AS- 2848

B.Sc (Hons) (Fifth Semester) Examination, 2013

LBC-504- Botany (Microbial Genetics)

1) (i) (a) Transposition

(ii) (a) Phenotype

(iii) (b) Transposase

(iv) (c) Transduction

(v) (d) Pilin protein

(vi) (a) Lactose permease

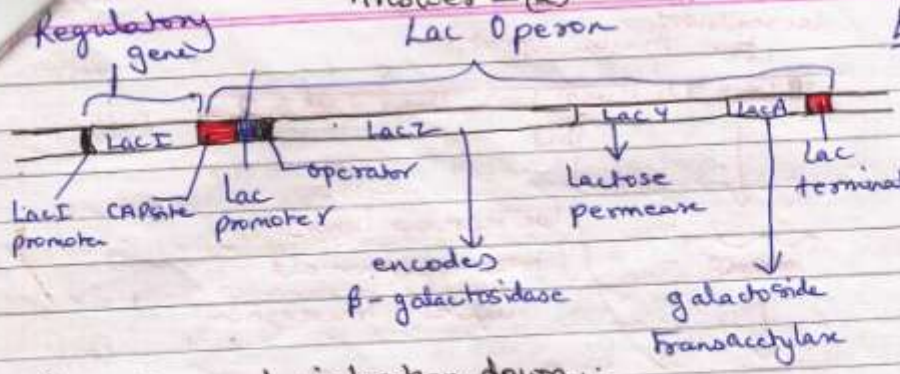
(vii) (c) Extensive DNA damage

(viii) (d) First

(ix) (a) Sensor kinase

(x) (c) cyanotoxin

Answer – (2)



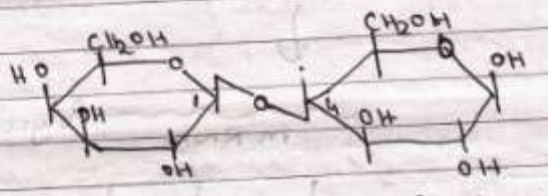
When a compd is broken down, the process is called Catabolic

Lactose Operon — Negative Transcriptional control of Inducible genes or Repression of Lac Operon

Lac operon expresses genes whose products catabolize lactose.

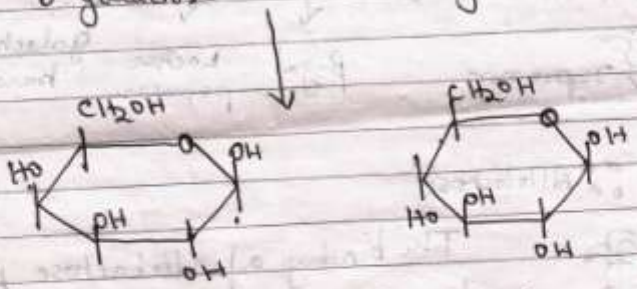
(a) Lactose permease — lac Y gene transports lactose into the cell.

(b) β-galactosidase (β-gal) encoded by lac Z gene — catalyzes the cleavage of β1,4-glycosidic linkage in lactose resulting in monosaccharides



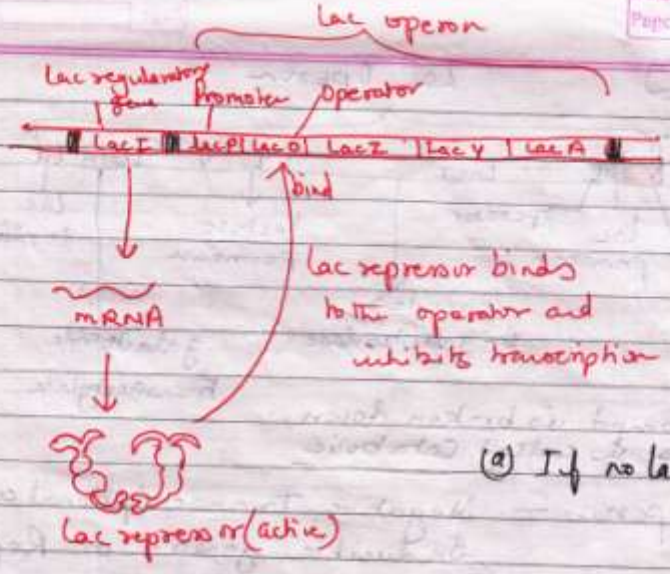
Lactose

D-galactose D-glucose



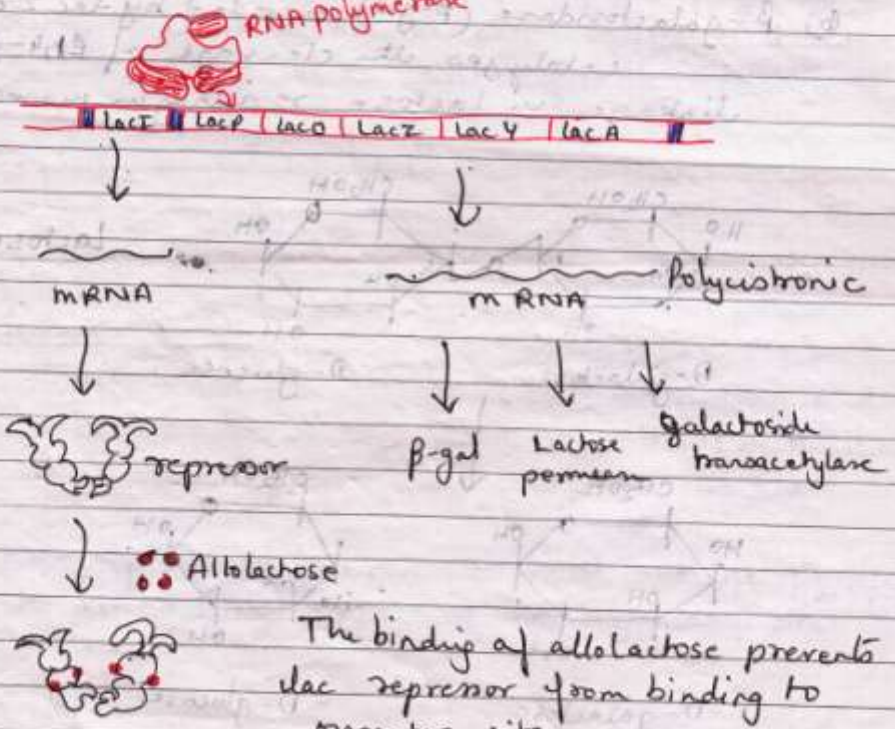
D-galactose D-glucose

(C) LacA encodes detoxifying enz β-galactoside transacetylase inactivate toxic thiogalactosides that are transported into cell along with lactose by permease.



