EVOLUTION: An Analysis of DNA Sequence Homology Among the Lambdoid Coliphages and Between the *trpB* and *tna* Genes of *Escherichia coli*

Rhonda Jan Myers

Doctor of Philosophy

University of Edinburgh

1980

The dissertation of Rhonda Jan Myers satisfying the requirements for the degree Doctor of Philosophy is approved.

University of Edinburgh

1980

DEDICATION

To those in my life, my family and my friends, who have made it enjoyable, gratifying and interesting.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF ELECTRON MICROGRAPHS

ACKNOWLEDGEMENTS

I am grateful to those who have helped, supported and advised me during my post-graduate education. My supervisor, Peter J. Highton was an integral part of this work, training me in electron microscopy and providing guidance and suggestions. Noreen Murray provided advice and bacterial and bacteriophage strains. Douglas Ward was extremely helpful by making available to me his expertise in computer programming. Joseph Lutkenhaus has been extremely helpful in providing scientific advice, artistic talent and critical comments. I am forever grateful to my parents, Charlotte S. Myers and Seymour L. Myers whose support knows no bounds and to my sister Robin and brothers Richard, Russell and Robert. I would like to express my gratitude to Marvin L. Tanzer for providing an atmosphere in which this thesis was completed and to the University of Connecticut Department of Biochemistry for their generosity in permitting me to use their typewritters. Finally, I am grateful to the following people: Algis Anilionis, Pamela Beattie, Jean Beggs, Elaine Borselle, Philip Ledger, Maria Millan, Debra Enriquez Pecaro, Herman Wolf and Margaret, Rena and Trina.

ABSTRACT

One way to approach the question of the evolution of bacteria and bacteriophages is by examining their DNA sequence homology. The electron microscope is a tool that allows the detection and measurement of regions of single- and double-stranded DNA. By using the techniques of DNA heteroduplex formation and DNA spreading I was able to use the electron microscope to seek regions of DNA sequence homology between various pairs of bacteriophages within the lambdoid family and between the tna and trp genes in the Escherichia coli (E. coli) chromosome.

Various phage DNA's were annealed in the presence of formamide and the resulting heteroduplex molecules were examined by electron microscopy (Davis and Davidson, 1968; Westmoreland et al, 1969; Davis et al, 1970). Regions of homology in such heteroduplexes appeared as double-stranded segments; regions of nonhomology gave two separate single-stranded segments. Regions of homology and nonhomology were mapped in heteroduplexes made between the DNA's of the various lambdoid coliphages and between λ-transducing phages carrying the trp and tna genes of E. coli. This technique was also used to determine the sizes of various DNA molecules and DNA segments.

The sizes of the following DNA molecules and DNA fragments were determined: the double-stranded DNA of plasmid pSC101 (19.0 ± 0.2%λ (s.d.; n = 25)); the single-stranded DNA of bacteriophage M13 (12.7%λ); the DNA of bacteriophage λ (49,150 ± 730 base pairs (s.d.; n = 25)); the DNA of bacteriophage PA2 (99.6 ± 2.0%λ (s.d.; n = 19)); the DNA of bacteriophage 424 (96.4 ± 1.3%λ (s.d.; n = 20)); the HindIII restriction fragment carrying the tna gene of E. coli (12.0 ± 0.5%λ (s.d.; n = 15)); the Hind III restriction fragment carrying the

trpA, *trpB* and *trpC* genes of *E. coli* (11.1 ± 0.4%λ (s.d.; n = 38)); the immunity region of λ (*imm*$^{\lambda}$) (8.0 ± 0.7%λ (s.d.; n = 15)); the immunity region of bacteriophage 21 (*imm*^{21}) (3.6 ± 0.4%λ (s.d.; n = 15)) and; the *nin* region of λ (5.4 ± 0.4%λ (s.d.; n = 15)). These values are expressed as the mean ± standard deviation. The number of base pairs comprising λ DNA was deduced from the two spreadings λ with pSC101 and pSC101 with ϕX174 (double-stranded); the size of ϕX174 was determined to be 5386 bases (Sanger *et al*, 1978). Other DNA segments within the heteroduplexes were also measured.

The following heteroduplexes between pairs of lambdoid phages were analyzed λ/424, λ/PA2, 434/424, 434/21, 434/PA2, 424/PA2 and 21/PA2. The total amount of homology between each pair was 48.3%, 56.7%, 45.4%, 42.7%, 27.8%, 56.6%, 53.3% and 37.4%, respectively. My results indicate that it is likely that the processes envisaged in the modular hypothesis and in the ancestral chromosome hypothesis were important in the evolution of the lambdoid phages.

Lambda-transducing phages carrying the *trpABC* and *tna* bacterial fragments, generated by restriction of *E. coli* and λ DNA's (with the endonuclease *Hin*dIII), followed by ligation and selection for complementation by these genes, were allowed to form heteroduplexes using various denaturing and spreading conditions. The following heteroduplexes were analyzed: NM540/λ*tna* *imm*$^{\lambda}$ *cI*857 *nin*$^{+}$; λ*trpABC*^{1}/λ*trpABC*^{r}; λ/λ*trpABC*^{1}; λ/λ*trpABC*^{r}; λ*trpABC*^{1}/λ*tna* *imm*$^{\lambda}$ *cI*857 *nin*$^{+}$ and; λ*trpABC*^{r}/λ*tna* *imm*$^{\lambda}$ *cI*857 *nin*$^{+}$. The DNA fragments containing the *trpABC* and *tna* genes were found to share no more than 58% homology by using the least denaturing conditions possible.

PART I

INTRODUCTION

THE LAMBDOID FAMILY

Lambda and its relatives are members of a group of Escherichia coli bacteriophages, the lambdoid family, which includes λ, 434, 424, 21, 82, 381, 466, ϕ80, ϕ81, PA2, ϕD326 and many more (Lederberg, 1951; Jacob and Wollman, 1956; Matsushiro, 1963; Schnaitman et al, 1975). Lambdoid phages are temperate; they can undergo vegetative replication or they can propagate as a prophage by integrating into and replicating as part of the host chromosome (under special conditions, the λ chromosome can survive and multiply in the host in a non-integrated form as a plasmid (Chow et al, 1974; Matsubara and Kaiser, 1968; Szybalski and Szybalski, 1974)). Cells carrying a prophage are called lysogens and they transmit the ability to produce these phage particles to their progeny (Lwoff and Gutmann, 1950; Lwoff et al, 1950). In the lysogen, most phage functions are repressed and the lysogen is immune to superinfection by another phage having the same immunity (Kaiser and Jacob, 1957). Also, the phage is replicated as part of the bacterial chromosome and segregates with it at cell division (Sharp et al, 1972). Spontaneous induction to the lytic phase occurs at low frequency (about 10^{-4} per cell generation). However, treatments such as ultraviolet (UV) irradiation can induce the prophage into the lytic phase of development (Roberts and Roberts, 1974). This stability of lysogeny depends upon repression of the phage genome (Sussman and Jacob, 1962). Repression is mediated by the repressor, which is synthesized after infection and prevents the expression of those genes necessary for development of the lytic pathway (Ptashne, 1971).

Each of the lambdoid phages has a unique immunity and it is the repressor that determines this specificity. The region of the DNA that specifies the immunity (imm) of a phage can be defined with respect to recombination with the λ immunity region; for example, the immunity region of phage 434 includes all the genes that are inseparable from the immunity of 434 in a cross between λ and 434. The immunity region is the length of non-homology between two DNA's in which the repressor gene and the sites of action of the repressor protein lies.

Phage of the lambdoid family are distinguishable from each other by having different immunities, different host ranges and by their sensitivity to anti-immune sera of other phages in the family. Dove (1968) classified lambdoid phages as those phages that share common cohesive DNA ends, undergo genetic recombination with each other to various extents and can be induced from the prophage state by UV-irradiation. Simon et al (1970) added that the lambdoid phages are capable of genetic complementation with each other to various extents.

Lambda has been studied extensively, more than any of the other lambdoid phages. In the following pages I discuss the general physical, morphological and genetic characteristics of λ and some of the similarities and differences among the phages of the lambdoid family. Other families of related phages are also discussed.

THE TEMPERATE PHAGE: TWO PATHWAYS UPON INFECTION

Upon infection, the linear λ DNA molecule is injected into the host cell and immediately circularizes because of the complementary, single-stranded, twelve nucleotide sequences at its ends (Hershey et al, 1963; Wu, 1970; Wu and Taylor, 1971; Yarmolinsky, 1971). The DNA is then converted into a covalently closed circle by the action of a

DNA ligase (Gellert, 1967). The phage DNA can then follow either of two pathways of development, lysogenic or lytic. In the former, the injected phage DNA directs the synthesis of gene products that repress the lytic cycle and promote its integration into the bacterial chromosome. These protein products bring about a single, reciprocal recombination event, which inserts the circular phage DNA molecule such that it becomes a linear sequence in the host chromosome (Campbell, 1962). The site on the λ DNA molecule at which insertion occurs is designated att on the λ genetic map (see Figure 1). The site on the bacterial chromosome at which the λ DNA molecule integrates is designated att^{λ}; the sites at which other lambdoid phage DNA molecules integrate are designated in an analogous manner.

The λ int gene product is required for integration (Zissler, 1967). Once the λ DNA is integrated, the host cell is immune to superinfection by other λ particles because of the synthesis of repressor. Establishment of repressor synthesis requires products of both genes cII and cIII (Kaiser, 1957). Transcription of the cI gene from promoter p_{re} then leads to the synthesis of repressor, which binds to the leftward and rightward operators and prevents further expression of all early genes (Ptashne, 1971). Maintanence of lysogeny requires the continuation of repressor synthesis originating from promoter p_{rm} (Heinemann et al, 1970). Sufficient repressor is synthesized by a prophage to act upon its own DNA as well as any homoimmune superinfecting phage DNA, preventing the superinfecting DNA from developing lytically or integrating into the host chromosome (Kaiser and Jacob, 1957; Ptashne, 1971).

Entry into the lytic pathway of development requires the synthesis of λ genes whose products promote phage DNA replication, phage

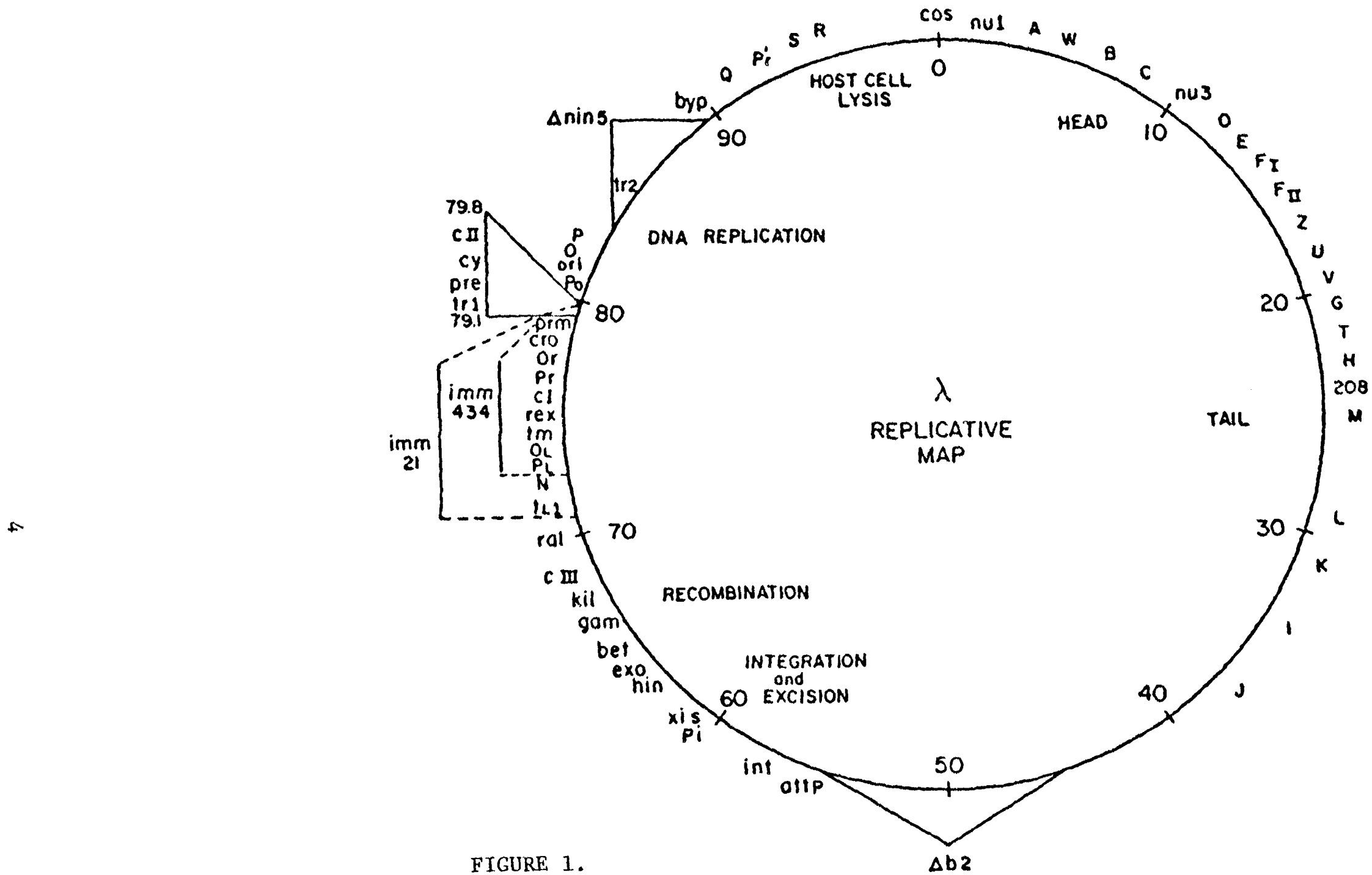
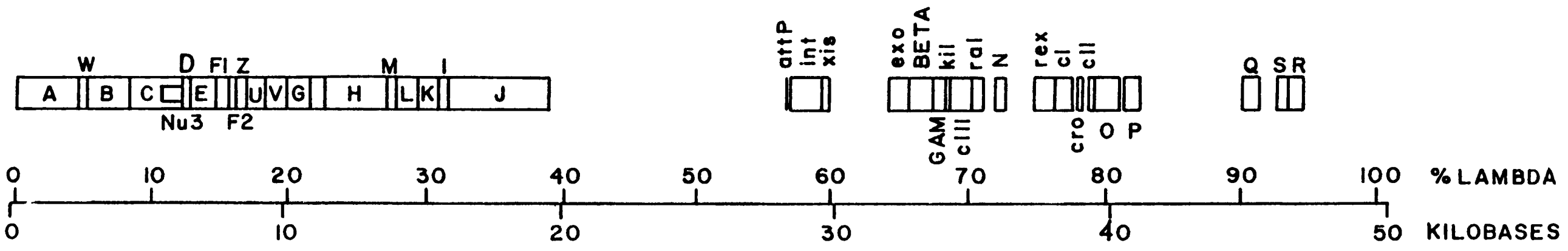

cos
nu1
A
W
B
C
nu3
O
E
FI
FII
Z
U
V
G
T
H
208
M
L
K
I
J
R
S
P'r
Q
byp
Δnin5
tr2
79.8
cII
cy
pre
tr1
79.1
P
O
ori
Po
prm
cro
Or
Pr
cI
rex
tm
OL
PL
N
tL1
imm
434
imm
21
ral
cIII
kil
gam
bet
exo
hin
xis
Pi
int
attP
Δb2
0
10
20
30
40
50
60
70
80
90
HOST CELL
LYSIS
HEAD
TAIL
DNA REPLICATION
RECOMBINATION
INTEGRATION
and
EXCISION
λ
REPLICATIVE
MAP

FIGURE 1.



λ VEGETATIVE MAP

FIGURE 1A.

coat protein synthesis, DNA packaging into phage particles and host cell lysis. To enter the lytic cycle, repressor synthesis must be prevented; this is achieved by the cro gene product, which prevents transcription from p_{rm} (Calef and Neubauer, 1968; Neubauer and Calef, 1970; Eisen et al, 1970). The sequence of gene expression in the regulation of lytic development of λ phage particles depends upon the sequential action of the two positive regulator genes, N and Q (Dove, 1966; Protass and Korn, 1966; Radding and Shreffler, 1966; Skalka et al, 1967; Radding and Echols, 1968; Oda et al, 1969; Echols, 1971).

Synthesis of repressor is a key event in determining the choice between the lytic or lysogenic pathway (Heinemann and Spiegelman, 1970b). This choice depends upon the expression of the cII and cIII genes. Their gene products are positive regulators of cI repressor synthesis (Echols and Green, 1971; Reichardt and Kaiser, 1971) and so they inhibit lytic gene expression (McMacken et al, 1970; Court et al, 1975).

LAMBDA: PHYSICAL PROPERTIES

The lambda phage particle consists of a single double-stranded DNA molecule about 49,150 base pairs in length, equivalent to a molecular weight of about 32.5×10^6 daltons. The DNA molecule is about 14.4 μ in length (this thesis). The DNA is encapsulated in an icosahedral head, 55 nm in diameter, out of which projects a tubular tail, about 153 nm in length and 12 nm in diameter (Eiserling and Boy de la Tour, 1965; Hershey and Dove, 1971). The tail terminates in a fine fiber, about 25 nm in length with a diameter of about 2 nm, which appears to be responsible for host range (Eiserling and Boy de

la Tour, 1965). The phage particles are about half protein and half DNA by weight (Kaiser, 1966; Campbell, 1969; Buchwald, Steed-Glaister and Siminovitch, 1970).

When a break is introduced into the middle of the linear λ duplex, the left and right halves of the molecule can be separated by their different buoyant densities. The left half has a G + C content of about 55% and has a greater buoyant density than the right half, which has a G + C content of about 45% (Skalka, 1966). The central region of the λ DNA molecule, from about 44% λ to 54% λ, has a G + C content of about 37%. The buoyant densities of various segments within the λ DNA molecule have been determined (Skalka et al, 1968). These segments and their respective buoyant densities are labelled in Figure 2.

LAMBDA: GENETIC MAP

The lambda DNA molecule consists of groups of genes coding for related functions. The map in Figure 1A is labelled with the λ genes that have been identified. The head and tail genes are located in the left arm of the λ molecule. The DNA recombination, positive and negative regulatory genes are all located around the center of the molecule. The DNA synthesis and host-cell lysis genes are located in the right arm of the λ molecule. The silent region, also known as the non-essential region, about 15% of the chromosome, is located to the left of the center of the molecule. A deletion, b, within this non-essential region does not affect any phage characteristics other than chromosome length and buoyant density (Kellenberger et al, 1961; Jordan, 1964; Parkinson and Davis, 1971; Shulman and Gottesman, 1971). An article by Echols and Murialdo (1978) reviews the genetic map of λ. All map positions quoted were taken from this paper.

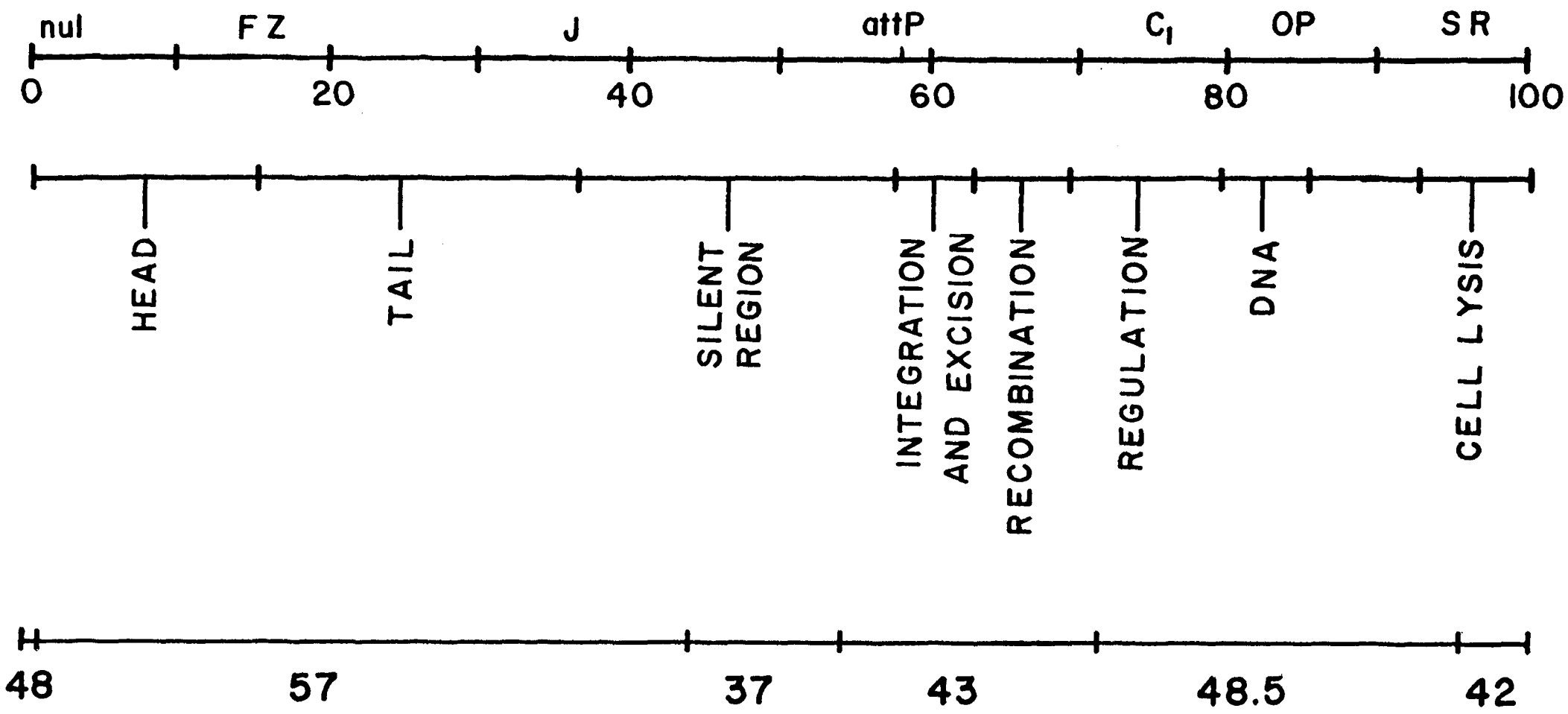


FIGURE 2. Comparison of the Genetic and Physical Properties of λ DNA.
The top line represents the λ genetic map. The middle line identifies the groups of λ genes coding for related functions. The bottom line indicates the ~~buoyant densities~~ % G+C of various segments within the λ DNA molecule.

Head and Tail Genes. The genes involved in head synthesis and assembly are clustered at the left end of the λ DNA molecule between 0 and 16.3% λ (Weigle, 1966; Mount et al, 1968; Parkinson, 1968). The A gene product cuts the DNA concatamers, which result from rolling circle DNA replication, at the cohesive end sites, cos (Wang and Kaiser, 1973). On the basis of biochemical and genetic analyses, it has been shown that the protein products of at least 9 λ phage genes and the protein product of at least one E. coli gene, groE, are required for proper λ phage head assembly (Casjens and King, 1975). These phage genes include A, W, B, C, Nu3, D, E, F_I, and F_{II}; two other genes may be involved, these are defined by the defective mutations, Nu1 and Nu2 (Parkinson, 1968; Buchwald, Murialdo and Siminovitch, 1970; Casjens et al, 1970; Murialdo and Siminovitch, 1972a, 1972b; Casjens and King, 1975; Ray and Murialdo, 1975). Genes D and E code for major capsid proteins (Casjens et al, 1970; Buchwald, Murialdo and Siminovitch, 1970; Murialdo and Siminovitch, 1971; Murialdo and Siminovitch, 1972; Bayer et al, 1973; Williams and Richards, 1974; Steinberg and Weisberg, 1977).

The tail genes are arranged to the right of the head genes, between 16.3 and 39.4% λ, and include the following genes: Z, U, V, G, T, H, 208, M. L, K, I and J (Campbell, 1961; Buchwald et al, 1970b; Murialdo and Siminovitch, 1971, 1972a; Parkinson, 1968). The product of gene H is the second major component of the tail (Buchwald, Murialdo and Siminovitch, 1970; Casjens and Hendrix, 1974). Gene U codes for a structural component of the tail and determines tail length (Mount et al, 1968; Murialdo and Siminovitch, 1972a; Katsura and Kühl, 1974). Gene V codes for the major protein of the tail tube (Buchwald, Murialdo and Siminovitch, 1970; Murialdo and Siminovitch, 1971, 1972; Katsura and Tsugita, 1977) and maps between 18.4 and 19.9% λ. Gene J codes for

a protein that forms the tail fiber and determines host range of adsorption; it maps between 31.8 and 39.4% λ (Dove, 1966; Mount et al, 1968; Buchwald and Siminovitch, 1969; Shaw et al, 1977)

Recombination Genes. Between about 57.3 and 68.7% λ are genes whose products determine the interplay between DNA molecules. The attP site at 57.3% λ is the location of site-specific recombination, a 15 base-pair sequence homologous with the bacterial site attB (Davis and Parkinson, 1971; Davies et al, 1977; Landy and Ross, 1977). The int gene lies between 57.4 and 59.4% λ and its product is an enzyme, which mediates the integration of the prophage during lysogenization (Echols et al, 1968; Echols, 1970; Ausubel, 1974). Int mutants are defective with respect to insertion into and excision from the bacterial chromosome (Gingery and Echols, 1967; Zissler, 1967; Gottesman and Yarmolinsky, 1968). The xis gene, mapping between 59.8 and 60.2% λ is required for excision from the bacterial chromosome (Guarneros and Echols, 1970). The promoter for int expression, pI, is under positive regulation by the cII and cIII proteins (Shimada and Campbell, 1974a; Shimada and Campbell, 1974b; Campbell, 1977; Enquist and Weisberg, 1977).

Lambda specifies its own recombination system. Two genes, redα (also known as exo), located between 64.7 and 66.1% λ and redβ (also known as akaβ), located between 66.1 and 67.8% λ determine the proteins exonuclease and β respectively (Manly et al, 1969) and are required for general recombination at normal frequency. These two proteins are the major influences on recombination between phage genomes. The β protein has been shown to increase the affinity of exonuclease for DNA (Radding and Carter, 1971). The gam (γ) gene maps between 67.8 and

68.7% λ and its protein product directly inhibits the host exonuclease V (product of the host genes recB and recC); it is necessary to protect the linear phage DNA concatamers from degradation by exonuclease V (Unger et al, 1972; Unger and Clark, 1972; Enquist and Skalka, 1973). Because of the inhibitory effect of the gam gene product, the host recombination system makes only a minor contribution in most phage x phage crosses (Echols and Gingery, 1968; Signer and Weil, 1968a, 1968b).

Control and Immunity Genes. Phage λ has a highly complex control region, composed of both positive and negative elements, in which the decision is made between the lytic and lysogenic pathways. The first of these elements to be described was the cI gene, located between 76.9 and 78.4% λ. As mentioned earlier, this gene codes for the repressor, which is responsible for the immunity phenotype and for the maintenance of lysogeny. The repressor inhibits transcription of genes necessary for vegetative development by binding to the operators o_L and o_R (Ptashne, 1971).

The operator, o_R maps between 78.4 and 78.5% λ and consists of three subsites o_{R1}, o_{R2},and o_{R3}, at which the cI and cro proteins act to prevent binding of RNA polymerase and thus transcription from p_R (Ptashne and Hopkins, 1968; Maniatis et al, 1975; Pirrotta, 1975). The promoter site, p_R, for initiation of early rightwards transcription from the cro gene through the Q region maps at 78.5% λ (Walz and Pirrotta, 1975). The promoter site p_E or p_{re} (promoter for repressor establishment), for transcription of the cI and rex genes during the establishment stage of lysogeny, maps at 79.2% λ. The promoter for transcription of the cI and rex genes during the maintenance stage of lysogeny, p_M or p_{rm}, is located at 78.4% and

the terminator for these genes during this stage of lysogeny, t_M, maps at 74.2% (Isaacs *et al*, 1965; Hayes and Szybalski, 1973).

The operator, o_L, located between 73.5 and 73.6% also consists of three subsites, o_{L1}, o_{L2} and o_{L3}. It is the site at which the *c*I and *cro* proteins act to prevent binding by RNA polymerase and thus transcription from p_L (Ptashne and Hopkins, 1968; Pero, 1971; Maniatis *et al*, 1975; Reichardt, 1975; Takeda, 1977).

The *cro* (control of repressor and other things) gene product depresses the level of transcription from p_L, p_R and p_M by binding to o_L and o_R (Eisen *et al*, 1970; Heinemann *et al*, 1970; Echols *et al*, 1973; Takeda *et al*, 1975; Folkmanis *et al*, 1977; Takeda *et al*, 1977; Johnson *et al*, 1978). The *cro* gene is also known as *tof*--turn off function; *ai*--anti-immunity; and *fed*. The *cro* gene maps between 78.6 and 79.0%.

Both the *c*II and *c*III gene products are required to induce repressor synthesis (Signer, 1969; Eisen *et al*, 1970). They act at p_{re} to initiate repressor mRNA synthesis--this message is transcribed leftward reading through the *cro* gene on the anti-strand into the *c*I gene (Echols and Green, 1971; Court *et al*, 1975; Chung and Echols, 1977). The *c*II gene extends from 79.2 to 79.8%; its protein product is necessary for the establishment of immunity (Kaiser, 1957). The *c*III gene maps between about 68.7 and 70.7%. The *c*III gene product together with the *c*II gene product are required for the establishment of lysogeny; both protein products activate transcription from the *c*I and *int* genes and repress transcription of genes of the lytic pathway (Eisen *et al*, 1970; Echols and Green, 1971; Reichardt and Kaiser, 1971; Spiegelman *et al*, 1972; Court *et al*, 1975; Katzir *et al*, 1976; Court *et al*, 1977).

The N gene extends from 72.5 to 73.3%. The N gene product provides for positive regulation of three regions of λ DNA: recombination, replication and late genes (Dove, 1966; Eisen et al, 1966; Joyner et al, 1966; Protass and Korn, 1966; Skalka et al, 1967; Radding and Echols, 1968; Butler and Echols, 1970; Heinemann and Spiegelman, 1970). The N gene product acts on the phage DNA at the termination sites t_{L1}, t_{R1} and t_{R2} and allows transcription initiated at p_L and p_R to proceed through these sites--hence its name, "anti-termination factor" (Kourilsky et al, 1968; Kumar et al, 1969; Heinemann and Spiegelman, 1970; Hopkins, 1970; Roberts, 1971; Thomas, 1971). The t_{L1} site maps at about 71.8% and is a rho-sensitive transcription termination site for the early RNA synthesis initiated at p_L (Roberts, 1969; Kourilsky et al, 1970; Roberts, 1970). The rho-sensitive transcription termination sites, t_{R1} and t_{R2} are the terminators for most of the early RNA synthesis initiated at p_R and map at 79.2% and 83.3% respectively (Roberts, 1970; ~~Roberts, 1971~~). The N protein interacts with RNA polymerase and renders it insensitive to a variety of termination signals (Adhya et al, 1974; Franklin, 1974). The N protein stimulates the transcription of the following genes: cII, O, P, Q, γ, redα, redβ, xis and int (Protass and Korn, 1966; Kourilsky et al, 1968; Radding and Echols, 1968; Roberts, 1969; Luzzati, 1970; Hendrix, 1971). The promoter for initiation of transcription from N through the int region, p_L, is located at 73.5%, between the genes rex and N (Heinemann and Spiegelman, 1971).

