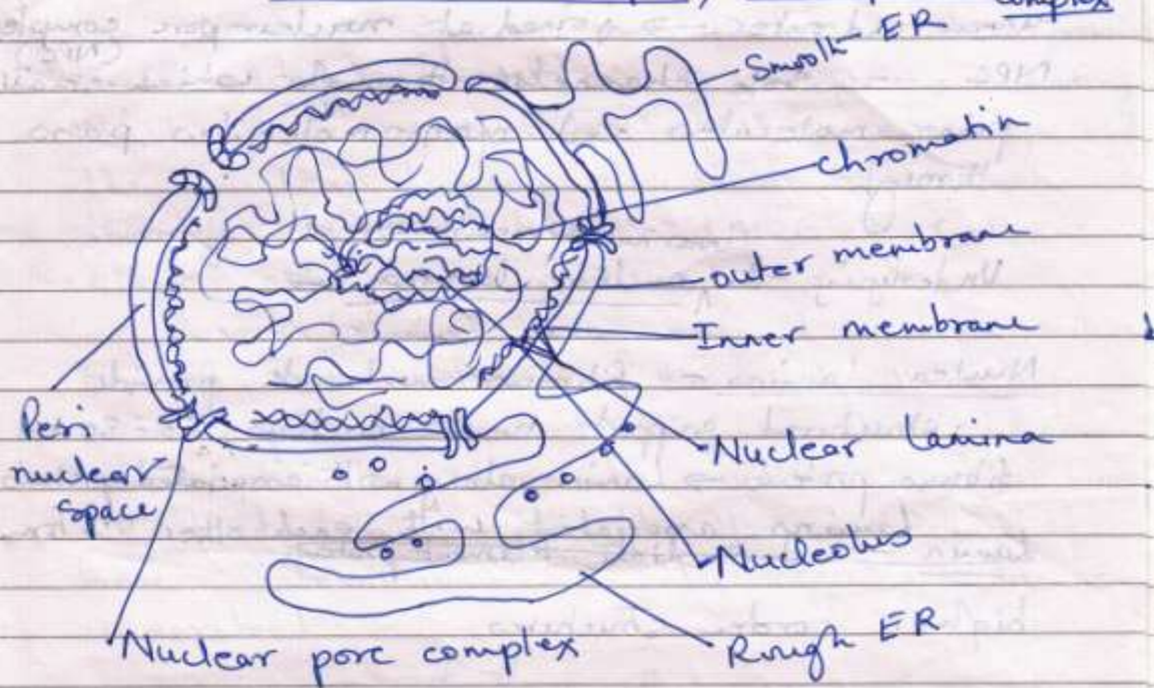
(viii) S phase – DNA replication takes place. DNA content 2n to 4n. In case of eukaryotes, each segment of genome is replicated once during this phase.

(ix) Cell signalling – in which information is relayed across the plasmamembrane to the cell interior and often transmitted to cell nucleus. It includes recognition of stimulus by specific receptor of the plasmamembrane, transfer of signal across the plasma-membrane to its cytoplasmic surface, transmission of signal to effector molecules that trigger cell's response and ultimately there is cessation of the response.

x) Saturated fatty acid – Such fatty acids have no double bonds between the individual carbon atoms of the fatty acid chain. The chain of carbon atoms is fully saturated with hydrogen atoms.

Answer 2. (i)

Nuclear Envelope, struc of Nuclear Pore Complex



Nuclear Envelope complex struc → 2 nuclear membranes (outer; an inner), underlying nuclear lamina and nuclearpore complexes.

outer membrane is continuous with ER, so the space between inner and outer nuclear membranes is directly connected with lumen of ER.

Func of OM similar to membrane of ER. Ribosomes bound to its cytoplasmic surface. but slight differences in protein comp. OM is rich in membrane proteins that bind the cytoskeleton.

IM – nucleus specific proteins e.g that bind the nuclear lamina.

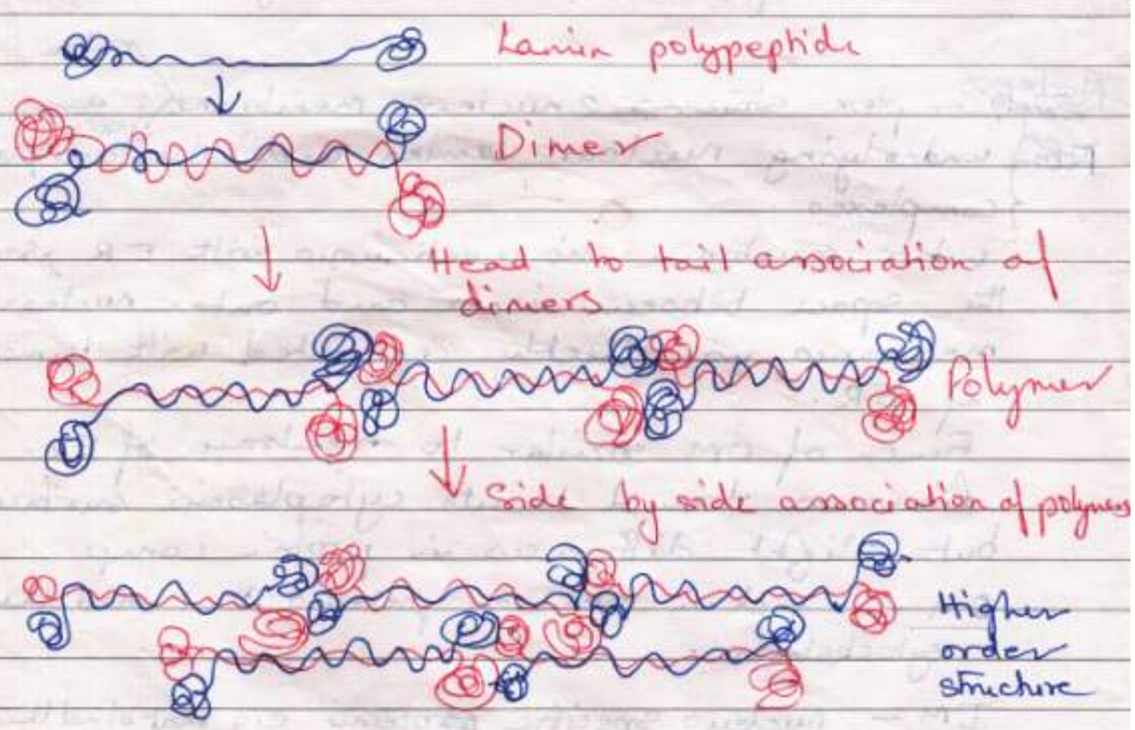
nuclear membrane act as barrier for nucleus and cytoplasm. nuclear membrane – phospholipid bilayer

nuclear membrane permeable only to small nonpolar molecules.

inner and outer. → joined at nuclear pore complex. (NPC)
NPC — sole channels through which small polar molecules and macromolecules pass through

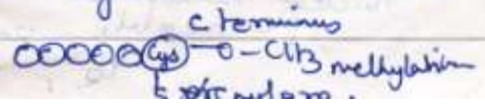
Underlying the inner nuclear membrane is nuclear lamina ~~is~~

Nuclear lamina - fibrous meshwork provide structural support to the nucleus, 60-80 kd fibrous proteins → Lamin along with associated proteins.
~~Lamin — intermediate filament protein~~ Lamins associated with each other to form higher order structures