(a) If no lactose in the environment

b) Lactose present in environment



The binding of allolactose prevents lac repressor from binding to operator site.

* When a compd is broken down, the process is called Catabolic. Lac operon expresses genes whose products catabolize lactose.

* ~~ONPG~~ In absence of Lactose, there is very low amount of enz. β gal and permease. When lactose is added all the three enzymes appear in the cell. Lactose is transported into the cell through permease. Some of the lactose is converted into allolactose by few molecules of β-gal found in uninduced cells.

* Allolactose is a rearranged lactose molecule and an inducer of lac operon.

Lac I has its own promoter and is constitutively expressed. It binds at Lac O which is located between LacI and lac Z.

In absence of inducer, lac repressor is bound to lac O preventing expression of the operon.

In presence of inducer, inducer binds to lac repressor ~~from binding~~ and prevents repressor from binding to lac O, leading to transcription of the operon.

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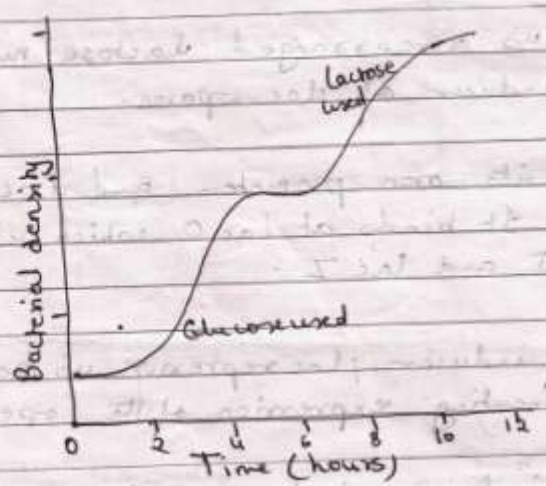
In absence of inducer, lac repressor is bound to lacO preventing expression of the operon.

In presence of inducer, inducer binds to lac repressor from binding and prevents repressor from binding to lacO, leading to transcription of the operon.

Activation of lac Operon by cyclic AMP CAP Protein

If glucose + lactose → E. coli uses glucose first until the sugar is exhausted. Then after a short lag, growth resumes with lactose as the carbon source. This biphasic growth pattern or response is called diauxic growth. The enz for glucose catabolism is constitutive. At this time lac operon is not induced. No lac mRNA or gene products are made.



This effect of glucose is the result of second regulatory mech Catabolite repression.

It affects not only lac gene expression but also other operons that catabolize specific

Diauxic growth – Glucose is first used then lactose. A short lag in growth is present while the bacteria synthesize the enzymes needed for lactose use.

sugars galactose, arabinose and maltose. Against its name, it describes an activating mechanism involving a complex between cAMP and Catabolite activator protein (CAP) or cyclic AMP receptor protein or (CRP).

Decreased level of glucose activates adenylyl cyclase

The level of cAMP is controlled by adenyl cyclase.

ATP $\xrightarrow{\text{Adenylcyclase}}$ cAMP + PP_i

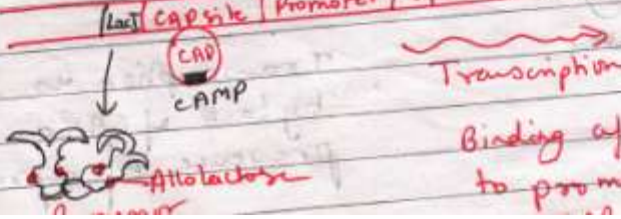
Adenyl cyclase is active only when little or no glucose is available. Thus, the level of cAMP varies inversely with that of glucose. When glucose is unavailable and the catabolism of other sugar might be needed, the amount of cAMP in cell increases allowing cAMP to bind to and activate CAP.

All catabolite operons contain CAP binding site, and CAP must be bound to this site before RNA polymerase can bind its promoter and begin transcription. Upon binding, CAP bends the DNA within two helical turns and increases the binding of RNA polymerase to promoter. Interaction of CAP with RNA polymerase then stimulates transcription.

* Thus all catabolite operons are controlled by two regulatory proteins:
  (a) regulatory protein specific to each operon (lac repressor)
  (b) CAP

Regulation of lac Operon by lac repressor and CAP

(i) lactose but no glucose



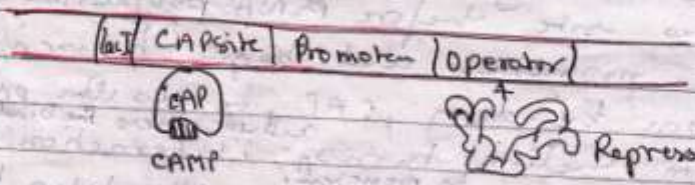
(ii) Lactose and glucose

Repressor (inactive)

CAP (inactive)

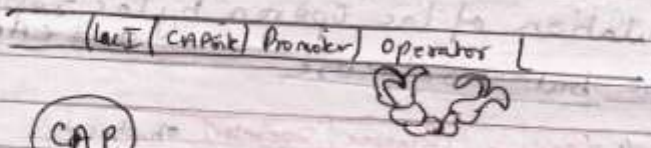
Transcription is inhibited by lack of CAP