Gene Q maps between 90.8 and 92.1% and is the positive control gene for late gene expression. The Q protein acts at the promoter p_R', which maps at about 93.1%, to allow transcription from p_R to continue through the late genes--lysis, head and tail genes--from gene S through gene J (Toussaint, 1969; Herskowitz and Signer, 1970a; Szpirer

and Brachet, 1970; Thomas, 1971; Roberts, 1975). In the absence of the product of gene Q little late mRNA is synthesized and the levels of endolysin and the structural proteins are low (Hendrix, 1971). The mutation, byp, makes the expression of gene Q independent of the product of gene N (Butler and Echols, 1970; Hopkins, 1970).

DNA Replication Genes. Phage λ replicates bidirectionally (Schnös and Inman, 1970). The origin of replication, ori, maps at 80.9% (Kaiser, 1971). The activation of the origin requires the products of genes O and P (Joyner et al, 1966; Tomizawa and Ogawa, 1968; Dove et al, 1971; Stevens et al, 1971; Takahishi, 1975).

The major rightward operon of phage λ contains the phage DNA replication genes O and P (Brooks, 1965; Joyner et al, 1966). Gene O maps between 79.9 and 81.9% and gene P maps between 81.9 and 83.3%. These genes are rarely transcribed in the absence of the N gene product (Takahishi, 1975).

Lysis Genes. The λ lysis genes S and R, map between about 93.1 and 93.9% and 93.9 and 95.0% respectively. They control the dissolution of the cell envelope that terminates the lytic cycle (Harris et al, 1967). Gene S codes for a protein involved in turning off phage DNA synthesis and affects the host membrane (Adhya et al, 1971; Reader and Siminovitch, 1971). Gene R determines the phage endolysin, an endopeptidase, which digests the host cell wall by splitting a bond between D-amino acids (Taylor, 1971). The morphogenetic genes (head and tail) are "late" genes continuous with genes S and R in the circular λ molecule.

Other Genes and Genetic Markers. The hin region affects host permeability and stabilizes phage mRNA (Court, in preparation). The λ kil function is responsible for killing the host cell prior to cell lysis during lytic phage growth (Greer, 1975). The kil gene extends from about 68.7 to 69.2%. The ral gene specifies a protein, which partially alleviates the restriction of unmodified phage DNA upon infection of a K-restriction host (DeBrouwere, Zabeau, Van Montagu, Schell, in preparation). The rex gene maps from about 74.2 to 75.9%. The rex gene product excludes phage T4rII mutants from developing in λ lysogens (Howard, 1967). The rex gene, part of the cI operon, is expressed concomittantly with the cI gene. The promoter for transcription of the 4s RNA, p_o, maps at 79.9% (Scherer et al, 1977). Nin5 signifies a deletion, which relieves the requirement of the N gene product for phage growth (Court and Sato, 1969). Imm434 is the substitution of the immunity region of λ by the corresponding DNA of phage 434 (Kaiser and Jacob, 1957; Davidson and Szybalski, 1971). Imm21 is the substitution of the immunity region of λ by the corresponding DNA of phage 21 (Davidson and Szybalski, 1971).

The b2 region of λ consists of genes nonessential to phage growth. The structural deletion mutant of λ, λb2, has an altered buoyant density (Kellenberger et al, 1961; Parkinson, 1971; Shulman and Gottesman, 1971). This mutant does not produce clear plaques (Zichichi and Kellenberger, 1963). Phage λb2 cannot lysogenize E. coli unless a $\lambda b2^+$ helper phage is present (Zichichi and Kellenberger, 1963; Campbell, 1965; Fischer-Fantuzzi, 1967; Gottesman and Yarmolinsky, 1968b). Bacteria carrying an integrated λb2 prophage yield very little phage upon UV induction (Zichichi and Kellenberger, 1963). This phage, however, can elicit the lytic response (Tonegawa and Hayashi, 1968).

RELATEDNESS AMONG THE LAMBDOID PHAGES

Heteroduplex Studies and Genetic Evidence. Evidence for relatedness among the lambdoid phages includes DNA sequence homology and genetic evidence. The electron microscope heteroduplex method was used to study and map regions of homology in the DNA molecules of the lambdoid group and other phage groups (Davis and Davidson, 1968; Westmoreland et al, 1969; Davis et al, 1970). Among phages of the lambdoid family, the following heteroduplexes have been described:

λ/21, λ/434, λ/82, 434/82, λ/ϕ80, ϕ80/ϕ81

and λ/ϕ81 (Simon et al, 1971; Fiandt et al, 1971; Niwa et al, 1972).

Phages 434, 82, 21 and ϕ80 share 65%, 55%, 38% and 25% homology respectively with phage λ (Simon et al, 1971; Fiandt et al, 1971). When λ was heteroduplexed with 434, 82 and 21 the largest nonhomologous region between them corresponded to the nonessential region of λ between about 40 and 57.4% in λ (Simon et al, 1971). However, in the 434/82 heteroduplex, the DNA's were homologous up to 64.2% λ along the 434 chromosome, which includes the DNA corresponding to the λ silent region. Thus, the DNA in the silent region is not unique for each phage. Most of the homology lies in the left arm. Lambda DNA is homologous with 434 and 82 DNA's up to 37.3%, which includes the λ genes A through I for head and tail formation. This correlates with the genetic evidence because phages 82 and 434 can complement λ in genes A, B, C and D (Dove, 1968).

The left arm of the λ/21 heteroduplex shows less homology with a series of bubbles, whose sizes and positions varied between molecules, up to 15.9% and 16.7% in the λ and 21 DNA molecules respectively (Simon et al, 1971). Following this, the DNA's of λ and 21 are identical up to 36.9% in the λ DNA molecule and 37.7% λ in the 21 DNA molecule, which includes the λ tail genes.

The heteroduplex data indicates that λ, 434, 21 and 82 DNA's are homologous in the region corresponding to the λ *exo* and *β* genes (Simon *et al*, 1971); the λ *exo* and *β* proteins are immunologically the same in λ, 434 and 82 (Radding and Schreffler, 1966; Radding *et al*, 1967). Lambda DNA is also homologous with 434 and 82 DNA's from 57.0 to 60.5%, which includes the λ crossover point at 57.4%; this implies that the sequences around the crossover points and the sequences specifying the *int* proteins may be identical in λ, 434 and 82. Lambda, 434 and 82 DNA's are nonhomologous in the region of the λ *xis* gene, but λ is homologous with 21 in this region.

Another homologous region around 95% exists among λ, 82, 434 and 21 DNA's implying that these phages may share a common *R* gene.

Regions of homology between λ and φ80 were found to total 25.8% λ; again, mostly in the left arm. Other regions of partial homology totaled 13% λ (Fiandt *et al*, 1971). Infectious particles were constructed from λ heads and φ80 tails and φ80 was found to complement λ in the head genes *A*, *B* and *C* (Sato *et al*, 1968; Deeb, 1970; Inokuchi and Ozeki, 1970). No homology was observed in the region of gene *J* between λ and φ80; this agrees with the known differences in the host range of these phages (Dove, 1968).

DNA sequence homology between φ80, φ81 and λ was examined by constructing and analyzing the φ80/φ81 and φ81/λ heteroduplexes (Niwa *et al*, 1978). The φ80/φ81 heteroduplex consists of two homologous regions--the left half and the right terminal region of 13%, including the late genes--interspersed by a long nonhomologous region, which consists of early gene functions. The φ81/λ heteroduplex has several regions of homology. In the left arm there is partial homology because more of the region denatured when the denaturing capacity was increased. These two partially homologous regions (between 0% and

10.1% λ and 11.9% and 14.1% λ) include the head gene cluster, A through E and are separated by a nonhomologous region of constant length (from 10.1 to 11.9% λ). The att-int-xis region is homologous between φ81 and λ.

Other Physical Studies. Skalka and Hanson (1972) determined that the order of relatedness of the lambdoid phages to λ was 82 and 434 > 21 > 424 > φ80. They found that the most common sequences were in segments high in G + C content, which in λ included the head and tail genes. DNA-DNA hybridization results indicated that the DNA of phages 424, 21 and 82 contain many sequences in common with λ, most of these sequences within the highest G + C containing fragments (Skalka and Hanson, 1972). This agrees with the heteroduplex results.

Highton and Whitfield (1974) examined the denaturation patterns of 424, 21 and λ DNA's. The denaturation maps were found to be similar indicating a similar base composition, even in nonhomologous regions.

The terminal DNA sequences of λ, φ80, 82, 424 and 21 are all the same (Murray et al, 1975). This sequence consists of twelve symmetrically arranged base pairs at the 5' termini. They found that the terminal sequence of phage φD326--a lambdoid phage closely related to φ80--differs from that of λ in two nucleotides (Murray et al, 1975; Rock et al, 1974). Still, the φD326 ter system, which codes for the gene product that interacts with and nicks the DNA resulting in the single-stranded projections of complementary base sequence, recognizes the corresponding λ DNA sequence (Murray et al, 1975).

LAMBDA AND P22: DIFFERENT AND SIMILAR

The temperate Salmonella typhimurium phage, P22, differs from λ, an Escherichia coli phage, in many ways. Unlike λ DNA, which is linear and has single-stranded cohesive ends, P22 DNA is circularly permuted and terminally repetitious (Hershey and Burgi, 1965; Thomas and MacHattie, 1967; Gough and Levine, 1968; Rhoades et al, 1968; Campbell, 1971). The P22 phage has a short tail and baseplate structure whereas, λ has an elongated tail (Kellenberger and Edgar, 1971). P22 uses two gene products to repress the prophage (Gough, 1968), in contrast to the single repressor employed by λ (Ptashne and Hopkins, 1968; Hopkins and Ptashne, 1971; Chan and Botstein, 1972). In addition, phage P22 carries out both generalized and specialized transduction (Ozeki and Ikeda, 1968; Wing, 1968) whereas, phage λ carries out only specialized transduction (Jacob, 1955; Morse et al, 1956a, 1956b; Arber, 1960; Kayajanian, 1962; Wollman, 1963; Fuerst, 1966).

Amidst these differences, λ and P22 share similarities, most notably their functional organization (Gough and Levine, 1968; Dove, 1971; Botstein et al, 1972). As with λ, genes specifying related functions are clustered within the P22 genetic map (Botstein et al, 1972). Regions concerned with control, recombination, integration, DNA replication and cell lysis are arranged in the same order on the genetic maps of λ and P22 (Botstein and Herskowitz, 1974). DNA-DNA hybridization studies showed 18% homology (Cowie and Szyfranski, 1967) and that most of this sequence homology between P22 and λ is in the right arm of λ, where the regions mentioned above are located (Skalka and Hanson, 1972). Hybrids between P22 and λ have been constructed; the immunity region from P22 was inserted into λ (Botstein and Herskowitz, 1974). This immunity substitution also replaced the λ genes N, O and P with the analogous P22 genes 24, 18 and 12 and the genes were

fully functional (Gemski et al, 1972). The repressor of phage P22 was found to be functionally identical to that of phage 21 and both P22 and 21 DNA's are homologous in part, but not all, of the immunity region (Botstein and Herskowitz, 1974). A low but significant amount of sequence similarity between λ and P22 DNA's was also found by Skalka and Hanson (1972). They also found that although phages ϕ80 and P22 were similar in G + C content they shared little DNA sequence homology.

Compositional heterogeneity of DNA can be measured by changes in the absorption spectrum at various wavelengths during thermal denaturation. Using this technique, it was confirmed that there is G + C clustering within the DNA's of P22, λ, ϕ80 and 434 (Falkow et al, 1969). They found that the DNA's of λ, ϕ80 and P22 had DNA base sequences in common with each other.

HOMOLOGY BETWEEN P2, 186 AND THE LAMBDOID PHAGES

Phages 186 and P2 are members of a family of temperate coliphages distinct from the lambdoid family. These two phages are non-inducible, have specific chromosomal attachment sites, have similar DNA lengths and have homologous cohesive ends, different from those of λ (Baldwin et al, 1966; Mandel, 1967; Mandel and Berg, 1968a; Mandel and Berg, 1968b). P2 and 186 recombine with each other, and form viable hybrids with each other, but not with the lambdoid phages (Bertani and Bertani, 1971). Hybridization tests showed that phages P2 and 186 have similarities to but few, if any, DNA sequences in common with λ (Skalka and Hanson, 1972). They showed that P2 and 186 have DNA sequences in common with each other, in G + C rich regions.

RELATEDNESS AMONG FEMALE-SPECIFIC COLIPHAGES

The female-specific coliphages T7, T3, ϕI, ϕII, W31 and H were all found to share some DNA sequence homology (Hyman et al, 1975). Phages T7 and T3 were isolated independently by Demerec and Fano (1945) and by Delbrück (1946). Phage ϕII was isolated by Wollman (1947); phage ϕI was isolated by Dettori et al (1961); and W31 was isolated by Watanabe and Okada (1964). Phage H was isolated as a lytic phage from Pasteurella pestis (Cavanaugh and Quan, 1953).

Phage T7 is morphologically, serologically and physiologically similar to phage T3 (Delbrück, 1946). Density gradient ultra-centrifugation experiments using a mixture of labelled and unlabelled DNA resulted in hybrid molecules of T7 and T3 DNA's indicating that there is sequence homology between them (Schildkraut et al, 1962). Examination of the external morphology of phages T7 and T3 by electron microscopy using a freeze-dry technique revealed that these phages are indistinguishable from each other in appearance and that they have a hexagonal cross-section (Fraser and Williams, 1953).

Davis and Hyman (1971) found that in most regions T7 and T3 DNA's are partially homologous with few sequences conserved. Their results support the interpretation of Simon et al (1971) that phages which recombine have maintained regions that are totally homologous. Phages, which are derived from a common ancestor but which no longer undergo genetic recombination with each other will be only partially homologous in DNA sequence. Without the selective pressure to maintain the ability to recombine, the DNA's of T7 and T3 may have accumulated random base changes giving rise to sequences, which are partially homologous. T7 and T3 do not recombine and do not destroy the host DNA and are therefore genetically isolated from each other and from their host.

The patterns of phage proteins synthesized in UV-irradiated E. coli cells after infection with either T7, ϕI, ϕII, W31, H or T3 indicated that T7, ϕI, ϕII, H and W31 are more similar to each other than they are to T3 (Hyman et al, 1974). Heteroduplexes between various pairs of these phages reveal that only a few discrete regions of their chromosomes have distinct base sequences (Hyman et al, 1973; Brunovskis et al, 1973; Hyman et al, 1974). The nonessential genes, in general, show more variation than do essential genes except for the major coat protein, which shows some variation among these phages.

HOMOLOGY AMONG THE T-EVEN PHAGES

The T-even bacteriophages T2, T4 and T6 are all virulent coliphages. The T-even phage DNA's are circularly permuted and terminally repetitious (Thomas and Rubenstein, 1964; Streisinger et al, 1967). Genetic recombination takes place between the T-even phages (Delbrück and Bailey, 1946). The T-even phages were found to have the same base composition and that 5-hydroxymethyl cytosine replaces cytosine in the DNA (Cohen, 1953). The T_m's of T2, T4 and T6 DNA's are 84.4°C, 84.5°C and 84.4°C respectively (Cowie et al, 1971). The T-even phages share similarities in their morphology and antigenic properties (Adams, 1959).

Sequence homology was found among the T-even phage DNA's although it was incomplete homology (Schildkraut et al, 1962). Results of DNA-DNA hybridization studies between T-even phages indicated that the molecular weights of their DNA's differ by as much as 10% (T2 > T6 > T4) and that T2 and T6 are evolutionarily more related than T2 and T4 ~~or T4~~ T4 and T6 (Cowie et al, 1971). They found a significant amount of partial DNA sequence homology. The average amount of homology between

T2 and T4, T4 and T6, T2 and T6 was found to be 87%, 84% and 91% respectively (Cowie et al, 1971).

The regions of homology between the T2, T4 and T6 DNA molecules were mapped by the electron microscope heteroduplex technique (Kim and Davidson, 1974). They found that the heteroduplexes T2/T4, T4/T6 and T2/T6 showed 87%, 88% and 89% homology respectively, which agrees with the DNA-DNA hybridization results (Cowie et al, 1971). Most of these homologous and nonhomologous regions were of gene-size or longer. Contrary to the results of Cowie et al (1971), their results showed little partial sequence homology, which would be expected for phages that undergo recombination; this will be discussed later.

HOMOLOGY BETWEEN BACILLUS SUBTILUS PHAGES SP02 AND ϕ105

SP02 and ϕ105 are two members of a group of temperate Bacillus subtilus phages whose relationship is analogous to that between the lambdoid phages of Escherichia coli. They are serologically related and morphologically indistinguishable (Boice et al, 1969). Evidence suggests that SP02 and ϕ105 are heteroimmune and adsorb to different receptor sites on the bacterial surface. It has been observed that these two phages occupy different locations on the bacterial chromosome (Rutberg, 1969). DNA sequence homology between SP02 and ϕ105 has been mapped using the electron microscope heteroduplex technique (Chow et al, 1972). The heteroduplex consists of a central region of partial homology--about 14% of the total molecular length--flanked by completely nonhomologous regions on each side. The amount of duplex in the central region decreases with increasing denaturing strength of the solvent indicating that these DNA's are only partially homologous within this region.

HOMOLOGY AMONG THE N PHAGES

The N phages are Micrococcus lysodeikticus bacteriophages, which were isolated from sewage (Naylor and Burgi, 1956). Phages N1 and N6 are serologically related and are capable of genetic recombination (Naylor and Burgi, 1956). They have very similar DNA base sequences determined by both DNA-DNA hybridization studies and by electron microscope heteroduplex studies (Lee and Davidson, 1975). They found that N6 and N1 shared 92.5% homology and N5 and N1 shared 1.72% homology. They also found that the cohesive end sequences of N1 and N6 are very similar and are capable of mutual cohesion. The contour lengths and the sedimentation coefficients ($s^{o}_{20,w}$) of N1 and N6 are also very similar (Lee and Davidson, 1970). The T_m's of N1, N6 and N5 in $[Na^+]$ = 0.0115 M are all virtually the same (Lee and Davidson, 1970).

The DNA of phage N5 shows very little homology with N1 and its ends are not capable of cohesion with those of N1. The contour length and sedimentation coefficient of N5 is significantly less than those of N1 and N6 and the buoyant densities of N1, N6 and N5 are all slightly different (Lee and Davidson, 1970).

HOMOLOGY AMONG OTHER PHAGES

The temperate coliphage P1 does not attach to the E. coli chromosome; it replicates autonomously as a plasmid (Ikeda et al, 1968). P1 DNA is about twice the length of λ DNA and is circularly permutted and terminally repetitious for about 14% of its length (MacHattie and Thomas, 1970; Ikeda and Tomizawa, 1968). The P1 genes and their control mechanisms are different from those of λ (Scott, 1969; Scott, 1970a; Scott, 1970b). Less than 1% hybridization was found between λ and P1 DNA's (Skalka and Hanson, 1972).

HOMOLOGY BETWEEN THE G PHAGES, ϕX174 AND S13

The G phages, ϕX174 and S13 are members of a family of small, tailless, icosahedral coliphages, 28 to 30 nm in diameter whose genomes consist of circular, single-stranded DNA of about 5500 bases (Godson, 1978). They are also known as isometric phages. They are present in sewage and no two isolates appear to be exactly the same.

The G coliphages have been isolated using a sucrose density gradient (Godson, 1974). Those phages sedimenting at 120S were found to be ϕX174-like; the phage particles had almost the same density and size as that of ϕX174. The four phages, G4, G14, G6 and G13 all code for the same number of proteins as ϕX174 and each protein coded for by the G phages has a ϕX174 counterpart of nearly the same molecular weight (Godson, 1974; Shaw et al, 1978). It was found that G4 and ϕX174 do not recombine or complement each other efficiently (Keegstra et al, 1979). In 1977, the entire DNA sequence of ϕX174 was determined and was found to consist of 5375 nucleotides (Sanger et al, 1977), which was later revised to 5386 nucleotides (Sanger et al, 1978). In 1978, the entire DNA sequence of G4 was determined and was found to consist of 5577 nucleotides (Godson et al, 1978).

DNA base sequence homologies between the G phages were studied by constructing heteroduplexes with ϕX174 DNA and analyzing them by electron microscopy (Godson, 1974). These heteroduplex molecules were mounted for electron microscopy in 45% formamide, which is equivalent to T_m-31°C for ϕX174 DNA. Thus, DNA that is single-stranded at T_m-31°C must contain at least 24% base sequence mismatch (See Appendix 1). DNA that is still double-stranded under these conditions can contain up to 24% base sequence mismatch. Phages G4 and G14, which appeared to be the most distantly related to ϕX174 by biological and serological criteria as well as by differences in their proteins,

produced DNA heteroduplex molecules (ϕX174/G4 and ϕX174/G14) with characteristic patterns of nonhomology bubbles. This nonhomology represented specific regions of the viral DNA. Godson (1974) showed that 62.1% of G4 DNA had more than 24% base sequences mismatch with ϕX174 DNA. He showed that about 25% of G14 DNA had a base sequence that was different from ϕX174 DNA by a mismatch of at least 24%. Phages G6 and G13, based on the same biological and serological criteria, appeared to be very closely related to ϕX174. Their heteroduplexes with ϕX174 resulted in random patterns of single-stranded bubbles indicating that the base sequence mismatch of 24% was distributed throughout the entire molecule (Godson, 1974). About 20% of G13 DNA had a base sequence mismatch of greater than 24% with ϕX174 DNA and about 50% of the G6 DNA molecule had a base sequence mismatch with ϕX174 of greater than 24%.

The amount of conservation of the amino acid and nucleotide sequences between ϕX174 and G4 is different for each gene as well as over the entire genome averaging 34.0% and 32.9% respectively (this does not include untranslated sequences or coding regions without homologous counterparts) (Godson, Fiddes, Barrell and Sanger, 1978). In the coding regions, there is an average of 33.1% base sequence difference between ϕX174 and G4 DNA's. By comparing the nucleotide sequences of G4 and ϕX174, it is obvious that the gene order in G4 is the same as that in ϕX174. Both DNA molecules contain two overlapping gene systems--gene E is encoded entirely within gene D and gene B is encoded entirely within gene A. Gene K was recently found to overlap genes B, A and C in G4 and ϕX174 (Shaw et al, 1978).

Mutants of bacteriophage G4 have been isolated and characterized (Borrias et al, 1979). The mutations were mapped and found to lie in 6 different genes (A, B, E, F, G and H). Complementation experiments

using amber mutants of G4 and ϕX174 revealed that ϕX174 was able to use the products of the G4 genes E, F, G and H. In phage G4, only the ϕX174 gene H product was cross-functional *in vivo*. It was suggested that ϕX174 and G4 have evolved independently from a common ancestor (Godson, Fiddes, Barrell and Sanger, 1978). They also suggested the possibility that the ϕX174 and G4 genomes evolved by a mechanism of intragenic recombination each using its own replicating DNA molecules as a source of new sequences.

Phage S13, one of the isometric phages was isolated from *Salmonella* cultures, but grows normally on *E. coli* C strains and behaves like a phage closely related to ϕX174 (Burnet, 1927; Spencer *et al*, 1978). Analysis of DNA restriction fragments suggest that the DNA base sequences of ϕX174, S13 and G6 are closely related (Godson and Roberts, 1976; Grosveld *et al*, 1976) and the DNA base sequences of G4 and G14 are distantly related both to ϕX174 and among themselves (Godson and Roberts, 1976). Both ϕX174 and S13 code for the same number of proteins ordered in a circular genetic map (Tessman, 1965; Baker and Tessman, 1967; Benbow *et al*, 1971). They complement each other in all but one gene and they can recombine (Tessman and Schleser, 1963; Jeng *et al*, 1970). Evidence for an overlap of genes A and B in S13 as in ϕX174 was presented by Spencer *et al* (1978). Godson (1973) showed that the DNA's of these phages contain an average of 36% base sequence mismatch. A more recent analysis by Compton and Sinsheimer (1977) using restriction fragments as reference points in heteroduplex studies, showed that three regions of the heteroduplex, totalling about 45% were double-stranded under mildly denaturing conditions of 40% formamide. Under more stringent denaturing conditions of up to 80% formamide only two duplex regions totalling between 2% and 4% were conserved.

HOMOLOGY AMONG TRANSFERABLE F-TYPE PLASMIDS OF *E. coli*

DNA sequence homology among transferable F-type plasmids of *E. coli* was studied by use of the electron microscope heteroduplex method (Sharp *et al*, 1973). Homology exists between the sex factor, F, between map positions in F of 50.0 and 94.5 and the R factors, R1 and R6-5 and the colicinogenic plasmid colV-K94. However, the other half of F is not homologous to the R factors and shows homology only in one small region with colV-K94. They speculated that the end of the large homologous region is the boundary between the fertility genes (50F to 94.5F) and other genetic functions not related to transfer (94.5F to 50F). They also showed that all the DNA sequences contained in R100 were present in R6 and about 85% of the DNA sequences of R1 were contained in R6. The homology is distributed over their genomes. Thus, these R-factors may be descendants of a single plasmid.

Various F-like plasmids were found to share DNA sequence similarities with R1drd DNA. R1drd is an F-like R factor (Guerry and Falkow, 1971). Other F-like R factors were also found to share DNA sequence similarities (Guerry and Falkow, 1971). Col-I factor, an I-like transmissible plasmid, and two other I-like R factors, N3 and R-144, were found to share only limited DNA sequence similarities with R1drd DNA (Guerry and Falkow, 1971). These sequences were found in regions of the R1 factor with a higher G + C content than the overall molecule; these may be in regions specifying drug-resistance genes.

HOMOLOGY BETWEEN PHAGES AND THEIR RESPECTIVE BACTERIAL HOSTS

Cowie and McCarthy (1963) reported that 33% of the λ genome was homologous with *E. coli* DNA using the DNA-DNA agar binding technique of Bolton and McCarthy (1962). Green (1963) found only 8% of λ DNA was homologous with *E. coli* DNA using a DNA-RNA hybridization method

(Hall and Spiegelman, 1961). Most, if not all, of the homology found by Green resided outside the λ b_2 region. The b_2 region of λ is between about 44.3% and 57.4% and contains no essential λ genes. Thermal dissociation studies of λ RNA-*E. coli* DNA hybrids indicated that there is DNA complementarity between λ and *E. coli* DNA's (Kiger and Green, 1964). The homology between λ DNA and *E. coli* DNA was found to be distributed throughout the length of λ DNA (Cowie and Hershey, 1965).

Phages ϕ80 and P22 both were found to have base sequences similar to those of their bacterial hosts, *E. coli* and *S. typhimurium*, respectively (Cowie and ~~Falkow~~ Szyfranski, 1967; Falkow *et al*, 1969). The similarity in DNA sequence between phage and host raises the possibility that the host may contain complete or partial genomes of the phage. Temperate bacteriophages may have evolved when a segment of the host chromosome acquired the ability to control its own duplication (Cowie and McCarthy, 1963).

No homology was found between *E. coli* and T3 DNA's (or between *E. coli* and T7 DNA's) in heating and annealing experiments (Schildkraut *et al*, 1962). DNA-DNA thermal elution chromatographic profiles show that the three lysogenic phages λ, P22 and 15(TAU$^-$) and the semilysogenic phage T3 are related to their respective hosts *E. coli*, *S. typhimurium*, *E. coli* strain 15(TAU$^-$) and *E. coli* (Cowie and Szyfranski, 1967). Phage 15(TAU$^-$) can be induced from its lysogenic host (Endo *et al*, 1965; Frampton and Brinkley, 1965; Gelderman *et al*, 1966).

Nearest neighbor frequency analysis indicated that the DNA's of the T-even phages are genetically related and are different from the host DNA (Adams, 1959). No homology was found between ~~the DNA's of~~ the DNA's of the virulent phages T2, T4 and the RNA phage MS2 and their host *E. coli* (Hall and Spiegelman, 1961; Doi and Spiegelman, 1962; Green, 1963).

CLUSTERING OF GENES WITH RELATED FUNCTIONS

The occurrence of clustering of genes related in function is common in bacteriophages. In the λ genome, the order of these clusters of genes is related to the order in which they function in the life cycle of λ. The λ genome consists of four distinct segments corresponding to genetically defined functional regions: DNA maturation and morphogenesis genes in the left arm; the silent region near the center; the recombination and regulation genes to the right of center; and the DNA replication, late, control and lysis genes in the right arm. These regions in λ differ considerably in base composition (Hershey and Burgi, 1965). Evidence that most genes within each segment are similar to each other in base composition has been presented (Hershey et al, 1967). The four segments of λ DNA contained: 43%, 10%, 23% and 24% of the DNA from left to right and consisted of an average of 57%, 37%, 43% and 48.5% G + C respectively (Skalka et al, 1968; Skalka and Hanson, 1972) (See Figure 2).

Skalka and Hanson (1972) suggested that intramolecular segmentation is a common feature of the DNA's of the lambdoid phages. They found that φ80 DNA had a distribution of functional regions similar to that of λ DNA and a physical map analogous to that of λ DNA. The arrangement of genes on the λ and φ80 genomes are very similar although certain gene products are not interchangeable or compatible with those of the other (Skalka, 1969; Szpirer et al, 1969; Deeb, 1970; Szpirer and Brachet, 1970). The head and tail genes of φ80 are clustered in the left arm, similar to the arrangement in λ.

Genes with related functions were found to be clustered on the T4 chromosome (Epstein et al, 1963). Genetic analysis of T2 and T4 bacteriophages indicated that the arrangement of genes on their chromosomes was similar (Streisinger and Bruce, 1960; Streisinger et al,

1967). Intramolecular G + C clustering was found in the sex factors F and R (Falkow et al, 1969).

EVOLUTION OF THE LAMBDOID AND RELATED PHAGES

The work on lambdoid phage DNA heteroduplex mapping led Hershey and Dove (1971) to three conclusions. First of all, the DNA molecules of these lambdoid phages contain segments either identical to or completely different from λ DNA segments; only a few partially homologous sequences were found. Secondly, the identical sequences in a given DNA pair add up to between 35% and 60% of the total molecular length. Thirdly, the identical segments occupy positions characteristic of the pair and are nearly equidistant from the left end of the DNA's of both molecules. These similarities together with the other similarities found among the lambdoid phages suggest the existence of a common genome organization and perhaps a common ancestral genome.

The modular or segmental hypothesis of viral evolution has been discussed by several authors (Hershey, 1971; Simon et al, 1971; Botstein and Hershowitz, 1974; Szybalski and Szybalski, 1974). This hypothesis professes that the viral genome is a collection of modules specifying particular functions such as insertion into the host chromosome, replication, virion formation, regulation and cellular lysis. Whole modules could undergo reassortment among various viral genomes as well as between viral and host genomes generating new combinations of these modules.