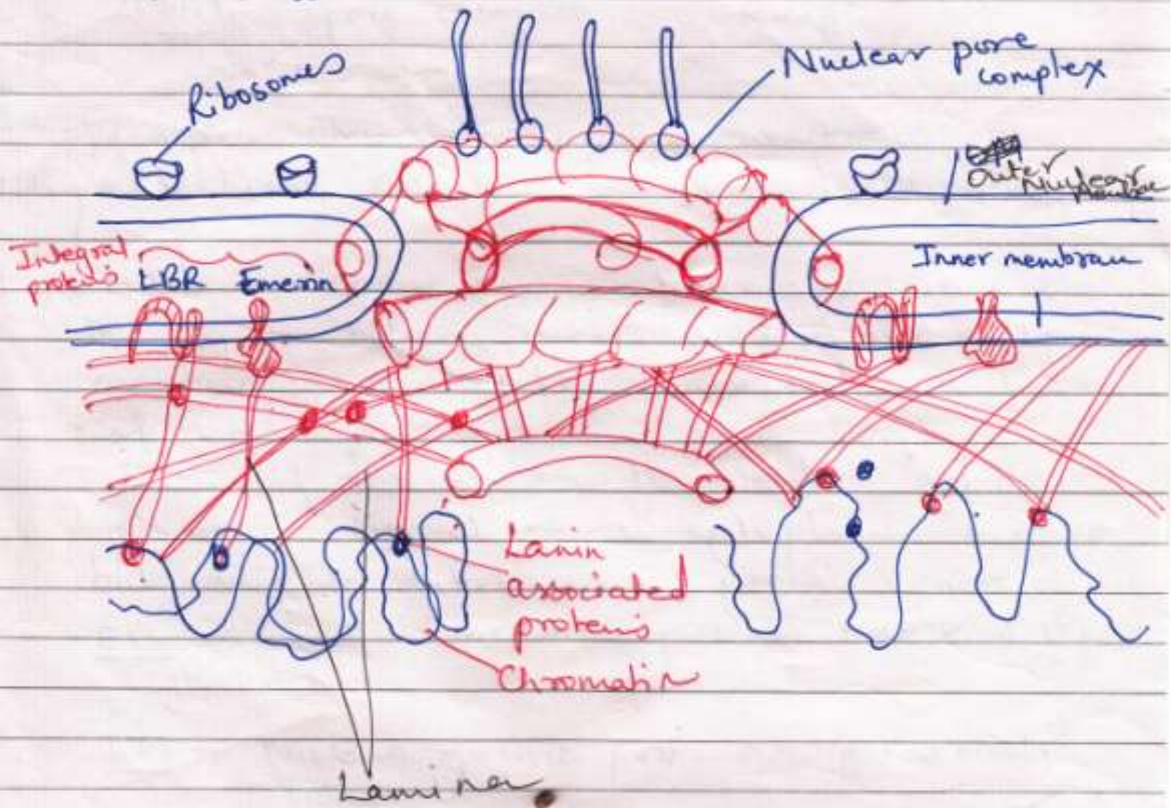
The association of lamins with the inner nuclear membrane is facilitated by post-translational addition of lipid — in particular prenylation of C-terminal cysteine residues.

* prenyl grp (farnesyl -15C
geranylgeranyl -20C)



Lamin binds to specific inner nuclear membrane proteins such as emerin and lamin B receptor, mediating their attachment to the nuclear envelope and The nuclear lamina also binds to chromatin through histones H2A and H2B as well as other chromatin proteins.

Lamins also extend in loose meshwork throughout the interior of the nucleus. Many nuclear proteins that function in DNA synthesis, transcription or chromatin modifications are known to bind lamins, although the significance of these interactions is only begining to be understood.



Answer 2 (ii)

Structure and Function of Nucleolus

25% nucleus volume

most prominent nuclear body of the nucleolus

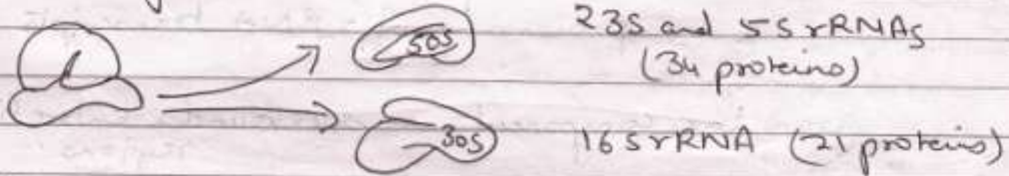
* site of rRNA transcription and processing, ribosome assembly ribosome production factory,

Nucleolus — is not membrane bounded, formed a highly specialized region of nucleus. It is associated with chromosomal regions that contain genes for 5S, 5.8S, 18S and 28S rRNAs

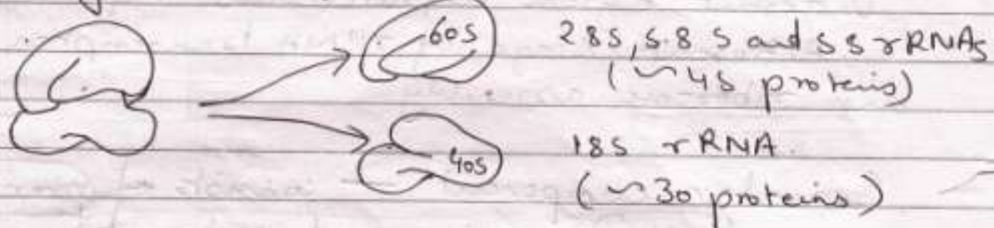
45S pre-rRNA is processed → 18S rRNA of 40S

based on sedimentation rates in ultracentrifugation.

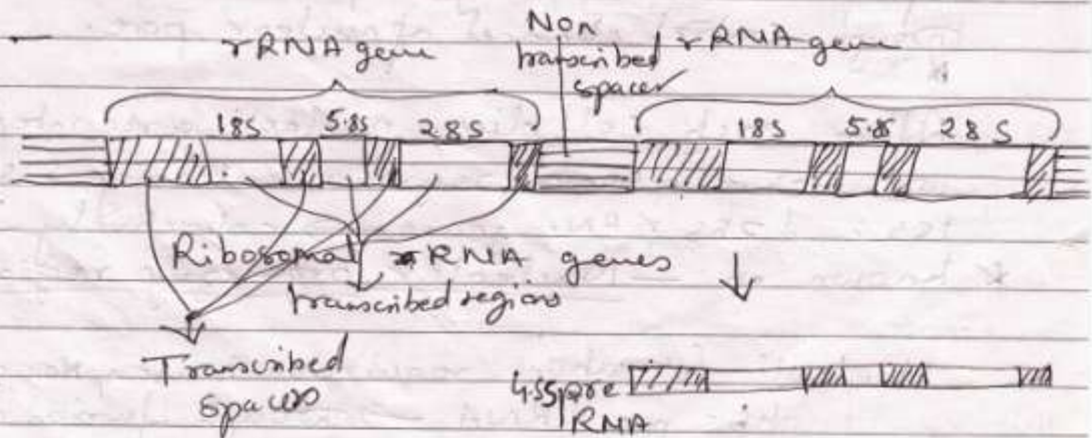
Prokaryotic 70S ribosome



Eukaryotic 80S ribosomes



5.8, 18 and 28S rRNAs → transcribed as single unit within nucleolus by — RNA Poly I ↑ yielding a 45S ribosomal precursor RNA



Transcription of 5S rRNA (60S ribosomal subunit) takes place outside the nucleolus, in (nucleoplasm) higher eukaryotes and catalyzed by RNA poly III

All cells contain multiple copies of rRNA genes. and multiple copies of rRNA nucleoli

Morphologically, nucleoli consist of 3 distinguishable regions

fibrillar center, — 1 or more, ribosomal transcription is believed to take place.

dense fibrillar component — surrounds centers processing of rRNA transcripts

granular component — surrounds entire fibrillar regions.

Different regions in nucleolus represent different stage of rRNA transcription, processing and ribosome assembly.

Granular component — consists of (preribosomal bodies) + (ribosomal subunits).

~~Ribosomal subunits~~ These components are smaller than mature ribosomes of the cytoplasm. After maturation, they export from the nucleus through central channel of nuclear pore.

