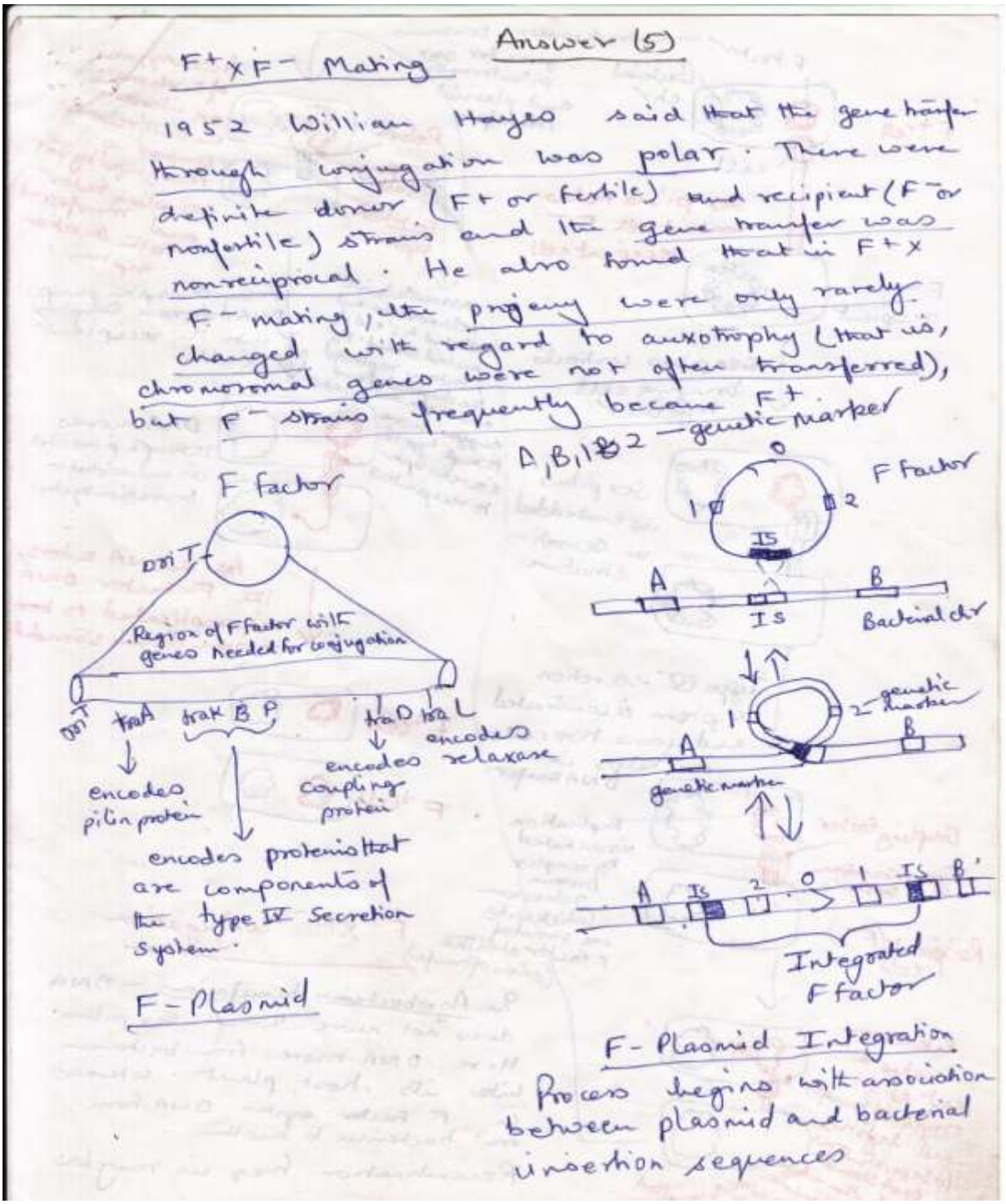
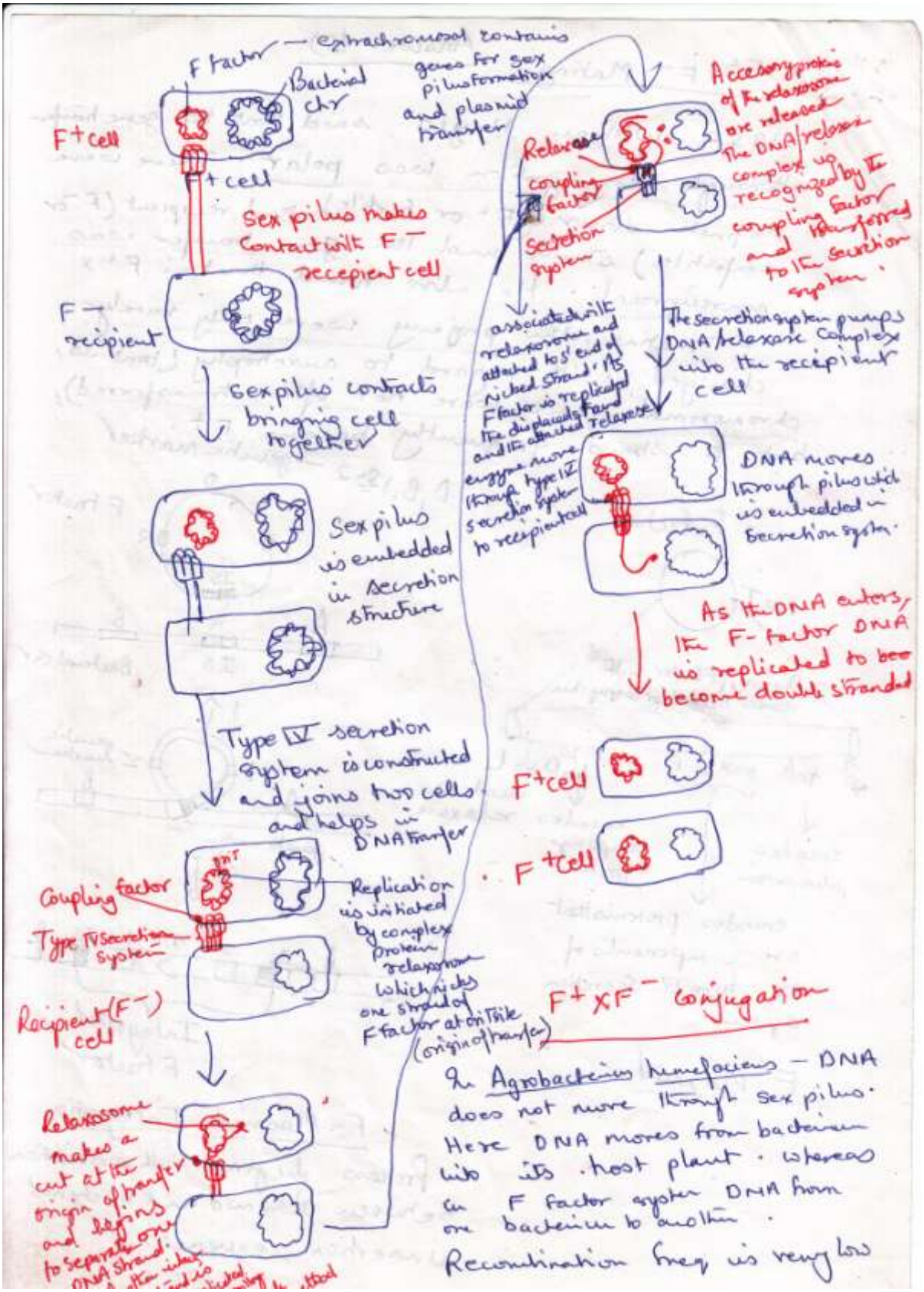
B.Sc Vth Sem 2014 AU-6885
LBC 502 Microbial Genetics