Campbell (1972) proposed that a phage such as λ could have been generated by the fusion of host genes with perhaps a nonviral plasmid. If a number of phages were constructed, each with a number of modules in common this could explain the homolgoies observed. A detailed discussion about the possible courses of viral chromosome evolution is

presented by Campbell (1977).

The clustering of genes with related functions, such as head, tail, recombination, regulation, replication and lysis genes, lends support to the modular hypothesis. Genes whose products are involved in transcription, replication, insertion and packaging are often located adjacent or near to their target sites (Thomas, 1963; Dove, 1968). That the regions of homology between two phage DNA's are often gene-sized or larger also lends support to the modular hypothesis; however, often regions of homology between any given pair of phages are less than gene-sized. All these observations, however, do not contradict the hypothesis that the lambdoid phage genomes are derived from a common ancestral genome.

Electron microscope DNA heteroduplex studies showed that non-homologous lengths within heteroduplexes between the lambdoid phages can differ (Fiandt et al, 1971; Simon et al, 1971). This could result if evolution from a common ancestor involved insertion and deletion events as suggested by Highton and Whitfield (1974). The similarity in denaturation patterns of 424, 21 and λ DNA's could indicate evolution from a common ancestor did occur.

The similarities between λ and P22 could be the result of a common evolutionary ancestry. The thermal stability of various phage/phage DNA sequences described by Falkow et al (1969) suggest that these phages may have had a common origin.

Cowie (1974) discussed the idea that the T-even phages diverged from a common ancestor. In the course of their evolution, genetic alterations, deletions, substitutions and random base changes may have occured, which could explain the occurrence of insertion/deletion loops seen in the T-even phage DNA heteroduplexes.

The results of the SP02/ϕ105 homology studies can lead one to suggest that perhaps these phages evolved from a common ancestor (Chow et al, 1972). Or, perhaps their unrelated ancestors underwent recombinational events resulting in a hybrid phage consisting of genes from both phages--possibly the central genes of one phage and the end genes of the other. This may explain the heteroduplex results, which show a central partially homologous region with completely nonhomologous regions on each side.

So, a variety of DNA species have been shown to be homologous. Most bacteriophages that are capable of recombination show some homology in their DNA sequences. Those that do not undergo recombination tend to share little, if any, DNA sequence homology. Most of those phages that share homology with their respective host are capable of becoming prophages. F-factors integrate into their host DNA and are related to the F-like R plasmids and the colicinogenic plasmid colV-K94. These plasmids all share some sequence homology. These findings lend support to the idea that related DNA species have enough DNA sequence homology to allow exchange of genetic material to occur freely between them. Genetic recombination tends to prevent sequences from diverging too far. In this thesis, I have undertaken to study DNA sequence homology between a variety of lambdoid phages and perhaps learn more about the regions, if any, of DNA, which are common and about their evolution.

It seems one cannot predict how closely related two DNA base sequences are from the amount of similarities of biological properties and of viral proteins. Based on base composition studies and transcriptional patterns, it was concluded that analogous arrangement and function of genes and similarity of transcriptional controls does not necessitate a high amount of DNA base sequence homology

(Szybalski et al, 1969). Duplex formation between homologous regions of two lambdoid phages is not necessarily conclusive proof that the sequences and genes in those regions are identical. For example, the exo genes of λ and ϕ80 are located in the exo-β homology regions common to both phages (Fiandt and Szybalski, unpublished results), however, the corresponding exonucleases are immunologically distinct (Szpirer et al, 1969). This seems also to be true for the O genes of λ and ϕ80 (Monnat, Szybalski, Lambert and Thomas, unpublished results). So, DNA sequences that code for distinct proteins, by at least some criteria, can still form duplexes that are stable enough to appear homologous by electron microscopic examination.

The lambdoid group of phages have been randomly selected from the wild and the presence of homologous regions suggests that the number of possible copies of some regions is small. Few, if any, regions of the DNA's of the lambdoid phages have been found to be common to all the DNA's, suggesting that there is more than one copy for most genes of the chromosome.

The work of Simon et al (1971), on the heteroduplexes λ/434, λ/82 and λ/21, in fact led to the hypothesis that there were certain regions common to all phages, which had resulted from some recombination process, which selected the best copy during evolution. As explained by Campbell (1972), these heteroduplexes could yield evidence to support the modular hypothesis of evolution by exchange of segments between phages. Subsequent work with the heteroduplexes λ/424 (~~this thesis~~; Highton and Beattie, unpublished results) and λ/ϕ80 (Fiandt et al, 1971; ~~Highton and Beattie, unpublished results~~) however, has shown that there is not more than 0.5% common to all the phages.

On the other hand, the heteroudplexes, all made with λ as one of the pair, suggested that in some regions all the phages might differ. To test this, I made heteroduplexes of all possible combinations of λ, 424, 434, 21 and PA2.

METHODS

Preparation of Plating Cells (Murray et al, 1973)

Cells were grown overnight in fresh L-broth. These were diluted 1:20 into fresh L-broth and grown for 2 hours (h) at 37°C with aeration (i.e. 10 ml in 100 ml flask, gently shaken). The cells were harvested by centrifugation 10 minutes (min) at about 5000 revolutions per minute (rpm). The supernatant was discarded and the cells were resuspended in half the volume of 10^{-2} to 10^{-3} M $MgSO_4$ and stored at 4°C.

Titration of Phage (Murray et al, 1973)

One-tenth ml of each of a range of dilutions of the phage in phage buffer was adsorbed to 0.2 ml of fresh plating cells at room temperature. After 20 min 2.5 ml of molten BBL soft agar was added and immediately poured onto a BBL agar plate. After about 12 h or overnight incubation at 30°C or 37°C, the plaques were scored and counted.

Isolation of a Single Plaque

Plating cells of the required host strain were prepared. Phage were plated as above to give about 200 plaques/plate. Single plaques were picked with a sterile hollow glass rod and blown into 1 ml of phage buffer; $CHCl_3$ was added to kill any cells carried over with the plaque.

Phage Preparation by Heat Induction of a Thermosensitive Lysogen

A thermosensitive lysogen carries a thermosensitive repressor, which, at the restrictive temperature (usually about 42°C) will not

bind to the DNA and therefore will not prevent transcription of the corresponding mRNA molecules. Cells lysogenic for a thermosensitive prophage were grown overnight at 32°C. These were diluted 50-fold into fresh L-broth, grown until the absorbance at 650 nm was 0.45 and then transferred to a shaking water bath at 42°C to inactivate the repressor. After 20 to 30 min the flask was transferred to 37°C with continual shaking in order to maintain good aeration for 2 to 5 h. To the lysed cells $CHCl_3$ was added (1 ml $CHCl_3$ in 500 ml culture) to lyse any remaining intact cells. The culture was allowed to shake for a few minutes and then the debris was spun down (10K rpm for 10 min). RNAase and DNAase (10 µg/ml final concentration of each) were added to the lysate, which was then left to stand at room temperature for at least 1 h. Further debris was removed by centrifugation (10,000 rpm for 10 min) at 4°C and the isolation of phage continued by plating the supernatant, selecting a single plaque, making a phage lysate, and then pelleting the phage or precipitating the phage with polyethylene glycol (PEG) or banding the phage on a CsCl step gradient (See following sections).

Ultraviolet (UV) Induction of a Lysogen (Miller, 1972)

Fresh plating cells of a lysogenic bacterium were prepared as described above. These were placed in a sterile petri dish or beaker and given a dose of UV radiation of 400 ergs/mm^2. The suspension was immediately diluted 4-fold into fresh warm L-broth in a lightproof culture flask and grown at 37°C in the dark with good aeration for 2 h. Surviving cells were lysed by the addition of 2 drops of $CHCl_3$ per 5 ml of culture. After 5 min, cell debris was removed by centrifugation (10,000 rpm for 10 min) and the supernatant was titrated.

Preparation of Phage Stocks by Plate Lysate

A single plaque was picked using a sterile glass rod and then was transferred into 1 ml of phage buffer containing a drop of $CHCl_3$. The phage were then brought into suspension using a whirlimixer. One-tenth ml of the suspension of phage was added to 0.2 ml of freshly prepared plating cells to give 5×10^5 to 5×10^7 plaque forming units (pfu) per plate depending on the size of the plaque. Larger plaques cover more area on the plate, therefore a more dilute suspension of phage producing such plaques was used. The ~~solution~~ suspension of plating cells and phage was allowed to incubate at room temperature, to allow the phage to adsorb to the bacterial cell surface, and then 2.5 ml of L soft agar was added and the solution was immediately poured onto freshly prepared, thick, moist L agar plates. The plates were incubated at 30°C or 37°C. After confluent lysis was achieved (usually after 5 to 8 h) phage were harvested in either of two ways. In one way, 3 to 4 ml of phage buffer was added to each plate and the plates were refrigerated overnight to get the phage into suspension; the broth was then poured off, $CHCl_3$ was added, the debris was spun down and the supernatant was titrated. Alternatively, 2 to 3 ml of phage buffer were added to the plates, the top layer of agar and buffer was removed, $CHCl_3$ was added, the suspension was centrifuged and the supernatant was titrated. The supernatant was stored at 4°C over a drop of $CHCl_3$. Phage immunity and temperature sensitivity were checked on appropriate indicator strains.

Phage Preparation by Liquid Lysate (Thomas and Abelson, 1966)

A fresh overnight culture of cells was diluted 20- to 50-fold into fresh L-broth supplemented with $MgSO_4$ (to give 10^{-2} M $MgSO_4$), using a flask large enough to allow vigorous shaking to give good

aeration (100 ml broth in a 1 liter flask). When the absorbance at 650 nm reached 0.45 to 0.6 (about 2 to 3 x 10^8 cells/ml) phage were added to give a multiplicity of infection (m.o.i.) of one. The absorbance at 650 nm was followed; it rose and then dropped as the cells lysed, until it reached a minimum when $CHCl_3$ was added. The lysate was allowed to continue to shake well for 5 to 10 min and was then left to stand 10 to 20 min in an ice bath. The lysate was clarified by centrifugation at 10,000 rpm at 4°C for 10 min and the supernatant was titrated. Phage immunity and temperature sensitivity were checked on appropriate indicator strains.

Concentration of Phage by Centrifugation (Thomas and Abelson, 1966)

Phage were harvested from a lysate by centrifuging at 21,000 rpm for 3 h at 6°C in a 10 x 65 ml rotor (Model 21 rotor) in a Spinco Model L centrifuge. The supernatant was titrated for unpelleted phage (less than 5% of the input of phage should remain). Phage pellets were resuspended overnight in 3 to 4 ml of phage buffer at 4°C by gentle rotary shaking (about 60 rpm). Resuspended pellets were combined and the debris was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatants were stored at 4°C and the pellets re-extracted in another 3 to 4 ml of phage buffer as above. These resuspended pellets were combined and the debris was centrifuged as above. The two supernatants were pooled and the volume was measured. RNAase and DNAase were added (to give 10 µg/ml final concentration of each) and allowed to digest the RNA and DNA in the suspension for at least 1 h at room temperature; DNA protected within the phage head would not be digested by the RNAase and DNAase. The residual debris was then centrifuged at 10,000 rpm for 10 min. Phage were further concentrated by banding in a CsCl gradient.

Concentration of Phage by Polyethylene Glycol (PEG) Precipitation

(Yamanoto et al, 1970)

To a clarified phage lysate prepared as above, RNAase and DNAase were added (to give a final concentration of each of 10 µg/ml). This was left to stand at room temperature for at least one hour after which the debris was centrifuged at 10,000 rpm for 10 min. To the supernatant was added NaCl and solid PEG 6000 to give final concentrations of 40 g/l and 10% respectively. The solution was stirred with a magnetic stirrer at very low speed for a few min and then stored at 4°C for at least 1 h (or overnight, if necessary). Phage were spun down at 10,000 rpm for 10 min; at this point the clear supernatant was titrated. The pellet was resuspended in phage buffer by gentle rotary shaking at 4°C overnight and the unresuspended material was centrifuged at 10,000 rpm for 10 min. The pellet was resuspended and the unresuspended material was centrifuged and the supernatants from both resuspensions were pooled. The phage were further purified and concentrated by banding on a CsCl step gradient.

Concentration of Phage by Banding on a CsCl Step/Shelf Gradient

Phage from a lysate were loaded onto a triple step gradient in three Spinco 25.1 centrifuge tubes. To each tube was first added 2.5 ml of CsCl with density 1.7 g/l. Onto this was gently layered 2 ml of CsCl with density 1.42 g/l and then a layer of 2 ml of CsCl with density 1.3 g/l. About 30 ml of the phage lysate was gently layered on the top of the 3-step gradient in each of the three tubes. These were centrifuged at 22,000 rpm for 3 h at 4°C in a SW 25.1 rotor in a L265B Beckmann ultracentrifuge with the brake off. Phage bands were removed with 5 ml syringes by puncturing the side of each tube. The bands were pooled. The weight and volume of the phage

solution recovered was recorded and the weight of CsCl present was calculated (wt CsCl present (g) = (1.3748 x wt solution) - (1.3811 x volume solution)). Solid CsCl was added to give a total of 6.01 g CsCl and the solution was transferred to a screw-top polycarbonate tube with a volume of 9.8 ml. The volume was made up to 9.8 ml with phage buffer. If more than one polycarbonate tube was required, each contained 6.01 g CsCl per 9.8 ml to maintain the proper density at which the phage would band. These were centrifuged in a Type 65 rotor in an L265B ultracentrifuge at 35,000 rpm for 48 h at 4°C with the brake off. The phage bands were removed from above with a syringe and were stored in EDTA-treated plastic tubes at 4°C.

Banding of Phage by Equilibrium CsCl Centrifugation

Solid CsCl was added to the phage solution to give 41.5% w/w (wt CsCl = 0.71 x wt phage solution). This solution was clarified by centrifugation at 15,000 rpm for 1 h at 4°C and decanted into a clean tube to remove the pellicle and the sediment. Phage were banded in a SW50.1 rotor at 33,000 rpm in a L265B ultracentrifuge for 24 h at 4°C in cellulose nitrate tubes. The bands were collected with a syringe from above and rebanded in preclarified 41.5% CsCl. Phage bands were collected from above and stored in clean plastic tubes at 4°C.

Construction of Lysogens

A mixture of 0.2 ml of a fresh overnight culture of the host strain and 2.5 ml of L soft agar was poured onto a fresh L agar plate. A drop of the phage was placed on top of the cells and the plate was incubated for 6 to 8 h at 37°C (or 30°C if the phage carried a thermosensitive repressor). Cells were picked from a turbid area of lysis

and streaked to produce single colonies on a fresh L agar plate. After lysogenization, the virus does not lyse the host cell and its DNA replicates with the host; lysogenic bacteria are found growing in the turbid plaques.

Individual colonies were tested for lysogeny on a dry BBL plate by cross-streaking a suspension of the colony against streaks of indicator phage, which were at concentrations of 2×10^4, 2×10^6 and 2×10^8 pfu/ml. These were examined after incubation overnight at the appropriate temperature.

Lysogens of a phage carrying $\underline{imm}^{\lambda}$ are immune to λ_{cI27} phage but are sensitive to $\lambda\underline{vir}$ phage. The phage $\lambda\underline{vir}$ is a virulent mutant of λ that is insensitive to repressor; it can grow in λ lysogens. The mutations resulting in the virulent phenotype map in the immunity region and are operator mutations (Ptashne, 1971). Lysogens carrying $\underline{imm}^{21}$, $\underline{imm}^{424}$, $\underline{imm}^{434}$ or $\underline{imm}^{PA2}$ are sensitive to $\lambda\underline{vir}$ and to all other phage with a different immunity region, but are immune to phage carrying their own immunity region.

Phage Crosses (Murray et al, 1973)

Freshly prepared plating cells usually of strain QR47 were infected with a mixture of the two phage each at a m.o.i. of 5 phage per bacterium. About 2×10^8 bacteria (directly counted with a Coulter counter) were mixed with 2×10^9 phage in 1.0 ml. The volume was adjusted to 1.0 ml with 10^{-2} M $MgSO_4$. Fifteen to 20 min was allowed for adsorption at room temperature. Cells were harvested by centrifugation (10 min at 5,000 rpm) and the supernatant was assayed for unadsorbed phage. Cells were resuspended in 1.0 ml of prewarmed L broth and diluted 10-fold if the frequency of recombination was very low (i.e. $\leq 10^{-6}$ for the $\underline{trpA}^r \times \underline{trpC}^r$) or 100-fold if the frequency

of recombination was fairly high, and grown at 37°C with vigorous aeration for 2 h. A few drops of $CHCl_3$ were added to lyse any remaining intact cells, the lysate was allowed to shake for about 10 min and then the debris was removed by centrifugation (10,000 rpm for 10 min). The supernatant was titrated on a permissive host for total progeny and on a selective host for the desired recombinants and stored at 4°C.

Complementation of E. coli Auxotrophs by λtrp Phages or Test for the Presence of λtrp Transducing Phages

These tests are based on the observations of Franklin (1971). Lambda-trp transducing phages form trp^+ plaques when grown on a trp^- host in a medium lacking tryptophan. The trp^+ plaques are characterized by a circle of lysis surrounded by a ring of bacterial growth, which was stimulated by tryptophan feeding from the phages. In the case of a phage producing a turbid plaque, the center of the plaque was studded with colonies of trp^+ lysogens. Clear plaque mutants elicit a poor response in the test, however, the addition of a drop of L broth to the soft agar improves the growth of the bacterial lawn.

A mixture of 0.1 ml of fresh plating cells in 2.5 ml of minimal soft agar was overlaid onto an appropriately supplemented minimal agar plate (ACH A). Ten-fold serial dilutions of λ transducing phages were spotted onto this top layer and the spots were allowed to dry. The plates were incubated at 37°C (or 30°C if the prophage carried the thermosensitive repressor). Complementation was scored after 36 to 72 h.

Test for the Presence of tna on $\lambda\ imm^{\lambda}\ nin^{+}$ Transducing Phages

C600 lysogens were made with λ transducing phages thought to

carry the tna gene. These were grown overnight at 30°C. Single colonies were picked and tested for lysogeny by streaking across a λ vir streak and a λ^+ streak at 30°C. All colonies should have been sensitive to λ vir whereas those carrying the λ with the λ immunity region should have been immune to λ^+. The colonies checking out as imm$^{\lambda}$ were grown overnight in fresh L broth and about 1 ml of each was mixed with an equal volume of indole reagent. Colonies lysogenic for λ tna transducing phage turned bright pink when mixed with the indole reagent. This is because a λ tna lysogen will break down tryptophan into indole and indole will be released into the medium. Indole reacts with the indole reagent and the solution turns pink.

Preparation of Phage DNA (Thomas and Abelson, 1966)

Phage stored in CsCl were dialyzed against 10^{-2} M Tris, 10^{-3} M EDTA (pH 8.0) for at least 1 h to remove the CsCl. Dialysis tubing was boiled for 20 min in 0.2 mM EDTA before use. The phage were then transferred to acid-washed repellcoated screw-capped tubes and diluted 2- to 6-fold with 10 mM Tris, 1mM EDTA (pH 8.0) depending on phage concentration. Freshly distilled phenol was pre-equilibrated with an equal volume of 0.5 M Tris (pH 8.0). The phases were allowed to separate and the Tris layer (top) was removed, except for a thin layer to prevent air getting in. DNA was extracted with an equal volume of phenol by mixing gently, allowing both phases to separate and finally removing the lower (phenol and protein) layer with a pasteur pipette. This extraction was repeated 3 to 4 times. Any contaminating phenol was then dialyzed out against 4 changes of 10^{-2} M Tris, 10^{-3} M EDTA (pH 8.0) during about 20 h. The DNA was stored in acid-washed plastic screw-cap tubes at 4°C. The absorbance at 260nm, 280nm and 235nm was measured.

DNA Heteroduplex Formation

The method followed in this thesis was that of Simon, Davis and Davidson (1971). Solutions of two intact phage strains or two phage DNA's were denatured in alkali and then neutralized and allowed to renature. Denatured DNA was prepared directly from the intact phage by simultaneous lysis and strand separation with alkali. Alternatively, when using DNA, the DNA was denatured directly with alkali. This was achieved by allowing a solution containing 3 to 6 x 10^{12} pfu/ml or 1.5 to 3.0 µg of DNA per ml, of each of two phages, to stand 20 to 40 min at 27°C. A solution containing 0.1 M NaOH and 20 mM EDTA was sufficient to denature the following phages: λ, λ trp, λ tna, 424 and 21. To ensure complete denaturation of ϕ80 phage and DNA, all heteroduplexes involving ϕ80 were given a chelation shock treatment before denaturation. This method is described by Deonier et al (1974). Phage PA2 was denatured in 0.25 M NaOH, but 0.1 M NaOH may have been enough. Phage 434 however, also required a chelation shock treatment. Phage were added to 0.375 ml of 0.2 M EDTA (pH 8.5) and allowed to stand at room temperature for one hour after which 0.125 ml of 1.0 M NaOH was added giving a final concentration of 0.25 M NaOH. The solution was allowed to stand at room temperature for 10 min.

Following denaturation, the DNA solution was neutralized by the addition of 50 µl of neutralization solution. This was then renatured, after the addition of an equal volume of formamide, for 2 to 3 h at 27°C. In the presence of formamide, random base interactions in single-stranded DNA (i.e. intrachain hydrogen-bonding) are melted out and single-strands can reanneal to form heteroduplexes or homoduplexes. After renaturing 2 to 3 h at 27°C about 50% of the molecules were renatured. The DNA was then ready to spread. To store

the heteroduplex at this point, the formamide was dialyzed away into a buffer solution (10 mM Tris, 1 mM EDTA, pH 8.5) overnight and the DNA was then stored at 4°C.

Standard Spreading Conditions

The standard procedure for spreading the DNA heteroduplexes was to dilute the DNA into a freshly prepared hyperphase solution and, after the addition of cytochrome C, 2 to 3 minutes later, to spread it on to the hypophase solution also freshly prepared. The hyperphase and hypophase solutions were isodenaturing, that is, a DNA molecule had about the same T_m in both. This is important when looking at regions of partial homology. This ensures that the regions with partial homology will not change from double- to single-stranded or from single- to double-stranded when spread on to the hypophase.

The DNA spreading solution was composed of 20 µl of the renatured DNA solution, 20 µl of cytochrome C (either 1 mg cytochrome C/ml or 2 mg cytochrome C/ml) and 200 µl of hyperphase. This gave a final concentration of each DNA of 1.45 µg/ml. The hyperphase used in the standard conditions consisted of 58.1% formamide, 0.12 M Tris, 0.012 M EDTA; thus, the final concentrations of formamide, Tris and EDTA in the DNA solution were 48.4%, 0.1 M and 0.01 M respectively. The standard hypophase solution consisted of 15% formamide, 0.01 M Tris and 0.001 M EDTA. The T_m (See Appendix 1) of the hyperphase was 50.9°C and the T_m of the hypophase was 57.8°C. These two phases are about isodenaturing. Cytochrome C was used to form the protein film, which binds the DNA on the surface of the hypophase. In the microscope, the structure visualized is a column of protein around the DNA. The structure is thicker than the DNA alone. The higher concentration of cytochrome C was used in some experiments to improve

the visualization of the DNA.

The standards used were double-stranded pSC101 DNA and single-stranded M13 DNA. These external standards were added because their lengths were known. Several of each of these DNA molecules could be photographed in the same field of view as a heteroduplex. As a grid is moved in the electron microscope, the specimen position changes and this results in a change in its magnification. The magnification is determined by the distance of the object from the final image plane. The presence of these standards allowed me to determine the relative sizes of the single- and double-stranded regions in each heteroduplex compared with the sizes of their respective standards.

The copper grids used to support the collodion or parlodion film (H4, 2.3 mm, 400 mesh grids) were first cleaned by sonicating them in 100% ethanol for 2 min. They were allowed to dry, and then were placed on to a wire mesh that rested in a petri dish filled with distilled water. A drop of either collodion (2% in amyl acetate) or parlodion (solid parlodion was baked for 24 h at 60°C in a vacuum oven and the appropriate weight was dissolved in amyl acetate to give a 2% w/v solution), both stored at room temperature in the dark, was dropped from 1 to 2 cm above the surface of the distilled water and allowed to dry into a film. This occurs because the amyl acetate evaporates leaving a film. A corner of the wire mesh was grasped with a pair of tweezers and lifted up leaving the grids coated with a wrinkle-free area of film. The grids were left to dry at room temperature in a petri dish lined with filter paper.

After the addition of the cytochrome C, if standards were spread with the DNA, 60 µl of the DNA/cytochrome C solution was removed, to which a small volume ($\leq$ 5 µl) of standard DNA was added (to give about 5 to 10 µg/ml final concentration of each standard). Then,

50 µl of this mixture was released down the quartz ramp. This was done under a draught-excluding hood on a stable surface with a 50 µl pipette held at a fixed angle to the ramp by metal clamps, the pipette tip about 1 mm from the ramp. Occasionally before running the DNA solution down the ramp, a small amount of talc was spread on to the hypophase. This was done to visualize the formation of the cytochrome C film on the hypophase because the talc was pushed away by the film.

The DNA-coated grids were shadowed with platinum. This was done by mounting them on a motor driven turntable, placing them in a vacuum chamber and evaporating platinum on to their surface at an angle of about 7^{o} while the mounted grids were spinning at about 30 rpm. The platinum wire (1 cm x 0.1 mm, or the equivalent) was wrapped tightly around a tungsten filament in the vacuum chamber (10^{-4} Torr) and was allowed 2 seconds to evaporate. Then a layer of carbon was evaporated on to the grids from an arc across two graphite electrodes to make the grids stable under the electron beam. The grids were finally rinsed in 100% ethanol to remove the collodion or parlodion and stored in a petri dish lined with filter paper. Grids were scanned with a Siemens Elmiskop 101 electron microscope at x 40,000 magnification, with an accelerating voltage of 80,000 Volts. Photographs were taken at x 20,000 magnification. The negatives were enlarged 5-fold and traced on to sheets of paper. The molecules were measured in inches with a Keuffel and Esser map measurer.

Negative-Staining

The intact phages were negatively stained using either sodium phosphotungstate or uranyl acetate. The negative stains outline the phage and reveal surface detail. A drop of phage (about 2 µl) was

placed on to a parlodion- or collodion-carbon coated grid. The excess moisture was removed with filter paper until a thin film remained. Then, several drops of the stain solution were dropped on to the grid, which was then drained and allowed to dry. The grids were viewed in the electron microscope and photographs were taken at a magnification of x40,000.

MATERIALS

The following were sterilized by autoclaving at 15 lb/in^2 for 15 minutes before use:

BBL Top Agar	NaCl	5 g
	Baltimore Biological Laboratory (BBL) Trypticase	10 g
	Difco Agar	6.5 g
	Distilled Water	to 1 Liter
BBL Bottom Agar (Parkinson, 1968)	NaCl	5 g
	BBL Trypticase	10 g
	Difco Agar	10 g
	Distilled Water	to 1 Liter
L-Broth (pH 7.2) (Lennox, 1955)	Difco Bacto Tryptone	10 g
	Difco Bacto Yeast Extract	5 g
	NaCl	10 g
	Distilled Water	to 1 Liter
L-Broth Top Agar (pH 7.2)	Difco Bacto Tryptone	10 g
	Difco Bacto Yeast Extract	5 g
	NaCl	10 g
	Difco Agar	12 g
	Distilled Water	to 1 Liter
L-Broth Bottom Agar (pH 7.2)	Difco Bacto Tryptone	10 g
	Difco Bacto Yeast Extract	5 g
	NaCl	10 g
	Difco Agar	15 g
	Distilled Water	to 1 Liter

Hammersmith Stabs	Difco Nutrient Broth	5 g
	Difco Agar	7.5 g
	NaCl	5 g
	Thymine	0.1 g
	Distilled Water	to 1 Liter
Water Top Agar	Davis New Zealand Agar	550 g
	Distilled Water	to 25 Liters
Spizizen Minimal Salts	$(NH_4)_2SO_4$	10 g
(Spizizen, 1955)	K_2HPO_4	70 g
	KH_2PO_4 (Analar	30 g
	Tri-sodium Citrate	5 g
	$MgSO_4$	1 g
	Distilled Water	to 1 Liter
Sugars (20%)	Sugar	20 g
Glucose	Distilled Water	to 100 ml
Glycerol		
Maltose		
ACH Agar "A"	Water Agar	300 ml
	Spizizen Salts	80 ml
	Glucose (20%)	4 ml
	Acid Hydrolyzed Casein	
	(ACH) 20%	1 ml
	Indole	to give 10 g/ml

ACH Agar "B"	Water Agar	300 ml
	Spizizen Salts	80 ml
	Glycerol (20%)	4 ml
	5-Methyl Tryptophan	20 mg
	ACH (20%)	1 ml
	Indole	to give 10 g/ml
Phage Buffer	Na_2HPO_4 (Anhydrous)	9 g
	KH_2PO_4 (Analar)	3 g
	NaCl	5 g
	$MgSO_4$ (0.1M Solution)	10 ml
	$CaCl_2$ (0.01M Solution)	10 ml
	1% Gelatin Solution	1 ml
	Distilled H_2O	1 Liter
Bacterial Buffer	KH_2PO_4	3 g
	Na_2HPO_4	7 g
	NaCl	4 g
	$MgSO_4-7H_2O$	0.2g
	Distilled H_2O	10 Liters

ENZYMES AND CHEMICALS

Indole Reagent	4-Dimethylaminobenzaldehyde	9 g
	dissolve in	
	Ethanol	200 ml
	then add	
	Concentrated HCl	45 ml
	then add	
	Ethanol	to 250 ml

Pancreatic DNAase and RNAase were obtained from Worthington Biochemical Corporation, Freehold, New Jersey, USA.

Caesium Chloride was obtained from BDH Ltd., Poole, Dorset, England.

Cytochrome C (Horse Heart) Type VI was obtained from Sigma Chemical Company.

Formamide (98%) was obtained from BDH Ltd., Poole, Dorset, England.

Uranyl Acetate (Analar) was obtained from BDH Ltd., Poole, Dorset, England.