*

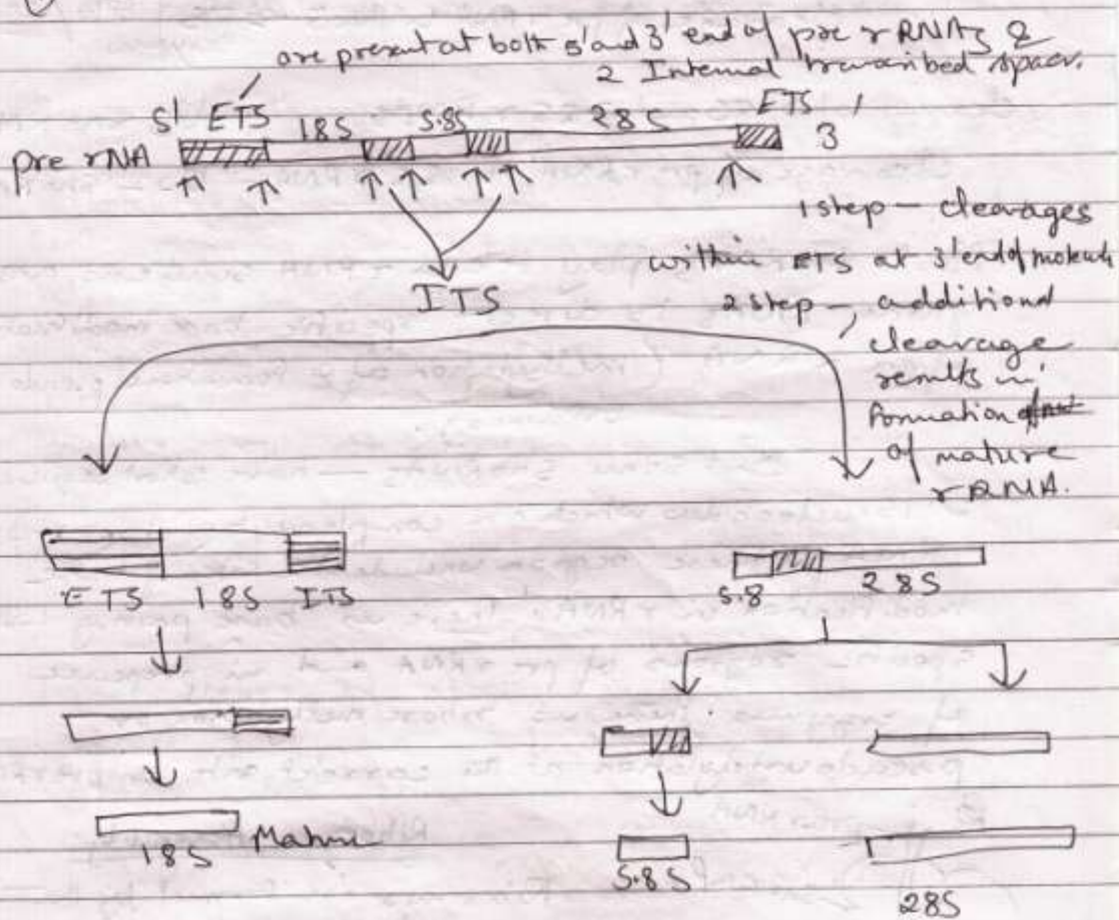
After each cell div, nucleoli associated with chromosomal regions containing 5.8S, 18S and 28S rRNA genes → collectively

* known as — Nucleolar organizing regions.

Nucleoli formation requires transcription of 45S pre rRNA → through fusion of small prenucleolar bodies ~~which having~~ processing factors and other components of nucleolus.

Initially, there are no. of nucleoli which ultimately fuse to form — single nucleolus.
size depends on metabolic activity.
large nucleoli — actively engaged in protein synthesis

(i) Transcription and Processing of rRNA →



(ii) substantial amount of base modification in pre RNA
addition of methyl to specific bases & ribose residues
conversion of uridine to pseudouridine.

processing of pre-rRNA requires proteins + RNAs (localized to nucleolus)
Nucleoli have < 3 no proteins and large no of (200) small nucleolar RNAs (snoRNAs) → play role in prerRNA processing.

It (snoRNAs) complexed with proteins → snoRNPs

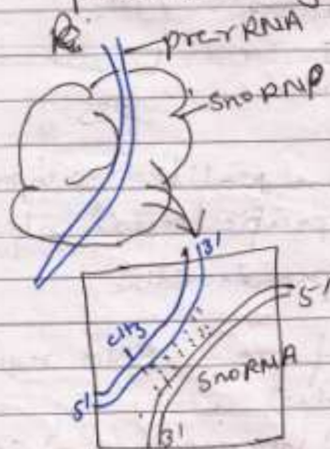
Individual snoRNPs → single snoRNAs + 8-10 proteins
↓
attach to pre-rRNA → for processing.

Most abundant nucleolar snoRNA → U3 200,000 copy/cell
cleavage of pre-rRNA within 5'-ETS sequences

cleavage of 5.8S and 28S rRNAs — U8 snoRNA
cleavage of pre rRNA to 18S rRNA — U22 snoRNA

Most snoRNAs play role in rRNA synthesis as guide RNAs to direct specific base modifications of pre-rRNA (methylation of 2' formation of pseudo-uridines).

Some snoRNAs — have short sequences of 15 nucleotides which are complementary to 18 or 28S rRNA. These region include the sites of base modification in rRNA. There is base pairing with specific regions of pre rRNA and in presence of enzymes there is ribose methylation or pseudouridylation at the correct site on prerRNA



Ribosome Assembly:

Ribosomes are formed by the assembly of ribosomal precursor RNA with both ribosomal proteins and 5S rRNA.

~~ribos~~

ribosomal proteins ~~are~~ are transcribed outside the nucleolus by RNA poly II which yields mRNA that are ~~RNA Poly II~~ translated on cytoplasmic ribosomes and from there, it transported to nucleolus where they assemble with rRNA's to form preribosomal particles.

5S rRNA. — from outside the nucleolus by RNA Poly III

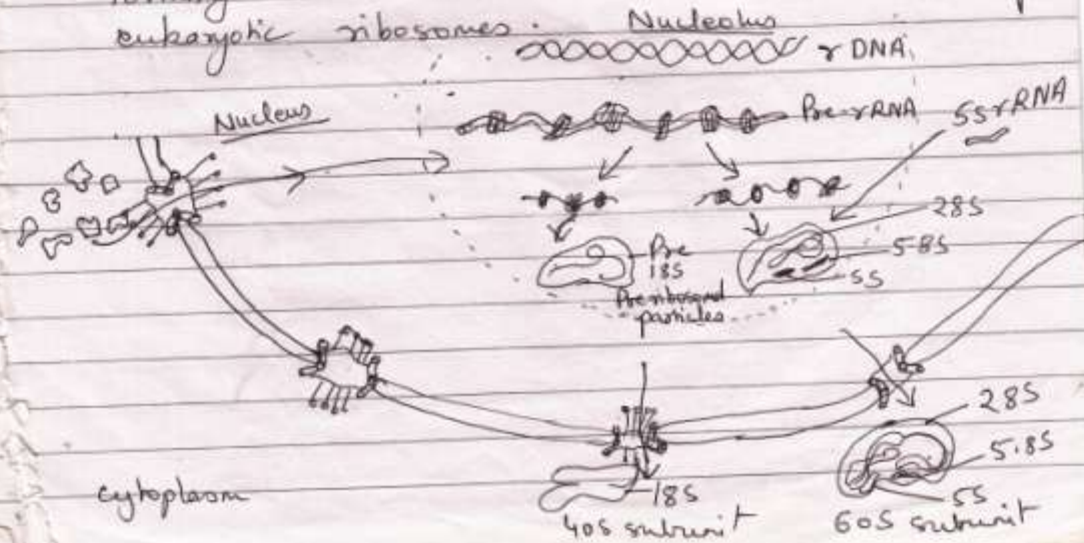
50% Ribosomal proteins attached with pre rRNA while it is still being synthesized or before its cleavage. Rest proteins and 5S rRNA are attached during cleavage of pre rRNA.

During early ribosome assembly, two nascent ribosomal subunits separate.

Processing of smaller subunit = 18S rRNA — simpler, 4 endonuclease cleavage

Processing of large subunit — — 28S, 5.8 and 5S with extensive endonuclear cleavage within nucleolus.

