M.SC. (Microbiology) 2nd semester ,
Paper 2- (Microbial Genetics) ,
Unit- 4th

“Lambda phage DNA and Its genetic organization and life cycle of lambda phage”

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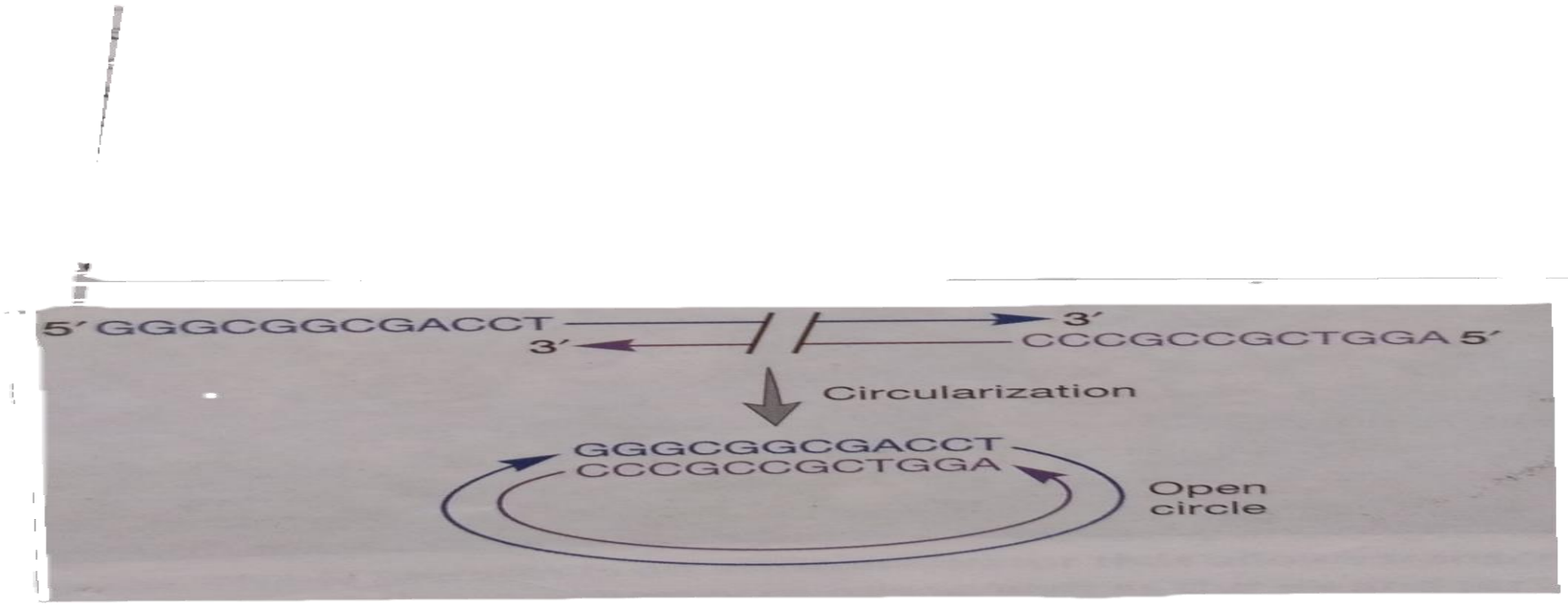
Lambda phage is a double stranded DNA phage that infects the K12 strain of *E.coli* . It has an icosahedral head 55 nm in diameter a non contractile tail with a thin tail fibre at its end **. Its DNA genome is a linear molecule with cohesive ends- single stranded stretches , 12 nucleotides long , that are complementary to each other and can base pair.**