HETERODUPLEX INGREDIENTS

Denaturation Solution "A"

0.1 M NaOH	NaOH	0.04 g
20 mM EDTA	EDTA	0.0744 g
	Distilled H_2O	10 ml

Denaturation Solution "B"

0.25 M NaOH	NaOH	0.1 g
20 mM EDTA	EDTA	0.0744 g
	Distilled H_2O	10 ml

Neutralization Solution "A"

2.0 M Tris	Tris	2.42 g
1.6 M HCl	Concentrated HCl (35%)	1.62 ml
	Distilled H_2O	8.38 ml

Neutralization Solution "B"

2.0 M Tris	Tris	2.42 g
1.7 M HCl	Concentrated HCl (35%)	3.16 ml
	Distilled H_2O	6.84 ml

Uranyl Acetate Stain

0.05 M Uranyl Acetate	Uranyl Acetate	21.2	mg
0.05 M HCl	Concentrated HCl (35%)	5	µl
	Ethanol (90%)	1	ml

Hyperphase Solution "A"

0.1 M Tris	Tris	0.605	g
0.01 M EDTA	EDTA	0.186	g
50% Formamide	dissolve in		
	Distilled H_2O	25	ml
	add		
	Concentrated HCl	to pH 8.5	
	add		
	Formamide	25	ml

Hyperphase Solution "B"

0.113 M Tris	Stock Solution		
0.0113 M EDTA	0.3 M Tris		
56.25% Formamide	0.03 M EDTA	3.95	ml
	Formamide	5.93	ml
	Distilled H_2O	0.60	ml

Hypophase Solution "A"

0.01 M Tris	Hyperphase "A"	10	ml
0.001 M EDTA	Formamide	10	ml
15% Formamide	Distilled H_2O	80	ml

Hypophase Solution "B"

0.013 M Tris	Hyperphase "B"	10	ml
0.0013 M EDTA	Formamide	9.35	ml
15% Formamide	Distilled H_2O	80.65	ml

Cytochrome C	Stock Solution (1 mg/ml)		

Dialysis Solution

0.01 M Tris	Stock Solution		
0.001 M EDTA	0.2 M Tris		
pH 8.5	0.02 M EDTA	25	ml
	Distilled H_2O	475	ml

(To remove formamide from heteroduplexes)

NEGATIVE-STAINS

Sodium Phosphotungstate	2% Phosphotungstic Acid in H_2O (w/v) adjusted to pH 7 with NaOH
Uranyl Acetate	2% Uranyl Acetate in H_2O (w/v), pH not adjusted

TABLE 1

BACTERIAL STRAINS

Strain	Relevant Characteristics	References	Source[a]
C600	tonA: resistant to phage ϕ80.	Appleyard (1954)	NEM
C600(λ)	as for C600; lysogenic for phage λ.	Baldwin (1966)	NEM
C600(λ*imm*^{21})	as for C600; lysogenic for phage λ*imm*^{21}.	Baldwin (1966)	NEM
C600(21)	as for C600; lysogenic for phage 21.	Baldwin (1966)	NEM
C600(434)	as for C600; lysogenic for phage 434.	Baldwin (1966)	NEM
C600(424)	as for C600; lysogenic for phage 424.	Baldwin (1966)	NEM
C600(82)	as for C600; lysogenic for phage 82.	Arber (1960)	NEM
C600(λ*tna*)	as for C600; lysogenic for phage λ*tna*.		RJM
C600 (λ*tna* *imm*$^{\lambda}$ *cI*857 *nin*$^{+}$)	as for C600; lysogenic for phage λ*tna* *imm*$^{\lambda}$ *cI*857 *nin*$^{+}$.		RJM
groN785	restricts growth of phages λ, 434 and 21; allows growth of phages with *nin* deletion.	Georgopoulos & Herskowitz (1971)	NEM
QR47		Signer & Weil (1968a)	NEM
*trpAC*9*su*III	*trpABC* deleted; *trpDE*$^{+}$; *tonB*.	Franklin (1971)	WJB

TABLE 1 (CONTINUED)

Strain	Relevant Characteristics	References	Source[a]
W1485		Franklin & Dove (1969)	NEM
W3110	*sup*^{0}.	Yanofsky & Ito (1966)	WJB
W3110(82)	*sup*^{0}; lysogenic for phage 82.		NEM
W3350	prototroph	Campbell (1961)	NEM

[a] NEM: Noreen E. Murray

RJM: Rhonda J. Myers

WJB: William J. Brammar

TABLE 2

BACTERIOPHAGE STRAINS

Strain	Relevant Characteristics	Reference	Source[a]
λvir	virulent strain of λ.	Jacob & Wollman (1954)	WJB
λclear	clear plaque mutant of λ.	Meselson (1954)	NEM
λ*c*I26	clear plaque mutant of λ.		NEM
λ*c*I857 *S*7	λ carrying a temperature sensitive mutation in the repressor and the *S*7 mutation, which causes a defect in cell lysis; *S*7 is an amber mutation, supressible by suIII^{+}.	Goldberg & Howe (1969)	NEM
λ*nin*^{5}	λ with *nin*^{5} deletion.	Court & Sato (1969)	NEM
λ*imm*^{21}	λ carrying the *imm* region of phage 21.	Liedke-Kulke & Kaiser (1967)	NEM
NM540	λ vector phage deleted for about 21% of the chromosome; *imm*^{21}.	Murray & Murray (1975)	NEM
λ*tna*	NM540 carrying the *tna* gene of *E. coli*.	Borck *et al* (1976)	WJB
λ*tna imm*$^{\lambda}$ *c*I857 *nin*$^{+}$	NM540 carrying the *tna* gene of *E. coli* and and *imm*$^{\lambda}$ cI857 and the *nin* region of λ.	this thesis	RJM

TABLE 2 (Continued)

Strain	Relevant Characteristics	References	Source[a]
λ*trpA*^{l}	NM540 carrying the *trpA* gene in the leftward orientation.	Hopkins *et al* (1976)	NEM
λ*trpC*^{l}	NM540 carrying the *trpC* gene in the leftward orientation.	Hopkins *et al* (1976)	NEM
λ*trpABC*^{l}	NM540 carrying the *trpABC* genes in the leftward orientation.	Hopkins *et al* (1976)	NEM
λ*trpA*^{r}	NM540 carrying the *trpA* gene in the rightward orientation.	Hopkins *et al* (1976)	NEM
λ*trpC*^{r}	NM540 carrying the *trpC* gene in the rightward orientation.	Hopkins *et al* (1976)	NEM
λ*trpABC*^{r}	NM540 carrying the *trpABC* genes in the rightward orientation.	This thesis	RJM
φ80 clear	derivitive of φ80 producing clear plaque morphology.		NEM
82		Arber (unpublished)	NEM
21		Baldwin *et al* (1966)	NEM
424		Baldwin *et al* (1966)	NEM
434		Baldwin *et al* (1966)	NEM
PA2		Schnaitman *et al* (1975)	CS
h^{80} *att*^{80} *imm*$^{\lambda}$ *c*I857 *nin*$^{+}$	φ80-λ hybrid phage	Müller-Hill *et al* (1968)	WJB

[a] NEM Noreen E. Murray WJB William J. Brammar RJM Rhonda J. Myers CS Carl Schnaitman

RESULTS

ISOLATION AND GROWTH OF THE LAMBDOID PHAGES

All the steps involved in the growth of cells, isolation of single plaques, concentration and purification of phages and isolation of phage DNA are described in detail in Materials and Methods. All DNA was isolated by phenol extraction. All phage and DNA stocks were stored at 4°C in plastic screw-top tubes.

Electron micrographs of the following negatively-stained bacteriophages: λ; 424; 21; 434; φ80; and; PA2 are shown in Appendix 4, Plates I through VIII.

424

This phage was induced by UV-irradiation of C600(424) lysogens and grown by infecting C600 cells in a liquid medium. Phage were pelleted and then banded and rebanded on a CsCl equilibrium gradient. The phage were used in the 424/21 heteroduplex from which came the molecules labelled 3902, 3783, 3786 and 9616. The DNA was used in the 434/424 heteroduplex from which came the eleven molecules labelled between 4446 and 4478 (See Appendix 3).

From the above phage stock, a single plaque was isolated from which a plate lysate of C600 cells was prepared. The single plaque was tested for the 424 immunity by superinfection of homoimmune and heteroimmune lysogens (The 424 phages will lyse only those host cells carrying heteroimmune phages--phages with immunity other than 424). The phage were purified by banding on a CsCl step gradient and finally by banding and rebanding on a CsCl equilibrium gradient. The phage were used in the λ/424, PA2/424 and the second 424/21 heteroduplexes.

The 434/424 heteroduplex producing molecules labelled between 6033 and 6208 was made with the DNA.

21

The phage resulting from UV induction of an overnight culture of C600(21) grown at 37°C in L-broth were used to infect C600 cells in a single-plate lysate. From this a single plaque was picked and used to prepare a large scale plate lysate (30 plates) using C600 cells. A single plaque was used to ensure a homogeneous population of phage. Phage were concentrated by pelleting and purified by banding and re-banding on CsCl equilibrium gradients. This phage stock was used to prepare the 424/21 heteroduplex that includes molecules labelled 3902, 3783, 3786 and 9616. This DNA stock was used to prepare the 434/21 heteroduplex.

A dilution of the above phage stock was plated on C600 cells on BBL-agar plates and single turbid plaques were picked. Phages carrying the wild-type $\underline{imm}^{21}$ are turbid in appearance. These plaques were tested for immunity by infecting a control plate of C600 and by superinfecting the following lysogens: C600(λ); C600(21) and; C600(434). Only those lysogens carrying phages with immunity different than $\underline{imm}^{21}$ will allow growth of phages carrying $\underline{imm}^{21}$. A single turbid plaque, carrying $\underline{imm}^{21}$, was used to prepare another stock of phage 21 by plate lysate using C600 cells. Phage were banded on a CsCl step gradient and were finally banded and rebanded on a CsCl equilibrium density gradient. The PA2/21 heteroduplex was made using this phage stock.

434

A single C600(434) colony was induced by UV-irradiation and plated on BBL-agar plates. A single plaque was used to infect C600 cells by plate lysis. Phage were pelleted and banded and rebanded on CsCl equilibrium gradients. This DNA was used to prepare the 434/424 heteroduplex that produced the molecules labelled between 4446 and 4478 and the 434/21 heteroduplex.

From the above phage stock, a single plaque was picked and used to make a plate lysate of C600 cells on L-agar plates. Phage were concentrated by banding on a CsCl step gradient and purified by CsCl equilibrium centrifugation. DNA from this phage stock was used in the 434/424 heteroduplex that includes molecules labelled between 6033 and 6208.

λ

A plate lysate was made using a single clear plaque and C600 plating cells. Phage were concentrated by banding on a CsCl step gradient and then purified by CsCl equilibrium centrifugation. The phage were used to make the λ/424 heteroduplex.

φ80

Phage φ80 clear was plated on the φ80-sensitive bacterial strain W3350. A single plaque was used to infect W3350 plating cells by plate lysis. Phage were banded on a CsCl step gradient and finally banded and rebanded on CsCl equilibrium gradients. Both phage and DNA were used to make the following heteroduplexes: φ80/434; φ80/21; φ80/424 and; φ80/PA2.

PA2

The strain CS214, which is lysogenic for the phage PA2 and carries a temperature-sensitive repressor was grown overnight at 30°C. Phage were heat-induced, concentrated by polyethylene glycol precipitation and banded on a CsCl step gradient. The band isolated was purified by CsCl equilibrium density centrifugation. The titer of this CsCl stock was about 10^{11} pfu/ml. To get a stock with a higher titer, a single plaque was isolated from the CsCl stock and used to prepare a plate lyste using QR47 plating cells and L-agar plates. Phage were harvested and then banded on a CsCl step gradient and finally banded and rebanded on CsCl equilibrium gradients. The phage were used to make the PA2/424, PA2/21, PA2/λ, and PA2/434 heteroduplexes.

82

Attempts were made to grow and concentrate phage 82, but with little success. Phage 82 lysogens, both W3110(82) and C600(82) were induced by UV-irradiation and either of several procedures were followed after this. I tried to grow the phage by liquid and plate lysates directly from the UV-induced stock or after isolating and purifying single plaques. I then tried to concentrate the phage by either pelleting, precipitating with polyethylene glycol or banding on a CsCl step gradient. It was after the concentration of the phage that the infectivity was lost; before this there was an adequate amount of phage to concentrate to get a reasonably high titer for CsCl banding (10^{12} pfu/ml). Perhaps during the concentration procedures the phage were inactivated and therefore lost the ability to lyse bacterial cells. I repeated these procedures but, each time the phage titers dropped to well below 10^{10} pfu/ml prior to banding on

CsCl equilibrium gradients. Because of this trouble in getting a high-titer stock of phage 82, I did not use the phage in heteroduplex studies.

LENGTH DETERMINATIONS: pSC101; λ; M13; 424 and; PA2 DNA MOLECULES

The lengths of double-stranded pSC101 and λ DNA molecules were determined as described in the trp/tna Results section; their respective lengths are 9361 ± 97 (s.d.; n = 10) and 49,153 ± 727 (s.d.; n = 25) base pairs. This value of the length of pSC101 DNA is based on the determination that pSC101 = 173.8 ± 1.8 (s.d.; n = 10)% ϕX174 (this thesis) and the determination that ϕX174 consists of 5386 bases (Sanger et al, 1978). Single-stranded M13 DNA was determined to consist of 6230 bases (David Finnegan, personal communication).

The length of the 424 DNA molecule was determined by spreading double-stranded 424 DNA with double-stranded pSC101 DNA under standard spreading conditions. Twenty 424 DNA molecules, each surrounded by between 6 and 13 pSC101 DNA molecules, were photographed, traced and measured. The ratio, 424 length/pSC101 length, was determined to be 5.1 ± 0.04 (s.d.; n = 20). The length of the pSC101 DNA molecule is 19.0 ± 0.2 (s.d.; n = 25)%λ (9361 ± 97 (s.d.; n = 10) base pairs) thus, the 424 DNA molecule is 96.4 ± 1.3 (s.d.; n = 20)%λ. Thus, the 424 DNA molecule consists of 47,369 ± 617 (s.d.; n = 20) base pairs based on the following: 424/pSC101 = 5.1 ± 0.04 (s.d.; n = 20); pSC101 = 173.8 ± 1.8 (s.d.; n = 10)% ϕX174 and; ϕX174 = 5386 bases.

The length of the PA2 DNA molecule was determined by spreading double-stranded PA2 DNA with double-stranded pSC101 DNA under standard spreading conditions. Nineteen PA2 DNA molecules, each adjacent to at least two pSC101 DNA molecules were photographed, traced and

measured. The ratio, length PA2/length pSC101, was determined to be 5.2 ± 0.1 (s.d.; n = 19). Thus, PA2 is 99.6 ± 2.0 (s.d.; n = 19)%λ. PA2 consists of 48,960 ± 983 (s.d.) base pairs based on the following: PA2/pSC101 = 5.2 ± 0.1 (s.d.; n = 19); pSC101 = 173.8 ± 1.8 (s.d.; n = 10)% ϕX174 and; ϕX174 = 5386 bases.

COMMON PROCEDURES IN HETERODUPLEX SPREADINGS

All six heteroduplexes described in the subsequent pages were prepared in the same way and spread under the standard conditions at room temperature (about 20°C). See Materials and Methods for details.

GENERAL DATA PROCESSING

With every heteroduplex molecule at least one nearby standard was photographed. The standards added were M13 DNA (single-stranded), pSC101 DNA (double-stranded) or renatured homoduplexes (double-stranded), and these had known lengths, either determined in the present work or by others; these are expressed in %λ units. The process by which original measurements within each heteroduplex were converted to final values is described below. All molecules were measured at least two times with a Keuffel and Esser swivel-handle map measurer. If homoduplexes were used as standards, all original measurements were divided by the length of the homoduplex. If M13 and pSC101 DNA's were used as standards, each single-stranded measurement was divided by the average of the measured lengths of all the nearby M13 standards and all double-stranded lengths were divided by the average of the measured lengths of all the nearby pSC101 standards. The next step was to multiply each measurement by the length of the standard in %λ (the standards, M13, pSC101, 434, 21, 424 and PA2 are 12.7, 19.0, 100, 89, 96.4 and 99.6%λ, respectively (See Tables 3, 3A). These

TABLE 3

LENGTHS OF PHAGE DNA'S[a]

Phage	λ	434	424	21	φ80	PA2	pSC101	M13
Length compared with λ (%)	100	100[b]	96.4[c] 97.2[d]	89[b]	92[e]	99.6[c]	19.0[c]	12.7[c]

[a] All measurements are of double-stranded DNA's except for M13, which is single-stranded.

[b] Simon et al, 1971.

[c] This thesis.

[d] Highton and Whitfield, 1974.

[e] Davis and Parkinson, 1971.

TABLE 3A

THE LENGTHS OF THE λ, pSC101 AND M13 DNA MOLECULES

	%ϕX174[a]	%λ	bases/base pairs
λ	912.6 ± 13.5 (25)	100	49,153 ± 727 (25)
pSC101	173.8 ± 1.8 (10)	19.0 ± 0.2 (25)	9,361 ± 97 (10)
M13	115.8	12.7	6,230[b]

All values quoted consist of the mean ± standard deviation. The number comprising the mean is in parentheses following the standard deviation.

[a] The length of ϕX174 DNA is 5,386 bases (Sanger et al, 1978).

[b] David Finnegan (personal communication).

two steps are equivalent to multiplying each original measurement by a scale factor (S), equal to the ratio, measured length of standard in %λ/measured length of standard in inches, which scales the measured lengths to %λ.

Each single strand within a bubble was then assigned to one of the two phages involved in the heteroduplex. This was done in a series of steps. First of all, I assumed that the base separation for double-stranded and single-stranded DNA was the same after scaling. Results of experiments in which M13 DNA and pSC101 DNA were spread together indicated that the ratio, measured length/known base pairs, for these single- and double-stranded standards are nearly the same. Two principal errors that can effect the results of comparing molecules spread by this heteroduplex technique are the distortion of the lengths of the molecules when immobilized on the grids and the variability in magnification over the grid. To minimize these errors, single- and/or double-stranded standards of known lengths have been photographed adjacent to every heteroduplex photographed. Secondly, each segment was averaged. The longer strands of each bubble were averaged together and the shorter strands of each were averaged together. The average difference in length between the single strands in each bubble was then determined and these differences were combined so as to equal the known difference in length of the two phages' DNA's (in %λ) as closely as possible. In this way, the single strands in each bubble could be assigned to one or other phage. Listed in Appendix 3 are the scaled values and the lengths of the standards for all molecules in all heteroduplexes studied. Thus, these scaled lengths can be converted back to the original measurements.

After assigning the single strands of each bubble in each molecule the scaled single-stranded lengths were "adjusted" to account for errors and to make the sum of the double- and single-stranded segments equal the known length of each strand. Each molecule will have two adjustment factors, one for each phage. I have assumed that the adjustment factor for double-stranded DNA is 1. I justify this because double-stranded DNA is a double helix, stabilized with hydrogen bonds and is thus less susceptible to distortion (i.e. local variation in spreading) than is single-stranded DNA. The adjustment factor (f) also accounts for errors in single-stranded length introduced by using only a double-stranded standard. The formula for f is:

$$f = \frac{L - \Sigma ds}{\Sigma ss}$$

where L = the known length of the phage DNA molecule (in %λ)

Σds = the sum of double-stranded segments in the heteroduplex (in %λ)

Σss = the sum of single-stranded segments in the heteroduplex (in %λ).

After adjustment, the individual molecules were drawn out and these are shown in Appendix 3 (Figures 13 - 20). Finally, the average values for the coordinates of the regions of homology and nonhomology were determined (See Table 5); these molecules are shown in Figure 3. Included in Figure 3 are the heteroduplexes I have constructed as well as those constructed by Fiandt et al, 1971; Niwa et al, 1971 and; Simon et al, 1971 (See Tables 5 and 5A). Each heteroduplex is represented twice in Figure 3, once with one phage DNA on 'top' and once with the other phage DNA on 'top'. (The average scaled coordinates, before adjustment, of the homologous and nonhomologous regions within the heteroduplexes between the lambdoid phages are found in Table 4).

TABLE 4

SCALED COORDINATES OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS IN THE HETERODUPLEXES BETWEEN THE LAMBDOID PHAGES

434	36.8-37.3	41.1-92.2	95.2-95.4	95.6-98.0	(98.8)					
424	36.8-37.3	41.1-86.9	89.9-90.2	90.3-90.4	(91.2)					
434	0 -15.9	35.8-36.2	36.8-39.1	39.7-55.9	63.5-69.9	77.5-90.4	94.3-95.2			
21	0 -16.7	36.6-37.0	37.6-38.5	39.1-59.1	66.7-71.3	78.9-81.6	85.5-87.8			
424	0 -15.9	38.0-40.1	40.9-86.5	89.1-89.5	89.8-92.8					
21	0 -16.7	38.8-39.8	40.6-81.9	84.5-84.9	85.1-87.6					
λ	37.3-37.5	37.9-39.0	39.2-39.4	41.4-41.5	41.8-42.5	43.1-43.3	45.0-92.4	(97.3)		
424	37.3-37.7	38.1-39.5	39.6-39.9	41.9-42.1	42.4-43.7	44.2-44.6	46.2-88.0	(92.8)		
λ	37.1-37.7	38.0-39.7	39.8-40.3	43.2-45.7	46.4-46.9	48.3-62.3	62.5-64.7	70.6-82.5	85.5-99.6	(105)
PA2	37.1-37.7	38.0-39.2	39.4-39.8	42.7-43.5	44.2-44.7	46.0-59.1	59.3-61.4	67.3-75.0	78.0-89.0	(94.1)
PA2	43.2-44.7	45.4-45.5	47.1-91.2	(97.9)						
424	43.2-45.6	46.3-46.4	48.0-88.8	(95.4)						
PA2	0 -15.9	37.6-38.5	39.3-60.7	66.5-74.0	76.8-89.8	93.4-96.4				
21	0 -16.7	38.4-40.2	41.0-60.4	66.3-73.7	76.5-81.3	84.9-87.5				

TABLE 4 (CONTINUED)

PA2	36.5-37.2	40.5-56.2	56.6-59.9	69.0-77.5	77.7-78.6	81.4-90.0	90.3-95.3	98.1-98.5	98.7-101.0 (101.8)
434	36.5-37.2	40.5-51.1	51.5-54.6	63.6-70.4	70.6-71.4	74.2-81.9	82.2-91.1	93.9-94.3	94.4- 94.7 (95.4)

Coordinates in a column define a nonhomologous region. Values of scaled measurements of each molecule within each heteroduplex are tabulated in Appendix 3 Tables 13 through 20. It is the overall "average" molecule from these scaled values that is represented by the coordinates in this table.

TABLE 5

SCALED AND ADJUSTED COORDINATES OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN HETERODUPLEXES BETWEEN THE LAMBDOID PHAGES

434	36.8-37.3	41.2-95.6	98.6-98.8	98.9-99.0	(100)				
424	36.8-37.3	41.2-89.5	92.5-92.8	92.9-95.5	(96.4)				
434	0 -15.9	35.8-36.2	36.9-39.5	40.0-58.1	65.7-72.9	80.5-94.9	98.7-100		
21	0 -16.7	36.6-36.9	37.6-38.7	39.2-59.6	67.2-72.0	79.6-82.4	86.3-89		
424	0 -15.9	37.9-40.2	41.1-89.5	92.1-92.7	92.9-96.4				
21	0 -16.7	38.7-39.8	40.7-82.8	85.4-85.9	86.2-89				
λ	37.3-37.5	37.9-39.1	39.3-39.5	41.5-41.6	41.9-42.7	43.2-43.5	45.2-94.8	(100)	
424	37.3-37.7	38.1-39.7	39.8-40.1	42.1-42.4	42.7-44.0	44.6-44.9	46.6-91.3	(96.4)	
λ	37.1-37.7	38.0-39.6	39.7-40.2	43.0-45.3	46.1-46.5	47.8-61.1	61.2-63.3	69.2-81.4	84.3-95.5 (100)
PA2	37.1-37.7	38.0-39.8	39.9-40.4	43.2-44.0	44.8-45.2	46.6-60.7	60.9-63.1	69.0-77.0	80.0-94.5 (99.6)
PA2	43.2-44.8	45.4-45.6	47.2-92.9	(99.6)					
424	43.2-45.6	46.3-46.5	48.0-89.6	(96.4)					
PA2	0 -15.9	38.0-39.9	40.8-63.0	68.8-76.6	79.4-92.9	96.5-99.6			
21	0 -16.7	38.8-39.8	40.6-61.0	66.9-74.8	77.6-82.7	86.3-89			

TABLE 5 (Continued)

PA2	36.5-37.2	40.5-56.0	56.4-59.8	68.8-75.5	75.7-76.5	79.3-87.8	88.1-93.0	95.9-96.3	96.4-98.7	(99.6)
434	36.5-37.2	40.5-51.9	52.2-55.5	64.6-73.7	73.9-74.9	77.7-85.9	86.2-95.7	98.5-99.0	99.1-99.4	(100)

Coordinates in a column define a nonhomologous region. Individual molecules within each heteroduplex are shown in Appendix 3, Figures 13 - 20. It is the overall "average" molecule from these scaled and adjusted values that is represented by the coordinates in this Table. Drawings of the above molecules are shown in Figure 3.

TABLE 5A

LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN HETERODUPLEXES BETWEEN LAMBDOID PHAGES

λ/434[a]	37.4	2.1 1.0 2.3	16.5 3.5 10.4	0 0.5 0.9	2.3 7.7 2.3	2.1 0.9 2.4	3.6 0.1 1.6	1.5 6.0 1.5	2.1 3.4 1.2	4.0 1.9 13.8	2.6 0.8 0.4									
λ/82[a]	37.3	1.9 1.0 2.4	16.8 3.5 10.3	0 0.5 1.1	2.2 5.7 2.2	11.2 0.1 6.1	0.8 3.0 0.9	9.8 3.0 13.4	2.5 0.7 0.2											
λ/21[a]		15.9 21.0 16.7	25.4 8.2 23.2	9.0 5.4 4.0	2.4 1.9 0.2	5.9 1.9 3.9	3.0 2.6													
434/82[a]	64.2	9.2 0.1 5.9	0.9 3.0 0.8	8.8 1.7 10.5	9.0 3.1 2.2															
λ/φ80[b]	10.0	1.0 6.5 1.0	1.0 2.5 1.0	0.5 7.5 0.5	22.0 0.5 15.5	14.0 5.5 10.0	9.8 1.8 13.3	7.0 2.0 3.7	4.4 0.5 6.2	4.5 0 5.0										
λ/φ81[c]	24.8	25.3 0.2 22.5	6.6 3.8 3.6	9.3 0.3 11.2	15.8 0.4 14.6	0.8 0.3 3.1	1.5 1.3 2.7	4.7 0.3 4.9	4.6 0 5.6											
φ80/φ81[c]	47.9	34.4 13.0 38.7																		

[a] Simon et al, 1971 [b] Fiandt et al, 1971 [c] Niwa et al, 1978

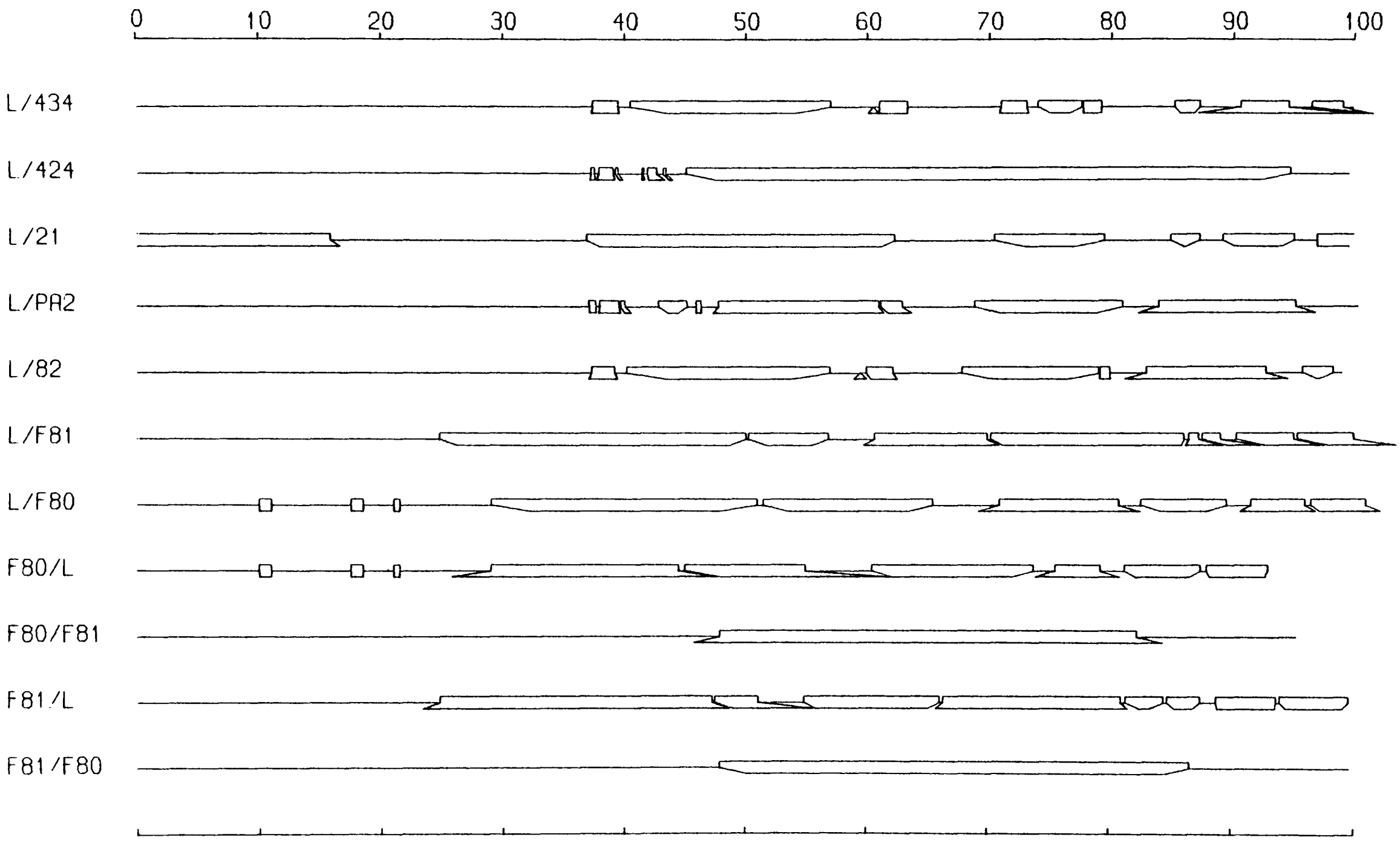


FIGURE 3. Scaled and Adjusted Heteroduplexes Between the Lambdoid Phages.