Final stages of ribosomal subunit maturation follow export of preribosomal particles to the cytoplasm, forming the active 40S and 60S subunits of eukaryotic ribosomes.



NOR (Nucleor Organizing Regions):

Nucleoli are formed around specific genetic loci called nucleolar organizing regions (NORs), first described by Barbara Mc Clintock.

Nucleolus is defined as a "genetically determined element".

NOR composed of tandem repeats of rRNA genes, which can be found in several different chromosomes

In typical eukaryote and sometimes in prokaryote, an rRNA gene consists of promoter, ITS, ETS, rRNA coding seq (18S 5.8 and 28S) and an external non transcribed spacer.

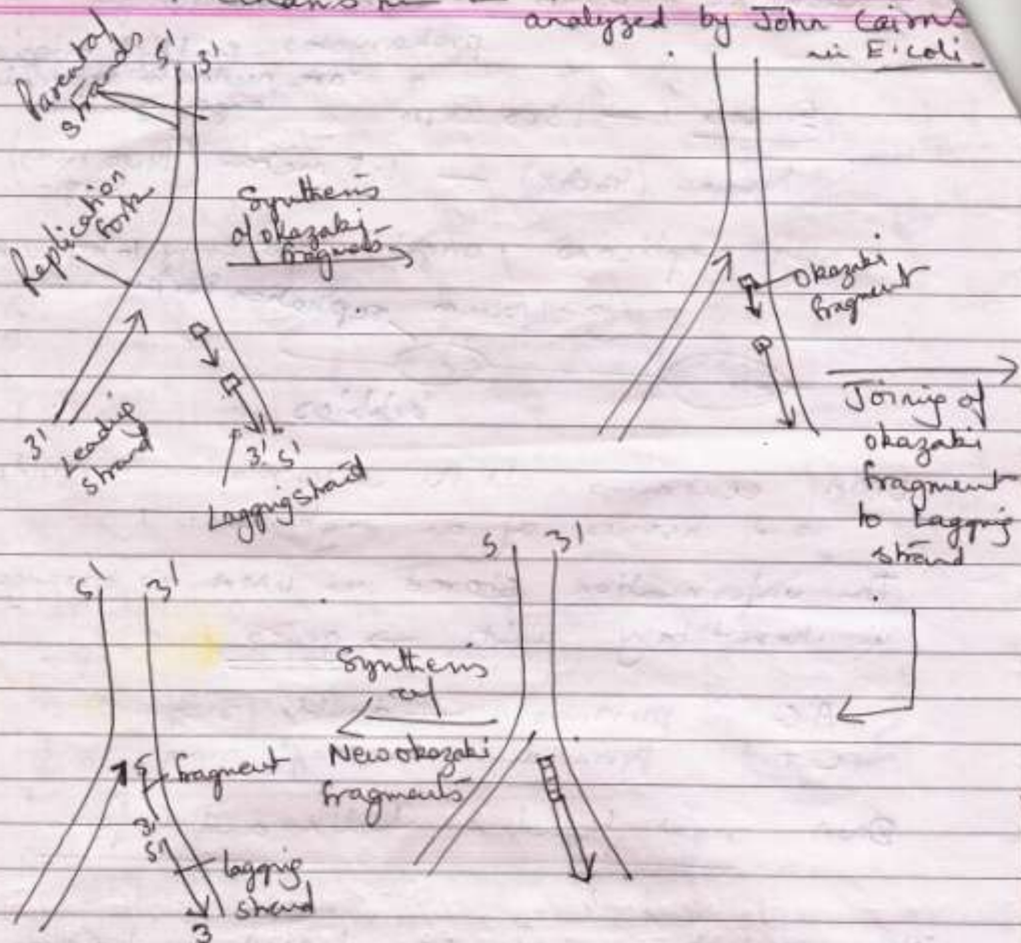
Answer 2. (iii)

Central dogma is the simplified presentation of DNA → RNA → protein. After the discovery of 1953, which was related to structure of DNA and also the discovery of the synthesis of DNA, its transcription and translation for assembling proteins is known as Central dogma. It does not reflect the role of proteins in synthesis of nucleic acid.

Mechanism of DNA replication —

DNA Polymerase — is an enzyme which read the sequence of bases on template in 3'–5' direction. It forms H-bonds between template and the arriving nucleotide. It also catalyzes phosphodiester bonds which links the nucleotides together in one strand.

Mechanism — process was first analyzed by John Cairns in E.coli



One strand of DNA is synthesized in continuous manner in 5-3' direction

Other 1-3kbp, discontinuous pieces of DNA that are synthesized backward with respect to direction of movement of replication fork.

Short fragments of RNA serve as primers for DNA replication. (3-10bp)

In Prokaryotes — removal of RNA by DNAPolyI acts as exonuclease 5'-3' or 3'-5' and removes ribonucleotides from 5' ends of okazaki fragments and replaced with DNTPs.

In case of Eukaryotes — DNA Poly I + RNase H (exonuclease)
5'-3' exonuclease / gaps are filled by polymerase δ and joined by Ligase → yield intact lagging strand.

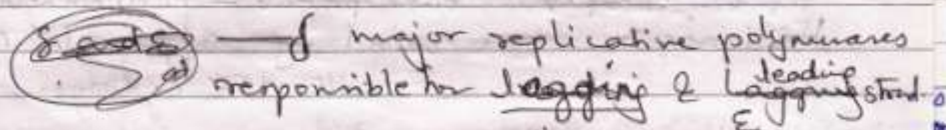
prokaryotes — 750 to 1000 bp/sec replication speed

eukaryotes — 50 to 100 bp/sec
* It needs much time to unwind DNA from nucleosomes.

In E. coli
DNA Poly III — major replicative polymerase

eukaryotes — α, δ and ε

α — formed complex with primase to synthesize RNA-DNA fragments during lagging strand synthesis.


δ and ε — major replicative polymerases responsible for lagging & leading strand (ε).

E. coli poly III and Eukaryotic poly δ and ε are associated with sliding clamp proteins (proliferating cell nuclear antigen (PCNA) in eukaryote)
↳ load polymerase on primer-template junction by clamp loading proteins (replication factor C in eukaryotes [RFC])
↓
open sliding clamp through ATP hydrolysis

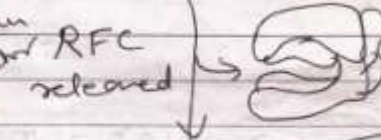
clamp loading protein.
Replication Factor C (RFC)

Proliferating cell nuclear Antigen PCNA

sliding clamp

clamp releases sliding clamp protein to form ring around template DNA. Then DNA Poly are loaded at primer — template junction.

Replication factor RFC released



maintains association of polymerases with its template as replication proceeds

~~Function of~~ ORC ~~is~~ required for initiation of DNA replication; func — to recruit other proteins — MCM DNA helicase
origin recognition complex

Helicases — unwinding of parental DNA with ATP hydrolysis

SSBs — Single Stranded DNA binding proteins
in eukaryotes — replication protein A
when synthesis complete, it is dislodged from Poly α and δ

Topoisomerases — catalyze reversible breakage and rejoining of DNA strands.

Top I — break one strand of DNA
Top II — simultaneous breaks in both the strands. and also avoid intertwined of newly synthesized DNA
both the strands rotate freely around each other so that twisting of DNA is avoided.

In Eukaryotes — also involved in mitotic chromosome condensation

DNA Polymerases: identified by Arthur Kornberg in 1956.
They have the ability to accurately copy a DNA template.
First time DNA poly I was isolated but it is involve in repairing of damaged DNA

Poly III — major polymerase for DNA replication in eukaryotes
α δ and ε — replication of nuclear DNA
γ — mitochondrial DNA replication

Functions: two fundamental properties -

(I) all polymerases synthesize DNA 5'—3' direction dNTP to the 3' hydroxyl group of a growing chain.
(II). DNA polymerases can add a new deoxyribonucleotide only to preformed primer strand that is hydrogenbonded to the template.
- They are not able to initiate DNA synthesis de novo by catalysing polymerization of free dNTPs.