1.

i) a; ii) b; iii) c; iv) a; v) c; vi) b; vii) b; viii) a; ix) b; x) a;

2.

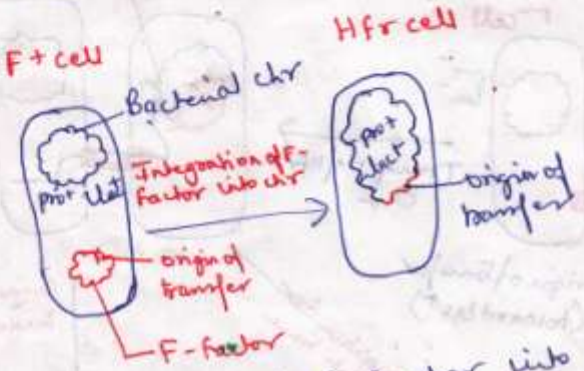



F factor — extrachromosomal contains genes for sex pilus formation and plasmid transfer
Bacterial chr
F+ cell
F+ cell
Sex pilus makes Contact with F− recepient cell
F− recipient
Sex pilus contracts bringing cell together
Sex pilus is embedded in secretion structure
Type IV secretion system is constructed and joins two cells and helps in DNA transfer
Coupling factor
Type IV secretion system
Recipient (F−) cell
Replication is initiated by complex protein relaxosome which nicks one strand of F factor at oriT site (origin of transfer)
Relaxosome makes a cut at the origin of transfer and begins to separate one DNA strand.
Relaxase
coupling factor
Secretion system
Accessory proteins of the relaxosome are released
The DNA/relaxase complex is recognized by the coupling factor and transferred to the secretion system.
associated with relaxosome and attached to 5' end of nicked strand. As F factor is replicated the displaced free and its attached relaxase
The secretion system pumps DNA/relaxase complex into the recipient cell
DNA moves through pilus which is embedded in secretion system.
As the DNA enters, the F- factor DNA is replicated to become double stranded
F+ cell
F+ cell
F+ X F− Conjugation
In Agrobacterium tumefaciens – DNA does not move through sex pilus. Here DNA moves from bacterium into its host plant. whereas in F factor system DNA from one bacterium to another.
Recombination freq is very low

Hfr Conjugation

Answer (7)

A second type F factor mediated conjugation was discovered. In this type donor transfers chromosomal genes with great efficiency but does not change the recipient bacteria into F+ cells. High frequency of recombinants are produced by this mating, so formed as Hfr conjugation and donor is known as Hfr Strain.



Insertion of F factor into chromosome

Hfr strains contain the F-factor integrated into their chr, rather than free in the cytoplasm. After the integration, F-plasmid's tra operon still functional. The plasmid direct the synthesis of pili, carry out rolling circle replication and transfer genetic material to an F⁻ recipient cell However, rather than transferring itself, F-factor directs the transfer of host chr. DNA transfer begins when the integrated F-factor is nicked at its site of transfer origin. As it is replicated, the chr moves to the recipient. Only part of F factor is transferred, F⁻ recipient does not become F+ unless the whole chr is transferred. Transfer of entire chr with the integrated F factor requires about 100 minutes in E. coli, and the connection breaks before this process. Thus a complete F-factor usually is not transferred and the recipient remains F⁻

3.

BACTERIOPHAGE

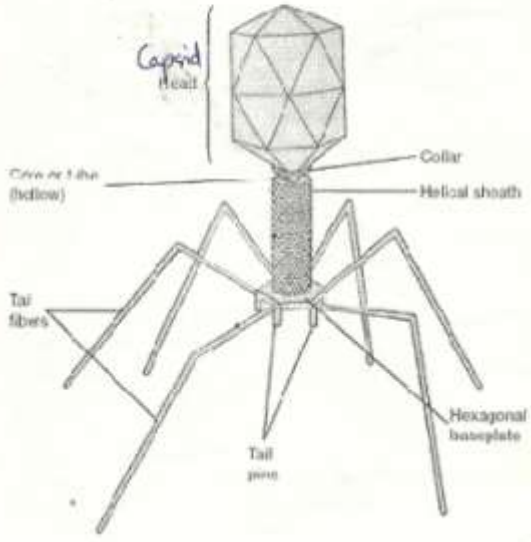
Bacteriophages are viruses that parasitize bacteria. Bacteriophages were jointly discovered by Frederick Twort (1915) in England and by Felix d'Herelle (1917) at the Pasteur Institute in France. Felix d'Herelle coined the term "Bacteriophage". Bacteriophage means to eat bacteria, and are called so because virulent bacteriophage can cause the compete lysis of a susceptible bacterial culture. They are commonly referred as "phage". Phages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. They occur widely in nature and can readily be isolated from feces and sewage. There are at least 12 distinct groups of bacteriophages, which are very diverse structurally and genetically.