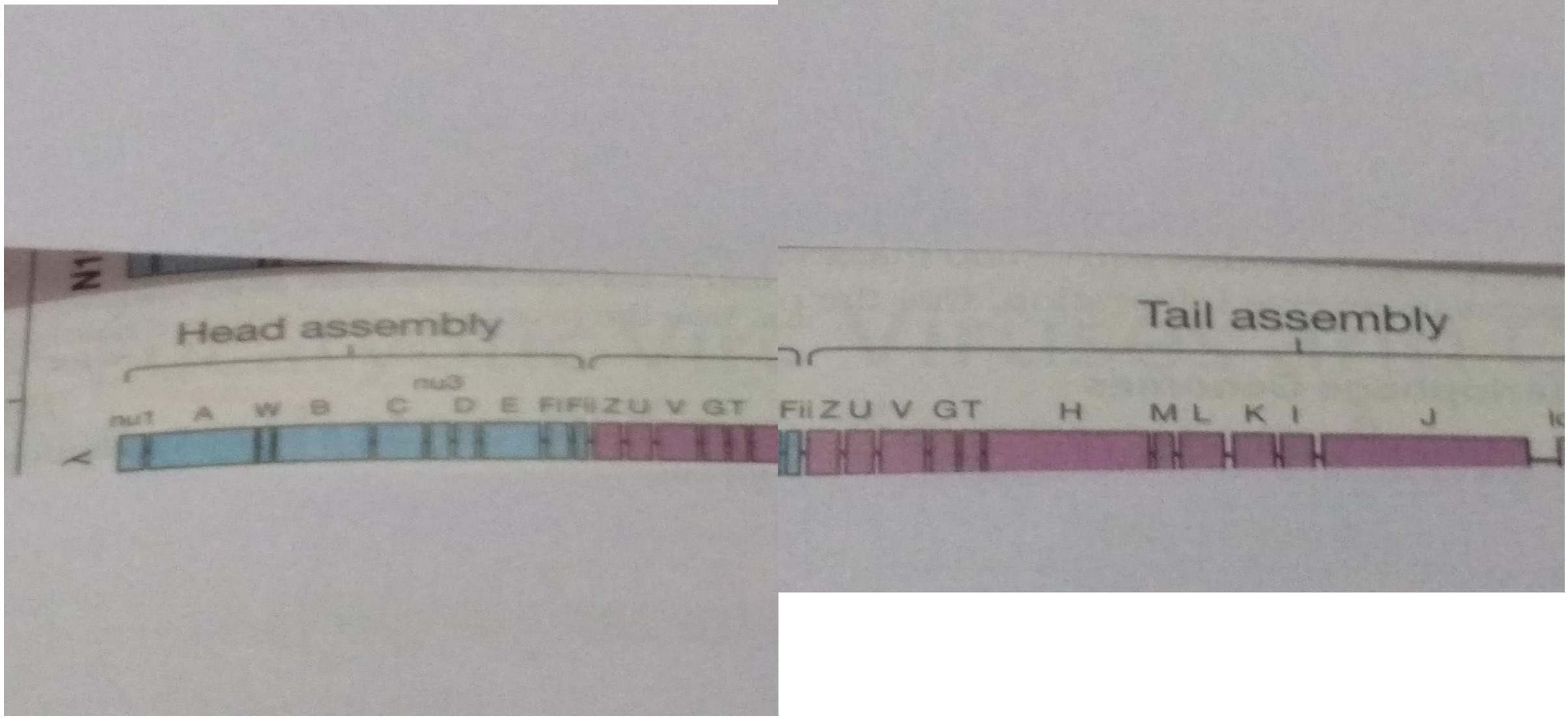


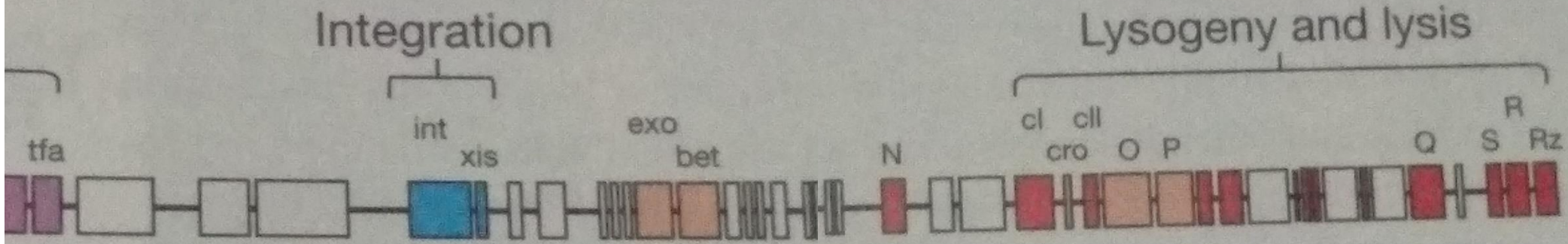
When lambda phage attaches to its host then injects its genome into the cytoplasm , leaving the capsid outside. **Once inside the cell , the linear genome is circularized when the two cohesive end base pair with each other** .The breaks in the strands are sealed by the host cell's DNA ligase.

The lambda genome has been carefully mapped , and over 40 genes have been located. Most genes are clustered according to their function, with separate groups involved in head synthesis, tail synthesis , lysogeny , DNA replication , and cell lysis .

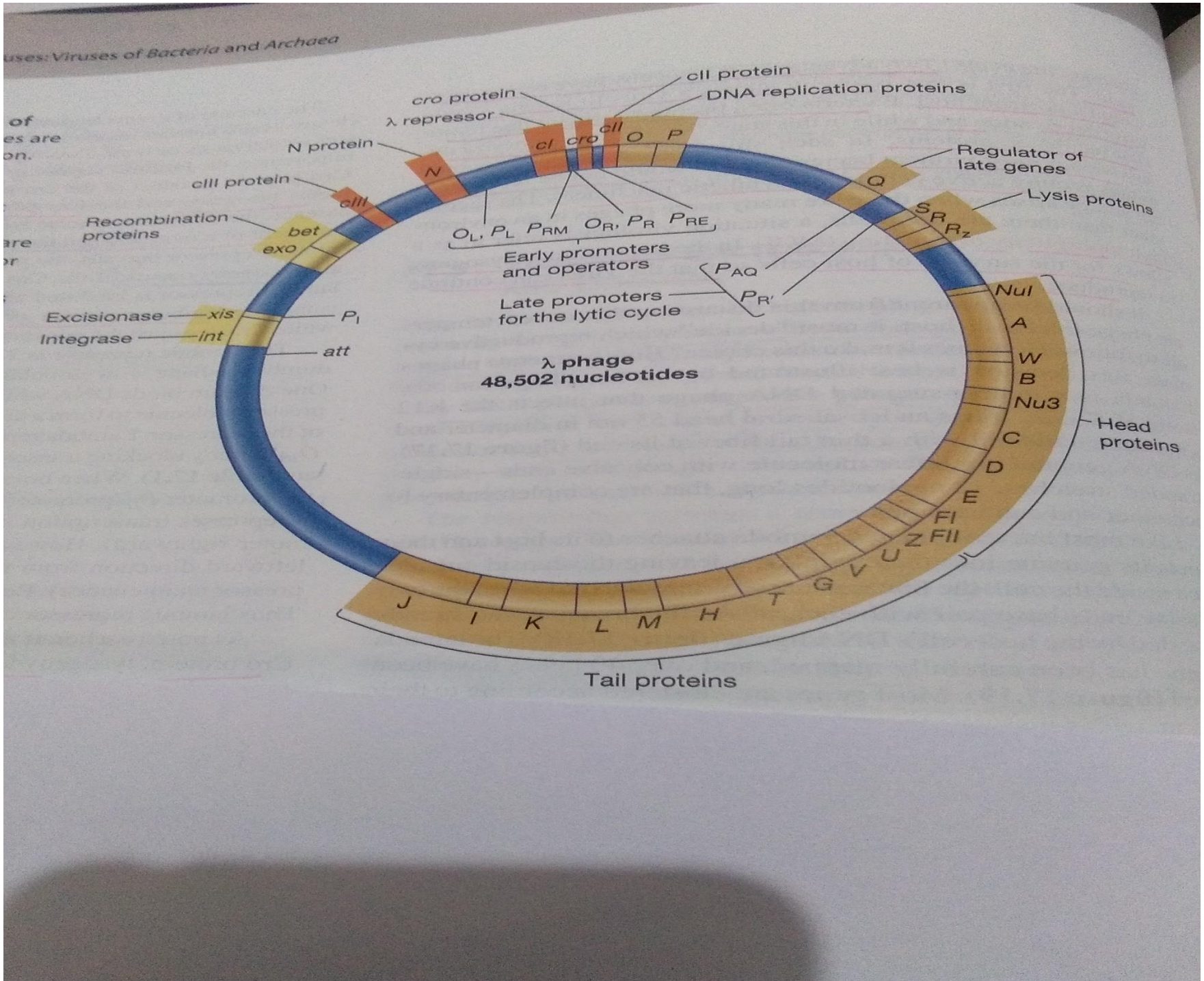
This organization is important because once the genome is circularized, **a cascade of regulatory events occurs that determine if the phage persue a lytic cycle or establishes lysogeny .**