(iii) −lactose, − glucose



Transcription is blocked by repressor

(iv) +Glucose −lactose



CAP

Inactive

Transcription is inhibited by lack of CAP and presence of repressor

Answer (3)

(1) Same Sense mutation: Although mutation has changed the nucleic acid sequence of a codon, but the altered codon still encodes the same amino acid. This is possible because the code is degenerate. Both GAU and GAC are codons for aspartic acid. So that a GC for AT in the third position of the DNA codon (fig. B) does not result in an amino acid substitution in the polypeptide. Thus same sense mutations are silent because expression of genes without altering their coding sequences

(2) Missense mutations. A single base pair substitution change a codon for one amino acid into a codon for a different amino acid, resulting in an amino acid substitution in the polypeptide. In fig. C an AT to GC transition in the DNA converts a UGG (Tryptophan) into a GGG (glycine) codon. Most missense mutations reduce or abolish gene expression by preventing the polypeptide from folding up into its active three dimensional configuration.

(3) Non-Sense mutations: A base pair substitution change the codon for an amino acid into a UAG, UAA or UGA chain termination triplex (fig. D). This causes the premature termination of translation, so that only an inactive polypeptide fragment is produced.

(4) Frameshift mutations: These are due to the addition or deletion of one or two base pairs (Fig. E). This shifts the reading frame so that all codons from one contain mutation to the end of the message are read out of phase and encode incorrect amino acids.

- Somatic Mutation – do not affect gametes and cannot be passed on to the next generation.

Answer (4)

Mu phage — Mutator phage

temperate like λ but has unusual property of replicating by transposition.

Transposable elements — sequences of DNA move one place to another on the host genome as discrete genetic units.

in both pro and eukaryotes, play role in genetic variation

Mutator phage → mutation in host genome in which it integrates. → mutation is due to integration of viral genome into the host genes, thus interrupting coding sequences → mutant phenotype.

It is a useful phage in bacterial genetics because it can be used to easily generate bacterial mutants.

Large virus, icosahedral head, a helical tail and six tail

genome consist of linear, double stranded DNA, Most Mu genes are involved in (i) synthesis of head and tail proteins

(ii) imp genes at the end which are involved in replication and host range.

(ii) 39 Kbp of DNA but only 37.2 Kbp constitute actual Mu genome.

Additional length is host DNA attached to the ends of Mu genome.

50 – 150 bp at left, 1–2 Kbp at right → These host sequences only represent DNA seq adjacent to location where Mu was inserted into the genome of its previous host.

When Mu virion is formed, a length of DNA containing Mu genome is excised from the host. But the DNA packaging time, host DNA at the right end varies from one virion to other.

Specific segment → G is invertible. G+ or G⁻ orientation
→ kind of tail fibers

. tail fibers + host cell surface →

G+ → phage make tail fibers that allow E. coli strain K12

G⁻ → E. coli strain C or several enteric bacteria

two alternative tail fiber proteins are encoded on opposite strands within this small G segment.

G+ orientation — promotor for gene S and U is active

G⁻ — different promotor directs transcription of genes S' and U' on the opposite strand.

~~C → Mu repressor for lytic cycle~~

Mu enters the lytic cycle either upon initial infection, if the Mu repressor is not made or by induction of a lysogen.

A → transposase enz

~~Mu DNA integrated~~

When Mu DNA is injected, it is protected from restriction endonuclease by modification system → several adenine residues are modified by acetylation.

Integration

Lytic cycle
When repressor is not made or induction of lysogens

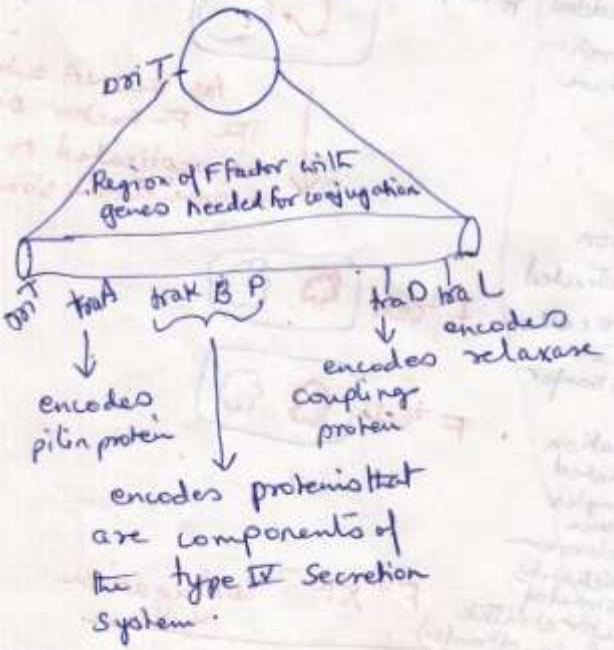
Lysogenic

Initially, only early genes are transcribed but when C protein is expressed (positive activator of late transcription)
↓
Mu head and tail proteins are synthesized.
↓
Eventually, cell is lysed and mature phage particles are released.

Answer (5)

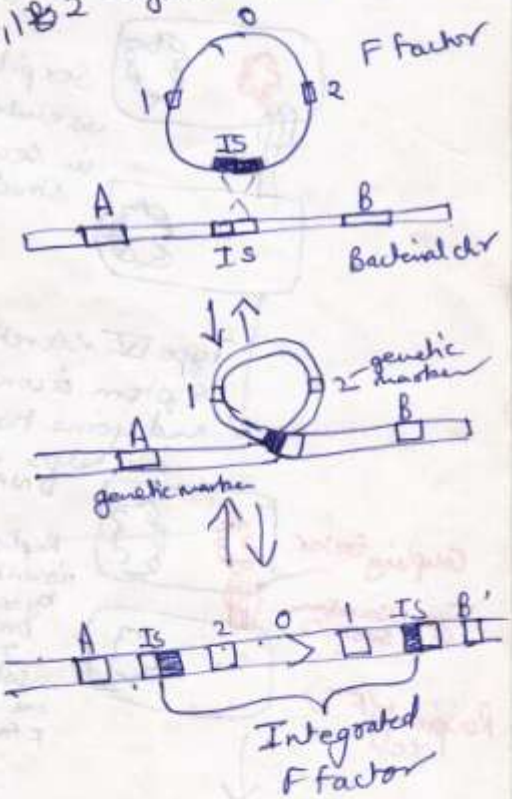
$F^+ \times F^-$ Mating

1952 William Hayes said that the gene transfer through conjugation was polar. There were definite donor (F^+ or fertile) and recipient (F^- or nonfertile) strains and the gene transfer was nonreciprocal. He also found that in $F^+ \times F^-$ mating, the progeny were only rarely changed with regard to auxotrophy (that is, chromosomal genes were not often transferred), but F^- strains frequently became F^+.