Examples of phages:

- T-even phages such as T2, T4 and T6 that infect *E.coli*
- Temperate phages such as lambda and mu
- Spherical phages with single stranded DNA such as PhiX174
- Filamentous phages with single stranded DNA such as M13
- RNA phages such as Qbeta

Composition:

Depending upon the phage, the nucleic acid can be either DNA or RNA but not both. The nucleic acids of phages often contain unusual or modified bases, which protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection. Simple phages may have only 3-5 genes while complex phages may have over 100 genes. Certain phages are known have single stranded DNA as their nucleic acid.



Morphology:

Most phages range in size from 24-200 nm in length. T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide. All phages contain a head structure, which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous.

The head encloses nucleic acid and acts as the protective covering. Some phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. T4 tail is surrounded by a contractile sheath, which contracts during infection of the bacterium. At the end of the tail, phages like T4 have a base plate and one or more tail fibers attached to it.

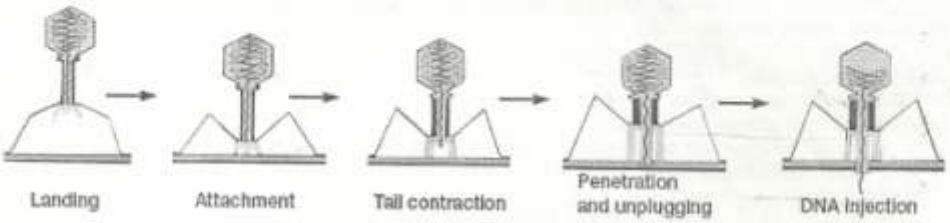
The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers.

Basic steps in life cycle of all viruses
Adsorption, Infection (penetration), replication, maturation and release.

Life cycle:

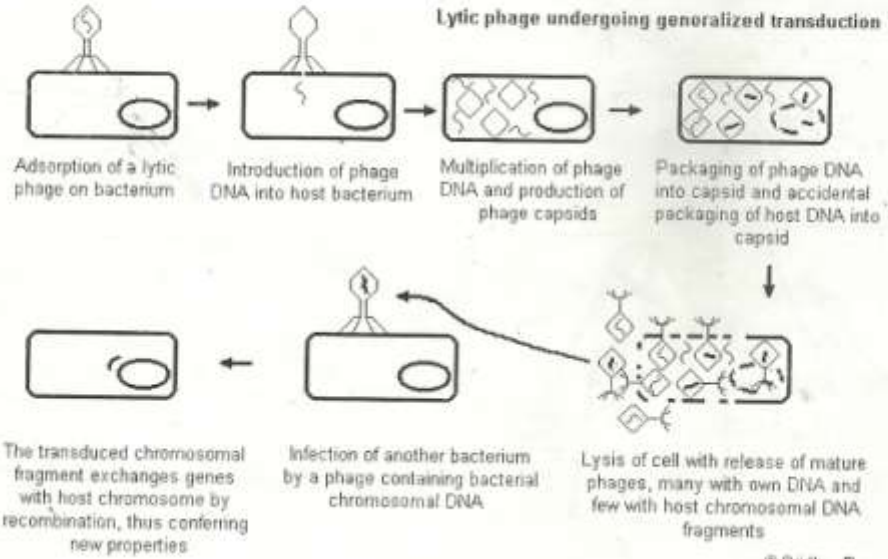
Adsorption: The first step in the infection process is the adsorption of the phage to the bacterial cell. This step is mediated by the tail fibers or by some analogous structure on those phages that lack tail fibers. Phages attach to specific receptors on the bacterial cell such as proteins on the outer surface of the bacterium, LPS, pili, and lipoprotein. This process is reversible. One or more of the components of the base plate mediates irreversible binding of phage to a bacterium.

Penetration: The irreversible binding of the phage to the bacterium results in the contraction of the sheath (for those phages which have a sheath) and the hollow tail fiber is pushed through the bacterial envelope. Some phages have enzymes that digest various components of the bacterial envelope. Nucleic acid from the head passes through the hollow tail and enters the bacterial cell. The remainder of the phage remains on the outside of the bacterium as "ghost". Even a non-susceptible bacterium can be artificially infected by injecting phage DNA by a process known as transfection.



Depending on the life cycle, phages can either by lytic (virulent) or lysogenic (temperate). While lytic phages kill the cells they infect, temperate phages establish a persistent infection of the cell without killing it. In lytic cycle the subsequent steps are synthesis of phage components, assembly, maturation and release.

Lytic phage undergoing generalized transduction



Lytic cycle:

Lytic or virulent phages are phages, which multiply in bacteria and kill the cell by lysis at the end of the life cycle. Soon after the nucleic acid is injected, the phage cycle is said to be in eclipse period. During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell. Eclipse phase represents the interval between the entry of phage nucleic acid into bacterial cell and release of mature phage from the infected cell. This phase is devoted to synthesis of phage components and their assembly into mature phage particles.

The phage nucleic acid takes over the host biosynthetic machinery and phage specified m-RNA's and proteins are made. In some cases the early phage proteins actually degrade the host chromosome. Structural proteins (head, tail) that comprise the phage as well as the proteins needed for lysis of the bacterial cell are separately synthesized. Nucleic acid is then packaged inside the head and then tail is added to the head. The assembly of phage components into mature infective phage particle is known as maturation. In Lysis and Release Phase the bacteria begin to lyse due to the accumulation of the phage lysis protein and intracellular phage are released into the medium. It is believed that phage enzymes weaken the cell wall of bacteria. The number of particles released per infected bacteria may be as high as 1000. The average yield of phages per infected bacterial cell is known as burst size.

Lysogenic cycle:

Lysogenic or temperate phages are those that can either multiply via the lytic cycle or enter a dormant state in the cell. In most cases the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. This integrated state of phage DNA is termed prophage. This process is known as lysogeny and the bacteria harboring prophage are called lysogenic bacteria. Since the prophage contains genes, it can confer new properties to the bacteria. When a cell becomes lysogenized, occasionally extra genes carried by the phage get expressed in the cell. These genes can change the properties of the bacterial cell. This process is known as lysogenic conversion or phage conversion.