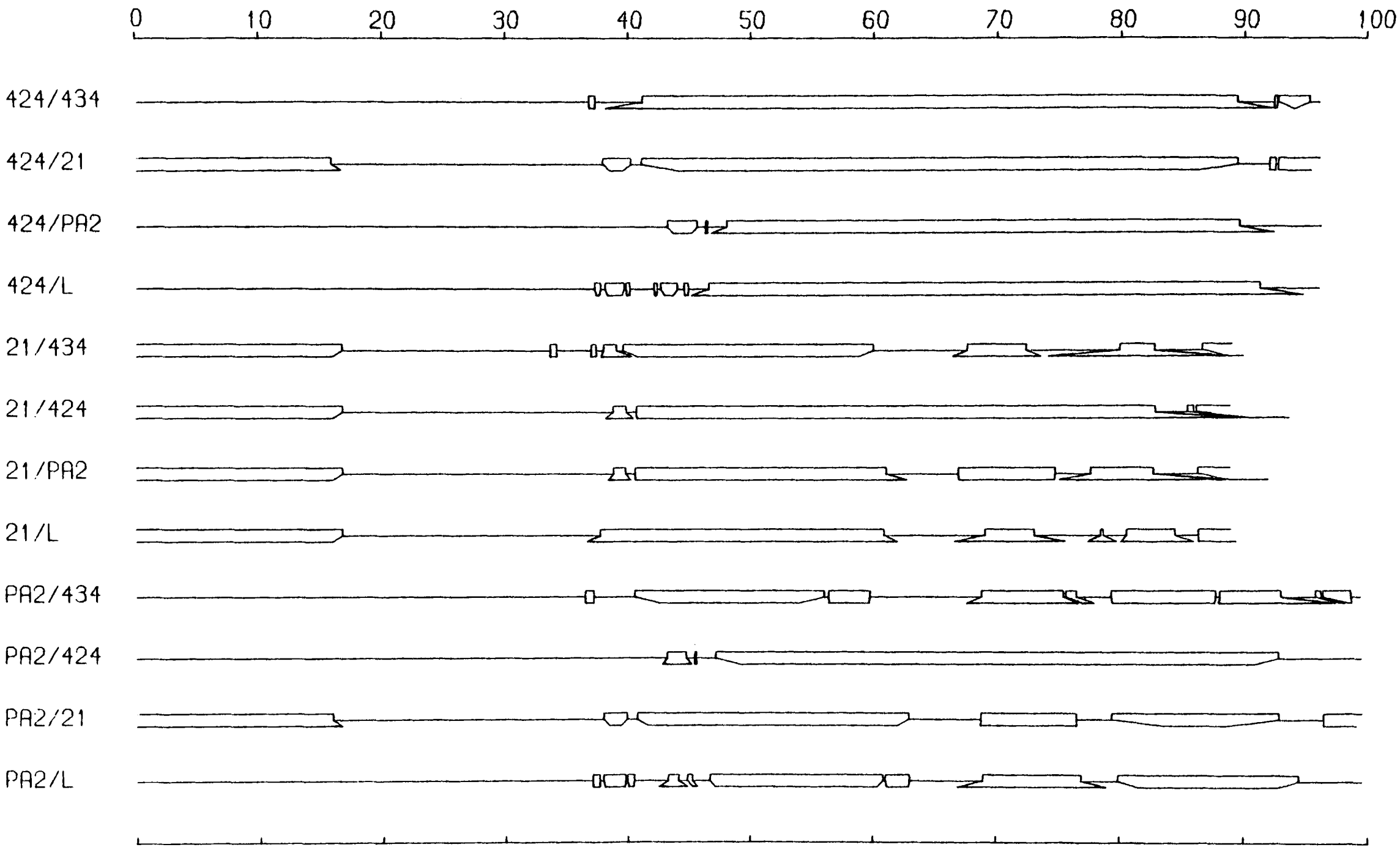


FIGURE 3 (Continued). Scaled and Adjusted Heteroduplexes Between the Lambdoid Phages.

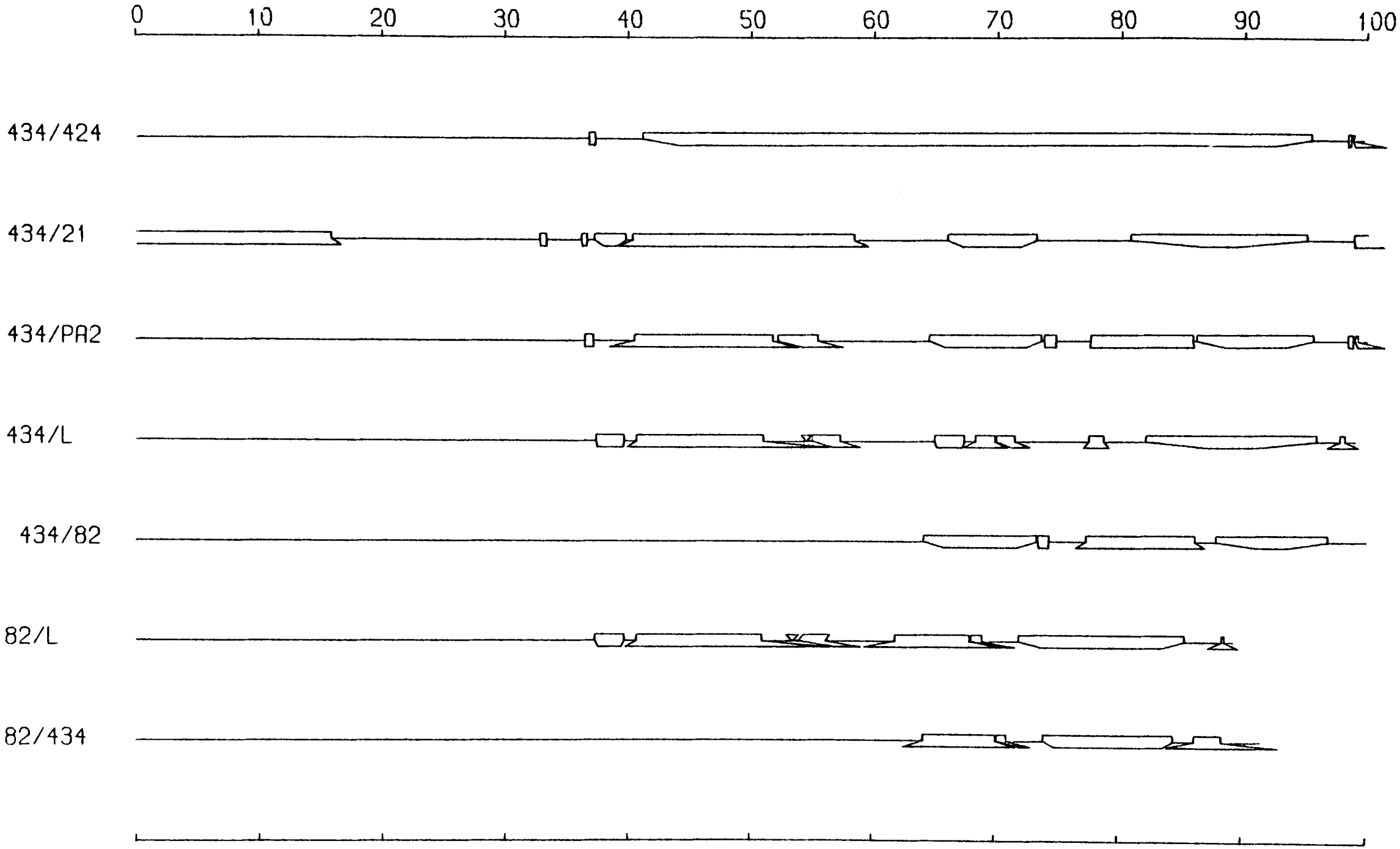
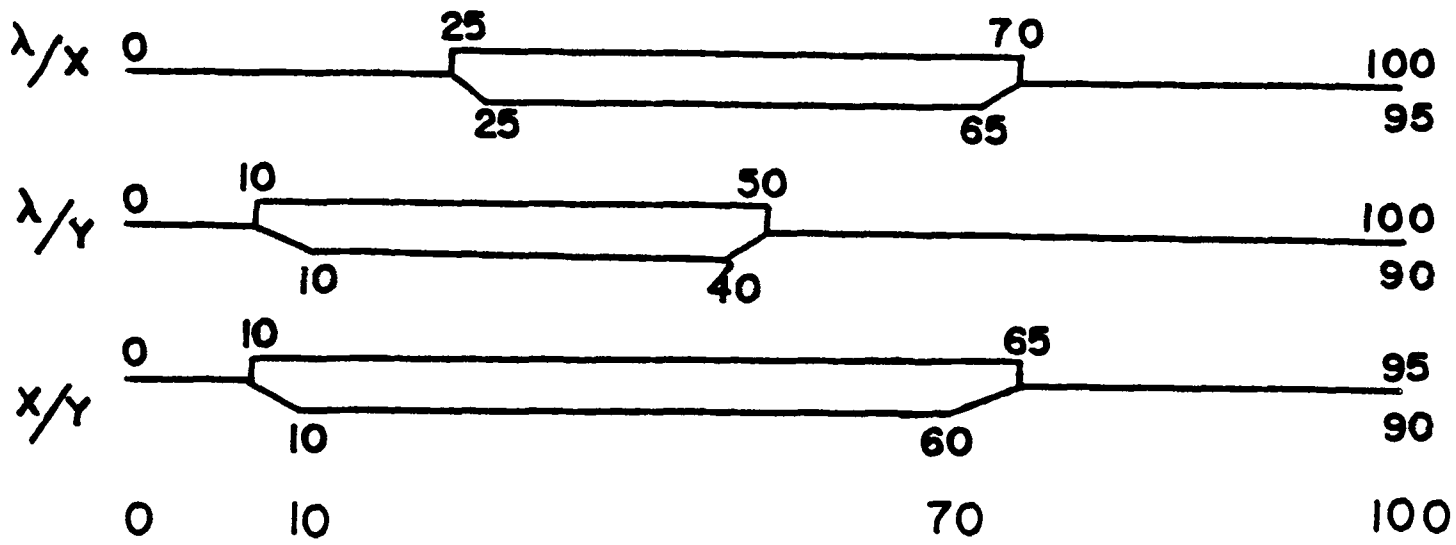


FIGURE 3 (Continued). Scaled and Adjusted Heteroduplexes Between the Lambdoid Phages.

PREDICTED HETERODUPLEXES

From any pair of heteroduplexes, which have one molecule in common, it is possible to predict some of the regions of homology and nonhomology in a heteroduplex between the other two molecules. For example, the heteroduplexes λ/21 and λ/424 were made and analyzed by Simon et al (1971) (See Table 5A and Figure 3) and from these data the 424/21 heteroduplex was predicted. Where both molecules in the predicted heteroduplex are nonhomologous with the common one they may be homologous or nonhomologous with eachother. Predicted molecules are shown in Figure 4. These were used to help the assignment of single strands of bubbles within the actual heteroduplexes.

A general example of using two heteroduplexes to predict a third is as follows.



Move along λ from left to right. Look for regions homologous to both X and Y. Only these are homologous in X/Y. The coordinates in X/Y are those corresponding to the coordinates of the regions in λ, which are homologous with both X and Y. The coordinate 60 on Y in X/Y is deduced as follows. Seventy percent in λ is 20% along a

double-stranded stretch from 50%. Fifty percent in λ is 40% in Y. Therefore 70% in λ is 60% in Y. The 10% position in X is calculated similarly. All the others come directly from λ/X and λ/Y. Where X and Y are both nonhomologous with λ they could be homologous to one another. There could be homology between X and Y in the regions corresponding to 25 to 50% in λ. (Because of the differences in length between λ and X and Y this region cannot be defined in X/Y). The lengths of the homologous and nonhomologous regions within the predicted heteroduplexes between the lambdoid phages are found in Table 6.

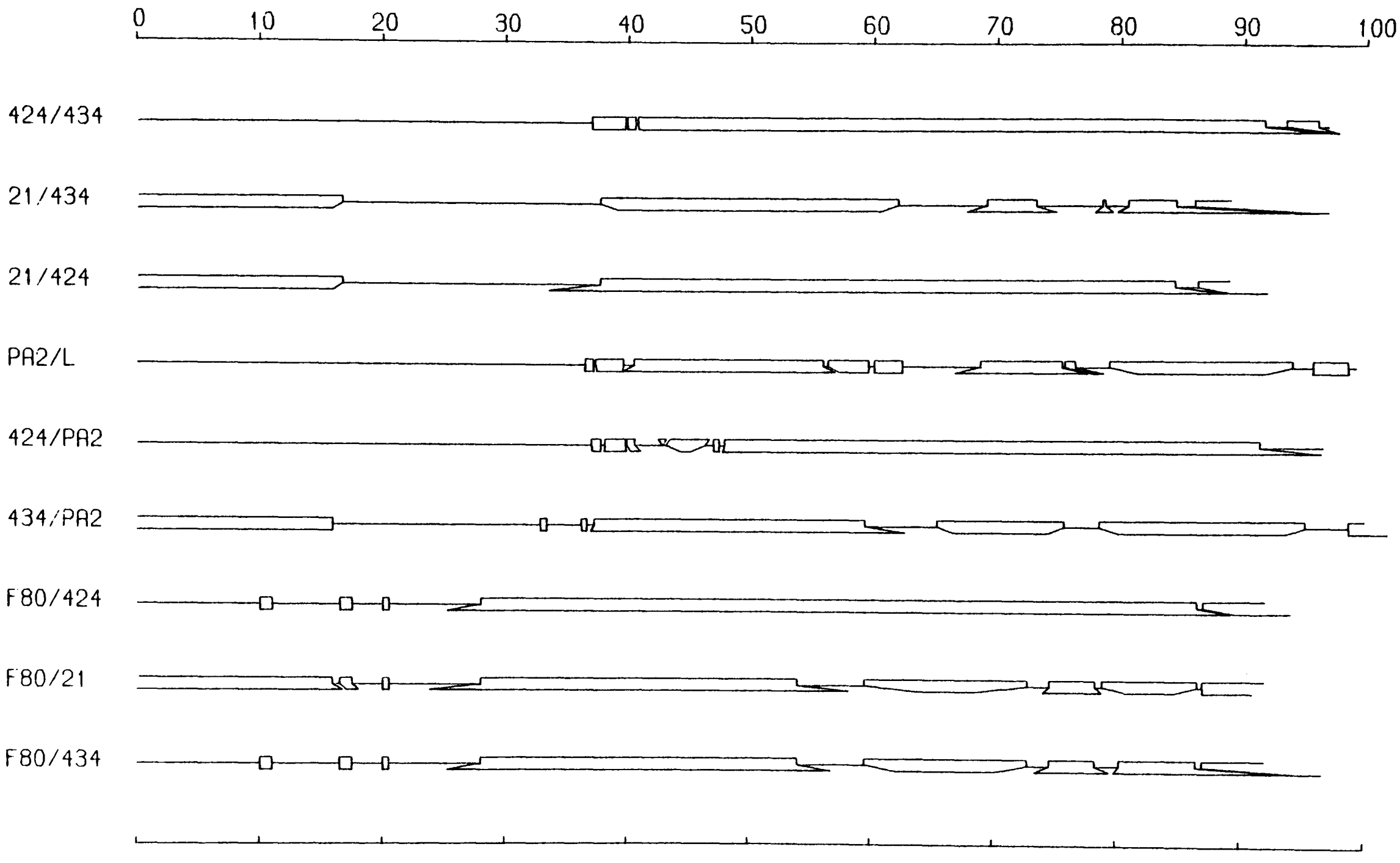


Figure 4 PREDICTED HETERODUPLEXES

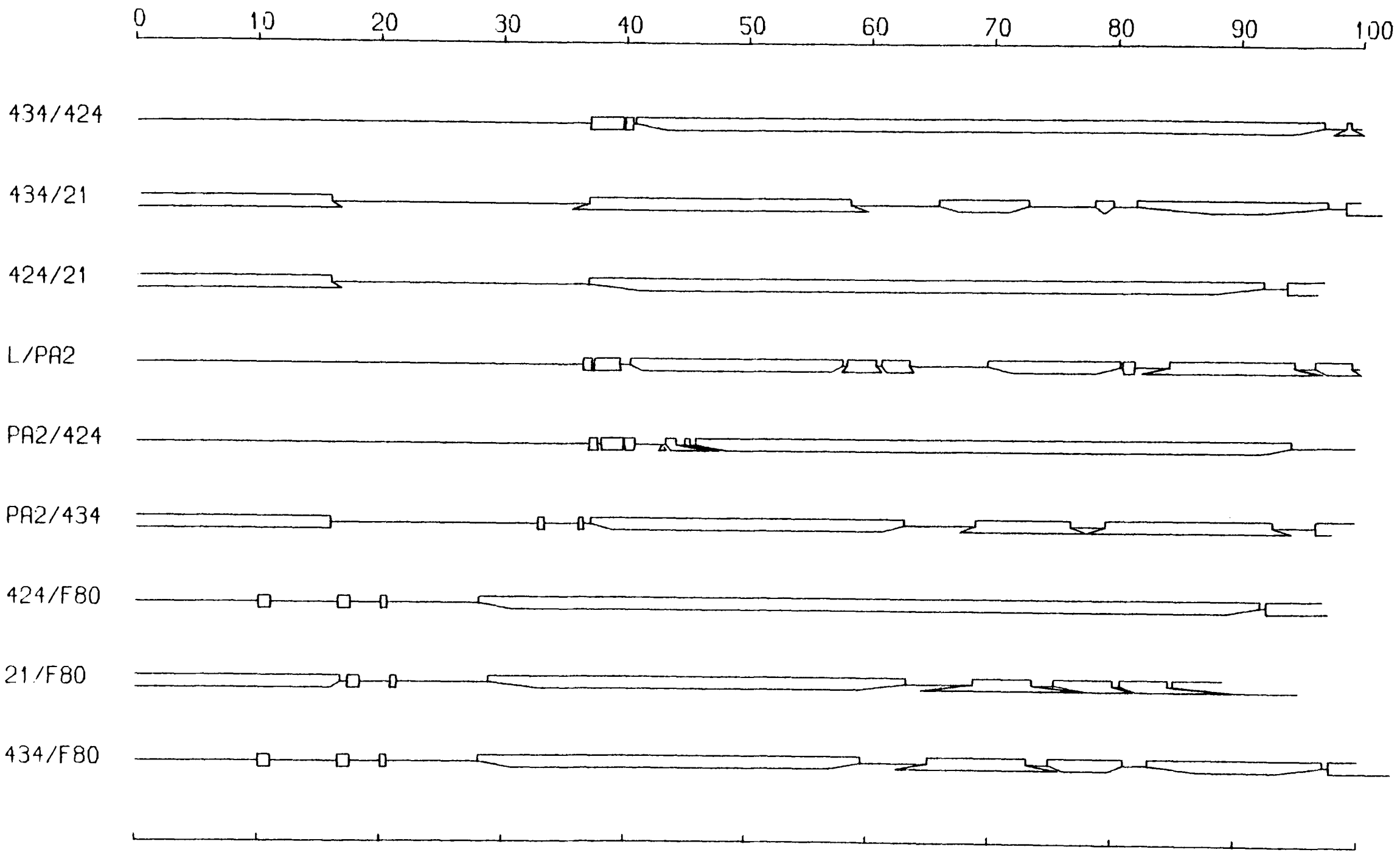


Figure 4 PREDICTED HETERODUPLEXES

TABLE 6

LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE PREDICTED HETERODUPLEXES BETWEEN THE LAMBDOID PHAGES

		2.7		0.6		56.3		0.4						
434/424	37.0		0.1		0.2		1.8		0.8					
		2.8		0.7		51.0		2.6						
	15.9		21.4		7.3		1.5		15.7		1.2			
434/21		21.0		7.2		5.4		1.9		1.5				
	16.7		24.3		4.0		0.2		3.9		2.9			
	15.9		55.2		3.0									
424/21		21.0		1.9										
	16.7		46.8		2.5									
		0.7		2.1		17.4		2.4		2.3		10.8		1.0		10.3		3.0	
λ/PA2	36.5		0.2		0.9		0.4		0.4		6.4		0.2		2.8		1.7		0.6
		0.7		2.3		15.5		3.3		2.3		6.6		0.8		14.8		2.8	
		0.7		1.8		0.9		0		0.8		0.4		48.7					
PA2/424	37.0		0.3		0.1		2.3		0.3		0.7		0.5		5.1				
		0.8		1.7		0.7		0.7		3.2		0.4		43.6					
	15.9		0.5		0.4		25.8		7.7		13.6		3.1						
PA2/434		16.9		2.8		0.6		5.9		2.9		3.5							
	15.9		0.5		0.4		22.2		10.3		16.7		1.3						
		1.0		1.0		0.5		64.0		4.5									
424/φ80	10.0		5.5		2.5		7.5		0.5										
		1.0		1.0		0.5		58.5		5.0									
	16.7		1.0		0.5		34.4		4.8		4.8		3.9		4.0				
21/φ80		0.6		2.5		7.5		5.5		1.8		0.6		0.4					
	15.9		1.0		0.5		26.0		13.3		3.7		7.7		5.0				

TABLE 6 (CONTINUED)

			1.0		1.0		0.5		31.5		8.1		6.1		14.2		2.3			
434/ 80		10.0		5.5		2.5		7.5		5.5		1.8		2.0		0.5				
			1.0		1.0		0.5		26.0		13.3		3.7		6.2		5.0			

There may be unpredicted regions of homology (i.e. between two strands, which are both nonhomologous with the third) and unpredicted regions of nonhomology between two strands only partially homologous with the third.

HETERODUPLEX RESULTS

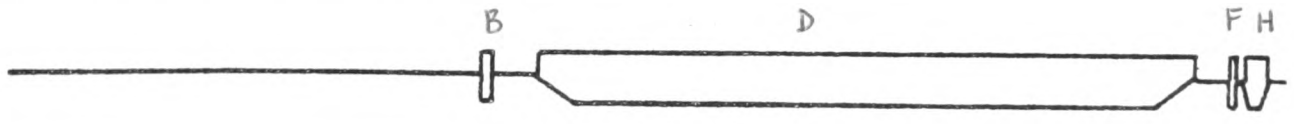
434/424. Heteroduplexes between the DNA extracted from the phages 434 and 424 were made and spread twice. The first heteroduplex was spread without the standards pSC101 and M13 DNA's. Homoduplexes were used as standards. The lengths of the 434 and 424 DNA molecules are 100%λ and 96.3%λ respectively. I assumed that it would be a simple task to distinguish between these DNA's. However, the lengths of the homoduplexes measured did not fall into two distinct groups and it was not possible to decide whether a homoduplex was 434 or 424. So, I decided to calculate an average scale factor by which every measured length was multiplied. The average scale factor (S_{avg}) was:

$$S_{avg} = \frac{\frac{100 + 96.4}{2}}{H_{avg}}$$

where H_{avg} = the average measured length of all homoduplexes. Nine heteroduplexes and nine homoduplexes were photographed.

The second 434/424 heteroduplex was spread with pSC101 and M13 DNA's. Seventeen heteroduplexes each with at least 2 adjacent pSC101 and 4 adjacent M13 DNA molecules were photographed and scaled against these standards.

The data from these two spreadings were then analyzed as one group. The scaled lengths of each segment in each molecule are tabulated in Appendix 3, Table 13. A picture based on the average scaled measurements is shown below.



An electron micrograph of this heteroduplex is shown in Appendix 4, Plate IX.

This heteroduplex contains 4 bubbles. The average differences in length of the single strands in the bubbles were: $\Delta B = 0$; $\Delta D = 5.2\%\lambda$; $\Delta F = 0$ and; $\Delta H = 2.3\%\lambda$. The difference in length between the 434 and 424 DNA molecules is about $3.7\%\lambda$, therefore, I assigned the longer strand of bubble D, and the shorter strand of bubble H, to 434. In several molecules the bubbles B and F were not seen and occasionally the region C was denatured. This suggests that these regions are partially homologous and have T_m's near 20^oC under these spreading conditions.

Some regions of homology between 434 and 424 can be predicted from the λ/424 and λ/434 heteroduplexes (Highton and Beattie, unpublished results; this thesis; Simon et al, 1971). This predicted 434/424 heteroduplex (See Figure 4) is very similar to the observed heteroduplex and predicts assignment of the strands in the bubbles as I have done above.

After the assignments and adjustments of the single-stranded regions of each molecule with appropriate factors (See previous sections), the average coordinates of the homologous and nonhomologous regions were calculated (See Figure 3 and Table 5).

434/21. Heteroduplexes were made from DNA extracted from the phages 434 and 21 (See Materials and Methods). Homoduplexes were used as standards and at least one homoduplex was photographed with each heteroduplex. The lengths of 434 and 21 DNA's are $100\%\lambda$ and $89\%\lambda$ (See Table 3), respectively, and are sufficiently different to allow unambiguous assignment of homoduplexes as either 434 or 21 DNA.

A picture based on the average scaled measurements is shown below.

An electron micrograph of this heteroduplex is shown in Appendix 4, Plate X.

This heteroduplex contains 6 bubbles in the right half of the molecule and a variable number of bubbles at the left end indicating partial homology here. This phenomenon was observed in the λ/21 heteroduplex (Simon et al, 1971). Because the bubbles in the left end were small and numerous, I have, like Simon et al, represented this left arm with split ends up to 16.7%λ in the 21 DNA molecule and 15.9%λ in the 434 DNA molecule. (The 434 DNA is homologous with λ in the left arm (Simon et al, 1971)). The average differences in the lengths of the single strands of each bubble are, from left to right along the molecule: $\Delta A = 0.8\%\lambda$; $\Delta C = 0$; $\Delta E = 1.4\%\lambda$; $\Delta G = 3.8\%\lambda$; $\Delta I = 1.8\%\lambda$; $\Delta K = 10.1\%\lambda$ and; $\Delta M = 1.4\%\lambda$.

The difference in length of 434 and 21 DNA's is 11%λ (See Table 3). The heteroduplex has a bubble at the right end so there must be a very small stretch of homology at the right end, which is undetectable. In order to allocate a proportion of this bubble to 434 and the rest to 21, the λ/21 and λ/434 heteroduplexes made by Simon et al, (1971) were consulted. The last common point of homology of 434 DNA with λ DNA is at 96.6% along λ DNA. Lambda DNA and 21 DNA are homologous up to 97.0% on λ therefore, 21 and 434 must be nonhomologous from 96.6% on λ (Note that Simon et al (1971) quote the small terminal stretch of single strand of 21 in the λ/21 heteroduplex as 3.0%λ however, that makes the total length of the 21 DNA molecule 89.4%λ and therefore, I decided to call this 2.6%λ in order to make the total length of 21 DNA 89%λ (See Table 3)). Therefore, from the point 96.6% on λ there must remain 3.0%λ of 21 DNA. So, in each terminal single-stranded loop, I have allocated 3.0/4.2 to 21 and 1.2/4.2 to 434.

The λ/434 and λ/21 heteroduplexes also allow some regions of homology between 434 and 21 to be predicted (See Figure 4). The resulting heteroduplex is very similar to that observed, and allows assignment of the strands in all the other bubbles. The long strands of bubbles G, K and M belong to 434 and that of I to 21. However, if I assign the single strands this way, the total difference is only 7.4%λ instead of 11%λ. An alternative assignment could have given better agreement, but I assigned the single strands as predicted, allowing the adjustment factor to take care of inconsistencies in total lengths. The average coordinates of the homologous and nonhomologous segments, calculated after single strand adjustment, are given in Table 5. The molecule is drawn in Figure 3 .

424/21. Molecules from two different heteroduplex preparations from the phages of 424 and 21 are included in these results. Homoduplexes were used as standards in the first spreading (4 heteroduplex molecules and 1 standard for each), and pSC101 and M13 DNA's in the second spreading (16 heteroduplexes). A picture of the heteroduplex based on the average scaled measurements is shown below.

An electron micrograph of this heteroduplex is shown in Appendix 4, Plate XI.

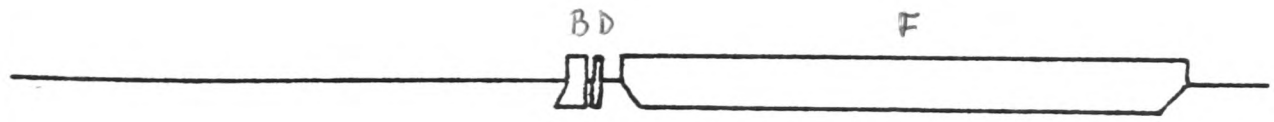
The average differences in length of the single strands in the bubbles are: ΔA = 0.8%λ; ΔC = 1.1%λ; ΔE = 4.3%λ; ΔG = 0 and; ΔI = 0.6%λ. The difference in length of the 424 and 21 DNA's is 7.3%λ (See Table 3). The DNA of phage 424 is homologous with λ DNA up to 37%λ (Highton, unpublished results; this thesis) and therefore the

424/21 heteroduplex looks like the λ/21 heteroduplex and the 434/21 (See previous section) up to about 37%λ. In all of the molecules in the second spreading there was a bubble at the right end, presumably with an undetectable amount of double strand at the very end, as in the 434/21 heteroduplex (434 and 424 are homologous at the right end). The DNA's of λ and 424 are homologous from 94.8% to 100% on the λ DNA molecule therefore, the 424/21 heteroduplex should look like the λ/21 heteroduplex from 94.8%λ to the right end. The λ/21 heteroduplex as represented by Simon et al (1971) however, has a split right end with λ DNA comprising 3.0%λ and 21 DNA comprising 2.5%λ. This is presumably because the λ and 424 molecules are not completely homologous at the very end and so the 21 DNA is less homologous with λ here than with 424. Therefore, of the final bubble in the 424/21 heteroduplex, 3.0/5.5 has been assigned to 424 and 2.5/5.5 has been assigned to 21.

The λ/424 and λ/21 heteroduplexes (Highton and Whitfield, 1975; this thesis; Simon et al, 1971) also allow some regions of homology between 424 and 21 to be predicted (See Figure 4). The observed heteroduplex differs from the predicted one by the presence of two regions of partial homology, D and G. The longer single strands in bubbles C and E were assigned to 424 on the basis of the difference in length between 424 and 21 DNA's (7.4%λ), which was consistent with the predicted heteroduplex.

The average coordinates of the homologous and nonhomologous segments calculated after single-strand adjustment are given in Table 5 and Figure 3.

PA2/424. This heteroduplex was made from DNA extracted from the phages PA2 and 424. The DNA's of pSC101 and M13 were added as standards. A picture based on the average scaled measurements is shown below.



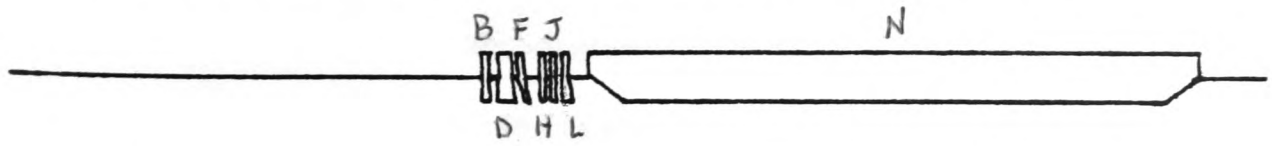
An electron micrograph of this heteroduplex is shown in Appendix 4 Plate XII.

The differences in the lengths of the single-stranded DNA within each bubble are: $\Delta B = 0.6\%\lambda$; $\Delta D = 0$; $\Delta F = 3.8\%\lambda$. The difference in the lengths of 424 and PA2 DNA's is $3.2\%\lambda$ (See Table 3). Based on this difference in length of $3.2\%\lambda$, the longer single strand of bubble F was assigned to PA2 and the longer single strand of bubble B was assigned to 424. The average coordinates of the beginning and end of each homologous and nonhomologous region calculated after single-strand adjustment are given in Table 5 and Figure 3.

λ/424. This heteroduplex was made directly from the λ and 424 phages. M13 DNA was added to the spreading solution as the single-stranded standard and homoduplexes were used as double-stranded standards.

At least two adjacent homoduplexes and two adjacent M13 molecules were photographed with each heteroduplex. I was able to separate these homoduplexes into two groups on the basis of their ratio to the average of the M13 molecules adjacent to them. Those homoduplex lengths giving a ratio less than 8.0 were considered to be 424 and those giving a ratio greater than 8.0 were considered to be λ. The length of λ is 100%, the length of 424 is $96.4\%\lambda$ and the length of M13 is $12.7\%\lambda$ (See Table 3). A picture of the heteroduplex

the average scaled measurements is shown below.