Details of lambda genome




Integration
Lysogeny and lysis
tfa
int
xis
exo
bet
N
cI
cII
cro
O
P
Q
S
R
Rz

Lambda phage genome



List of Genes and their function of lambda phage

TABLE 1. *Genes of phage λ and their function*

Gene symbol	Approx map coordinates[a]	Gene function and/or protein activity	References[b]
m	0	Left cohesive end of the mature DNA molecule; the first 12 nucleotides of the 5′ end of the *l* (transcribed leftward) strand protrude as a single-stranded chain, complementary to *m′*.	13, 31, 38, 57, 60, 61, 69, 87, *146*, *147*, 151, 206, 218, 228, *229*, *237*, *238*
Nu1	0/0.5	Involved in DNA packaging and cohesive end formation; may activate A protein.	**8**, 145, D
A	0.5/4.7	DNA packaging into proheads and formation of cohesive ends; protein is 79,000 daltons (79K).	**5–7**, 19, 67, 90, **95**, 103, 109–111, 132, 139, 144, 145, 155, 197, 204, 206, 210, 226, 229, 231
W	4.7/5.0	Modifies DNA-filled heads in an unknown way to allow FII action; protein is 5–10K.	**28**, 93, 132, 155, 205, 226
B	5.0/8.3	Structural component of the capsid; B protein and a cleaved derivative B* form the head-tail connector; B is 59–62K; B* is 53–56K.	7, 19, 67, 85, 86, 91, 120, 139, **140**, **141**, 142, 145, 155, 169, 204, 223, 231, 242
C	8.3/11.3	Structural component of capsid; the 56–61K C protein is present in the capsid as two cleaved derivatives, fused to a cleaved derivative of E; the two cleavage-fusion products, termed X1 and X2, are 29 and 27K, respectively.	7, 19, 67, 84–86, 91, 120, 139, **140**, **141**, 142, 144, 145, 155, 169, 204, 223, 231, 242
Nu3	11.3/12.3	Transient morphopoietic core for capsid assembly; protein is 19K.	7, 86, 91, 103, **140**, **141**, 144, 145, 169, 242
D	12.3/12.8	Major component of the phage head; the 11–12K protein is incorporated into the capsid during or after DNA packaging.	4, 7, 19, 29, 91, **92**, 95, 98, **111**, 129, 132, 139, 143–145, 204, **207**, 226, 231, 234
E	12.8/14.8	Major component of the capsid; 10 to 12 of the 420 molecules of the 37K E protein are present in the capsid fused to C, forming X1 and X2 (see *C*).	4, 7, 16, 19, 27, 29, 85, 86, 91, 94, 98, 101, 103, 120, 132, 139, **140**, **141**, 144, 145, 155, 169, 204, 223, 226, 231, 234
FI	14.8/15.7	Packaging and maturation of DNA; the 17K protein may confer specificity on A protein.	7, **8**, 14, 19, 142, 144, 155
FII	15.7/16.3	Structural component of DNA-filled heads; the 11.5K protein mediates tail attachment.	12, 14, 15, 19, 24, **25**, 28, 132, 144, 145, 155, 226
Z	16.3/17.4	Structural component of the proximal end of the tail; the 20K protein is involved in proper positioning of the right end of the DNA molecule in the tail.	**113–116**, 155, 218, 219
U	17.4/18.4	Structural component of the tail; the 14–16K protein is the tail length determination factor.	**113–117**, 120, **125**, 139, 144, 155
V	18.4/19.9	Major protein of the tail tube; protein is 25–32K.	6, **113–117**, **125**, 143, 144, 155
G	19.9/21.7	Involved in the assembly of the tail initiator; the 33K protein is possibly a structural component.	67, **116**, 120, **125**, 139, 144, 155, 231
T	21.7/22.6	Structural component of the tail; the 16K protein is involved in the assembly of the tail initiator.	67, 139, 144
H	22.6/27.3	Cleaved form of the 87–90K H protein, the 78–79K H*, is a structural component of the tail, involved in DNA injection.	19, 83, 103, **113**, **116**, 120, **125**, 139, 143, 144, 155, 183, 231
M	27.3/27.9	Structural component of the tail; the 10K protein is involved in tail initiator assembly.	19, **113**, **114**, **116**, **117**, 120, 139, 155, 231
L	27.9/29.6	Structural component of the tail initiator; protein is 29K.	19, 103, **114**, **116**, 120, **125**, 139, 144, 155, 231
K	29.6/31.2	Temporarily associated with the tail initiator, function unknown; protein is 27K.	19, 103, **114**, **116**, 120, **125**, 139, 144, 155, 231
I	31.2/31.8	Involved in assembly of the tail initiator.	19, 103, **113**, 116, 155, 223, 231
J	31.8/39.4	Structural component of the tail; the 130–140K protein forms the tail fiber and determines host range of adsorption.	17, 19, 42, 67, 82, **113**, **114**, **116**, 120, **125**, 139, 143, 144, 155, 188, 231