Significance of lysogenic conversion includes:

- Lysogenic phages have been shown to carry genes that can modify the Salmonella O antigen.
- Toxin production by *Corynebacterium diphtheriae* is mediated by a gene carried by a beta phage. Only those strains that have been converted by lysogeny are pathogenic.
- *Clostridium botulinum*, a causative agent of food poisoning, makes several different toxins, 2 of which are actually encoded by prophage genomes.
- Lysogenised bacteria are resistant to superinfection by same or related phages. This is known as superinfection immunity.

The lysogenic state of a bacterium can get terminated anytime when it exposed to adverse conditions. This process is called induction. Conditions that favor the termination of the lysogenic state include: desiccation, exposure to UV or ionizing radiation, exposure to mutagenic chemicals, etc. The separated phage DNA then initiates lytic cycle resulting in cell lysis and releases of phages. Such phages are then capable of infecting new susceptible cells and render them lysogenic.

Phage Genetics:

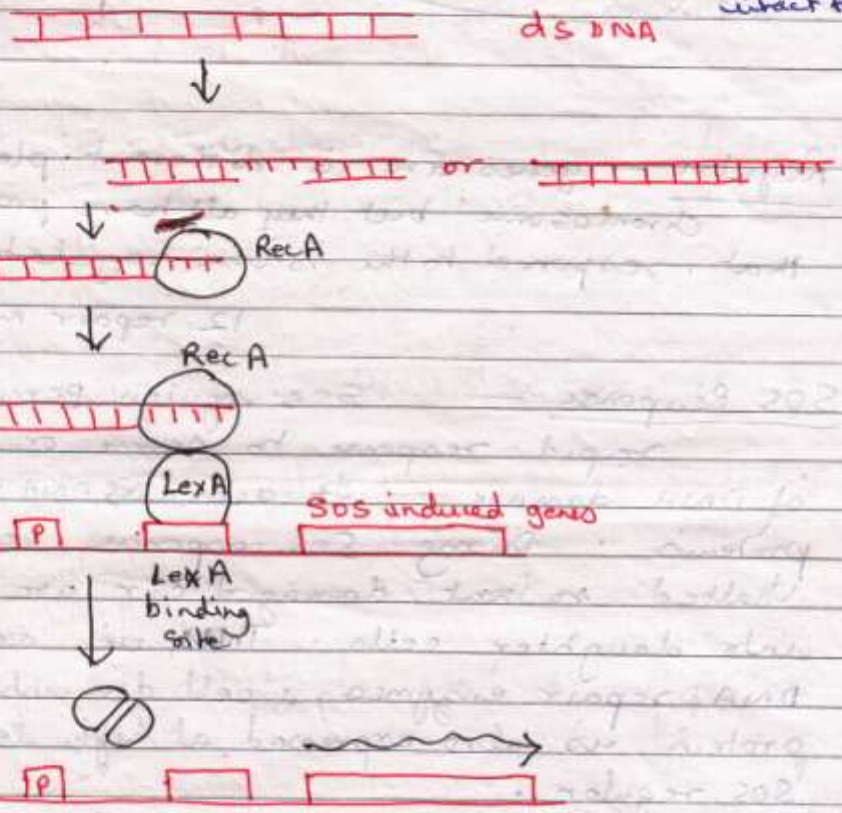
The transfer of genetic elements from one bacterium to another by a bacteriophage is termed as transduction. Transduction can be generalized or specialized. The generalized transduction is seen in lytic cycle where segments of bacterial DNA are packaged inside phage capsid instead of phage DNA. When such phages infect new bacterial cells, the bacterial DNA is injected inside. This piece of DNA may then transfer genes to the host chromosome by recombination. Any bacterial gene may be transferred in generalized transduction. Generalized transduction is usually seen in temperate phages that undergo lytic cycle. Only those genes that are adjacent to the prophage are transferred in specialized transduction.

4.

Answer(6)

Regulation of SOS regulon by proteolytic cleavage of repressor

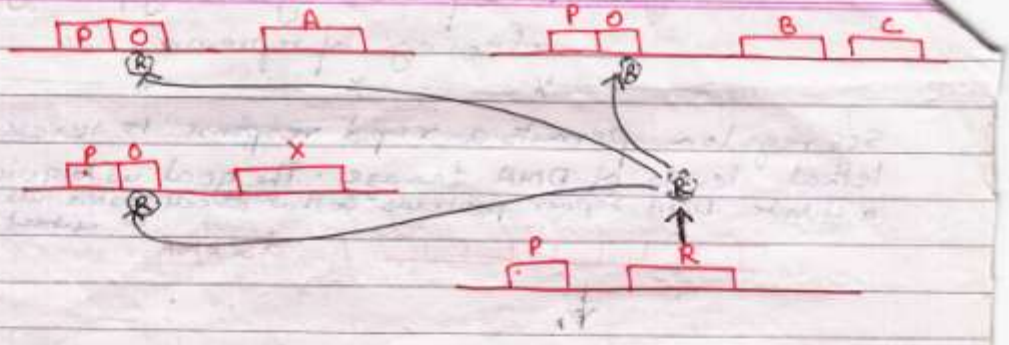
SOS regulon permits a rapid response to severe or lethal levels of DNA damage. The goal is to quickly activate DNA repair proteins so that the cell restore its DNA in intact form.



During the SOS response, cell div is halted so that damaged chromosomes are not segregated into the daughter cells.

SOS response. (a) DNA damage leads to single stranded DNA (b) RecA binds to SS DNA.
(c) RecA - SSDNA binds to LexA.
(d) LexA undergoes autocleavage relieving repression of the SOS genes.

Regulon is a group of genes all needed for the same process but physically located in different parts of the chromosome and containing their own promoters. In a regulon, the promoters are all regulated in the same fashion and



Regulon — genes are in different places on the chromosome but they all have promoters that respond to the same regulators.

12 repair mech

SOS Response — SOS regulon permits a rapid response to severe or lethal levels of DNA damage. It activates DNA repair proteins. During SOS response, cell div is halted so that damaged chr are not segregated into daughter cells. Thus, in addition to DNA repair enzymes, a cell div inhibitor protein protein is also expressed at high levels during SOS regulon.