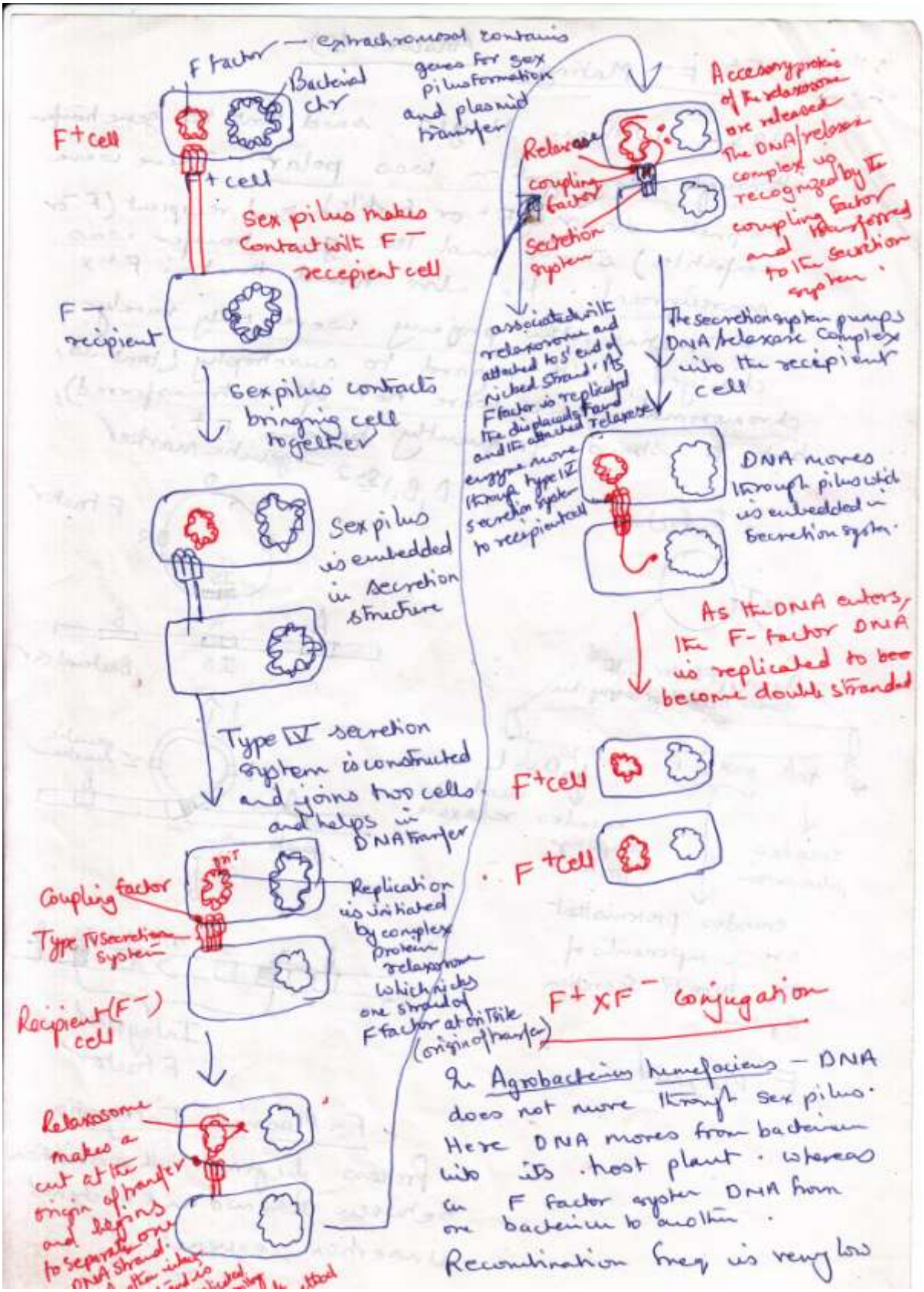
F-Plasmid

A, B, 1, 2 – genetic marker



F-Plasmid Integration

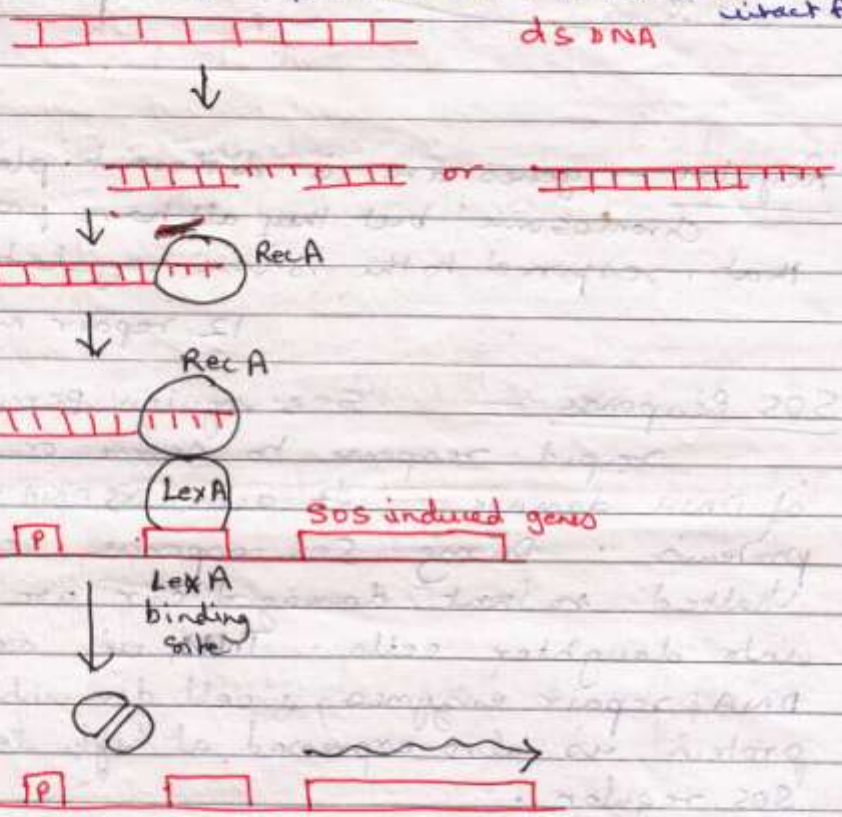
Process begins with association between plasmid and bacterial insertion sequences


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

Answer(6)

Regulation of SOS regulon by proteolytic cleavage of repressor

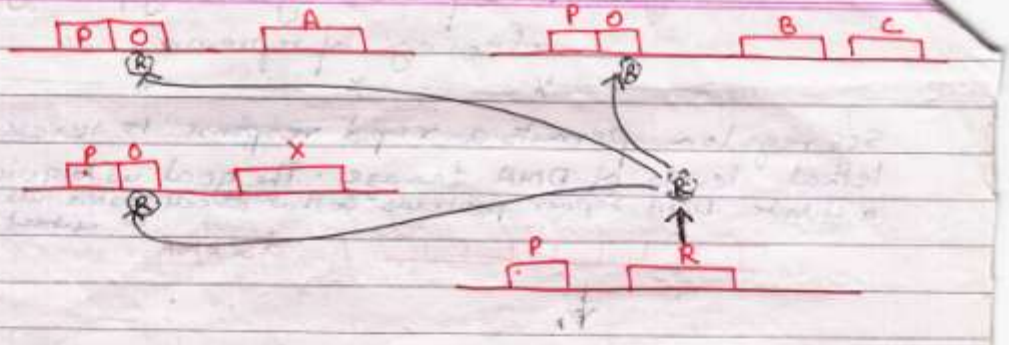
SOS regulon permits a rapid response to severe or lethal levels of DNA damage. The goal is to quickly activate DNA repair proteins so that the cell restore its DNA in intact form.



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.

Answer (7)

Transduction

Third mode of bacterial gene transfer. It is frequent mode of horizontal gene transfer in nature and is mediated by virus.

"Transduction is the transfer of bacterial genes by viruses. Bacterial genes are incorporated into a phage capsid because of errors made during the virus life cycle. The virus containing there genes then injects them into another bacterium completing the transfer.