TABLE 1.—*Continued*

Gene symbol	Approx map coordinates[a]	Gene function and/or protein activity	References[b]
b	39.4/57.3	Although this region of the chromosome is called the silent region, it codes for several proteins of unknown function.	**32**, 40, 81, 82, 119, 128, 134, 144, 157, **173**, 177, B
a·*a*′/(*P*·*P*′ or *att*P)	57.3	Determines location and specificity of site-specific recombination; 15-base homology with host site *b*·*b*′.	*39*, 40, 50, 99, *127*, 192, 194
int	57.4/59.8	Integrative and excisive recombination; the 40K protein provides sequence recognition for *a*·*a*′.	3, 45, 50, 59, 70, 71, 73, 74, **121**, **122**, 138, 148, 194, 243
xis	59.8/60.2	Excisive recombination.	45, 59, 76, 108
p_I	(60.2)	Promoter for transcription from the *int* gene under positive regulation by cII and cIII proteins.	59, 190, 191
*red*X	(64.7/66.1)	General recombination; the 24K protein ("λ-exonuclease") is a 5′-exonuclease active on double-stranded DNA.	**23**, 49, **131**, 165, **167**, 193, 194
*red*B	66.1/67.8	General recombination; the 28K protein ("*β*-protein") associates with λ-exonuclease.	**23**, 49, 165, 193, 194
gam	67.8/68.7	Regulation of DNA replication; the 16K ("gamma") protein inhibits the RecBC DNase, an antagonist of the rolling-circle mode of replication.	58, **112**, **180**, 195, 225, 244
kil	(68.7/69.2)	Loss of host cell viability, associated with an inhibition of cell division.	75
cIII	(68.8/70.7)	Establishment of lysogeny (together with cII); the cII and cIII proteins activate transcription from the *c*I and *int* genes and repress transcription from the lysis, head, and tail (and probably replication) genes (see cII, p_E, and p_I).	33, 34, 36, 51, 55, 106, 118, 122, 133, 170, 201
ral	(70.7/71.6)	Partial alleviation of restriction of λ DNA by K-12 restriction endonuclease.	A
t_L	(71.8)	Termination for the earliest (immediate-early) stage of RNA synthesis initiated at p_L, a *ρ*-mediated event.	123, 126, **174**
N	72.5/73.3	Positive regulation of early development; the 13K protein activates delayed-early transcription from recombination, replication, and regulation genes; N protein prevents termination events at t_L, t_{R1}, and t_{R2}.	1, 19, 66, 80, 124, 126, 149, 166, 174, 189, 196, 221, 222
p_L	73.5	Promoter for transcription of the *N* gene during the immediate-early stage of development and for the *N* through recombination region during the N-activated delayed-early stage of development (see *N*); repressed by Cro during the late stage of lytic development and by cI during the maintenance stage of lysogeny (see *c*I and *cro*).	**10**, 11, 124, *136*, **174**, 217
o_L	73.5/73.6	Operator for regulation of transcription from p_L; the o_L sequence defines three 17-base binding sites, o_{L1}, o_{L2}, o_{L3}; the cI and Cro proteins bind at o_L to prevent binding by RNA polymerase and thus transcription from p_L.	52, 96, 102, 107, *135*, *136*, 159, **163**, 172, 199, **203**, **213**, **214**, **235**, **236**
t_M	(74.2)	Terminator for transcription of the *c*I and *rex* genes (initiated at p_M) during the maintenance stage of the lysogenic pathway.	79, 102
rex	(74.2/75.9)	Restricts growth of T4rII mutants and helps cell growth in limiting carbon sources; protein is 29K.	77, 97, 130, B

TABLE 1.—*Continued*

Gene symbol	Approx map coordinates[a]	Gene function and/or protein activity	References[b]
cI	76.9/78.4	Maintenance of lysogeny through a repression of RNA from early genes; the 26K protein binds as a dimer (or tetramer) to o_L and o_R, repressing transcription from p_L and p_R and regulating transcription of the *cI* and *rex* genes, positively at low cI levels and negatively at high cI levels (see o_L and o_R).	30, 53, 55, 102, 106, 107, **137**, **161**, **162**, 171, 172, *182*, **203**, **236**
p_M (p_{rm})	78.4	Promoter for transcription of the *cI* and *rex* genes during the maintenance stage of lysogeny; the cI and Cro proteins bind at o_R to regulate this transcription (see o_R, *cI*, and *cro*).	55, 79, 102, *135*, *160*, 170, 171, 241
o_R	78.4/78.5	Operator for regulation of transcription from p_M and p_R; the o_R seqeuence defines three 17-base binding sites, o_{R1}, o_{R2}, o_{R3}; the cI and Cro proteins bind at o_{R1} and o_{R2} to prevent binding of RNA polymerase and thus transcription from p_L; cI binding at o_{R1} (and/or o_{R2}) enhances transcription from p_M, whereas cI and Cro binding at o_{R3} prevents transcription from p_M.	52, **64**, 96, 102, **104**, 107, *135*, **137**, *160*, **163**, 171, **203**, **213**, **214**, **235**, **236**
p_R	78.5	Promoter for transcription of the *cro* gene (and limited *cIIOP* transcription) during the immediate-early stage of development and for the *cro* gene onwards during the N-activated delayed-early stage of development (see *N*); repressed by Cro during the late stage of lytic development and by cI during the maintenance stage of lysogeny (see *cI* and *cro*).	**10**, 11, 124, *135*, **174**, 217, *227*
cro (*tof*, *fed*)	78.6/79.0	Regulation of late stage of lytic development; Cro represses early transcription and is also required directly or indirectly for normal late replication; the 7K protein binds as a dimer to o_L and o_R, repressing transcription form p_L, p_R, and p_M (see o_L and o_R).	18, 35, 52, 55, 63, **64**, 65, *100*, **104**, 123, 154, 158, 159, *176*, *186*, 199, 200, **213**, **214**, 215
t_{R1}	79.2	Terminator for most of the earliest ("immediate-early") stage of RNA synthesis initiated at p_R, a ρ-mediated event.	80, 149, **174**, *178*, *186*
p_E (p_{re})	79.2	Promoter for transcription of the *cI* and *rex* genes during the establishment stage of lysogeny; positively regulated by cII and cIII proteins.	51, 170, 201, C
cII	79.2/79.8	Establishment of lysogeny (together with *cIII*); the 11K cII protein (aided by cIII) activates transcription from the *cI* and *int* genes and represses transcription from the lysis, head, and tail (and probably replication) genes (see *cIII*, p_E and p_I).	33, 34, 36, 51, 55, 106, 118, 122, 133, 170, *186*, 201, 240, B
p_O	79.9	Promoter for transcription of the 4S or "OOP" RNA, an 81-base RNA, terminating in the absence of ρ, that so far lacks a clearly defined function (it has been postulated to be involved in initiation of replication or the establishment of repression).	*37*, 79, *184*
O	79.9/81.9	Initiation of the early (simple-circle) mode of DNA replication (with *P*); the 34K protein probably interacts with the origin sequence and P protein, and the complex directs the host DNA propagation enzymes to replicate λ DNA; O and P proteins may also be required for the propagation stage of replication (see *P* and *ori*).	19, 56, 68, 105, 152, 164, *186*, 212, 224, 240, B