During normal cell growth — low expression of SOS genes

Extensive DNA damage — high level expression

Weigh and coworkers first reported during reactivated UV irradiated λ that some DNA repair systems are inducible. Many of these repair mechanisms are activated as part of SOS regulon.

SOS genes contain a common seq that in some cases overlaps the promotor region

In other cases, adjacent to promoter.

The common seq is bound by repressor protein called LexA

When SOS promoters bind with LexA
↳ cannot initiate transcription.

At the DNA damage — LexA repressor should be inactivated and removed so that SOS genes are expressed.

Upon exposure to DNA damage agents.
↓
large amount of single stranded DNA accumulate.
↓
They are bound with RecA protein
↓
which participates in homologous recombination and in post replication DNA repair.

Now this bounded RecA protein binds to LexA and induces cleavage of LexA
↓
There is autocleavage of LexA between two specific a.as that separate repressor into two domains
- DNA binding domain
- dimerization domain

Two LexA molecules remain bind with seq found in SOS regulon promoters.

↓

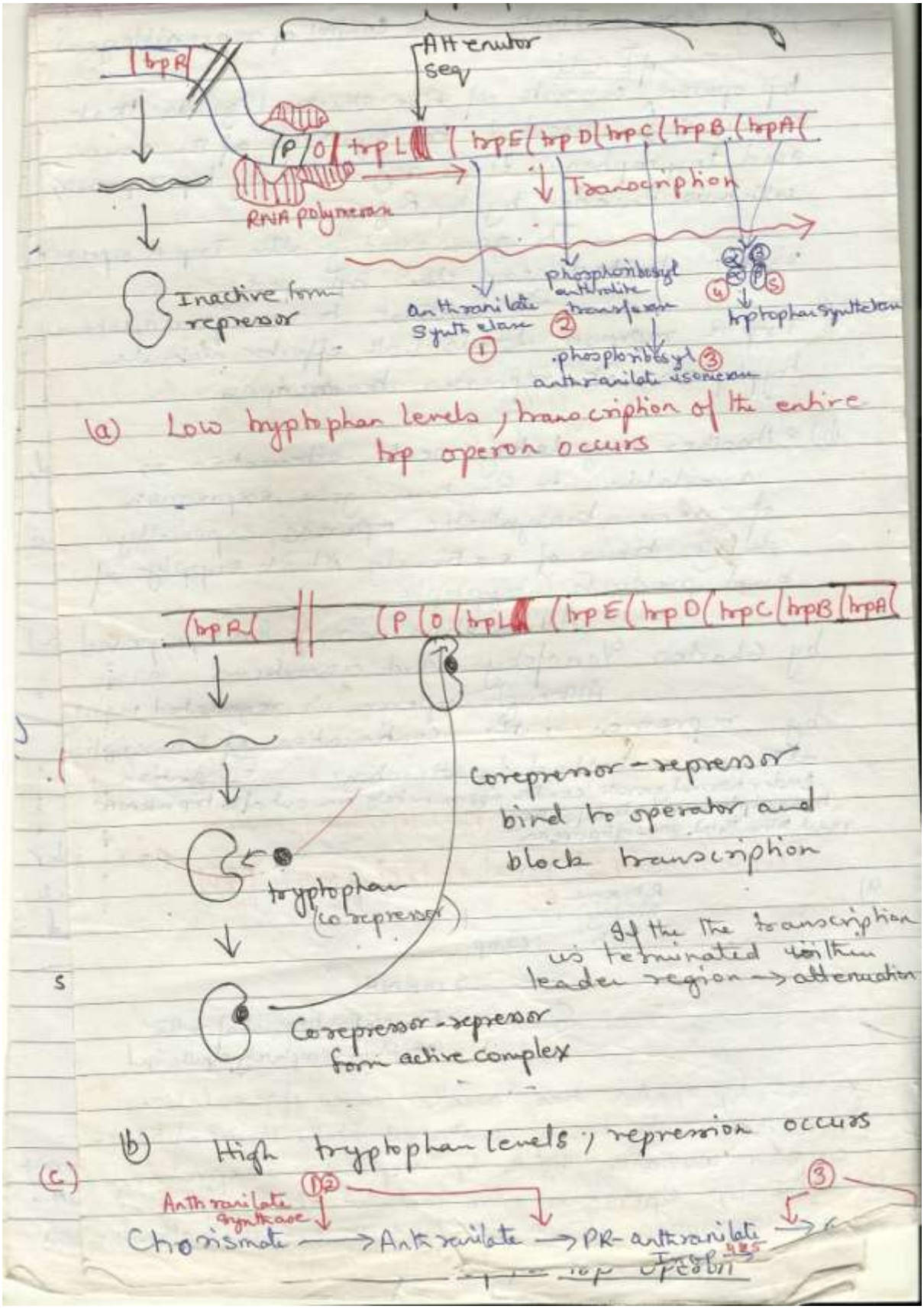
Disruption of dimerization results in the removal of LexA from SOS promoters.

Once LexA is removed, SOS genes are expressed at high levels.

Eventually, the induction signal (RecA) complexed with single stranded DNA, drops because of completion of repair of DNA, and LexA is no longer able to undergo autocleavage. This returns the regulon to its prestimulus or uninduced state.

* It means cell div inhibitor protein is also expressed at high levels during SOS response.

5.


trpR
Attenuator seq
P
O
trpL
trpE
trpD
trpC
trpB
trpA
RNA polymerase
Transcription
Inactive form repressor
Anthranilate Synthetase
phosphoribosyl anthranilate transferase
phosphoribosyl anthranilate isomerase
tryptophan synthetase
(a) Low tryptophan levels, transcription of the entire trp operon occurs
corepressor-repressor bind to operator and block transcription
tryptophan (corepressor)
If the the transcription is terminated within leader region → attenuation
Corepressor-repressor form active complex
(b) High tryptophan levels / repression occurs
(c)
Anthranilate synthase
Chorismate → Anthranilate → PR-anthranilate
trp operon

Negative Transcriptional

*1959, Cohen & Jacob — Control of repressible genes

trp operon of *E. coli consists of five structural genes that encode enz needed for synthesis of the amino acid tryptophan. It is regulated by trp repressor, which is encoded by trp R gene.