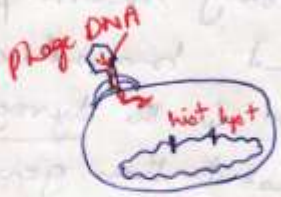
Two types transduction < Generalized / Specialized

Generalized: only during lytic cycle of virulent and some temperate phages and can transfer any part of the bacterial genome.

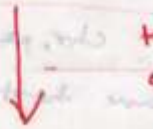
During the assembly stage of phages some part of partially degraded bacterial chromosome may be packaged by mistake. The quality of bacterial DNA carried depends primarily on the size of the capsid.

Answer (7)

Joshua Lederberg and Norton Zinder in 1951

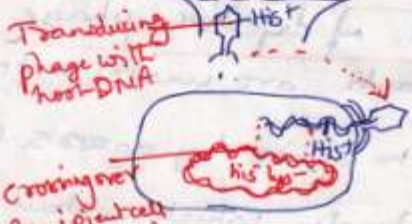


Phage infects Bacterial cell



Host DNA is hydrolysed into pieces and phage DNA and proteins are made

Phages assemble; occarionally a phage carries a piece of the host cell chromosome.



Transducing phage injects ~~into~~ its DNA into a new recipient cell

The transduced DNA is recombined into the chr of the recipient cell.

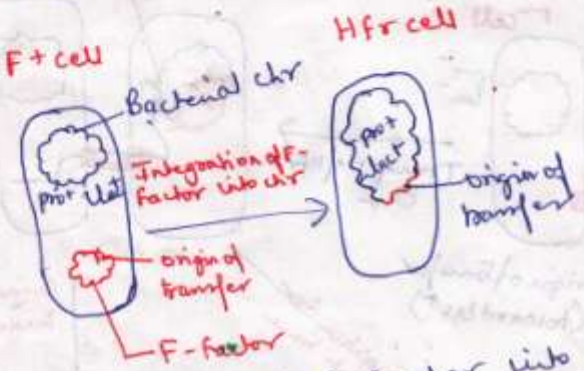
The recombinant bacterium has a genotype (his+ lys⁻) that is different from recipient cell (his⁻ lys⁻).