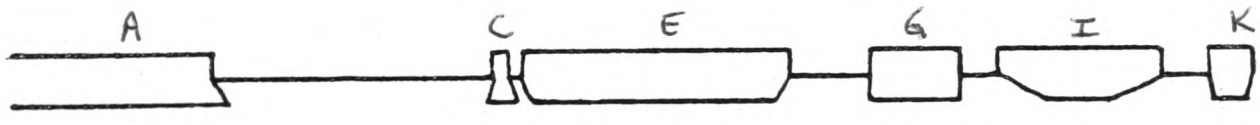


An electron micrograph of this heteroduplex is shown in Appendix 4 Plate XIII.

The region just left of the large bubble is partially homologous. The average differences in length of the single strands comprising the bubbles are: $\Delta B = 0.2\%\lambda$; $\Delta D = 0.3\%\lambda$; $\Delta F = 0$; $\Delta H = 0$; $\Delta J = 0.6\%\lambda$; $\Delta L = 0$ and; $\Delta N = 5.7\%\lambda$. The longer strand in the large bubble, N, was assigned to λ as were all the shorter strands in the bubbles B, D, F and J. The difference in length between λ and 424 DNA's is $3.6\%\lambda$. The regions C, E, F, H and L are partially homologous.

The average coordinates of the beginning and end of each homologous and nonhomologous region after single-strand adjustment are given in Table 5 and Figure 3. The coordinates of this heteroduplex are in agreement with the unpublished results of Highton and Beattie.

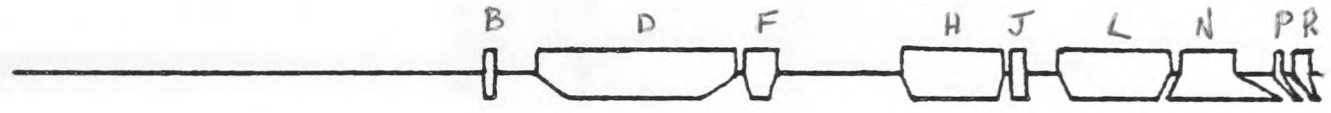
PA2/21. This heteroduplex was made directly from the phages PA2 and 21. Both double-stranded pSC101 and single-stranded M13 DNA molecules were added as standards. At least three adjacent pSC101 DNA molecules and six adjacent M13 DNA molecules were photographed with each heteroduplex. Seven heteroduplex molecules were analyzed. A picture of the heteroduplex based on the average scaled measurements is shown below.



An electron micrograph of this heteroduplex is shown in Appendix 4 Plate XIV.

The DNA of phage PA2 is homologous with phage 424 up to 43.2%λ, therefore, the PA2/21 heteroduplex resembles the 424/21 heteroduplex up to 43.2%. The average differences in length of the single strands in each bubble are: ΔA = 0.8%λ; ΔC = 1.0%λ; ΔE = 2.0%λ; ΔG = 0; ΔI = 8.2%λ and; ΔK = 0.4%λ. The heteroduplex also has a bubble at the right end as in the 424/21 heteroduplex because 424 and PA2 are homologous in this region. Because PA2 and 424 are homologous up to 43%λ the longer strand of bubble C belongs to PA2 (See the 424/21 heteroduplex results). The difference in length of the PA2 and 21 DNA's is 10.6%λ. The longer strands of bubbles E and I were assigned to PA2. The average coordinates of the homologous and nonhomologous regions after single-strand adjustment are given in Table 5 and Figure 3 .

PA2/434. This heteroduplex was made directly from the phages PA2 and 434. Both double-stranded pSC101 and single-stranded M13 DNA molecules were added as standards. At least one adjacent pSC101 and four adjacent M13 DNA molecules were photographed with each heteroduplex. Three heteroduplex molecules were used in this analysis. A picture of the heteroduplex based on the average scaled measurements is shown below.



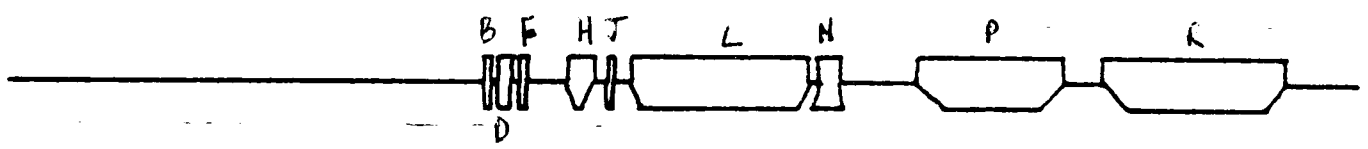
An electron micrograph of this heteroduplex is shown in Appendix 4 Plate XV .

The average differences in the lengths of the single-stranded DNA within each bubble from left to right along the molecule were: ΔB = 0; ΔD = 5.1; ΔF = 0.3; ΔH = 1.8; ΔJ = 0; ΔL = 0.8; ΔN = 3.8; ΔP = 0 and; ΔR = 2.1. I examined the 434/21 and PA2/21 heteroduplexes

to predict the assignment of single strands of the bubbles in the 434/PA2 heteroduplex (Drawings of the predicted heteroduplexes are shown in Figure 4). The predicted differences in the lengths of the single strands of each bubble and the phages to which these differences were assigned are: $\Delta B = 0$; $\Delta D + \Delta F = +3.6$ (PA2); $\Delta H = +2.6$ (434); $\Delta J = 0$; $\Delta L + \Delta N = +3.1$ (434); $\Delta P = 0$; $\Delta R = +1.8$ (PA2). On the basis of these differences I assigned the longer strands of bubbles D, F, L and R to PA2 and the longer strands of bubbles H and N to 434.

After the assignments and adjustments of the single-stranded regions of each molecule with appropriate factors (See previous sections) the average coordinates of the beginning and end of each homologous and nonhomologous region were calculated. These are given in Table 5 and represented in Figure 3.

λ/PA2. This heteroduplex was made directly from λ and PA2 phages. Phage M13 DNA and plasmid pSC101 DNA were added to the spreading solution as single- and double-stranded standards, respectively. At least one adjacent pSC101 and four adjacent M13 DNA molecules were photographed with each heteroduplex. Fourteen heteroduplex molecules were analyzed. A picture of the heteroduplex based on the average scaled measurements is shown below:



An electron micrograph is shown in Appendix 4 Plate XVI.

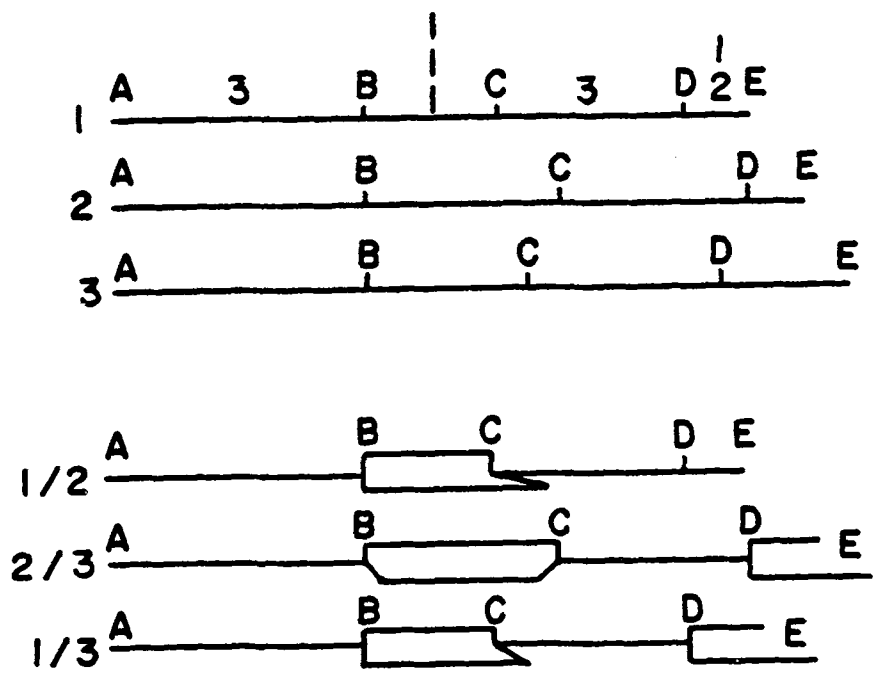
The average differences in length of the single strands comprising the bubbles were: $\Delta B = 0$; $\Delta D = 0.5$; $\Delta F = 0$; $\Delta H = 1.7$; $\Delta J = 0$; $\Delta L = 1.0$; $\Delta N = 0.1$; $\Delta P = 4.1$ and; $\Delta R = 3.2$. I examined the λ/434 and PA2/434 heteroduplexes to predict the assignment of single strands of the bubbles in the λ/PA2 heteroduplex (See Figure 4). The predicted differences in

the lengths of the single strands and the phages to which these differences were assigned are: $\Delta B = 0$; $\Delta D + \Delta F = +0.2$ (PA2); $\Delta H + \Delta J + \Delta L = +1.0$ (λ); $\Delta N = 0$; $\Delta P = +4.2$ (λ) and; $\Delta R = +4.6$ (PA2). The total difference between the actual lengths of the single strands in all bubbles but N is 1.1%λ. Therefore, because λ is only 0.4% longer than PA2, I assigned the longer strand of bubble N to PA2.

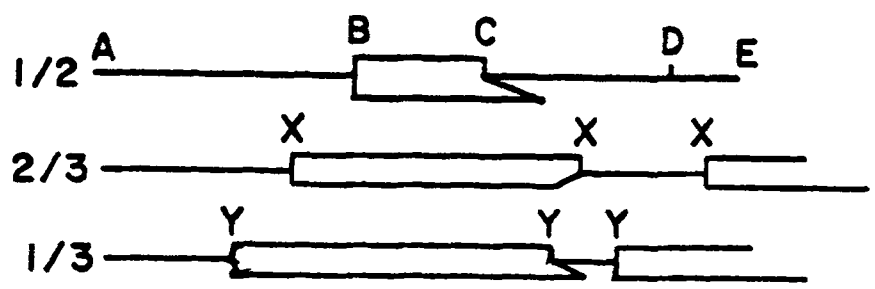
After the assignments and adjustments of the single-stranded regions of each molecule with appropriate factors (See previous sections) the average coordinates of the beginning and end of each homologous and nonhomologous region were calculated. These are given in Table 5 and represented in Figure 3.

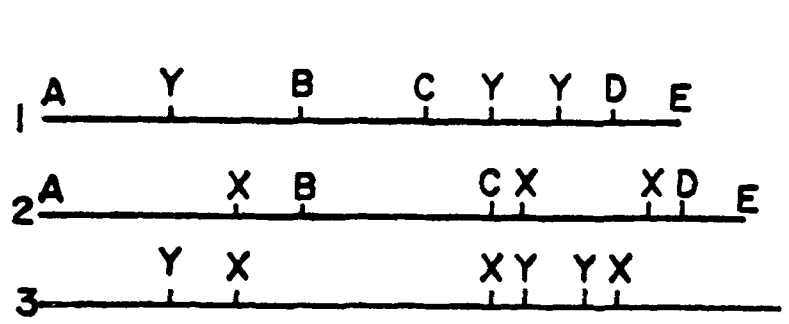
INTREPRETATION OF HETERODUPLEXES

Inspection of the heteroduplexes suggests that there are a number of fixed points along each phage genome, which define the regions that may differ in a particular pair. (Several contiguous regions may differ in a particular pair). The distances between the points are not the same in all the phages (in other words, each phage has its own copy of the region, which may or may not be the same as in another phage). For example,



where A, B, C and D represent points where regions begin or end. (Phages are represented by the numbers 1, 2 or 3 at the left of the molecule, and the heteroduplexes between them are represented by the numbers 1/2, 2/3 and 1/3). The designation $\begin{matrix}1\\1\\1\end{matrix}$ represents a region in which each of the three phages has its own sequence, $\begin{matrix}1\\2\end{matrix}$ represents a region in which two phages have the same sequence and the third has a different sequence and 3 represents a region in which all three phages have the same sequence. An alternative is that there is no relation between the lengths of the regions that are the same in one pair, and those that are the same in another. For example,





where A, B, C, D, E, X and Y represent points where regions begin or end. The first possibility gives a pattern much more like that observed.

To analyze the phage genomes in these terms I have used drawings of the heteroduplexes studied in this thesis and those studied by Simon et al (1971) (See Figure 3 and Table 5A). I have included phage 82 in this even though I do not have all the possible heteroduplexes with it. I first arranged the drawings of the lambda heteroduplexes one above the other, with lambda on top, in descending

sequence λ/434, λ/82, λ/PA2, λ/21 and λ/424. Below this I had 434/82, 434/PA2, 434/21, 434/424 and then PA2/21, PA2/424 and finally 424/21. I then determined the positions of the homology/nonhomology boundaries in the heteroduplexes between λ and all the other phages. I have used λ as the 'reference' for convenience only--any other phage should give the same results. As I did this I checked that all the non-λ heteroduplexes had homology and corresponding boundaries where predicted by the λ heteroduplexes, and looked for any unpredicted homology. The regions were given the approximate average values of the boundaries that defined them (See Table 7).

I summarized across the phage (See Table 7) the relationships between the phages in each region. Below are given all the available values for the coordinates of the right boundary of the region. First, all the positions in λ (below the corresponding partner, and with the coordinates in that partner in brackets), then 434 and so on. Un-bracketed numbers in a row should be the same and bracketed numbers in a column should be the same. Numbers in a 434 row (un-bracketed) should equal numbers in a 434 column (bracketed) and so on. This gives a measure of the consistency of the heteroduplexes. Then a plot was produced showing the number of different copies for each region and the number assigned to each region (See Figure 6). Then, Table 8 was deduced from Table 7 and groups together the regions along the chromosome (i.e. 1 through 33) that are the same with respect to the number of phage copies. Finally, from inspection of Table 8, Figure 7 was produced. This figure shows a possible sequence of events to explain the evolution of the phages. These results are discussed in the following section.

TABLE 7 Positions of the Homology/Nonhomology Boundaries in the Heteroduplexes Between λ and the Lambdoid Phages

(1)	0	-37	λ	=	434	=	82	=	PA2	=	21	=	424
			λ						37.1(37.1)		36.9(37.7)		37.3(37.3)
			434						36.5(36.5)		35.8(36.6)		36.8(36.8)
(2)	37	-37.5	λ	=	434	=	82	≠	PA2	=	21	=	424
			λ						37.7(37.7)				37.5(37.7)
			434						37.2(37.2)		36.2(36.9)		37.3(37.3)
(3)	37.5	-38	λ	=	434	=	82	=	PA2	=	424	(=)	21
			λ		37.4(37.4)		37.3(37.3)		38(38)		37.9(38.1)		
			434										36.9(37.6)
			PA2										38.0(38.8)
			424										37.9(38.7)
(4)	38	-39.5	λ	≠	434	=	82	=	PA2	=	424	≠	21
			λ		39.5(39.7)		39.2(39.7)		39.6(39.8)		39.1(39.7)		
			434										39.5(38.7)
			PA2										39.9(39.8)
			424										40.2(39.8)
(5)	39.5	-40.5	λ	=	434	=	82	(=)	PA2	(=)	424	(=)	21
			λ		40.5(40.7)		40.2(40.7)		40.2(40.4)		39.5(40.1)		
			434						40.5(40.5)		41.2(41.2)		40.0(39.2)
			PA2										40.8(40.6)
			424										41.1(40.7)

TABLE 7 (Continued)

(6)	40.5-43	λ	=	PA2	=	424	≠	434	=	82	≠	21
		λ		43(43.2)		41.5(42.1)						
		PA2				43.2(43.2)						
(7)	43 -44.5	λ	≠	434	=	82	≠	PA2	≠	21	≠	424
		λ						45.3(44.0)				42.7(44)
		PA2										44.8(45.6)
(8)	44.5-45.5	λ	=	PA2	=	424	≠	434	=	82	≠	21
		λ		46.1(44.8)		43.2(44.6)						
		PA2				45.4(46.3)						
(9)	45.5(46)	λ	≠	434	=	82	≠	PA2	≠	21	≠	424
		λ						46.5(45.2)				43.5(44.9)
		PA2										45.6(46.5)
(10)	46 -47.5	λ	=	PA2	=	424	≠	434	=	82	≠	21
		λ		47.8(46.6)		45.2(46.6)						
						47.2(48)						
(11)	47.5-57	λ	≠	434	=	82	≠	PA2	≠	21	≠	424
		λ		57(51.1)		57(51)						
		PA2		56(51.9)								
(12)	57 -57.4	λ	=	434	=	82	=	PA2	≠	21	≠	424
		PA2		56.4(52.2)								

TABLE 7 (Continued)

(13)	57.4-61	λ	=	434	=	82	≠	PA2	≠	21	≠	424	
		λ		61(56)		61(56.1)		61.1(60.7)					
		PA2		59.8(55.5)									
(14)	61 -63	λ	≠	434	=	82	=	PA2	≠	21	≠	424	
		λ		63.3(58.3)		63.2(58.3)		63.3(63.1)		62.3(60.9)			
		434								58.1(59.6)			
		PA2								63(61)			
(15)	63 -69	λ	=	434	=	82	=	PA2	=	21	≠	424	
		λ				68.9(64)		69.2(69)					
		434				64.2(64.2)		64.6(68.8)					
		21						66.9(68.8)					
(16)	69 -71	λ	=	434	=	21	≠	(82)	≠	PA2	≠	424	
		λ		71(66)		70.5(69.1)							
		21		69.2(65.7)									
(17)	71 -73	λ	≠	434	≠	(82)	≠	PA2	≠	424	≠	21	
		λ		73.1(68.4)									
(18)	73 -74	λ	=	434	≠	(82)	≠	PA2	≠	424	≠	21	
		λ		74(69.3)									
(19)	74 -79	λ	≠	434	≠	(82)	≠	PA2	≠	424	≠	21	
		λ		79.2(73.5)									

TABLE 7 (Continued)

(20)	79	-80	λ	=	434	≠	(82)	≠	PA2	≠	21	≠	424
			λ				80.1(70.1)				79.5(73.1)		
			434				73.4(70.1)		73.9(75.7)		72.9(72.0)		
(21)	80	-81	λ	=	434	=	21	≠	(82)	≠	PA2	≠	424
			λ						81(71.1)		81.4(77)		
			434						74.4(71)		76.9(76.5)		
			21								74.8(76.6)		
(22)	81	-84	λ	=	434	=	82	=	PA2	=	21	≠	424
			λ				84(74.1)		84.3(80)				
			434				77.4(74)		77.7(79.3)				
			21						77.6(79.4)				
(23)	84	-85	λ	=	434	=	21	≠	(82)	≠	PA2	≠	424
			λ		85.2(78.5)		84.9(78.5)						
(24)	85	-87.5	λ	≠	434	=	21	≠	(82)	≠	PA2	≠	424
			λ		87.3(79.7)		87.3(78.7)						
(25)	87.5	-89	λ	=	434	=	21	≠	(82)	≠	PA2	≠	424
			λ				89.2(80.6)						
			434				80.5(79.6)						
(26)	89	-91	λ	=	434	≠	(82)	≠	PA2	≠	21	≠	424
			λ		90.7(83.1)								

TABLE 7 (Continued)

(27)	91	-92	λ	≠	434	≠	(82)	≠	PA2	≠	21	≠ 424
			434				86.2(84.5)		85.9(87.8)			
(28)	92	-93	λ	≠	434	=	82	=	PA2	≠	21	≠ 424
			434				87.9(86.2)		86.2(88.1)			
(29)	93	-94	λ	≠	434	≠	(82)	≠	PA2	≠	21	≠ 424
			λ				93.8(87.5)					
			434						95.7(93)		94.9(82.4)	95.6(89.5)
			PA2								92.9(82.7)	92.9(89.6)
			21									82.8(89.5)
(30)	94	-95	λ	=	82	≠	434	=	PA2	=	21	= 424
			λ				94.7(96.9)		95.5(94.5)		95.1(84.5)	94.8(91.3)
			434		96.9(88.4)							
(31)	95	-97	λ	=	434	=	82	=	PA2	=	21	= 424
			λ		96.6(98.8)		96.8(90.5)				97(86.4)	
			434						98.5(95.9)		98.7(86.3)	98.6(92.5)
			PA2								96.5(86.3)	
(32)	97	-99	λ	=	PA2	=	424	≠	434	=	82	≠ 21
			λ						99.2(99.2)		99.3(90.7)	
			434		99.4(98.7)		99(95.5)					

TABLE 7 (Continued)

(33)	99	-100	λ	=	434	=	82	=	PA2	=	424	≠ 21
			λ		100(100)		100(91.5)		100(99.6)		100(97)	100(89)

Region

(1) 21 has only weak homology with all the others in the region defined as 0-15.9% in λ.

(3) 434 = PA2 = 424 = 21. However, λ = 434 = PA2 = 424 but λ ≠ 21. See explanation for (5).

(5) 434 = PA2 = 424 = 21. However, λ = 434 but not PA2, 424 or 21. This is possible if 434 is only partially homologous with all the others. λ could be more like 434, than like the others. In fact, the region is double-stranded in some λ/424 heteroduplexes. This could occur if λ, PA2, 424 and 21 diverged from a '434' region (in other words, '434' in ancestors (1) and (2) in Figure 7. This is not really a type (c) region.

(7) There is 0.5% homology between λ and 424 in this region.

(7)-(10) Better agreement between the λ/424, λ/PA2 and PA2/424 heteroduplexes in these regions is achieved if the strands in 424/λ in region (7) beyond the 0.5% homology are reversed. However, this makes the agreement between the observed and predicted differences of the strands in the big bubble less good.

(12) λ = 434 and PA2 = 434 but λ ≠ PA2 (Same explanation as for (5)).

(13) 434 and 82 are 1% longer than λ.

(13-14) The λ/PA2 heteroduplex suggests 0.2% λ = 434 = 82 = PA2 between these regions (type i region).

(20) (See Figure 6). The cII is known to be between the imm^{434} and imm^{21}. Szybalski and Westmoreland (1969) got 79.8 for the right end of imm^{21}. Szybalski and Szybalski (1974) quote 71.1 for the left end of imm^{21} (See (16)).

(21) For 0.1% at the left end, PA2 = 434, 434 (and 82) = λ but λ ≠ PA2. Same explanation as for (5).

TABLE 7 (Continued)

Region

(28) This should probably be two regions.

(32) 0.1% 434 (and 82) = PA2 = 424, but λ≠ 434 (and 82), which predicts 0.1% in the λ/PA2 and λ/424 heteroduplexes to be nonhomologous. This is not observed.

(19) 0.1% λ = 434.

(82) This means that 82 could be homologous with PA2, 424 or 21.

Numbers 1 through 33 represent regions along the chromosome as indicated in Figure 6 .
Following each number (1 - 33) are the coordinates of the homology/nonhomology boundary in the heteroduplexes between λ and all the other phages. These were given the rough average values of the coordinates of the boundaries, which defined them.
Across the page are summarized the relationships between the phages in each region. Below are given all the available values for the coordinates of the right boundary of the region. See the text for a detailed discussion of this Table.

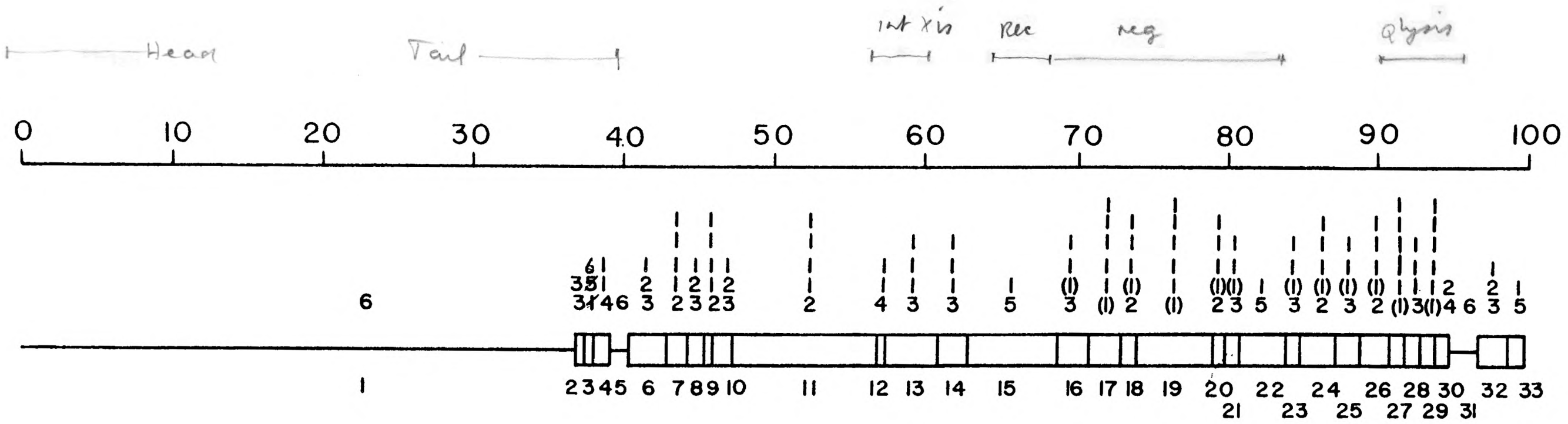


FIGURE 6. Plot showing the number of different copies for each region along the chromosome.

The top scale (0 - 100) represents the length of the phage chromosome.

The numbers below the scale represent the number of copies of a particular sequence in a region of the chromosome.

The bottom row of numbers (1 - 33) represents regions along the chromosome.

TABLE 8 Regions Along the Phage Chromosome That are the Same With Respect to the Number of Phage Copies.

	6[1]	33	51	411	321	2111	411	3111	3111	51	2(1)111	(1)11111	3(1)11	2(1)111	42
Phage	c[2]	d	e	f	g	h	i	j	k	l	m	n	o	p	q
λ	λ	λ	λ	λ	λ^a	λ	λ	λ	λ	λ	λ	λ	λ	λ	λ^B
434	λ	λ	λ	434	434^A	434	λ	λ	434	λ	434	434	λ	λ	434^b
82	λ	λ	λ	434	434^A	434	λ	λ	434	λ	(82)	(82)	(82)	(82)	λ^B
PA2	λ	PA2	λ	434	λ^a	PA2	λ	PA2	434	λ	PA2	PA2	PA2	PA2	434^b
21	λ	PA2	21	21	21	21	21	21	21	λ	434	21	λ	21	434
424	λ	PA2	λ	434	λ	424	424	424	424	424	424	424	424	424	434
Regions[3]	1	2	~~3~~	4	6	7	12	13	14	15	24	17	16	18	30
	5	33			8	9			28	22		19	21	20	
	31				10	11						27	23	26	
	3				32							29	25		

Designations: λ - like λ; 434 - like 434, unlike λ; PA2 - like PA2, unlike λ or 434;
82, 21, 424 - found only in 82, 21, 424.

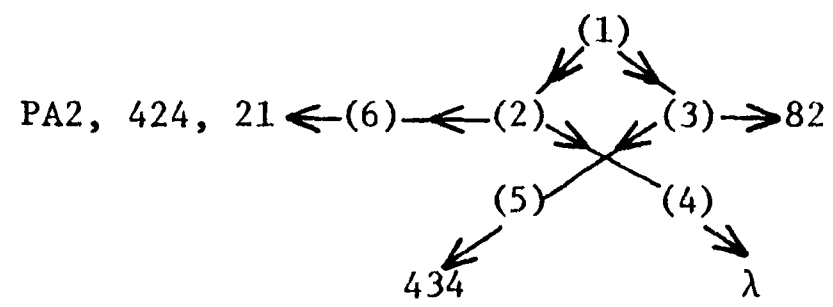
[1] These numbers indicate the number of copies of a particular sequence in a particular region of the chromosome.

[2] Letters c through q are assigned to the different regions 1 through 33. Regions that are the same with respect to the number of copies of a given sequence have been grouped together and assigned a letter. (For example, regions 1, 5 and 31 have been grouped together for in these regions all the phages have a λ copy of that region.

[3] Numbers 1 through 33 represent regions along the chromosome as indicated in Figure 6.

FIGURE 7 A Possible Sequence of Events to Explain the Evolution of the Lambdoid Phages.

	c[1]	d	e	f	g	h	i	j	k	l	m	n	o	p	q
(1)[2]	λ	?	λ	434	?	434	λ	λ	434	λ	434	λ'	λ	λ	?
(2)	λ	?	λ	434	λ	434	λ	λ	434	λ	434	λ'	λ	λ	434
(3)	λ	?	λ	434	434	434	λ	λ	434	λ	434	λ'	λ	λ	λ
(4)	λ	?	λ	434	λ	434	λ	λ	434	λ	434	λ'	λ	λ	λ
(5)	λ	?	λ	434	434	434	λ	λ	434	λ	434	λ'	λ	λ	434
(6)	λ	PA2	λ	434	λ	434	λ	λ	434	λ	434	λ'	λ	λ	434



This figure shows a possible sequence of events to explain the evolution of the phages. This is deduced by inspection of Table 8. The regions designated λ' are different in each phage.

[1] Letters c through q are assigned to the different regions of the phage chromosome 1 through 33. Regions that are the same with respect to the number of copies of a given sequence have been grouped together and assigned a letter (c through q).

[2] Numbers (1) - (6) represent 6 different phage molecules.

A more detailed explanation of this Figure is presented in the text.

DISCUSSION

Under the standard conditions used in this thesis (50% formamide in the hyperphase, 15% formamide in the hypophase, spreading temperature 20°C), those DNA regions appearing homologous (double-stranded) had greater than 79% homology and those appearing nonhomologous (single-stranded) had less than 79% homology. By spreading under less denaturing conditions (30% formamide in the hyperphase and 5% formamide in the hypophase, spreading temperature 4°C), it has been shown that the nonhomologous regions of the λ/424 heteroduplex have less than 58% homology. Within a population of a particular heteroduplex, most regions were consistently double- or single-stranded. Those regions that had about 79% homology appeared double-stranded in some molecules and single-stranded in others. In other words, these regions were partially homologous. The left end of phage 21 containing the head and some tail genes has regions, which are partially homologous to those of λ, 434, 424 and PA2.

In some regions, all the lambdoid phages studied differ in their DNA sequences. In others, the sequences are the same in all the phages. In yet others, the sequences may be the same for only some of the phages. This was deduced by making heteroduplexes between all possible combinations of λ, 424, 434, 21 and PA2. My results indicate that among those lambdoid phages studied here and possibly among all lambdoid phages, which exist in nature the number of copies of most DNA regions is small. In the five phage DNA's studied in this thesis, λ, 434, 424, 21 and PA2, one copy of most regions occurs at least twice.

My results reveal how many times any copy of a region of the DNA molecule is present in the population of phages. For λ, the results

indicate that about 40% of the λ DNA molecule is common to the four other lambdoid phages studied and to 82 (I have included in the 40% the left arm of λ from 0 to about 37%; all six phages share this DNA sequence except for phage 21, which is partially homologous with the others in the region between 0 and 15.9%λ). Perhaps mutations are less tolerated in these genes and therefore these "common" regions are the most highly conserved. Thus, these regions may be less active in terms of evolutionary change than other regions of the chromosome such as most of the right half, which shows only one short common region. A further 31% of the λ DNA molecule is common to at least one other phage. In only about 10% of the genome has no homology been found between any pair, and phage 82 has not been checked against all others here.