The gene encoding the Trp R repressor is not located near the trp operon.

(a) In contrast to lac operon, when Trp R repressor binds with effector molecule tryptophan, it represses transcription.

(b) * Another regulatory mech. attenuation is available to fine tune gene expression of some biosynthetic operons, especially during times of extremely short supply of end products (tryptophan)

Attenuation is first proposed by Charles Yanofsky and coworkers (1970s)

Although operon is regulated mainly by repression, the continuation of transcription or elongation is controlled by attention.

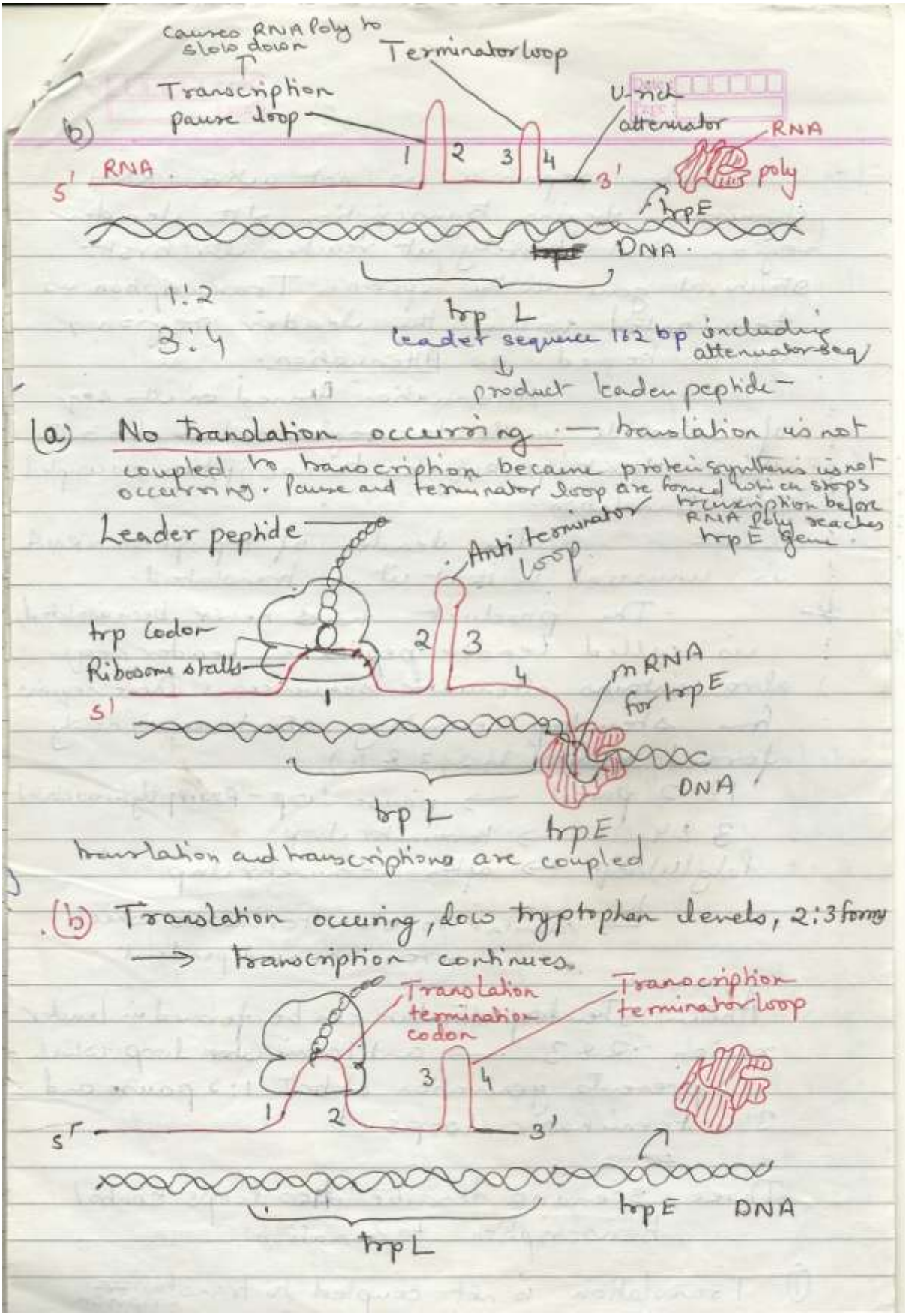
Under normal growth conds approximately nine out of 10 trp mRNA transcripts terminate before they reach structural genes of trp operon. Rho-independent terminator

(a)

→ mRNA

→ Translation begins before the mRNA is completely synthesized

* trp operon has another layer of regulation. In addition to being controlled at the level of transcription initiation by the trp repressor, expression of the trp operon is also controlled at the level of transcription elongation by a process called attenuation


causes RNA Poly to slow down
Terminator loop
Transcription pause loop
U-rich attenuator
RNA
(b)
RNA
5'
1
2
3
4
3'
poly
trpE
DNA
1:2
3:4
trp L
leader sequence 162 bp including attenuator seq
product leader peptide
(a) No translation occurring — translation is not coupled to transcription because protein synthesis is not occurring. Pause and terminator loop are formed which stops transcription before RNA poly reaches trpE gene.
Leader peptide
Anti terminator loop
trp codon
Ribosome stalls
2
3
4
1
mRNA for trpE
5'
DNA
trp L
trpE
translation and transcriptions are coupled
(b) Translation occuring, low tryptophan levels, 2:3 forms
→ transcription continues.
Translation termination codon
Transcription terminator loop
3
4
1
2
5'
3'
trpE
DNA
trpL

When repressor is not active, RNA polymerase begins transcription of the leader region but rarely it reaches the first structural gene in the operon. Transcription is terminated within the leader region, it is termed as Attenuation.

Attenuation based on the seq of nucleotide in leader region and on the fact that in prokaryotes transcription is coupled with translation.

The leader of trp operon mRNA is unusual in that it is translated.

* The product has never been isolated, is called leader peptide. Leader seq also contains attenuator sequences. These seques form stem loop secondary structures in newly formed mRNA (1, 2, 3 & 4).