Generalized Transduction in Bacteria

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⁻

Answer (7)

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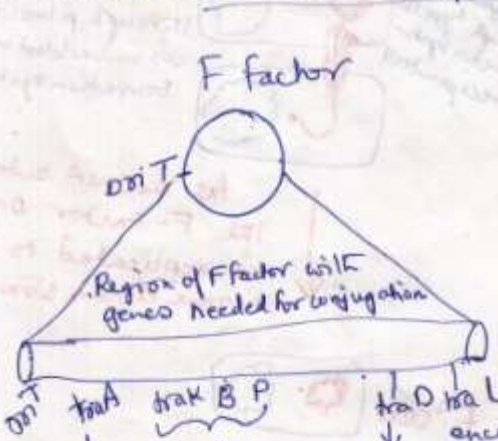
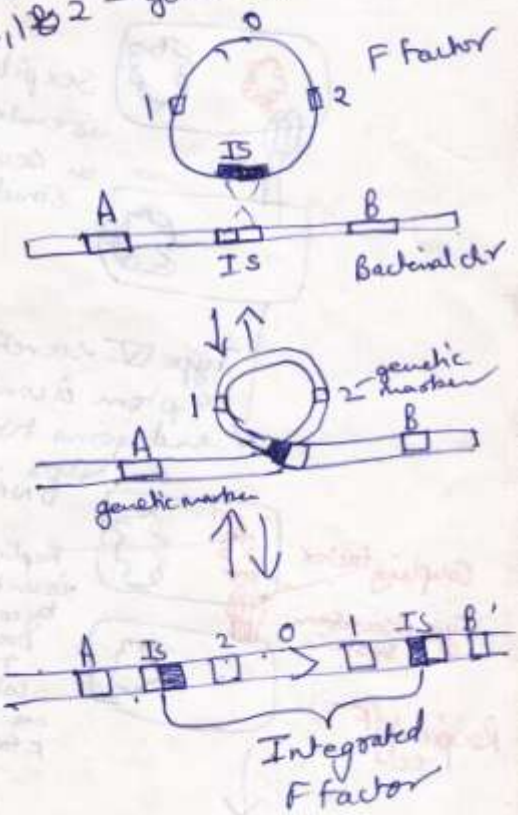


F-Plasmid

A, B, 1, 2 — genetic marker



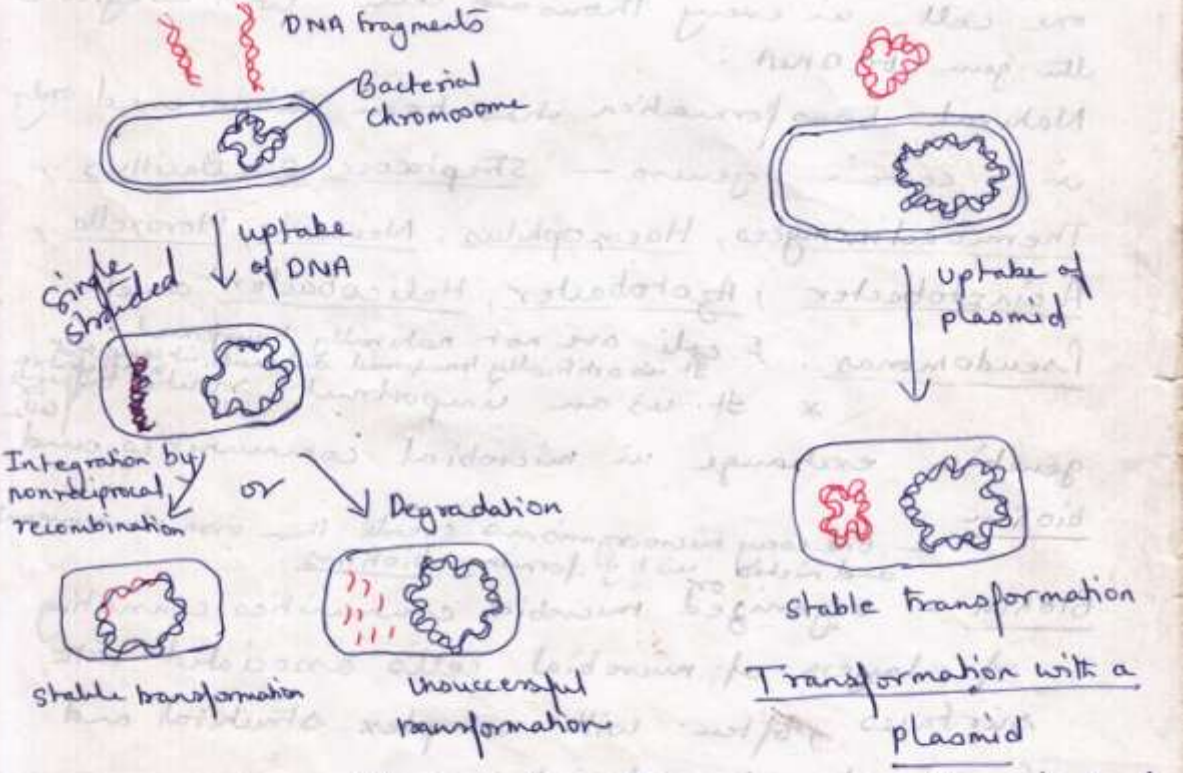
F-Plasmid Integration

Process begins with association between plasmid and bacterial insertion sequences

Answer (8)

Transformation — DNA can move between the two strains of bacteria (Streptococcus (Diplococcus) pneumoniae) through transformation and is discovered by Fred Griffith in 1928.

In other words uptake of naked DNA molecule or fragment from the medium by a cell and incorporation of this DNA molecule into the recepient chromosome in a heritable form



Transformation with DNA fragments

Transforming DNA is integrated at a homologous region of the genome.