Hypotheses have been presented to explain the homologous and nonhomologous DNA sequences that exist between the lambdoid phages. Mutations in certain DNA base sequences or protein amino acid sequences may not be well tolerated. These sequences, therefore, would be conserved. Such conserved sequences are known to exist in proteins inter- and intraspecially. The ~~present~~ amounts of homologies between various pairs of phages could reflect some of these regions where mutations are not tolerated and thus remain the same in all the phages. The heteroduplex analyses of the following pairs of lambdoid phage DNA's; λ/434, λ/82, λ/21 and 434/82 (Simon et al, 1971) led to the hypothesis that homology existed because there were certain regions common to all the lambdoid phages, which may have resulted from some recombination process that selected the best gene copies during evolution (Davis and Hyman, 1971). Subsequent work with PA2, 424 and λ (this thesis; Highton and Beattie, unpublished results) and ϕ80 and λ (Fiandt et al, 1971; Highton and

Beattie, unpublished results) however, has shown that there is not more than about 15% common to all seven of these phage DNA's, whereas most pairs have much more homology than this.

My data could suggest that the present-day phages were assembled from a choice of previously evolved modules. This hypothesis (Campbell, 1972) predicts that genes from one module, which are related in function, should be situated near to each other. The fact that the clustering of functions exists in the DNA's of the lambdoid phages is in agreement with the modular hypothesis. In any two phages there are segments of homology separated by segments of nonhomology. Perhaps the genome is composed of separate modules within which recombination cannot occur in heterologous matings. Regions of homology might lie between modules. Alternatively, recombination could occur within modules common to both partners.

DNA exchanges between phages are possible provided there is some sequence homology. Viable hybrid phages among the lambdoid phages and between the lambdoid and other phages have been isolated. These have characteristics of both parental phages. This can be interpreted to support the modular hypothesis, where the functional segments (modules) such as head, tail, DNA replication, lysis and immunity genes are allowed to reassort among the phages resulting in viable phages with different combinations of these functional segments. This ability for DNA exchanges to occur between phages can however, also be interpreted to support the theory of phage evolution from a common ancestor. If a common ancestral chromosome were allowed to evolve with spontaneous mutations occuring without restriction and some of these mutations were selected for, the result could be a family of phages with different copies of some

common ancestral genes, and identical copies of others, which is necessary for DNA exchange to occur.

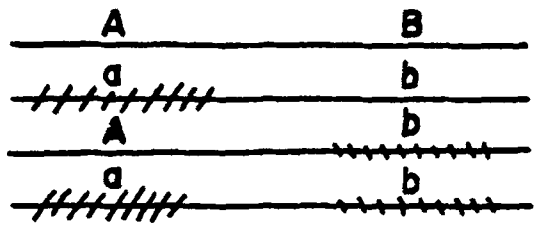
It is possible to explain the existing relationships between λ, 434, 82, PA2, 424 and 21 in terms of direct evolution from a common ancestor, apart from one DNA exchange. This is explained in the following paragraphs.

In Table 8 all the regions have been arranged according to the copies present. Thus, a region designated 6 would be represented in each of the six phage DNA's. A region designated 51 would be represented in five of the phage DNA's and the sixth phage DNA would have a different sequence in this region. There are also regions designated 42, 33 and 321. The 42 designation implies that four phage DNA's share the same DNA sequence and two phages share another but different sequence.

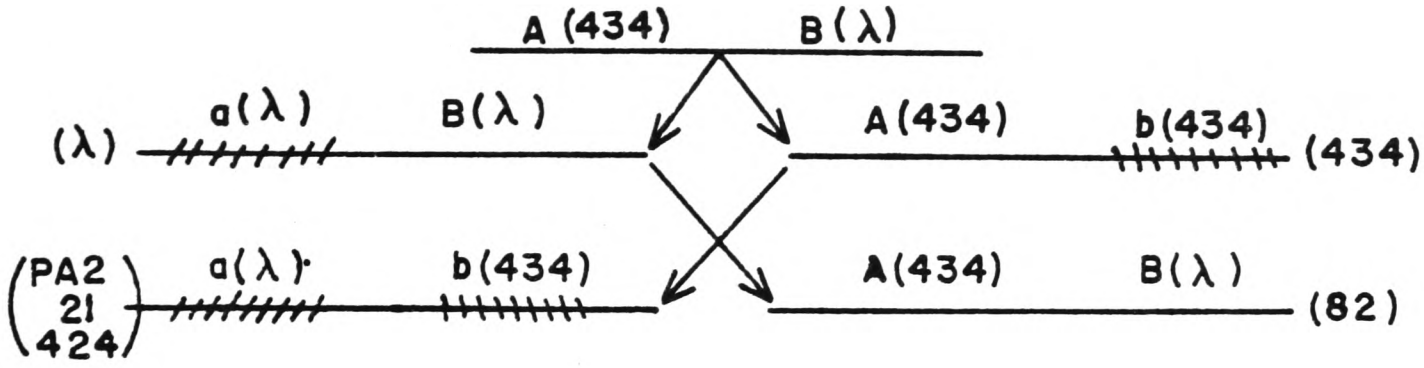
Figure 7 is a representation of one way to explain the evolution of these six DNA molecules from a common ancestor. It is deduced from Table 8 . Regions such as 111111, 21111, 3111, 411, 51 and 6 can evolve from a common ancestor if regions can change in some lines, but not in others. A hypothetical common ancestor can be deduced by first picking the copy for each region that occurs more than once. If this is done the result is ancestor (1). Queries indicate that 2 copies occur more than once. In g and q regions this can be explained if the original ancestor splits into 2 lines with changes at g and q, giving ancestors (2) and (3). However, because '434' and 'λ' copies occur in g and q in all 4 possible combinations, ancestors (4) and (5), which can be derived from (2) and (3) by an exchange, are required (See Campbell, 1972). If region d is 'λ' then λ, 434 and 82 can be derived directly from (4), (5) and (3), respectively. If d changes to 'PA2' in (2)

giving ancestor (6), PA2, 21 and 424 can be derived directly from it. This scheme requires that some regions change while others do not, and then stop changing while yet others start. This is necessary to explain how two copies can occur more than once in d, g and q regions. The alternative explanation is that all six phages have evolved directly from an ancestor such as (1), but with some exchanges. For example, region d could start as λ but change to 'PA2' in PA2 sometime after evolution of the six lines. Exchange with 424 and 21 could change them to 'PA2' in this region. The g and q regions could be similarly derived. We still have to postulate that regions can change in some lines, but not in others, unless all homologies are the result of exchanges. The d, g and q regions are the only ones, which could indicate an exchange because only in these do two copies occur more than once.

If there has been an exchange of segments, Campbell (1972) shows that we should expect to find arrangements such as



In Table 8 all the regions have been arranged according to the copies present. Of interest are types d, g and q. The boxed areas indicate an exchange. Following Campbell's (1972) notation a possible sequence of events would be



There are a number of such possible schemes, but this one fits the data. (as does Fig. 7)

Let us look at the lambdoid chromosome in terms of left and right halves. The left half contains the head and tail genes--genes coding for proteins, which are involved in interactions with eachother. The right half of the chromosome consists of the genes coding for proteins that are involved in regulatory functions. The proteins coded by the genes in the left half are more intimately involved with other proteins than are those coded by the genes in the right half. Perhaps mutations, involving even only a single base pair, in a gene involved in phage head or tail formation would change the protein sufficiently to cause a deleterious effect and thereby make the phage unable to make a functional head. The mutant would not survive, would never be found in nature.

Phage gene J codes for the phage tail fiber. My results indicate that the homology that exists between the phages in the tail genes ends within the J gene. The protein product of gene J is involved

in an interaction with other proteins--both at the tail/tail fiber junction and with proteins on the bacterial cell surface. Only the left half of this gene seems to remain constant among this family of phages. Beckendorf (1973) suggested that the left end of the gene J, the NH_2-terminus, codes for the part of the protein that is involved with the tail/tail fiber interaction and interacts with other phage proteins and that the right half of the gene J, the COOH-terminus, codes for the part of the protein involved with the tail fiber/bacterial cell surface protein interaction. Thus, the right end of gene J has evolved more rapidly to accomodate new receptors whereas the left end is invariant since the product of this portion of the gene would still be involved with the same interactions. My heteroduplex results support this suggestion.

The genes in the right half of the phage chromosomes code for proteins of a regulatory nature. These proteins interact with DNA rather than (or in addition to) proteins. Perhaps not being intimately involved with protein structures allows the genes in the right half the freedom to acquire mutations in their DNA and amino acid sequences without these mutations being deleterious. Thus, a mutated, but viable phage would result. Thus, the spontaneous mutations occurring in the DNA sequences could be manifested throughout the replication and propagation of the phage and its progeny. The progeny would also be able to acquire such mutations. Throughout the many generations since the ancestor evolved to its present stage, the various lambdoid bacteriophages could have acquired many such mutations and thus the more different they have become from each other in these more evolutionarily active regions of the chromosome.

As indicated above, ϕ80 was found to have only about 15% homology with the other six phages studied under the standard conditions.

This has actually only been shown for λ and 434, but the 15% lies at the left end, which is homologous in λ, 434, 82, 424 and PA2. In fact, only the first 5% of ϕ80 appears truly homologous with λ, the rest being partially homologous, and because 21 is only partially homologous with the other five throughout this region there can be no region that is exactly the same in all seven molecules, in the "common" region from 0 to 37% discussed above. Of the other three "common" regions defined in Figure 7, regions 3, 5 and 31, none are homologous to ϕ80, and in fact regions 3 and 5 are probably not completely homologous in the other six phages (See Table 7 notes 3 and 5).

The fact that these regions are not completely homologous does not affect the process of evolution proposed, and it will be of interest to see, by varying the conditions of denaturation, just how close any regions in the six phages are to one another, and how far they are from ϕ80. Fiandt et al (1971) detected more homology between λ and ϕ80 than did Highton and Beattie (unpublished results), presumably because their conditions were less denaturing. Also, the amount of homology observed between λ and ϕ81 at the left end, where ϕ81 and ϕ80 are homologous, increases as the denaturing capacity of the solutions is reduced (Niwa et al, 1978).

PART II

INTRODUCTION

EVOLUTION OF THE *ESCHERICHIA coli* CHROMOSOME

The circular chromosome of *E. coli* contains enough DNA to code for about 3000 polypeptide chains with average molecular weight of 40,000 daltons. Over 600 of these genes have been assigned specific functions and positions on the *E. coli* chromosome. Genes coding for proteins with related functions, such as isoenzymes catalyzing the same reactions or groups of enzymes involved in sequential metabolic conversions, may be either close together or far apart on the circular chromosome. Some related genes are close together because they form part of the same operon and are transcribed together. Other related genes are close together but do not share a common control mechanism.

Lewis (1951) discussed the idea of new genes arising from pre-existing genes. He envisioned a new gene originating by a two-step process: one or more duplications of a gene would occur after which the duplicate genes would mutate to a new function. This could be related to the function of the original gene. Clustering of genes with related functions may be the result of such a tandem gene duplication followed by evolution of a series of related genes (Lewis, 1951; Horowitz, 1965; Dixon, 1966).

Hopwood (1967) found that related genes on the circular map of *Streptomyces coelicolor* were separated by 180°. He speculated then that a genome duplication event may have been a mechanism of evolution of this organism. Sparrow and Nauman (1976) proposed that a process of chromosome doubling was a major event in the evolution of all major phylogenetic groups.

Primitive organisms most likely would have had fewer genes and corresponding enzymes than their more highly developed,

biosynthetically sophisticated present-day descendants. One can then say that the present-day bacterial genome evolved from a simple to a more complex form. The evolution of the bacterial genome must have involved the increase of total genetic information as well as the increase in the complexities of metabolic function and cell structure. Duplications of genes, of longer genetic segments and of the entire chromosome may have been important in increasing the size of the bacterial chromosome. Retention of nonfunctional genes would provide genetic plasticity that is probably evolutionarily advantageous.

Zipkas and Riley (1975) proposed a mechanism for the evolution of the E. coli K12 chromosome. This, they said involved two successive duplications of an ancestral chromosome--one quarter the size of the present chromosome--followed by mutation and divergence of the duplicated genes. They found that many pairs of genes that are biochemically related are located either 90^{o} or 180^{o} apart on the E. coli K12 circular chromosome. This proposed theory of chromosome duplication is supported by the observations of Wallace and Morowitz (1973). These authors tabulated the chromosome sizes of about 70 different species of bacteria. They found that the sizes, which vary from 0.5 to 3.0 x 10^{9} daltons do not vary randomly. Mycoplasma species have chromosome sizes clustered about the peaks 1.4 x 10^{9} and 2.8 x 10^{9} daltons. This is consistent with the idea that smaller chromosomes give rise to larger ones by duplication.

Among the 460 genes listed by Taylor and Trotter (1972), Zipkas and Riley (1975) selected those for which sufficient information was available about their gene products. They listed pairs or groups of genes considered to be related. Gene pairs were considered to be related if their gene products fell into one of the following three categories: 1) isoenzymes catalyzing the same reaction;

2) functionally related genes sharing some specificity such as sequential enzymes in metabolic conversions and enzymes catalyzing similar reactions involving the same or similar small molecules and; 3) genes coding for related cellular structures or functions such as ribosomal proteins and proteins involved in K^+ transport. Pairs within a single operon were rejected, however, individual members of an operon were paired with other biochemically related genes that lie outside the operon (for example, thrC and ilvA).

Zipkas and Riley (1975) discussed three possible modes of chromosome duplication. The first involves homologous crossing over between identical circular chromosomes. Another involves head-to-tail joining of two identical linear chromosomes having complementary cohesive ends. The third involves the production of concatamers followed by cleavage at specific points resulting in unit chromosomes. The latter would involve mutation at a cleavage site yielding a dimer chromosome, which, following circularization and subsequent replication would yield concatamers that would eventually be reduced to dimers by cleavage.

In 1976, a revised genetic map of E. coli was published (Bachmann et al, 1976) after which the concept of biochemical relatedness of genes was more strictly defined (Riley et al, 1978). Genes were considered to be related if their enzyme products either catalyzed similar reactions according to the Enzyme Commission classification scheme or interacted with the same small molecule either as a substrate or as a product. Related gene pairs were statistically analyzed but showed no tendency to be located 90^o or 180^o apart. The tendency to lie 90^o or 180^o apart resided almost entirely in the group of genes concerned with the central pathways of glucose catabolism. However, nonrandom distribution of genes may not be confined

to those genes involved with glucose degradation. Spatial relationships may exist between pairs of genes that descended from a single ancestral gene.

Mizobuchi and Saito (1975) investigated gene and chromosome duplication and the principle of gene diversification. They classified 246 genes of E. coli K12 into functionally related groups and inspected the relationships between the positions on the circular genetic map of genes in the same group. Genes they considered to be related fell into one of the following three groups: 1) genes encoding isoenzymes; 2) genes encoding identical or similar products other than proteins (for example, the two tyrosine tRNA genes, which have identical base sequences and; 3) genes encoding enzymes with similar reaction mechanisms (i.e. genes that are functionally related). This last criterion is different from that used by Zipkas and Riley (1975) who defined functionally related genes as those involved in one sequential metabolic pathway.

Of the genes classified by Mizobuchi and Saito (1975), 63% were found to be separated from homologs by a multiple of 12.4 $\pm$ 1 min on the 100 min circular map of E. coli. They have therefore adopted the 12.4 min map distance as a duplication unit of the chromosome. If the ancestor of the modern E. coli underwent total chromosome duplication three times this theory predicts that each gene will have seven sister genes positioned every 12.5 min on the 100 min map, or every 45^{o}. These authors do not dismiss the possibility that the duplication unit may be further divided into a smaller unit. Thus, Zipkas and Riley predicted that the present-day chromosome is the result of two duplications of an ancestral E. coli chromosome and Mizobuchi and Saito predicted that the chromosome is the result of at least three previous duplications.

TWO RELATED GENES: *argF* AND *argI*

One example of biochemically related genes is the pair *argF* and *argI*. These genes are not 90°, 180° or 270° apart, but both code for the enzyme ornithine transcarbamoylase (Glansdorff *et al*, 1967). Ornithine transcarbamoylase (OTC) catalyzes the carbamoylation of ornithine into citrulline in *E. coli*, the sixth step of arginine biosynthesis. Zipkas and Riley (1975) suggested that although these genes are biochemically related in that they specify proteins that carry out the same reaction, they may have had independent origins; as a consequence they may be expected to have different DNA sequences. Mizobuchi and Saito (1975) classified *argF* (8.1 min) and *argI* (94.7 min) into the same group because they have similar catalytic reaction mechanisms, they are separated by 13.4 min and thus may be sister genes. Kikuchi and Gorini (1975) have shown that these genes have similar base sequences, suggesting that one of the genes arose by duplication of the other. This is not consistent with the suggestion that functionally related genes not 90°, 180° or 270° apart evolved independently (Zipkas and Riley, 1975). It does, however, lend support to the theory that duplicate genes will be separated by a multiple of 12.4 $\pm$ 1 min. Kikuchi and Gorini (1975) found that the homology detected by electron microscope heteroduplex formation extended over a length equivalent to that of the genes. This homology between *argF* and *argI* does not support the theory that the chromosome arose by two duplications. They are too far apart from each other on the chromosome to be the result of tandem gene duplication. It seems reasonable to suggest that they arose by an early chromosome duplication event assuming that the unit of duplication was about one-eighth the size of the present chromosome. The work of Sens *et al*

(1977) established that between 25 and 40% of the base pairs in the argF and argI genes have changed since they diverged from a hypothetical ancestral gene. The extent of divergence of these genes was determined by mRNA-DNA hybridization. They suggest that the argF gene is new and probably arose as a result of the duplication of the argI gene.

Perhaps gene duplication followed by translocation can explain the argF and argI homology. The acquisition of multiple copies of a gene on the bacterial chromosome by gene duplication is one way to improve the potential yield of that gene product. The duplication will be selected for and maintained only under conditions in which the duplication will be advantageous or essential to the growth and survival of the cell. Otherwise, the duplicated gene will be lost by recombination between the two homologous segments with loss of one of them. This would most likely occur if the duplicate genes are very near to the original gene.

In the strains E. coli B and E. coli W, only the argI gene is present. Also, in B. subtilus and Proteus mirabilis only one OTC gene locus has been found (Masters and Pardee, 1965; Zaharia and Soru, 1971). This indicates that perhaps the presence of both the argI and argF genes in E. coli K12 strains could be the result of a duplication of the argI gene locus. However, the fact that not even all E. coli strains have these two genes suggests that this duplication was rather recent and did not occur during duplication of an ancestral genome.

Elongation factor Tu (EF-Tu) of E. coli promotes the binding of aminoacyl-tRNA to ribosomes in the form of an equimolar complex with GTP and aminoacyl-tRNA (Lucas-Lenard and Lipmann, 1971). Two copies of the EF-Tu have been identified, one near rif (tufB) and the other

near fus (tufA) (Jaskunas et al, 1975). Both are the same size and of the same antibody specificity. They are very similar in their physical properties; there is, however, a slight difference of one spot in the tryptic fingerprint (Furano, 1977). Although the proteins have practically identical amino acid sequences (Miller et al, 1978; Furano, 1977), they have been separated by ion exchange chromatography on DEAE-Sephadex A50 (Geiser and Gordon, 1978a and 1978b). Duplication of the tuf genes has been conserved in both E. coli K12 and E. coli B strains as well as in Salmonella typhimurium (Furano, 1978).

NEW GENES THROUGH GENE DUPLICATION

The significance of gene duplication in evolution has been discussed by Dixon (1966). As an evolutionary mechanism, gene duplication is important in that it results in the addition of information. Bacteria and viruses and other microorganisms contain much less DNA than higher organisms. If higher organisms evolved from simpler ones, an increase in information would be required. Gene duplication would provide a double dose of a particular piece of information. Subsequent mutations in the duplicate region could occur, be selected for and eventually expressed resulting in a modified protein. The original gene would still be able to produce its product according to selective pressures. Dixon (1966) suggested that gene duplication could be responsible for evolution of closely linked genes in biosynthetic pathways, for example the histidine genes in Salmonella typhimurium (10 enzymes) and the galactose pathway of E. coli. Gene duplication with subsequent independent evolution of the genes could be responsible for the clustering of genes that code for groups of functionally related enzymes. Partial gene duplication would result in a

modified gene increased in length and incorporating a repeated sequence. Jackson and Yanofsky (1973b) have discussed duplication and translocation events and their roles in evolution. A gene duplication event followed by a translocation event may play an important role in evolution by generating new copies and arrangements of genetic segments while keeping the original genes intact. Gene duplications have been found of many genes and operons including the following: the ribosomal RNA genes in the oocytes of several amphibians, of an echiuriod worm and of a surf clam (Brown and Dawid, 1968); genes of the E. coli arginine operon (Glansdorff and Sand, 1968); genes of the E. coli galactose operon (Morse, 1967; Ahmed, 1975); E. coli tRNA genes (Hill et al, 1969; Russell et al, 1970); the E. coli glycyl-transfer RNA synthetase gene (Folk and Berg, 1971); the genes of the E. coli tryptophan operon (Jackson and Yanofsky, 1973b); the bacteriophage T4 rIIB cistron (Freedman and Brenner, 1972); the early genes A and B of the bacteriophage P2 (Bertani and Bertani, 1974; Chattoraj and Inman, 1974); the tetracycline-resistant determinant on the α-plasmid of Streptococcus faecalis strain DS-5C1 (Clewell et al, 1975); the dihydrofolate reductase structural gene (Schimke et al, 1977; Alt et al, 1978); the tryptophanase gene of E. coli (Yudkin, 1977); the amp region of the E. coli K12 chromosome (Normark et al, 1977a; Normark et al, 1977b; Edlund et al, 1979). Anderson and Roth (1977) present a review of tandem genetic duplications in bacteriophage and bacteria.

The gene dosage of an operon may be increased by introducing into the cell an episome carrying that operon. For example, the E. coli F-factor can carry bacterial genes; it can be introduced into an F^- cell. By introducing into the cell an F factor carrying a duplicate region of the chromosome, the gene dosage would be

doubled and so would gene expression (Müller-Hill *et al*, 1968). An F factor carrying a duplicate region of the chromosome could integrate into the host and upon excision leave the duplicated region intergrated into the host.

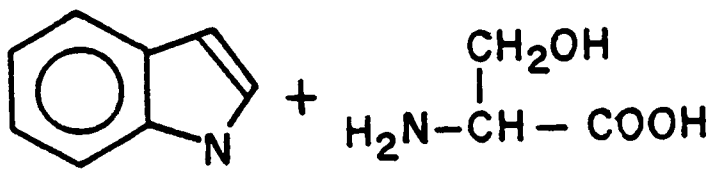
It is possible that primitive bacteria containing duplicated genes and chromosomes may have had a selective advantage over the others. Several possible suggestions explaining the advantage of having a duplicated chromosome have been put forward by Zipkas and Riley (1975). The selective advantage may be because of an increased gene dosage effect, or because of a reduced frequency of deleterious mutations in the multiploid bacterium or because of the increased genetic capacity, which would provide DNA for future evolutionary changes, leading to an increased genetic capability. Mizobuchi and Saito (1975) found very few genes on the *E. coli* K12 genetic map to be individually duplicated. They argued that chromosome duplication was more important than individual gene duplication in increasing the size of the *E. coli* chromosome.

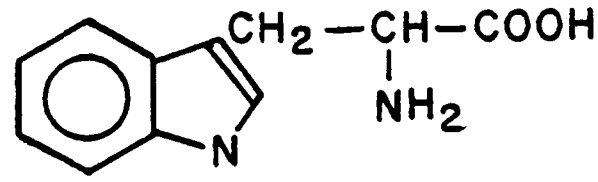
The theories of evolution by chromosome duplication would be supported by the detection of further examples of homology between pairs of genes classified as functionally and spatially related by both Zipkas and Riley (1975) and Mizobuchi and Saito (1975). A complete list of gene pairs examined by Mizobuchi and Saito has not been published; it is therefore not possible to make a complete comparison of the related gene pairs of these two groups. One pair of genes classified as functionally and spatially related by both groups is the tryptophan synthetase B gene (*trpB*) and the tryptophanase gene (*tna*) of *E. coli*.

THE E. coli TRYPTOPHAN SYNTHETASE B AND TRYPTOPHANASE GENES AND GENE PRODUCTS

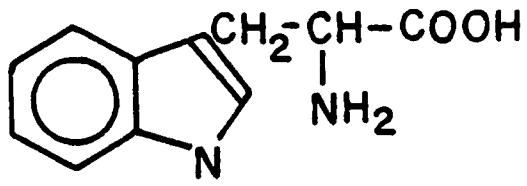
E. coli tryptophan synthetase B and tryptophanase are two enzymes that catalyze similar but not identical reactions involving the same small molecules. Tryptophanase degrades tryptophan to indole, the reverse of the reaction catalyzed by tryptophan synthetase. Tryptophanase can be used by a cell with a complete deletion of the tryptophan operon to convert indole to tryptophan (i.e. it can work in the reverse direction to catalyze the same reaction as tryptophan synthetase). The exact reactions catalyzed by these two enzymes are:

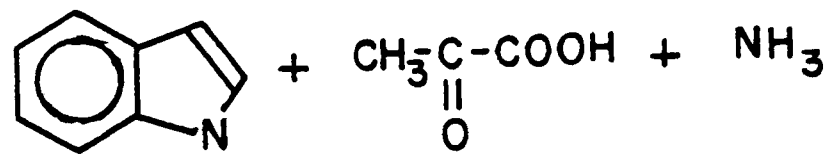
Indole + Serine $\rightleftharpoons$ Tryptophan





Tryptophan $\rightleftharpoons$ Indole + Pyruvate + Ammonia





Both enzymes require the coenzyme pyridoxal phosphate. It is relevant here to discuss briefly the tryptophan operon of E. coli. Tryptophan synthetase B participates in the biosynthetic pathway of tryptophan synthesis. The tryptophan gene cluster consists of five structural genes: trpA; trpB; trpC; trpD and trpE reading clockwise round the circular linkage map (Yanofsky and Lennox, 1959; Taylor and Trotter, 1972; Bachmann et al, 1976). The proteins coded by these genes catalyze the conversion of chorismic acid (an intermediate common to the biosynthesis of the amino acids phenylalanine, tyrosine and tryptophan) to tryptophan as shown in Figure 8 . The role of

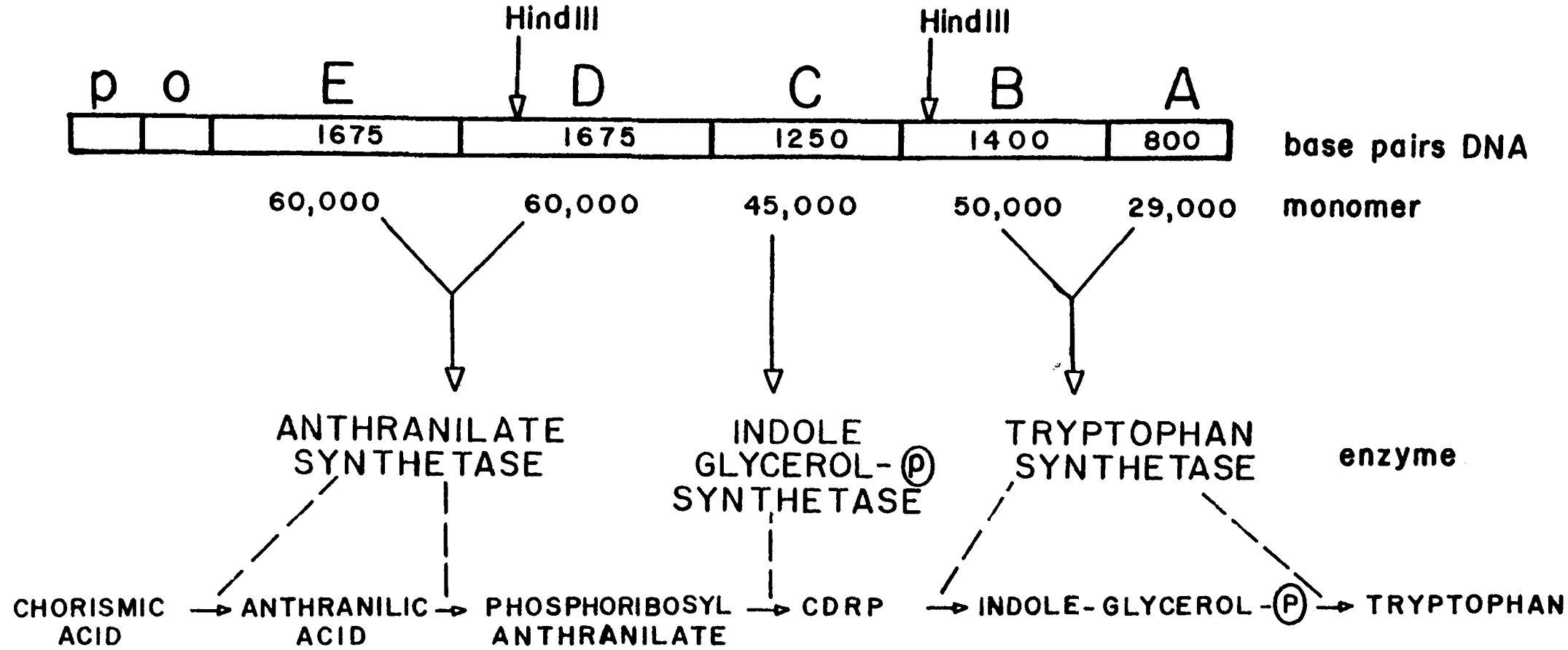


Figure 8. THE TRYPTOPHAN OPERON. (Adapted from Yanofsky, 1971).

The HindIII restriction sites within the tryptophan operon are indicated. Abbreviations: CDRP, 1-(o-Carboxyphenylamino)-1-deoxyribulose-5-phosphate; (P), phosphate.

each of the five enzymes has been identified and biochemical assays exist for all five (Smith and Yanofsky, 1962; Creighton and Yanofsky, 1969a).

TrpE is the structural gene for the enzyme anthranilate synthetase component I. In the absence of component II, component I converts chorismate to anthranilate but does so inefficiently compared with its activity when complexed with component II (Ito and Yanofsky, 1966; Ito et al, 1969).

TrpD determines component II of anthranilate synthetase, which is known as phosphoribosyl transferase (Ito and Yanofsky, 1969). Component II alone is required for the transfer of a phosphoribosyl group to anthranilate from 5-phosphoribosyl 1-pyrophosphate (Henderson et al, 1970; Henderson and Zalkin, 1971; Jackson and Yanofsky, 1974).

TrpC gene product, indoleglycerolphosphate synthetase, is responsible for the conversion of N-(phosphoribosyl) anthranilate to indole-3-glycerolphosphate (Smith, 1967; Creighton and Yanofsky, 1969b).

TrpB and trpA genes code for the two distinct components α and β, respectively, of tryptophan synthetase. These act as a complex, $\alpha_2\beta_2$ (two A and 2 B gene monomers) and catalyze the conversion of indole-3-glycerolphosphate to tryptophan (Carlton and Yanofsky, 1962; Wilson and Crawford, 1965; Goldberg et al, 1966).