* The difference between RNA and DNA polymerases
RNA polymerases — can initiate synthesis of a new strand of RNA in absence of primer.

E.Coli | Mammal
Leading — Pol III | Leading — Pol ε
Lagging — Primase ↓ Pol III | Primase-pol α
Short RNA-DNA fragments are synthesized by this complex and then extended by pol δ

Fidelity of Replication

freq of error estimated due to mutation rate for variety of genes — 1 base / 10^9 nucleotides

Polymerase does not simply catalyze incorporation of nucleotide in newly strand by hydrogen bonding with template strand.

① Binding of correctly matched dNTPs induces conformational changes in DNA polymerase that lead to incorporation of nucleotide into DNA.

This ability of DNA polymerases to select matched nucleotides for incorporation appears to increase the accuracy of replication about a thousand fold, reducing the expected error frequency to approximately 10^{-5}.

⑤ proof reading activity of DNA poly δ and ε and poly III — replicative DNA polymerases also have an exonuclease activity which can hydrolyse DNA into 3'–5' direction. increase accuracy 100 to 1000 folds

DNA repair

Control of Replication

With their multiple origins, how does the eukaryotic cell know which origins have been already replicated and which still await replication.

2 control — positive / negative

Positive — Origin Recognition

Answer 2IV

Function of Carbohydrate ;

1) Development and functioning of all organisms.
2) used as fuels
3) information rich molecules
4) Cell survival ; cell to cell communication
5) formation of tissues
6) biochemical functions and also helps in defining reality ; functionality and uniqueness of cell.

Carbohydrates are built from small molecules known as monosaccharides containing 3 to 9 Carbon atoms that are bound to hydroxyl groups that vary in size and in stereochemical configuration at one or more carbon centers. Simplest carbohydrate is monosaccharides having two or more hydroxyl groups. Simple monosaccharides with four carbon atom are known as tetroses, five carbon are known as pentoses, six carbon are hexoses and seven carbon are known as heptoses respectively. Monosaccharides are linked to form complex carbohydrates → oligosaccharides. Oligosaccharides are built by the linkage of two or more monosaccharides by glycosidic bonds.

Polysaccharides are made up of multiple monosaccharides which play central role in energy storage and maintenance of structural integrity of an organism.

Lipids are heterogenous group of compounds related to fatty acids and include fats, oils, waxes and other related substances. These are oily or greasy organic substances relatively insoluble in water and considerably soluble in organic solvents like ether, chloroform and benzene. They are hydrophic in nature. They are present in small quantities (except phospholipids and sterols) but play crucial roles as enzyme cofactors, electron carriers, light absorbing pigments, hydrophobic anchors for proteins to help membrane proteins fold, emulsifying agents in the digestive tract, hormones and intracellular messengers.

Proteins are linear polymers built of monomer units called amino acids, which are linked end to end. The sequence of linked amino acid is called Primary structure. Three dimensional structure is formed by H-bonds between amino acids

near one another is called secondary structure. Three dimensional structure of functional protein is known as tertiary structures. Hydrogen bond, disulphide bond, ionic and hydrophobic interaction play role in 3D structure. Functional protein is composed of several distinct polypeptide chains. The spatial arrangement of subunits of protein is known as its quarternary structure.

Proteins are most versatile macromolecules in living systems and function as catalyst, transport and store other molecules, provide mechanical support, immune protection, generate movement, transmit nerve impulses and control growth and differentiation.

Protein contain wide range of functional groups which are chemically reactive and essential to the function of enzymes. Proteins can interact with one another and with other biological macromolecules to form complex assemblies. Some proteins are quite rigid, whereas others display a considerable flexibility.

Amino acids are the building blocks of proteins. Amino acid residue joined to its neighbour by specific covalent bond to form proteins. Amino acids exist as dipolar ions at neutral pH where amino group is protonated and carboxyl group is deprotonated

All twenty common amino acids are α-amino acids which consists of a central carbon atom, called α-carbon. They have a carboxyl group and an amino group bonded to the same carbon atom (α-carbon). They differ from each other in their side chains or R-groups. R groups are varied in structure, size and electric charge and they also influence the solubility of amino acids in water.

Answer 2 V

Clamp loading proteins open sliding clamp through ATP hydrolysis. It releases proliferating cell nuclear antigen to form ring around template DNA. Thereafter, DNA Polymerase is loaded at primer - template junction. It maintains association of polymerase with its template as replication proceeds.

The stacked base pairs in duplex DNA absorbs less UV light than unstacked bases in single stranded DNA. So abrupt increase in the absorption of UV light is known as Hyperchromicity.

DNA strands are denatured at Tm which depends on several factors -

(1) GC ratio, if GC is high then there is high Tm

(2) Ion conc also affect Tm - because negatively charged phosphate groups of the two strands are shielded by positively charged ions.

(3) pH — low pH → low Tm,

The unwinding and separation of DNA strands, referred to as denaturation or melting. It can be induced by increasing temp. There is a break in hydrogen bonds and other forces which stabilize the double helix. The strands are separated due to electrostatic repulsion of the negatively charged deoxy-ribose phosphate backbone of each strand.

When lowering the temperature, increasing the ion conc and neutralizing the pH causes two complementary strands to reassociate into a perfect double helix

The method relies on thermal cycling, consisting of cycles of repeating heating and cooling of the reaction for DNA melting and enzymatic replication of DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with DNA polymerase are key components to enable selective and repeated amplification.

During PCR, generated DNA is itself used as a template for replication in exponential amplification (2^n where n → no. of cycle)

The thermal cycling steps are necessary first to physically separate the two strands in a DNA double helix at a high temp in process called DNA melting. At lower temp, each strand is then used as the template in DNA synthesis by DNA polymerase, to amplify specific target DNA.

How PCR works

(1) Initialization step — heating the reaction at high temp (upto 94 — 96°C) 1–9 minutes. It is only required for DNA polymerases that require heat activation by hot-start PCR.

(2) Denaturation step: First regular cycling event and consists of heating the reaction to 94 – 98°C for 20 – 30 seconds. It causes DNA melting of DNA template by disrupting H-bonds between complementary

bases, yielding single stranded DNA molecules

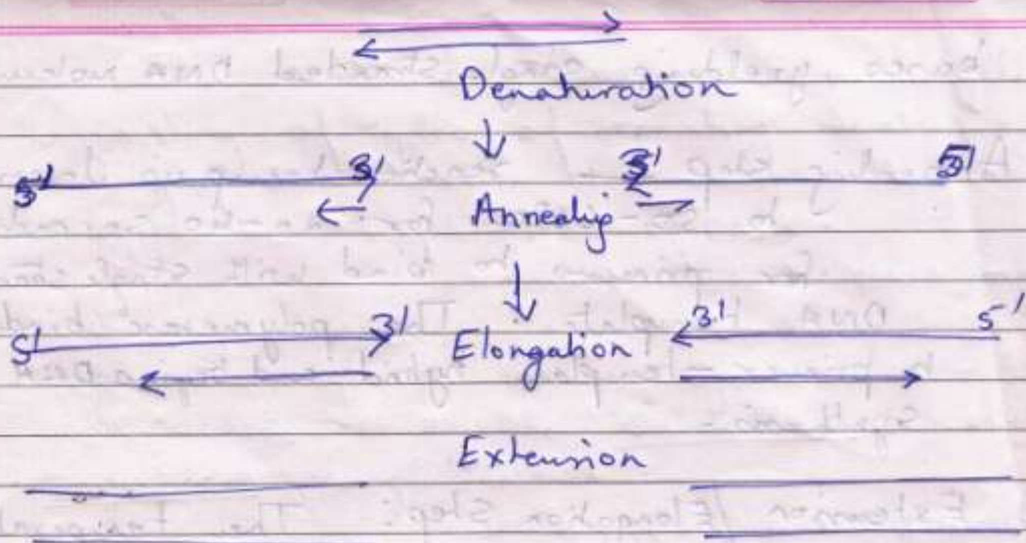
(3) Annealing step :— reaction temp is lowered to 50–65°C for 20–40 seconds for primers to bind with single stranded DNA template. The polymerase binds to primer–template hybrid and begins DNA synthesis.