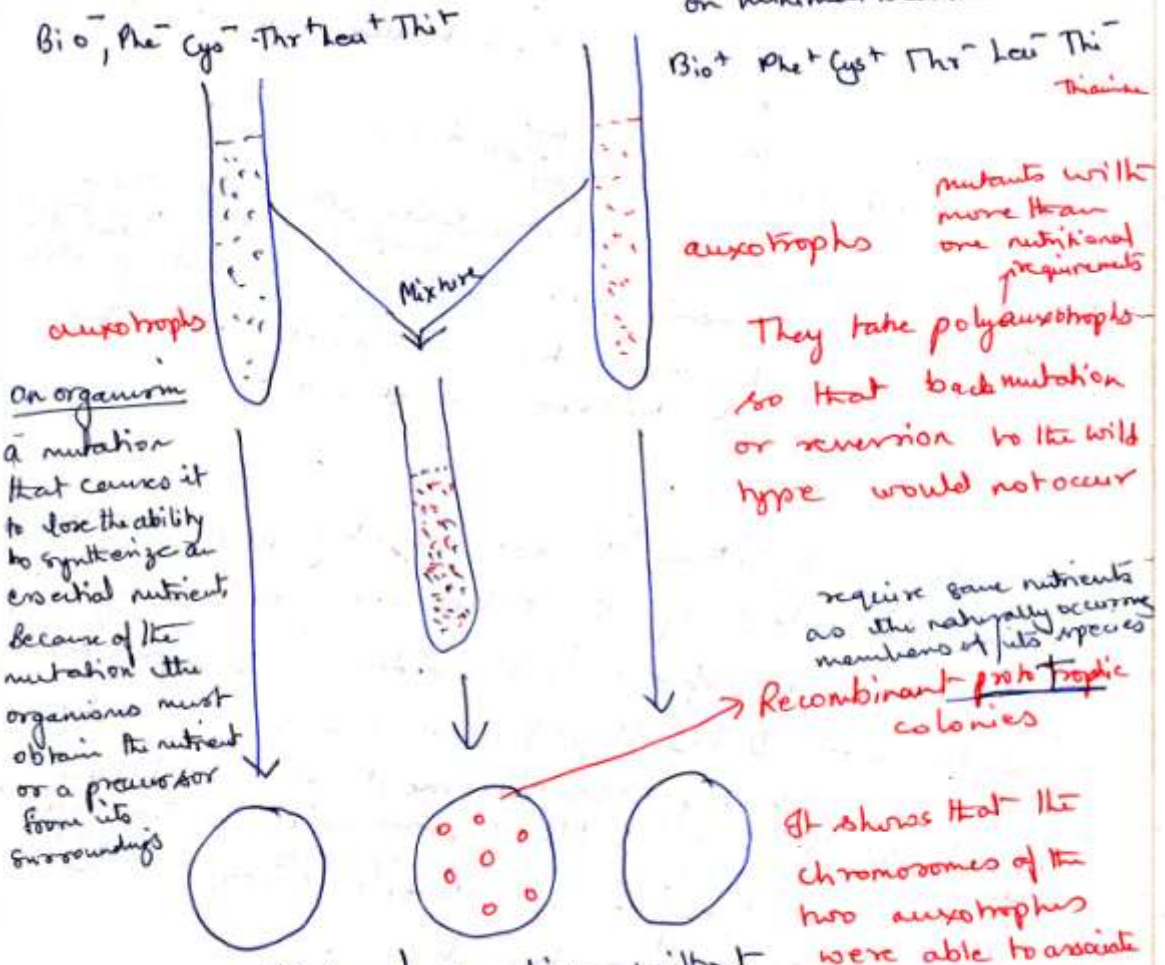
Transformation with a plasmid

is often induced artificially in the laboratory.

Conjugation

The transfer of DNA by direct cell to cell contact, came from an elegant experiment performed by Joshua Lederberg and Edward Tatum (1946).

They mixed 2 auxotrophs → incubated for several hours in nutrient medium → then plated on minimal medium



An organism a mutation that causes it to lose the ability to synthesize an essential nutrient, because of the mutation the organisms must obtain the nutrient or a precursor from its surroundings

It shows that the chromosomes of the two auxotrophs were able to associate and undergo recombination.

Evidence For Bacterial Conjugation

Answer (B)

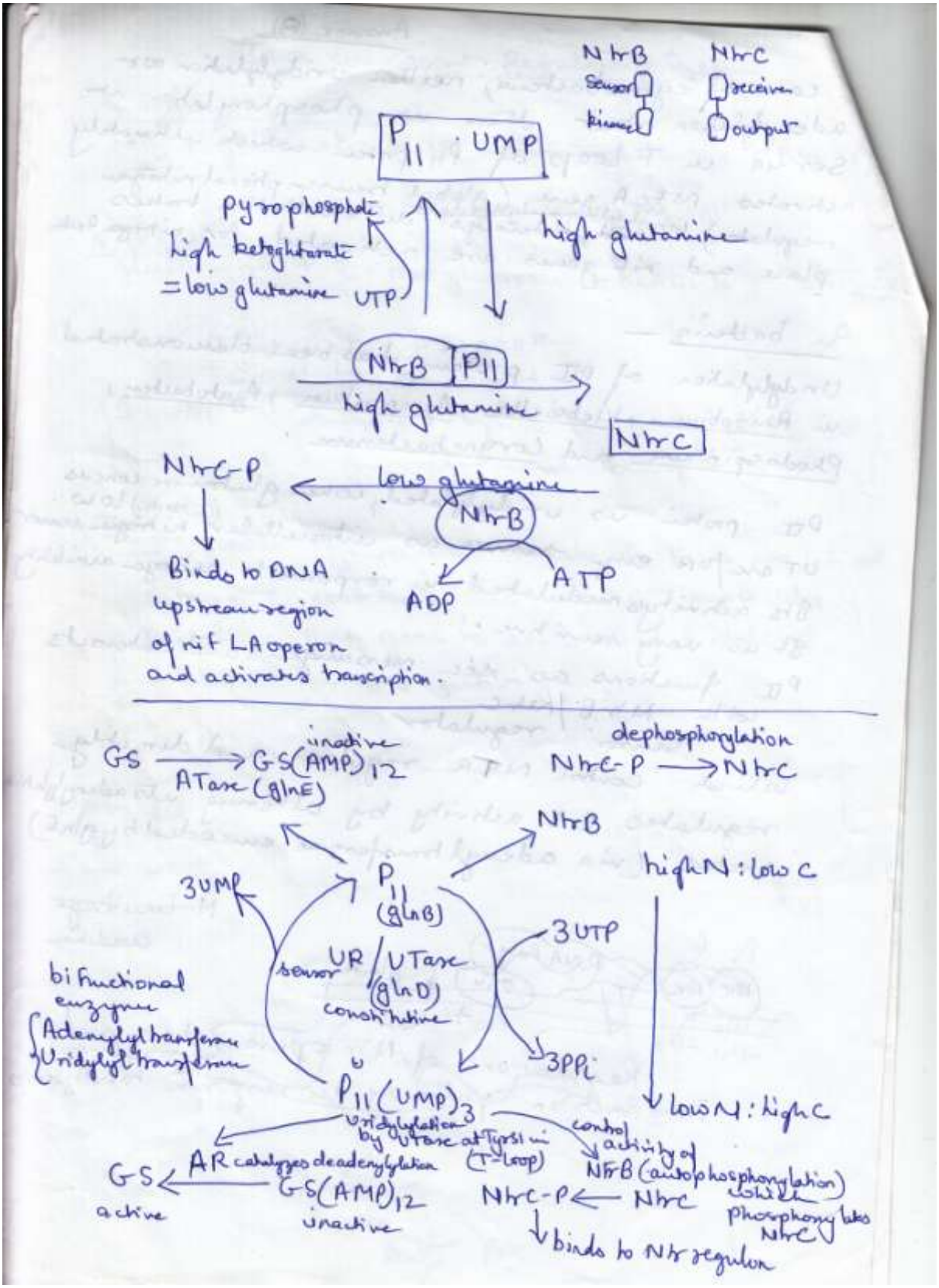
In case of cyanobacteria, neither uridylylation nor adenylylation. but there is phosphorylation at Ser 49 in T loop of P_{II} protein which ultimately activates NtcA gene (global transcriptional nitrogen regulator) ~~which ultimately leads to~~ Heterocyst differentiation takes place and nif genes are 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 is UT are/UR enz (C:N ratio) low. serves as intracellular nitrogen sensor Its activity is modulated in response to nitrogen availability It is very sensitive.