TABLE 1.—*Continued*

Gene symbol	Approx map coordinates[a]	Gene function and/or protein activity	References[b]
ori	80.9	Origin (initiation site) for the bidirectional early (simple-circle) mode of DNA replication; activation of the origin requires O and P proteins (and probably RNA synthesis through or near the site) (see *O* and *P*).	*41*, 44, 168, 185, 208
P	81.9/83.3	Initiation of the early (simple-circle) mode of DNA replication (with *O*); the 24K protein probably interacts with O protein, and the resultant *O/P/ori* complex directs the host propagation enzymes to replicate λ DNA; O and P proteins may also be required for the propagation stage of replication (see *O* and *ori*).	56, 105, 152, 153, 212, 224, B
t_{R2}	(83.3)	Terminator for the residual fraction (after t_{R1}) of the earliest (immediate-early) stage of RNA synthesis initiated at p_R, a ρ-mediated event.	80, 149, 178
Q	90.8/92.1	Positive regulation of the late stage of lytic development; the 23K protein activates late transcription (initiated at p'_R) from the lysis, head, and tail genes; Q might provide for new initiation events or prevent termination of a small 198-base 6S RNA synthesized (at least in vitro) from the p'_R region (see p'_R)	19, 42, 89, 105, 149, 150, 175, 196, 198
p'_R	(93.1)	Promoter used for transcription of the head, tail, and lysis genes, subject to positive regulation by Q protein (see *Q*).	89, 175, *198*
S	(93.1/93.9)	Cell lysis (together with *R*); the S protein is probably involved in a turnoff of certain host functions as a necessary prerequisite for cell lysis, perhaps through a direct effect on the cell membrane; S^- mutants accumulate large numbers of intracellular phage.	2, 72, 78
R	(93.9/95.0)	Cell lysis (together with *S*); the 18K (endolysin) protein is an endopeptidase that produces lysis through hydrolysis of the cross-linking bond in murein.	**9**, 22, 54, 62, **216**
m'	100	Right cohesive end of the mature DNA molecule; the first 12 nucleotides of the 5′ end of the *r* strand protrude as a single-stranded chain, complementary to *m*.	13, 31, 38, 57, 60, 61, 69, 87, *146*, *147*, 151, 206, 218, 228, *230*, *237*, *238*

[a] Approximate map coordinates were determined mainly from electron microscopic data on heteroduplex structures of genetically characterized deletions and from molecular weights of proteins; the extensive set of heteroduplex data by Szybalski and co-workers has been particularly helpful in this effort (11, 38, 99, 211). Coordinates likely to be off by more than ±0.2 units are in parentheses.

[b] Reference numbers in roman type denote studies of gene function; boldface numbers denote studies of activity in vitro with purified proteins; italic numbers denote nucleotide or amino acid sequences. Letters refer to manuscripts in preparation or submitted for publication but not yet in press: (A) L. De Brouwere, M. Zabeau, M. Van Montagu, and J. Schell; (B) C. Epp and M. L. Pearson; (C) M. Jones, R. Fischer, I. Herskowitz, and H. Echols; (D) R. A. Weisberg and N. Sternberg.

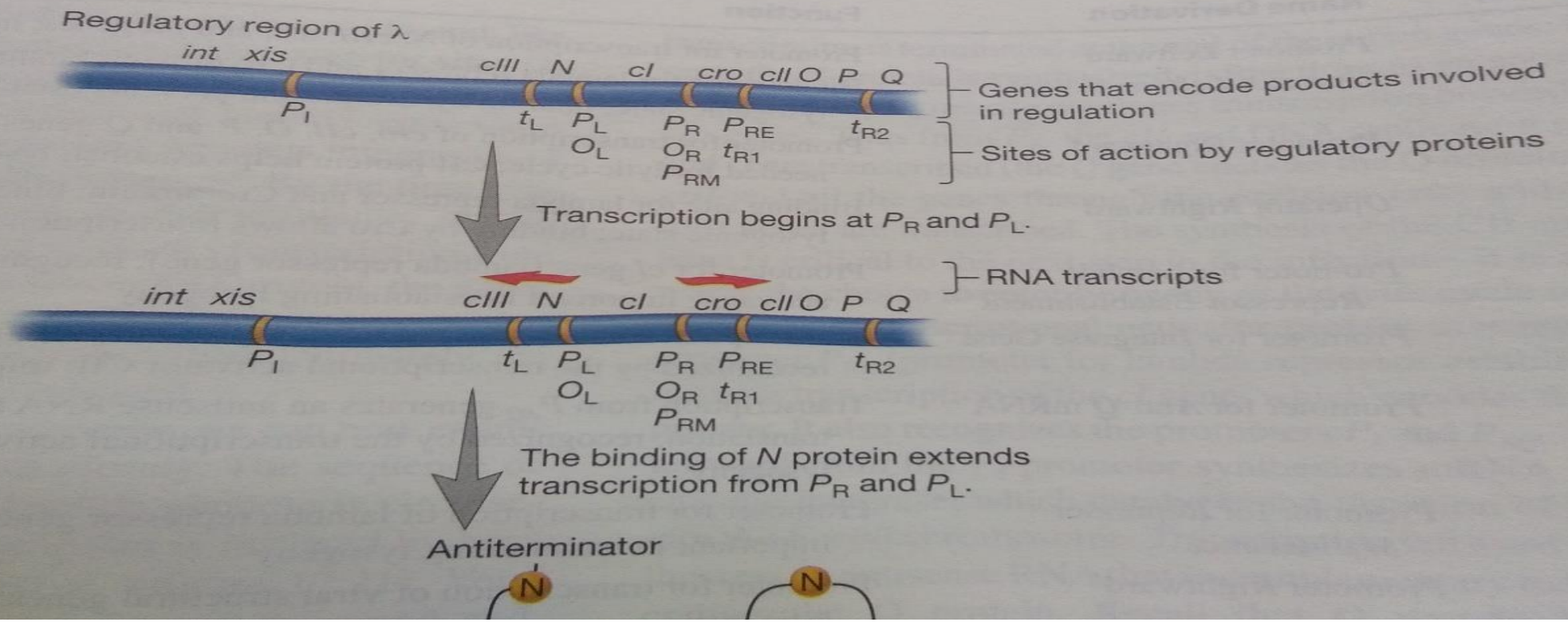
percase designations (19); genes for regulatory proteins needed for lysogeny were given lowercase designations (106); and "other" genes nonessential for productive growth (or thought to be) were given three-letter designations (e.g., *int, red*). We consider this situation unfortunate, but suspect that an effort for complete consistency at this stage will cause more confusion than it will remedy. Thus we have adopted most of the current nomenclature and have only sought to explain it (Table 1). In order not to have the same name for two things (e.g., gene *P* and attachment site P), we have used lowercase italic letters throughout for sites of protein activity and retained uppercase italic letters and italic three-leter designations for genes that code for proteins. The proteins themselves are designated by nonitalic letters, generally followed (in the text) by protein; in the field of bacteriophage morphogenesis, gene products are more usually