(4) Extension /Elongation Step: The temperature depends on DNA polymerase used. Taq polymerase has its optimum activity temp at 75–80°C, commonly 72°C. During extension, DNA polymerase synthesizes a new DNA strand complementary to DNA template strand by adding dNTPs that are complementary to template in 5'–3' direction.

The extension time depends both on DNA polymerase used and on the length of DNA fragment to be amplified. At its optimum temp, DNA polymerase will polymerize a thousand bases per minute. ~~Under optimum condts,~~

(5) Final Elongation — 70–74°C for 5–15 minutes

to ensure that any remaining single stranded DNA is fully extended.



Exponential growth of short product

Artificial gene synthesis — used in synthetic biology to create artificial genes in laboratory.
Synthesis of first complete gene, a yeast tRNA was demonstrated by Har Gobind Khorana in 1972.
Nowadays, commercial gene synthesis services are available.
There is no need of preexisting DNA sequences. They are used in vaccine development, gene therapy and Molecular engineering.
Oligonucleotides are chemically synthesized using some modified nucleosides → nucleoside phosphoramidites. The chain grows in 3'—5' direction.
Currently, upto 200bp of oligonucleotides are being synthesized for biological application.

Answer2(VI)

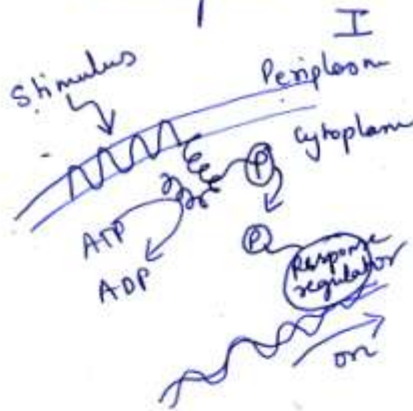
Two component system

It is known as two component because

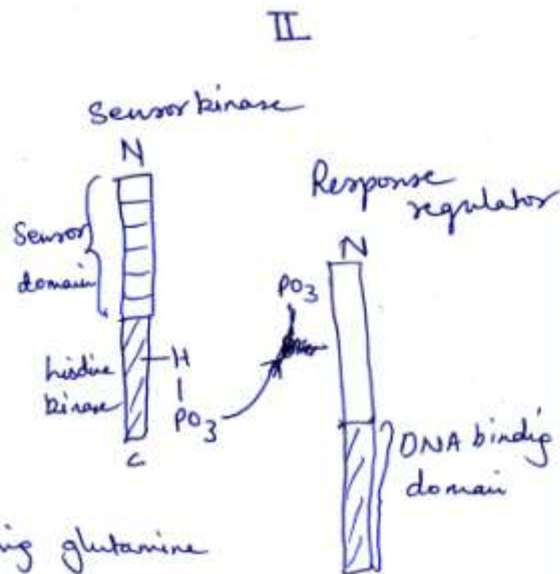
a) one is sensor histidine domain } proteins
b) second " response regulator } activator or repressor
c) signalling involved phosphorylation of sec component by the first.
d) the phosphorylated form of second component is the active state of that regulator

In NTR regulon, NtrB is apparent kinase. that phosphorylated NtrC and that NtrC-P is the form of regulator that stimulated transcription.

I



II



glnA — GS synthesizing glutamine

glnB — P_{II}, P_{II}-UMP — inhibit phosphatase of NtrB, — activate adenylyl transferase

glnD — Uridylyl transferase (UTase) — transfer UMP to and from P_{II}; Uridylyl removing enzyme (UR)

glnE — Adenylyl transferase (ATase) — transfer AMP to GS

glnF rpoN σ^{54} — RNA polymerase recognition of promoters of Ntr operons

ntrC glnG NtrC, NtrC-PO_4 — activator of promoters of Ntr operons

ntrB glnL NtrB, NtrB-PO_4 autokinase, phosphatase, PO_4 transferred to NtrC

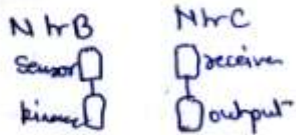
Answer VI

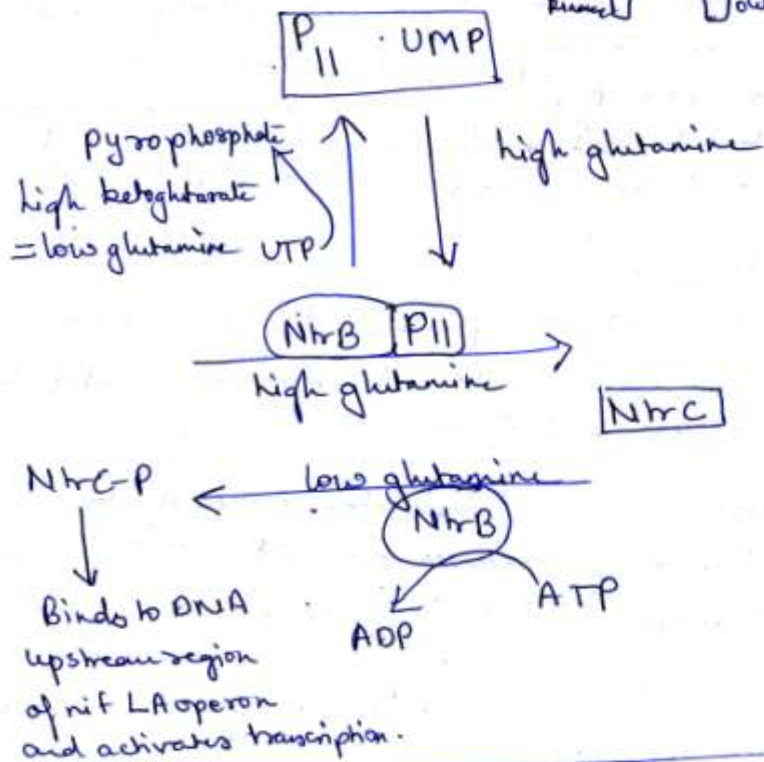
In case of cyanobacteria, neither uridylylation nor adenylylation. but there is phosphorylation at Ser 49 in T loop of P_{II} protein which ultimately activates NtcA gene (global transcriptional nitrogen regulator) Heterocyst differentiation takes place and nif genes are activated for nitrogen fixation.