1 & 2 pair. → pause loop - RNA polymerase stored

3 & 4 → terminator loop

Poly(U) seq → after terminator loop

↳ similar to others terminators which are rho independent.

Another stem loop structure can be formed in leader region. 2 & 3 → antiterminator loop which prevents generation of both 1:2 pause and 3:4 terminator loops.

Three scenario describe these loops control transcription termination →

① Translation is not coupled to transcription because protein synthesis is not occurring. no ribosome is associated with mRNA.

— Pause & terminator loops. form, stopping transcription before RNA polymerase reaches trp E gene transcription end.

② & ③ translation and transcription are coupled

ribosome associates with leader mRNA as the rest of mRNA is being synthesized. RNA polymerase and nearest ribosome determine the formation of stem loop struc.

During translation, ribosome follows RNA polymerase.

~~two Trp~~ Among the first region (1) there are two Trp codons. This is unusual because normally there is only one Trp per 100 amino acids in E. coli proteins.

If tryptophan level low —

ribosome stall due to paucity of charged $tRNA^{Trp}$ molecules which delays the filling of A site of the ribosome. Meanwhile ~~Ribosome~~ RNA polymerase continues to transcribe mRNA, moving away from stalled ribosome.

The presence of ribosome on region 1 will prevent it from base pairing with region 2. As RNA polymerase continues / region 3 is transcribed, enabling the formation of 2:3 antiterminator loop. This prevent the formation of 3:4 terminator loop.

Because the terminator loop is not formed ~~is~~ RNA polymerase is not ejected from DNA and transcription continues into trp biosynthetic genes.

(c)

→ PK- [illegible]

b) If more tryptophan in the cell —

then there abundant charged tRNA^{trp} and so ribosome translate there two trp codons in leader peptide seq without hesitation. Thus ribosome remains close to the RNA polymerase. As ribosome & RNA polymerase continue through leader region 1 region 1 & 2 are transcribed and readily form a pause drop. Similarly, 3 & 4 form terminator loop and RNA is ejected from the DNA template. Finally, UGA stop codon between 1 & 2 will cause premature termination of translation. Although leader peptide will be synthesized, it appears to be rapidly degraded.

Repression decreases transcription 70 fold and attenuation slows it another 8 to 10 fold.

When both mech operates together, transcription can be slowed 6000 fold.

6.

Answer (B)

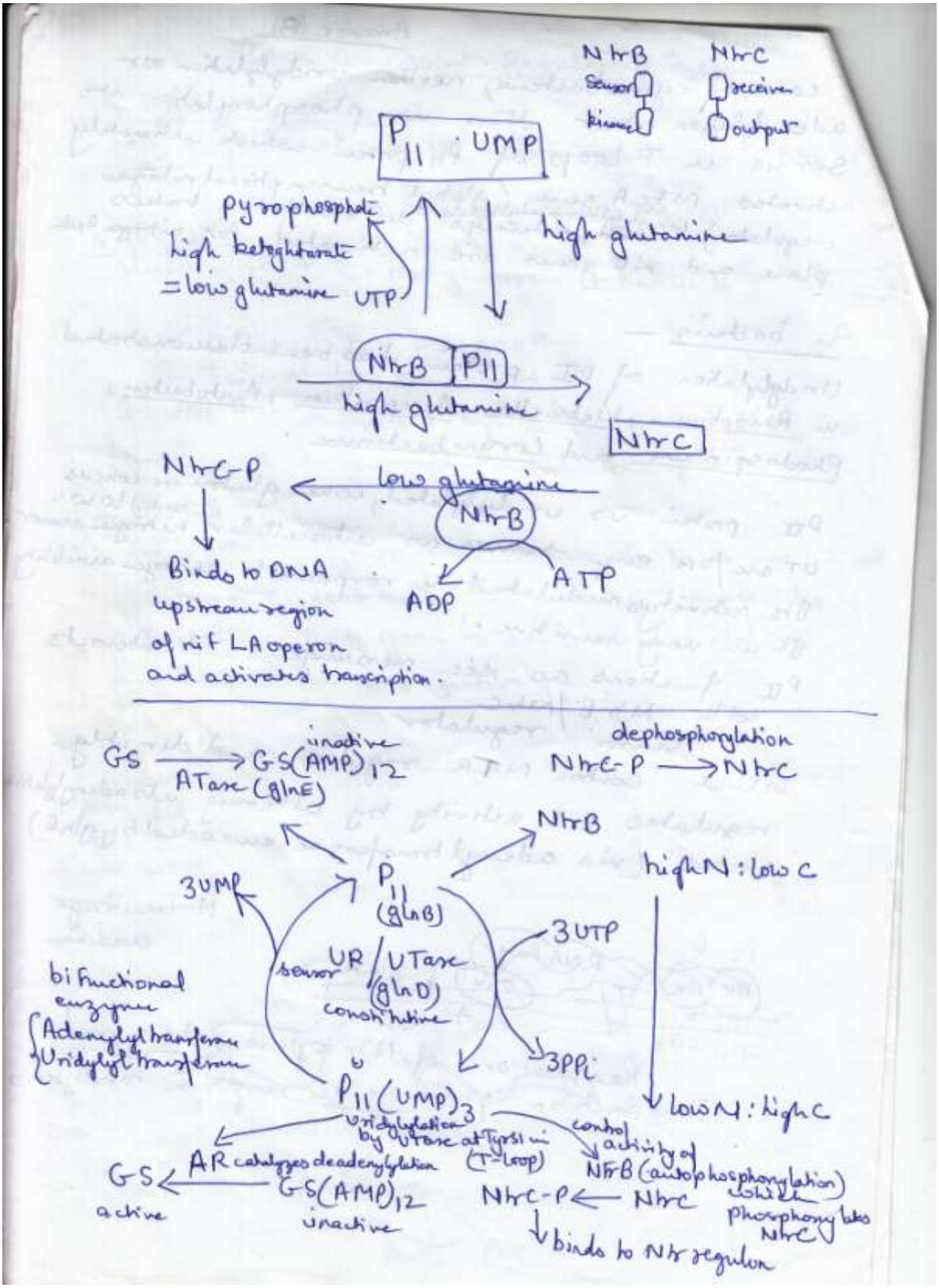
In case of cyanobacteria, neither uridylylation nor adenylylation · but there is phosphorylation at Ser 49 in T loop of P_{II} protein which ultimately activates NtcA gene (global transcriptional nitrogen regulator) ~~which ultimately leads to~~ Heterocyst differentiation takes place and nif genes are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