Life-Cycle of LAMBDA PHAGE

Leftward genes are responsible for Lysogenic cycle and rightward gene are responsible for Lytic cycle of lambda phage.

Two regulatory proteins are important
The lambda repressor which is the product of c I gene and Cro protein that is the product of cro gene.

The lambda repressor protein promotes lysogeny and the cro protein promote the lytic cycle.
If lambda repressor prevails, the production of Cro protein is inhibited and lysogeny occurs.
If the cro protein prevails , the production of lambda repressor is inhibited and the lytic cycle occurs this is because lambda repressor prevents transcription of viral genes.


Regulatory region of λ
int xis
cIII N cI cro cII O P Q
P_I
t_L P_L O_L
P_R P_{RE} O_R t_{R1} P_{RM}
t_{R2}
Genes that encode products involved in regulation
Sites of action by regulatory proteins
Transcription begins at P_R and P_L.
RNA transcripts
int xis
cIII N cI cro cII O P Q
P_I
t_L P_L O_L
P_R P_{RE} O_R t_{R1} P_{RM}
t_{R2}
The binding of N protein extends transcription from P_R and P_L.
Antiterminator
N
N

The lambda repressor is 236 amino acids long and folds into a dumbbell shape with globular domains at each end.
One domain binds DNA while the other binds another lambda repressor molecule to form a dimer . The dimer is most active form of lambda repressor .

Lambda repressor binds two operator sites , OL and OR thereby blocking transcription of most viral genes.

When bound at OL , it represses transcription from the promoter PL. Likewise , when bound at OR it represses transcription in the rightward direction from PR .It also activates transcription in the leftward direction from the c I promoter PRM (promoter for repressor maintenance.)

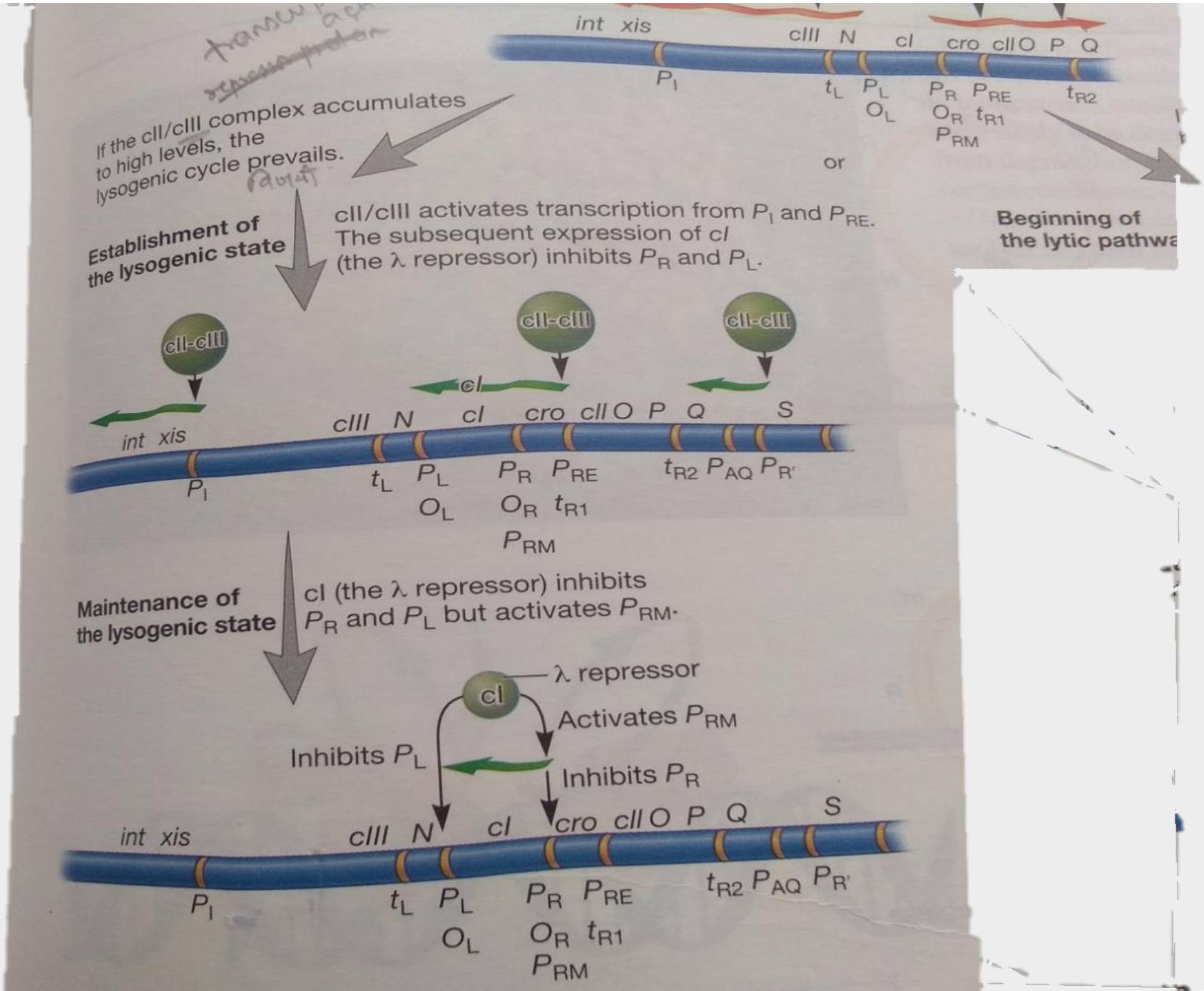
PL (promotor leftward) – promoter for transcription of N , c III , xis , and int genes; important in establishing lysogeny .
OL (Operator leftward)- binding site for lambda repressor and cro protein ; binding by lambda repressor maintains lysogenic state ; binding by cro protein prevents establishment of lysogeny .