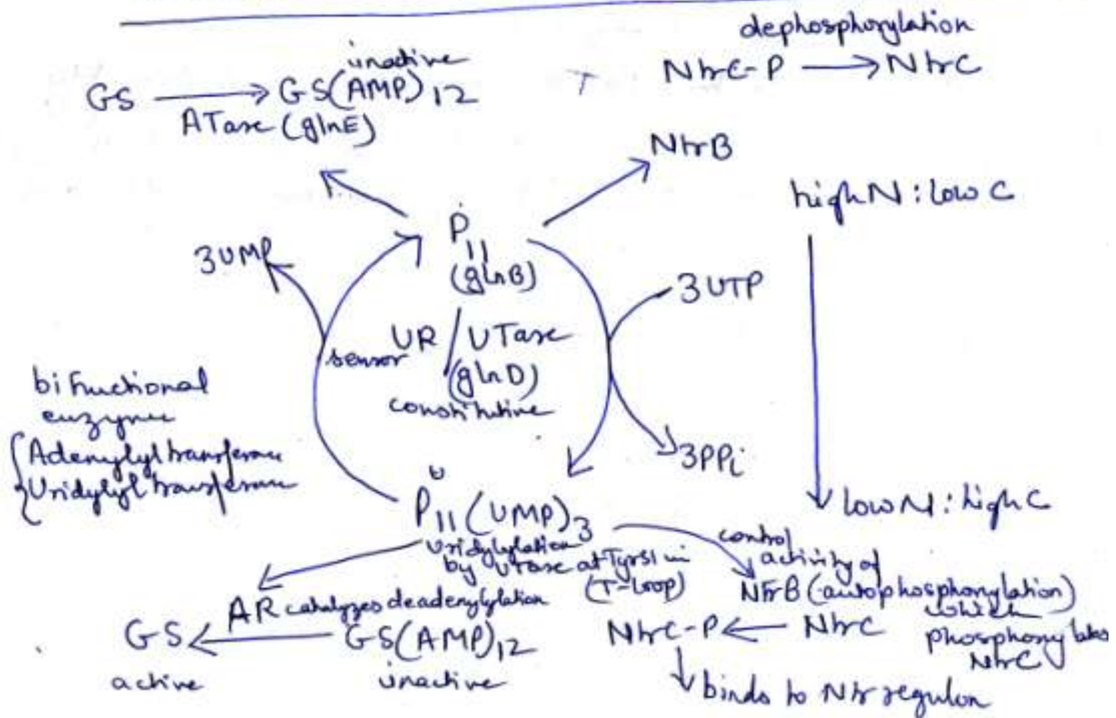
In bacteria —

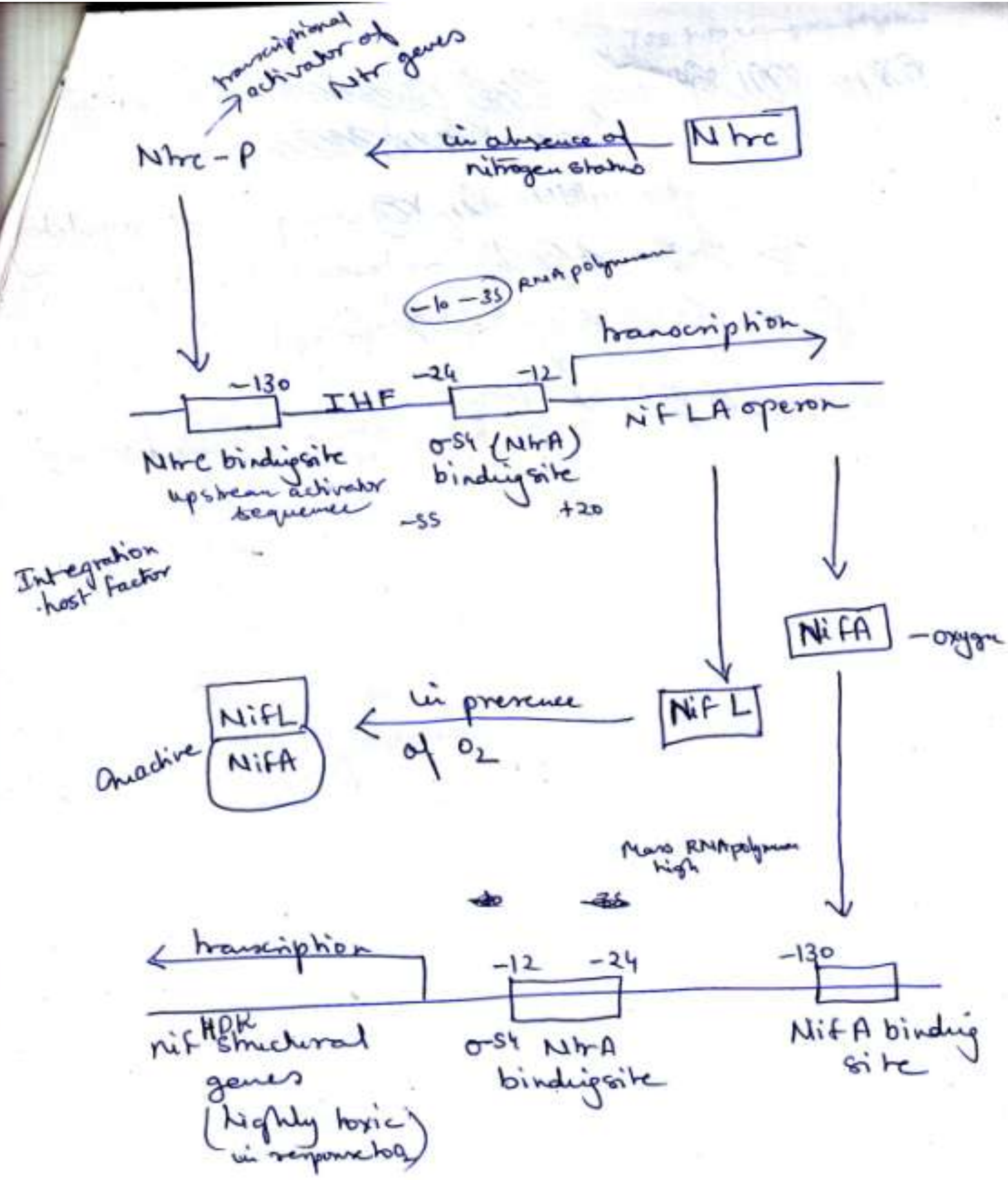
Uridylylation of PII protein has been demonstrated in *Rhizobium*, *Klebsiella*, *Azospirillum*, *Azotobacter*, *Rhodospirillum* and *Corynebacterium*.

PII protein is uridylylated, when glutamine concn (C:N ratio) low.
UTase/UR enz serves as intracellular nitrogen sensor
Its activity is modulated in response to nitrogen availability
It is very sensitive.!
PII functions as sec messenger and interacts with NtrB/NtrC (sensor / regulator) which control NTR regulon and directly regulates GS activity by altering its adenylylation status (via adenyltransferase encoded by glnE)


NtrB
NtrC
Sensor
kinase
receiver
output


P11 · UMP
pyrophosphate
high ketoglutarate
= low glutamine UTP
high glutamine
NtrB P11
high glutamine
NtrC
NtrC-P
low glutamine
NtrB
ATP
ADP
Binds to DNA
upstream region
of nif LA operon
and activates transcription.


dephosphorylation
NtrC-P → NtrC
GS → GS(AMP)12
inactive
ATase (glnE)
NtrB
high N : low C
P11
(glnB)
3UMP
3UTP
sensor UR / UTase
(glnD)
constitutive
3PPi
bifunctional
enzyme
Adenylyl transferase
Uridylyl transferase
P11(UMP)3
low N : high C
Uridylylation by UTase at Tyr51 in (T-loop)
control activity of NtrB (autophosphorylation) which phosphorylates NtrC
AR catalyzes deadenylylation
GS ← GS(AMP)12
active
inactive
NtrC-P ← NtrC
binds to Ntr regulon



NifLA operon is absent in cyanobacteria.

Leghaemoglobin —
Cyt oxidase —

Enzyme Inhibition Answer 2(VII)

Enzyme inhibitors are molecular agents that interfere the enzyme activity either slowing or halting the reactions.

Inhibition can be either irreversible or reversible.

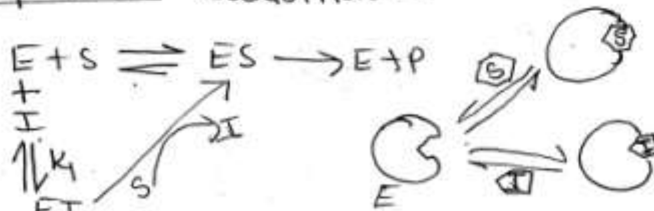
Irreversible : – inhibitor has become tightly bound to the enzyme, either covalently or noncovalently so that it dissociates very slowly from its target enzyme.

e.g Penicillin covalently modifying the enzyme transpeptidase so the synthesis of bacterial cellwalls are prevented and thus bacteria are killed.

e.g Aspirin acts by covalently modifying the enz cyclooxygenase, reducing the synthesis of signaling molecules in inflammation. Inhibitors bind covalently with or destroy a functional group on an enz that is essential for enz activity.