PII functions as sec messanger 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
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active
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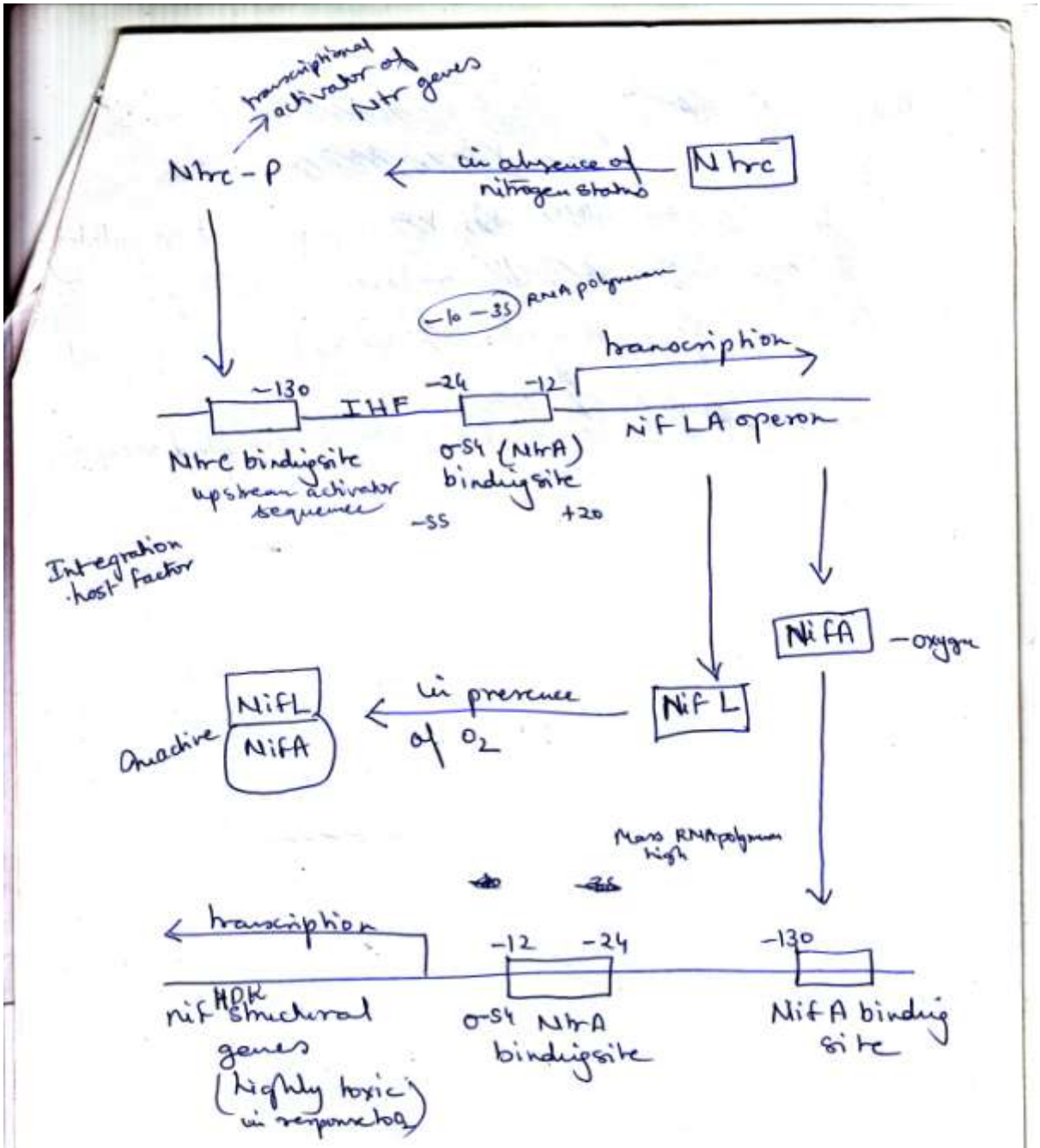
inactive
NtrC-P
NtrC
binds to Ntr regulon



NifLA operon is absent in cyanobacteria.

Leghaemoglobin —

Cyt oxidase —

Answer(9)

Antibiotic – is chemical substances secreted by micro organisms 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.