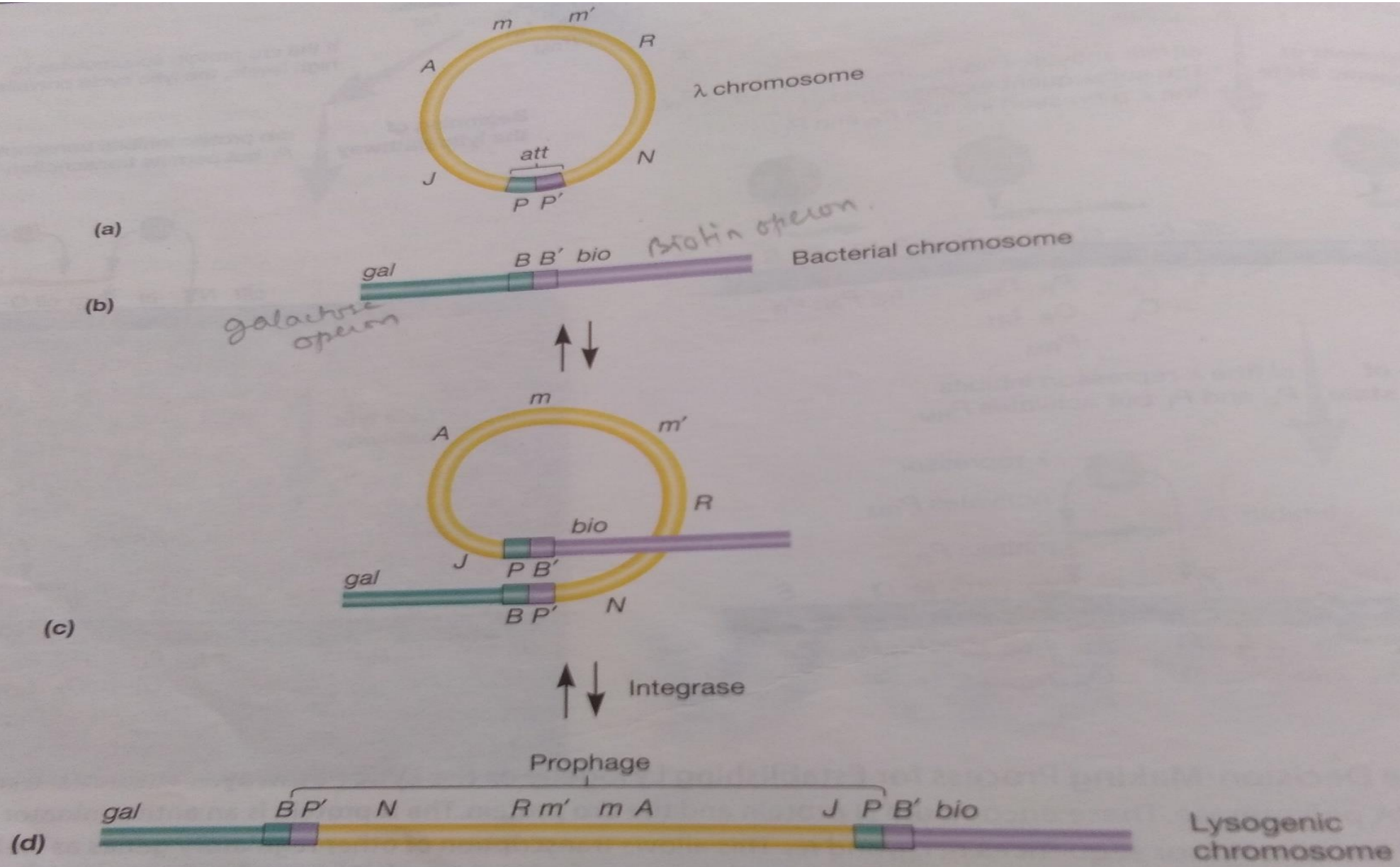
Once the lambda genome is circularized within the host cytoplasm , transcription is initiated by host RNA polymerase at promoter PL. Very early in the infection , only the N and cro genes are expressed and transcription is terminated at the end of these two genes. However, once the N protein is synthesized , it function as an antiterminator so that RNA polymerase continues transcription beyond the N and cro genes. Thus from PR the cII and DNA replication genes O, P, and Q are transcribed from PL ,c III and all the gene through the excision (xis) and integration (int) genes are transcribed.

Role of C II and C III gene product

CII is a transcriptional activator protein that recognizes the promoter PRE promoter for lambda repressor establishment . And initiates transcription of CI gene , which encode the lambda repressor .
It also recognizes the promotors PI and PAQ. Transcription leftward from the PI promotor synthesizes mRNA encoding the enzyme integrase , which catalyzes the insertion of lambda DNA into the E. coli chromosome. Integration takes place at the site in the host chromosome called attachment site (att) . A homologous site is found on the phage genome , so the phage and bacterial (att) site can base pair with each other . The bacterial site is located between the galactose (gal) and biotin (bio) operon , and as a result of integration , the circular lambda genome becomes a linear stretch of DNA located between this two host operons . The prophage can remain integrated indefinitely, being replicated as the bacterial genome is replicated.