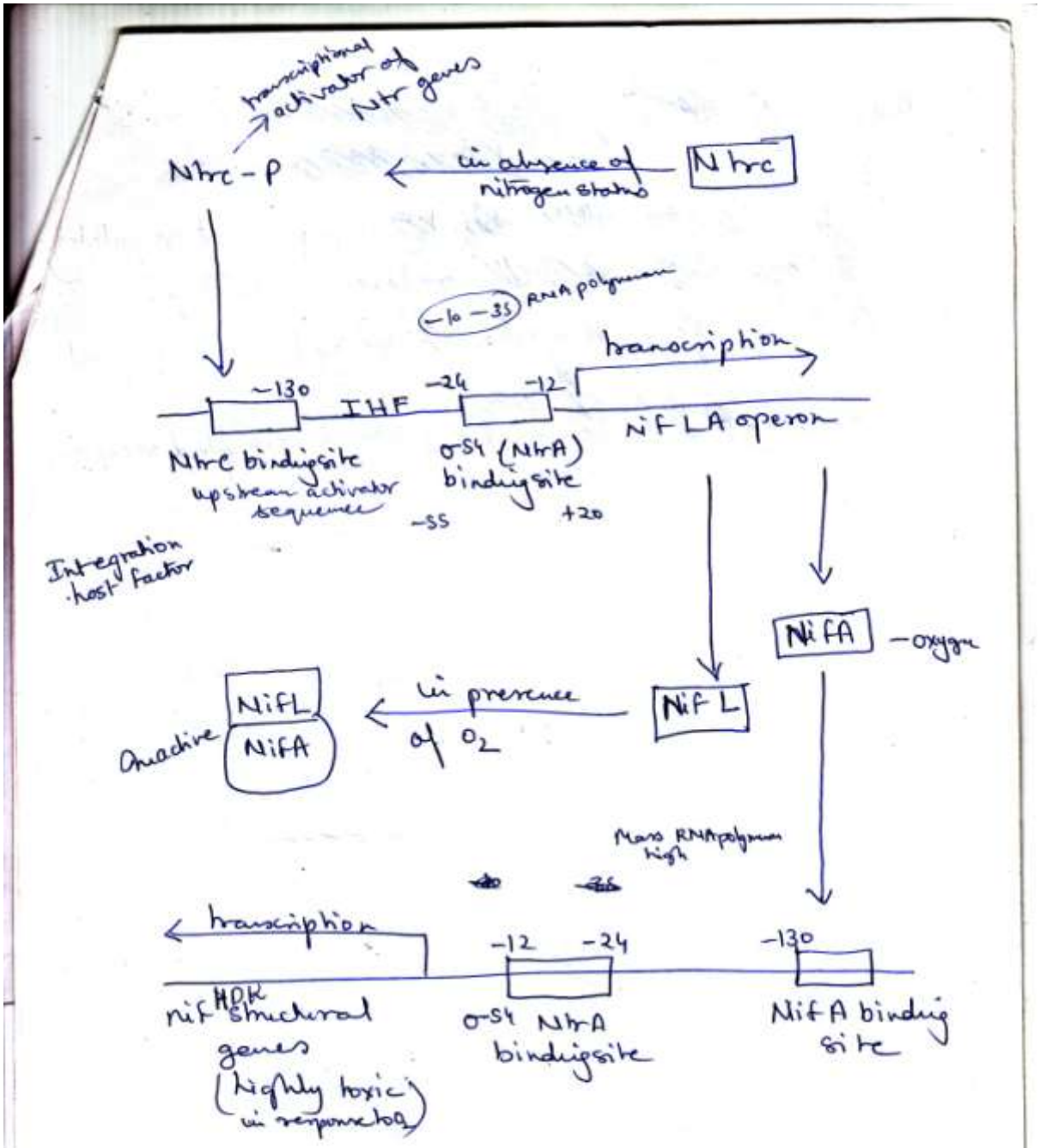
In bacteria —

Uridylylation of PII protein has been demonstrated in Rhizobium, Klebsiella, Azospirillum, Azatobacter, 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
sensor
kinase
NtrC
receiver
output
P II · UMP
Pyrophosphate
high ketoglutarate
= low glutamine
UTP
high glutamine
NtrB P II
high glutamine
NtrC
NtrC-P
low glutamine
NtrB
ATP
ADP
Binds to DNA
upstream region
of nif LA operon
and activates transcription.
GS
ATase (glnE)
GS (AMP) 12
inactive
dephosphorylation
NtrC-P → NtrC
NtrB
high N : low C
3UMP
P II
(glnB)
3UTP
UR / UTase
(glnD)
sensor
constitutive
bifunctional
enzyme
{Adenylyltransferase
{Uridylyltransferase
3PPi
low N : high C
P II (UMP) 3
uridylylation
by UTase at Tyr51 in
(T-loop)
control
activity of
NtrB (autophosphorylation)
while
phosphorylates
NtrC
AR catalyzes deadenylylation
GS
active
GS (AMP) 12
inactive
NtrC-P
NtrC
binds to Ntr regulon



NifLA operon is absent in cyanobacteria.

Leghaemoglobin —

Cyt oxidase —

7.

Answer(9)

Antibiotic – is chemical substances secreted by microorganisms which inhibits the growth and development of other microbes. Most of them are produced by actinomycetes; especially the genus Streptomyces and Filamentous fungi.

Mycotoxin — Toxins produced by fungi

Phycotoxin — Toxins produced by algae

Exotoxin — are mainly proteins that are secreted by a bacterial cell into surrounding fluids and are produced by both Gram negative and Gram positive bacteria. Most are readily destroyed by heat, but they can be converted into toxoids that are used as vaccines. Exotoxins are extremely powerful biological

Endotoxins — are part of the cell wall of Gram negative bacteria. Only small amounts may escape into surrounding fluids from living bacteria. Greater amounts are released when the bacteria die and their cell walls disintegrate. Endotoxins are less potent, and larger amounts are needed to induce disease symptoms. Also, they are heat resistant and cannot be converted into toxoids.