Reversible inhibition – is characterized by a rapid dissociation of the enzyme - inhibitor complex. It is of many types

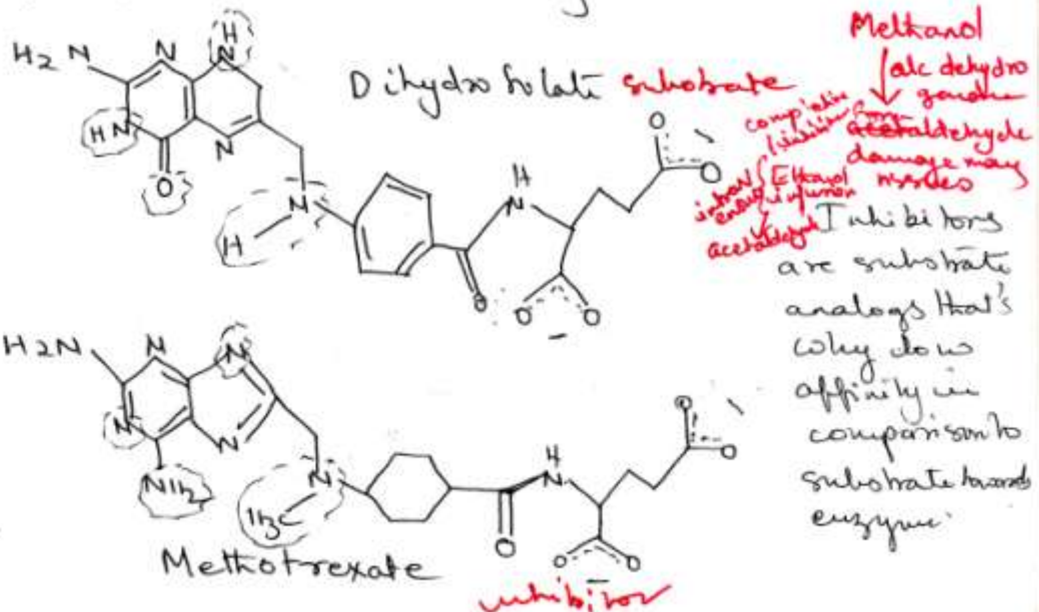
Competitive inhibition –



In Competitive inhibition,
An enzyme can bind substrate (forming ES) or inhibitor (EI) but not both (ESI) complex. This inhibitor resembles the substrate (structural analogs) and binds to the same active site. It diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to substrate.

At any given inhibitor concentration, comptetive inhibition can be relieved by increasing the substrate concentration. The substrate successfully competes with inhibitor for active site.

e.g Methotrexate — potent comptetive inhibitor for enz dihydrofolate reductase — responsible for biosynthesis of purines and pyrimidines.
Methotrexate (inhibitor) is structural analog of dihydrofolate (substrate) a substrate for dihydrofolate reductase (enz).
It binds to enz 1000 times tightly as natural substrate binds and inhibits nucleotide base synthesis.
It is used in treating cancer.

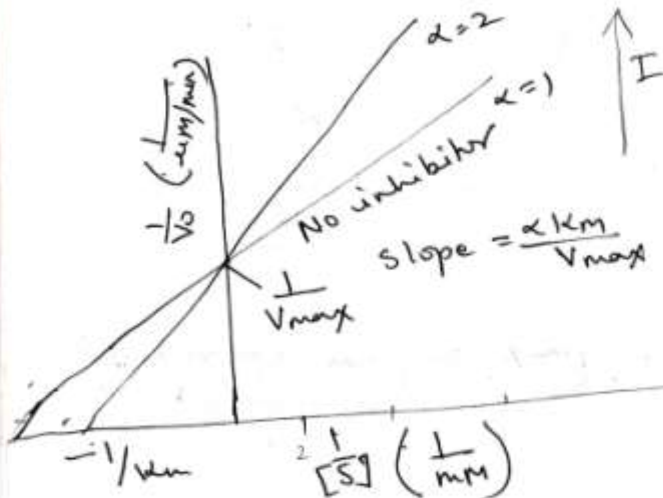


In presence of competitive inhibitor, M Meq equation

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$$

where $\alpha = 1 + \frac{[I]}{K_I}$ and $K_I = \frac{[E][I]}{[EI]}$

αK_m — K_m observed in presence of inhibitor is known as apparent K_m.



$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

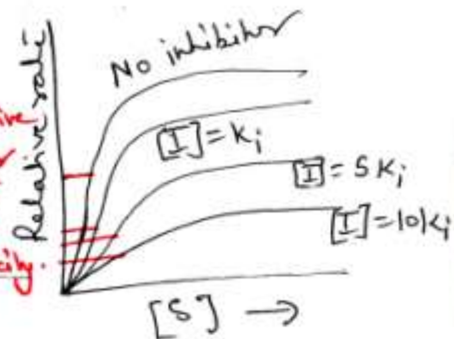
* V_{max} is unchanged by the presence of competitive inhibitor
* K_m changed and increased

K_I – inhibitor constant for inhibitor binding value of α can be calculated from change in slope at any given [I]

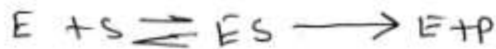
If we know [I] and α then K_I can be calculated

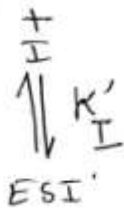
$$\alpha = 1 + \frac{[I]}{K_I}$$

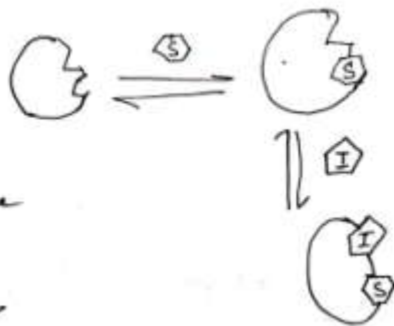
As the conc of competitive inhibitor increases, higher concentrations of substrate are required to attain a particular reaction velocity.

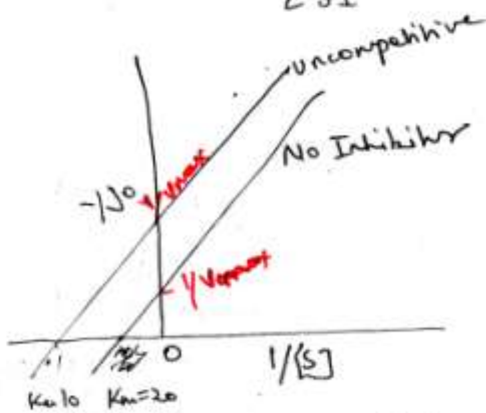


Uncompetitive inhibition — inhibitor binds only to enz-substrate complex. The uncompetitive inhibitor's binding site is created only on interaction of the enz and substrate. This type of inhibition cannot be overcome by the addition of more substrate





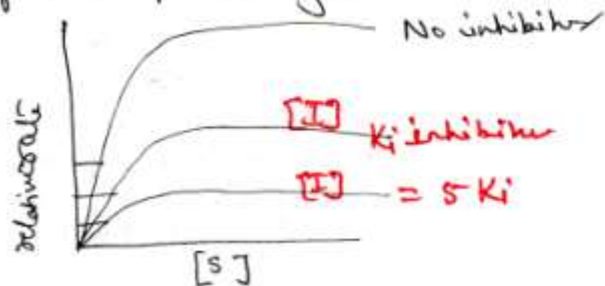




*Vmax and Km are reduced by equivalent amount.

This enzyme substrate inhibitor complex does not go on to form any product. Some unproductive ESI complex is always present that's why Vmax is lowered or Vmax cannot be attained

herbicide glyphosate — uncompetitive inhibitor of an enz in biosynthetic pathway for aromatic amino acids.



$\frac{1}{.1} = 10$

$\frac{1}{.05} = 20$

Noncompetitive

The inhibitor and substrate can bind simultaneously to an enzyme molecule at different binding sites. Unlike uncompetitive inhibition, a noncompetitive inhibitor can bind free enzyme.

Noncomptetive inhibitor acts by decreasing the conc of functional enzyme rather than by diminishing the proportion of enzyme molecules that are bound to substrate. It decreases the turnover no. Like uncomptetive inhibition, it cannot be overcome by increasing the substrate concentration