The activity of CII protein is influenced by environmental factors.this protein is susceptible to proteases. When *E*. *coli* is in nutrient rich conditions , many proteases are formed and CII is more likely to be degraded. The role of CIII protein is to protect the CII from degradation. If its protection is not completed, CII protein is inactivated whether CIII Protein is present or not.


int xis
cIII N cI cro cII O P Q
P_I
t_L P_L O_L
P_R P_{RE} O_R t_{R1} P_{RM}
t_{R2}
or
If the cII/cIII complex accumulates to high levels, the lysogenic cycle prevails.
Establishment of the lysogenic state
cII/cIII activates transcription from P_I and P_{RE}. The subsequent expression of cI (the λ repressor) inhibits P_R and P_L.
Beginning of the lytic pathwa
cII-cIII
cI
int xis
cIII N cI cro cII O P Q S
P_I
t_L P_L O_L
P_R P_{RE} O_R t_{R1} P_{RM}
t_{R2} P_{AQ} $P_{R'}$
Maintenance of the lysogenic state
cI (the λ repressor) inhibits P_R and P_L but activates P_{RM}.
λ repressor
Activates P_{RM}
Inhibits P_L
Inhibits P_R

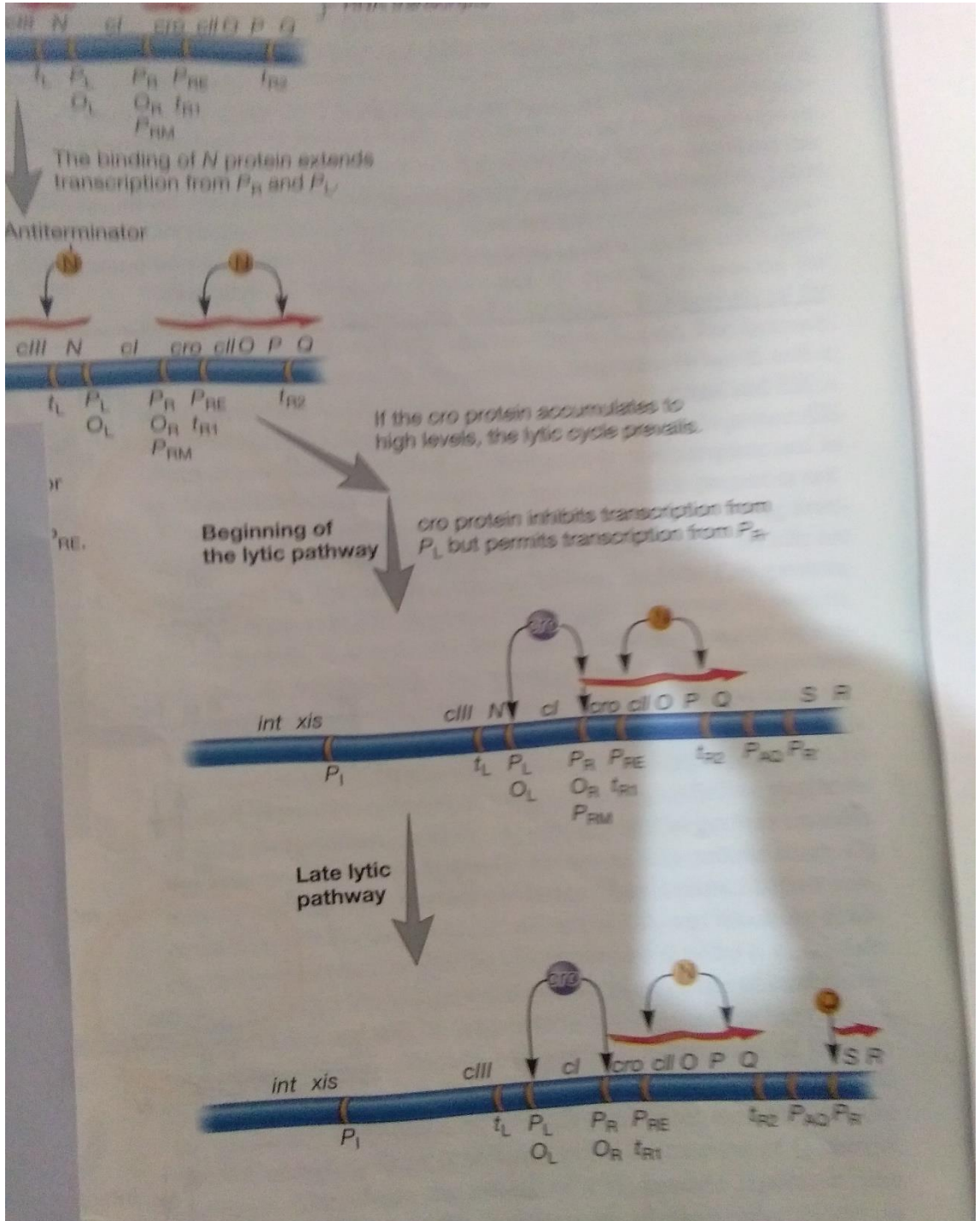


22 Reversible Insertion and Excision of Lambda Phage. After circularization, the *att* site P, P′ **(a)** lines up with a bacterial sequence B, B′ **(b)** and is integrated between the *gal* and *bio* operons to form the

When CII protein is in inactivated condition , several genes are being transcribed and their protein accumulated in the cell . These proteins included Cro protein , lambda repressor , CII Protein , CIII Protein , integrase , N protein and Q protein.

Both Cro protein and lambda repressor binds the regulatory site OR. If the cro protein binds OR, it blocks synthesis of lambda repressor.
If lambda repressor binds OR it promotes its own synthesis but block synthesis of the cro protein. Because synthesis of cro protein begins before synthesis of lambda repressor , initially the amount of cro protein exceeds the amount of lambda repressor. However, cro protein binds OR less tightly than does lambda repressor. Thus it takes a higher concentration of cro protein in the cell to bind OR and block the binding of lambda repressor .

The cro protein is composed of 66 amino acids and like lambda repressor , forms a dimer that binds the operator sites OR and OL , blocking transcription from the PR and PL promoters. If cro protein wins the race with lambda repressor it blocks synthesis of lambda repressor and prevents integration of the lambda genome into the host chromosome . By the time synthesis of lambda repressor is blocked , another regulatory protein called Q protein has accumulated. Q promotes transcription from a promotor called P’R , and in the presence of Q protein , the genes encoding viral structural proteins, as well other proteins needed for virus assembly and host lysis , are transcribed. Ultimately, the host is lysed and the new virions are released.



LYTIC PATHWAY