The five genes are transcribed as a single polycistronic mRNA from the tryptophan promoter (Imamoto et al, 1968). Expression of the operon is repressed by high levels of tryptophan (Jacob and Monod, 1961) acting with the product of the unlinked trpR gene (Cohen and Jacob, 1959).

The *trpB* gene is located at 27.4 min and the *tna* gene is located at 82.2 min on the 100 min *E. coli* linkage map. So, *trpB* and *tna* are 55 min apart. This pair of genes, therefore, are biochemically related and about 180° apart. Thus, they make an appropriate pair to study for evidence to test the proposed theories of the evolution of the *E. coli* chromosome by duplications of an ancestral chromosome followed by mutation and divergence of the duplicate genes. Duplicate copies of an ancestral gene concerned with tryptophan metabolism could have evolved to yield the gene for tryptophan synthetase and 180° apart from it, the gene for tryptophanase.

If this were the case, one would expect that there may be some sequence homology between the *trpB* gene and the *tna* gene. If such homology existed it would lend support to either the theory proposed by Zipkas and Riley (1975) or the theory proposed by Mizobuchi and Saito (1975). Because both gene products involve the same substrates it would be likely in view of the proposed theories that their DNA sequences would not have mutated as much as pairs of related genes not involving the same substrates.

I have used the technique of heteroduplex formation to determine whether any DNA sequence homology exists between the *trpB* gene and the *tna* gene. Basically, DNA of transducing phages carrying either the *trpB* gene or the *tna* gene were denatured and renatured together, and spread for electron microscopy by the method of Davis *et al* (1971). The resulting heteroduplexes were photographed and analyzed by measuring with a map measurer.

PHAGES AND STANDARDS

The parental vector phage used in these heteroduplex studies is a derivative of phage λ (Murray, 1975). It carries the wild-type phage 21 repressor and thus forms turbid plaques. It has a single HindIII restriction target at which the bacterial fragments carrying the trpABC genes and the tna gene were inserted. The transducing phages used in this research are: NM540; λtrpA^{r}; λtrpC^{r}; λtrpA^{1}; λtrpC^{1}; λtrpABC^{r}; λtrpABC^{1}; λtna and; λtna imm$^{\lambda}$ nin^{+}.

The construction of all phage vectors and transducing phages used in this research was expedited by using restriction endonucleases (commonly called restriction enzymes). Restriction endonucleases recognize and bind to a very specific sequence of nucleotides, breaking internal phosphodiester bonds and leaving two tails with complementary DNA sequences, which at some later stage can hydrogen-bond to other complementary DNA sequences.

The orientation of the inserted DNA fragment is designated either 1 or r. The fragment with 1 orientation is transcribed from the 1 strand of the λ transducing phage from the λ promoter p_L The fragment with r orientation is transcribed from the r strand of the λ transducing phage from the λ promoters p_R and p_R'. The tryptophan operon contains two targets for the restriction endonuclease Haemophilus influenzae serotype Rd (HindIII), one in the trpD gene and one in the trpB gene (See Figure 8). It was therefore not possible to generate a HindIII fragment consisting only of the trpB gene. The trpABC fragments consist of genes trpA, trpB and trpC and include DNA to the right of gene trpA and to the left of gene trpC. The tryptophan operon is normally transcribed from trpE ----> trpA.

The transducing phage λtrpABC^{1} was constructed in vivo by making the following phage cross: λtrpA^{1} x λtrpC^{1} (Hopkins, PhD

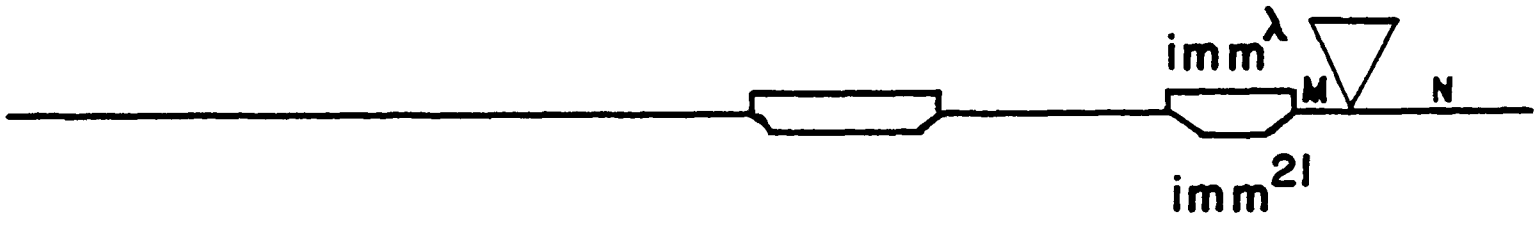
thesis, 1975). I constructed the λ*trpABC*^{r} transducing phage by making the following phage cross: λ*trpA*^{r} x λ*trpC*^{r} *in vivo* using the host QR47. Lambda-transducing phages carrying the *E. coli* tryptophanase gene have been produced *in vitro* (Brammar *et al*, personal communication).

Local standards were needed in these heteroduplex studies because of magnification variation, which results principally from variation of the vertical specimen (grid) position. Single-stranded and double-stranded standards were both needed because their nucleotide spacing may be different. The transducing phage λ*tna imm*$^{\lambda}$ *nin*$^{+}$ was constructed to provide internal standards when heteroduplexed with the phage NM540. This heteroduplex consists of three regions of nonduplexed DNA (See Figure 9-1). The left single-stranded loop represents the *tna* gene. The bubble represents the immunity regions of λ (*imm*$^{\lambda}$) and 21 (*imm*^{21}). The right loop represents the *nin* region. The genes comprising the immunity region of λ are different from the genes comprising the immunity region of phage 21. Therefore, when these two segments of DNA are aligned, they will not hybridize and will appear as two single strands. The λ*tna imm*$^{\lambda}$ *nin*$^{+}$ transducing phage contains the genes comprising the *nin* region of λ; the NM540 vector phage does not carry these genes. When these two phages are annealed, there will be a loop projecting from the λ*tna imm*$^{\lambda}$ *nin*$^{+}$ phage at the point where the *nin* genes are inserted. In Table 9 are listed values of *imm*$^{\lambda}$, *imm*^{21} and *nin* lengths determined by various persons.

The small circular DNA molecules of the following: M13 (single-stranded); pSC101 (double-stranded) and; ϕX174 (double-stranded) were obtained from David Finnegan and were used as standards in most of my later heteroduplex experiments. David Finnegan

determined the size of M13 DNA, measured relative to ϕX174 DNA, using gel electrophoresis methods (David Finnegan, personal communication). I used his value of 6230 bases as the size of M13 DNA in my experiments in which M13 DNA was used as a single-stranded standard.

The sizes of <u>imm</u>$^{\lambda}$, <u>imm</u>^{21}, <u>nin</u>, M (the double-stranded segment of DNA between the <u>imm</u>$^{\lambda}$/<u>imm</u>^{21} nonhomology bubble and the <u>nin</u> loop in appropriate heteroduplexes) and N (the double-stranded segment of DNA to the right of the <u>nin</u> loop in appropriate heteroduplexes) were also determined by DNA heteroduplex spreadings with M13 and pSC101 DNA's as standards. These values are tabulated in Table 9. These segments of DNA are labelled below



where HindIII represents the bacterial DNA fragment (produced by restriction with <u>Hind</u>III) inserted at the <u>Hind</u>III restriction target.

RESULTS

THE CONSTRUCTION OF λ*trpABC*^{r}

I constructed the λ*trpABC*^{r} transducing phage by crossing λ*trpA*^{r} with λ*trpC*^{r} *in vivo* in the host QR47. The λ*trpA*^{r} and λ*trpC*^{r} phages were isolated from a pool of DNA's of *E. coli* and the λ vector, NM540, which had been digested with the endonuclease R. *Hin*dIII and subsequently ligated (Hopkins *et al*, 1976). The λ*trpA*^{r} and λ*trpC*^{r} phages were selected for by their abilities to complement *trpA* and *trpC* mutants, respectively. A dilution of the cross between these two phages was incubated with *trpAC9suIII* plating cells. Single turbid recombinant plaques were picked after incubating at 37°C for 48 h on fresh ACH plates. The host cells, *trpAC9suIII*, lack the genes *trpA*, *trpB* and *trpC* and therefore require the addition of tryptophan or the complementation of the *trpA*, *trpB* and *trpC* genes in order to grow in this medium. These plaques were purified by plating again for single turbid plaques on ACH plates using the host *trpAC9suIII*.

THE CONSTRUCTION OF λ*tna imm*$^{\lambda}$ *cI857 nin*$^{+}$

A λ transducing phage carrying the *E. coli* tryptophanase (*tna*) gene has been produced *in vitro* (W. J. Brammar, S. Dunbar and S. Muir, personal communication). This transducing phage was crossed with a phage of the genotype *h*^{80} *att*^{80} *imm*$^{\lambda}$ *cI857 nin*$^{+}$. The cross was made in the host QR47. Recombinants were selected for their ability to form clear plaques on the host C600 (phage carrying the *imm*$^{\lambda}$ *cI857* temperature-sensitive allele produce clear plaques at 37°C). The desired recombinant phage is λ*tna imm*$^{\lambda}$ *cI857* nin^{+} (See Figure 9). Single clear plaques were picked and dilutions were spotted on various lysogens to check the immunity carried by the phage. Those plaques

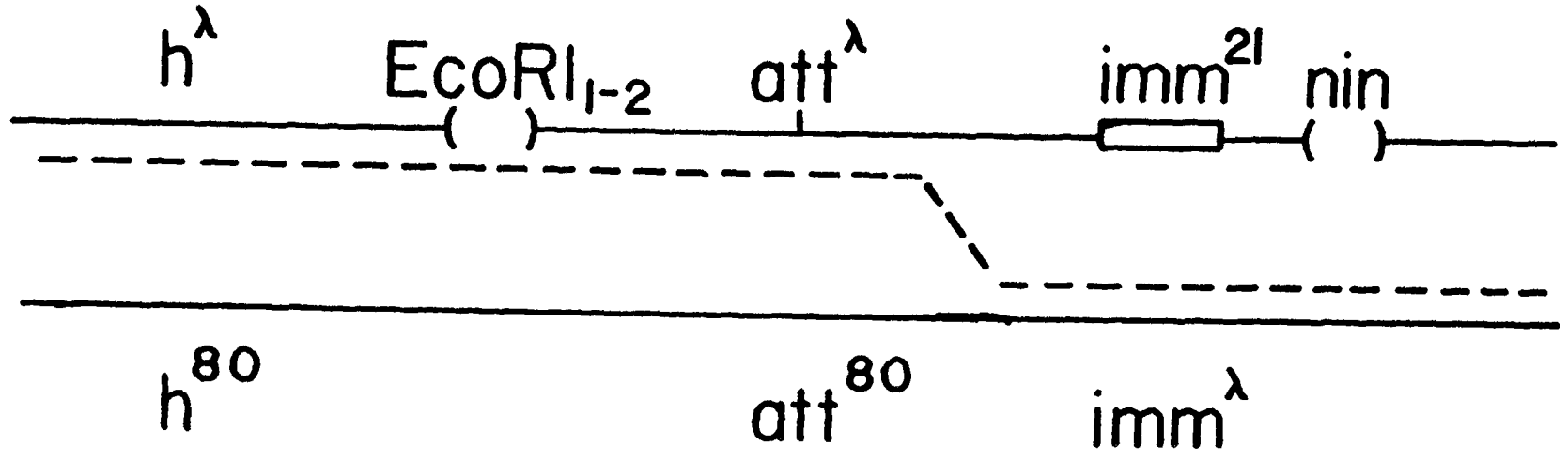


Figure 9. Recombinant Bacteriophage ($\underline{h}^{\lambda}$ $\underline{att}^{\lambda}$ $\underline{imm}^{\lambda}$ $\underline{nin}^{+}$).

being imm$^{\lambda}$ cI857 (i.e. clear plaque morphology) were further screened for the presence of the nin region by spotting dilutions on to a groN host. The strain groN carries a mutation in RNA polymerase, which will not allow a complex to form between RNA polymerase and the N protein. The RNA polymerase will therefore fall off when it sees the termination sequence. A wild-type λ phage (λ^+) requires the N protein to bind to the RNA polymerase to allow transcription to continue in to the late genes. A λ^+ phage will not grow on a groN host because the late genes will not be expressed. A phage with the nin region deleted will grow on the groN host because this deletion removes the termination sequence.

To screen for the presence of the tna insertion, C600 lysogens of the recombinant phage were isolated by picking from the center of several recombinant plaques and streaking these on to fresh L plates. Single colonies were picked and screened for lysogeny by streaking across streaks of λvir and λ^+ phages. Single lysogens were grown in L broth overnight at 30°C, the permissive temperature. These were then added to an equal volume of indole reagent. If a lysogen carries a λtna transducing phage, the solution will turn red, otherwise it will remain yellow. A high-titer phage stock was prepared by plate lysate from a single λtna imm$^{\lambda}$ cI857 nin^{+} plaque.

DETERMINATION OF THE SIZE OF pSC101 DNA

I determined the size of double-stranded pSC101 DNA by spreading it with double-stranded ϕX174 DNA under the standard conditions (See Materials and Methods). Ten sets of photographs were traced and measured; each set consisted of photographs of between 8 and 31 ϕX174 and pSC101 DNA molecules lying within about 20μ of each other. There were about equal numbers of each pSC101 and ϕX174 DNA molecules in

each set. Within each set, all photographs were taken without changing the magnification of the microscope. The ratio of the mean pSC101 DNA length to the mean ϕX174 DNA length was determined for each set. The length of pSC101 DNA was determined to be 173.8 ± 1.8 (s.d.; n = 10)% of the length of ϕX174 DNA. Because the size of ϕX174 DNA is 5386 base pairs (Sanger et al, 1978) the size of pSC101 DNA was deduced to be 9361 ± 97 (s.d.; n = 10) base pairs.

DETERMINATION OF THE SIZE OF WILD-TYPE λ DNA

Wild-type λ DNA was spread with pSC101 DNA under the standard conditions. Twenty-five λ DNA molecules, each with between 4 and 29 pSC101 DNA molecules lying within about 20μ of it were photographed. The ratio of the mean pSC101 DNA length to the mean λ DNA length was determined to be 19.0 ± 0.2 (s.d.)% of the length of λ DNA (%λ) and from the preceding section, the length of λ DNA was hence deduced to be 912.6 ± 13.5 (s.d.)% ϕX174, i.e. 49,150 ± 730 (s.d.) base pairs.

DETERMINATION OF THE SIZES OF THE INTERNAL STANDARDS AND OF THE *tna* BACTERIAL FRAGMENT

A heteroduplex was made between the λ vector, NM540 and λ*tna* *imm*$^{\lambda}$ *c*I857 *nin*$^{+}$. This was spread under standard spreading conditions. Before spreading, pSC101 and M13 DNA's were added to the spreading solution. At least 1 adjacent pSC101 DNA molecule and between 5 and 11 adjacent M13 DNA molecules were photographed with each heteroduplex. The averages of these were used as the double- and single-stranded standards, respectively for the heteroduplex. The lengths of these standards are listed in Table 3. The heteroduplex is shown in Figures 10 and 11. An electron micrograph is

FIGURE 10

10-1 NM540/λ*tna imm*$^{\lambda}$ *cI857 nin*$^{+}$

10-2 λ*trpABC*^{1}/λ*trpABC*^{r}

10-3 λ/λ*trpABC*^{1}

10-4 λ/λ*trpABC*^{1}

10-5 λ/λ*trpABC*^{r}

10-6 λ/λ*trpABC*^{r}

10-7 λ*tna imm*$^{\lambda}$ *cI857 nin*$^{+}$/λ*trpABC*^{1} (spread at 20°C)

10-8 λ*tna imm*$^{\lambda}$ *cI857 nin*$^{+}$/λ*trpABC*^{r} (spread at 20°C)

10-9 λ*tna imm*$^{\lambda}$ *cI857 nin*$^{+}$/λ*trpABC*^{1} (spread at 4°C)

10-10 λ*tna imm*$^{\lambda}$ *cI857 nin*$^{+}$/λ*trpABC*^{r} (spread at 4°C)

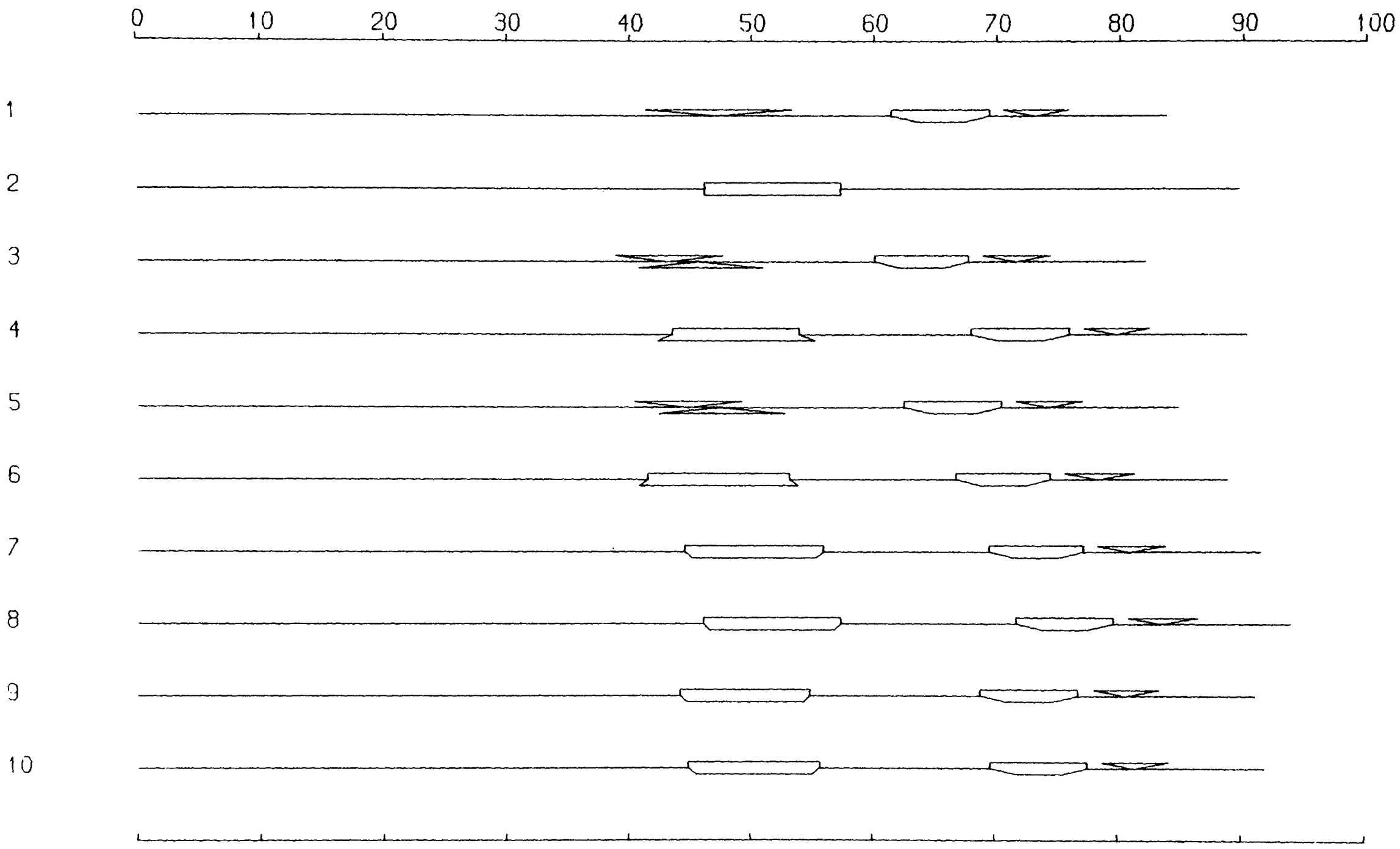


FIGURE 10. Heteroduplex Molecules Between λ-Transducing Phages.

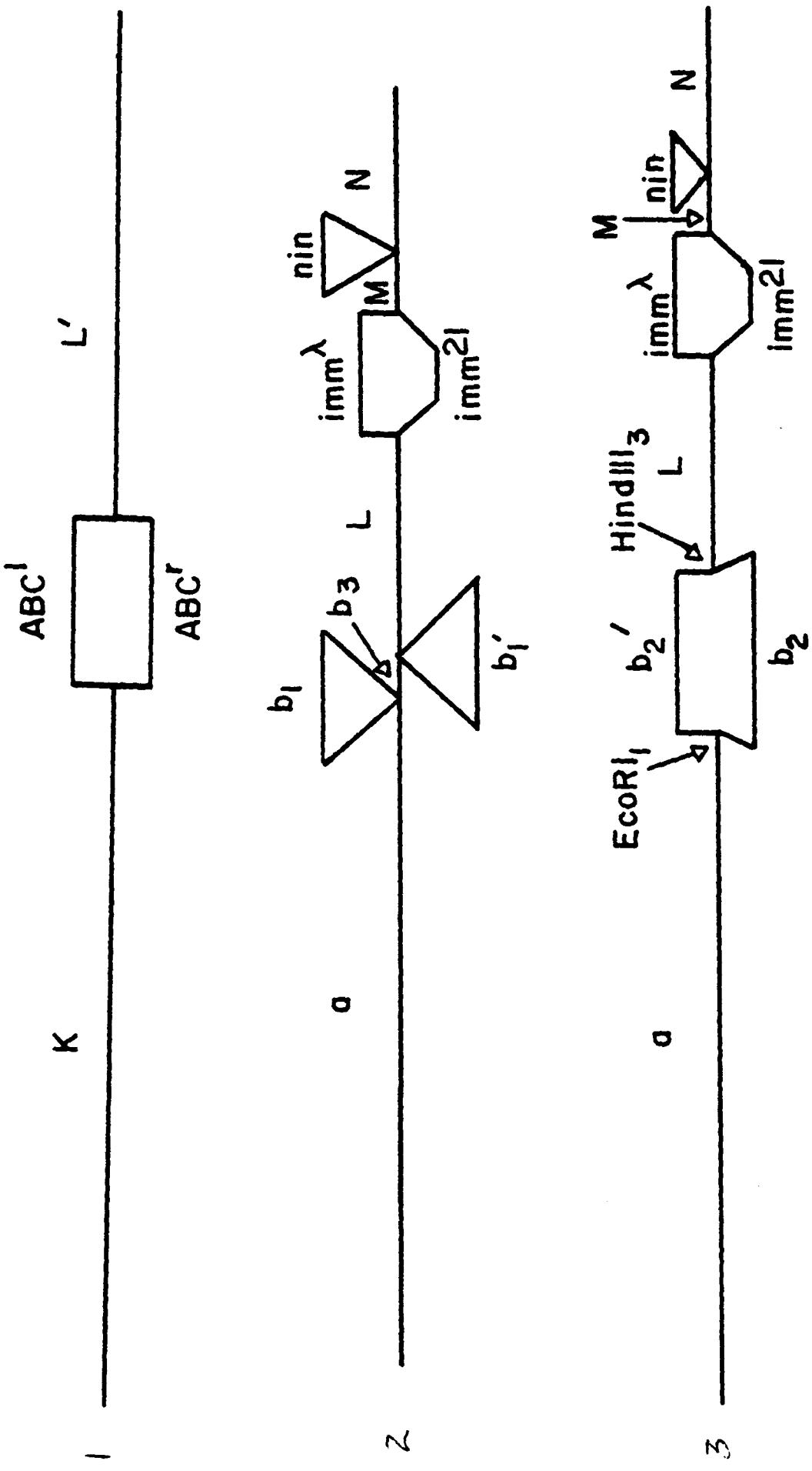


FIGURE 11. trp/tna HETERODUPLEXES. (1) λtrpABC^{l}/λtrpABC^{r}; (2) λ/λtrpABC; (3) λ/λtrpABC; (4) λtrpABC/λtna imm$^{\lambda}$ cI857 nin^{+}. All DNA segments identified in this figure are explained in detail in the text (Part I Results).

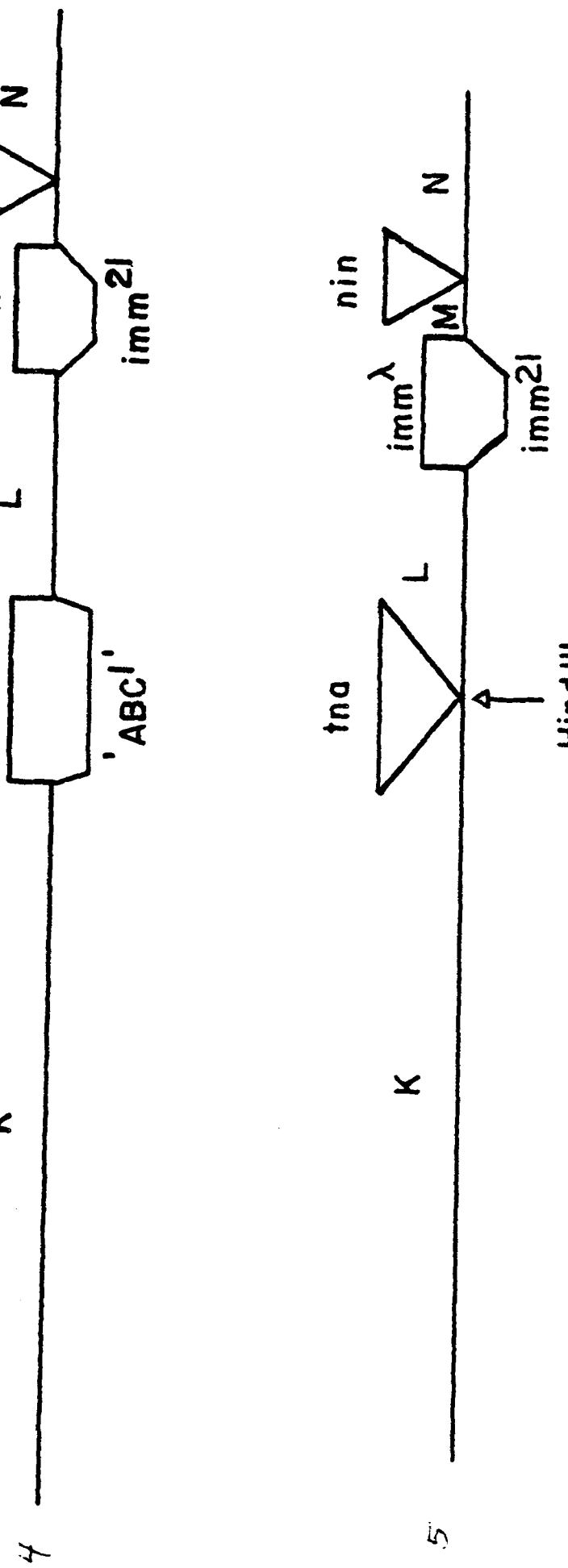


FIGURE 11. trp/tna HETERODUPLEXES. (1) λtrpABC^{1}/λtrpABC^{r}; (2) λ/λtrpABC; (3) λ/λtrpABC; (4) λtrpABC/λtna imm$^{\lambda}$ cI857 nin^{+}. All DNA segments identified in this figure are explained in detail in the text (Part I Results).

shown in Appendix 4 Plate XVIII. The lengths of its segments are tabulated in Table 9. The molecule is double-stranded from the left end up to the R. HindIIIλ3 (shnIIIλ3) restriction target because the strands are identical up to this point. I determined the size of this double-stranded segment, K, to be 47.3 $\pm$ 1.3 (s.d.; n = 15)%λ in length. The single-stranded insertion loop that follows is the bacterial fragment containing the gene coding for tryptophanase (tna). This loop is 12.0 $\pm$ 0.5 (s.d.; n = 15)%λ in length. This is followed by a double-stranded stretch of DNA, L, 14.1 $\pm$ 0.4 (s.d.; n = 15)%λ in length. The bubble that follows consists of the imm^{λ} and the imm^{21} DNA fragments; the λ vector, NM540, contains the imm^{21} and the λtna imm^{λ} cI857 nin^{+} transducing phage contains the imm^{λ}. The imm^{λ} strand is 8.0 $\pm$ 0.7 (s.d.; n = 15)%λ in length. The imm^{21} strand is 3.6 $\pm$ 0.4 (s.d.; n = 15)%λ in length. Rightward of the immunity bubble is a double-stranded segment, M, 2.8 $\pm$ 0.3 (s.d.; n = 15)%λ in length. The loop that follows is the nin segment, which is deleted in the nin mutant, 5.4 $\pm$ 0.4 (s.d.; n = 15)%λ in length. This size of the nin segment agrees with the previously quoted value of 5.4%λ (Fiandt et al, 1971) determined by electron microscopy. The right end of this heteroduplex is a double-stranded segment, N, 10.5 $\pm$ 0.5 (s.d.; n = 15)%λ in length.

The lengths used as internal standards were imm^{λ} + imm^{21} + nin (single-stranded) = 17.0 $\pm$ 0.1 (s.d.; n = 15)%λ. Segments were summed in each molecule before determining the mean and standard deviation.

For comparison, I have included in Table 9 values for each double- and single-stranded segment of this heteroduplex based on previously reported values for the internal standards.

TABLE 9

THE LENGTHS OF DOUBLE- AND SINGLE-STRANDED DNA SEGMENTS IN THE NM540/λ*tna imm*$^{\lambda}$ *c*I857 *nin*$^{+}$ HETERODUPLEX[a]

	b	c
K	47.3 ± 1.3 (15)	49.1 ± 1.4 (14)
tna	12.0 ± 0.5 (15)	13.0 ± 0.9 (15)
L	14.1 ± 0.4 (15)	14.5 ± 0.6 (15)
imm$^{\lambda}$	8.0 ± 0.7 (15)	8.6 ± 0.5 (15)
imm^{21}	3.6 ± 0.4 (15)	3.9 ± 0.3 (15)
M	3.8 ± 0.3 (15)	3.9 ± 0.2 (15)
nin	5.4 ± 0.4 (15)	5.9 ± 0.5 (15)
N	10.5 ± 0.5 (15)	10.9 ± 0.2 (15)

All values quoted consist of the mean ± standard deviation. The number comprising the mean is in parentheses following the standard deviation.

[a] See Figure 10 and Figure 11.

[b] Single-stranded standard used was M13 = 12.7%λ.

Double-stranded standard used was pSC101 = 19.0 ± 0.2 (25)%λ.

[c] Single-stranded standard used was *imm*$^{\lambda}$ + *imm*^{21} + *nin* = 17.9%λ

where *imm*$^{\lambda}$ = 8.7%λ (Szybalski and Szybalski, 1974)

imm^{21} = 3.8%λ (Westmoreland *et al*, 1969) and

nin = 5.4%λ (Fiandt *et al*, 1971).

Double-stranded standard used was M + N.

DETERMINATION OF THE LENGTH OF *trpABC*

A heteroduplex was made between λ*trpABC*^{1} and λ*trpABC*^{r} and spread under the standard conditions with the standards pSC101 and M13 DNA's. The heteroduplex is shown in Figures 10 and 11 it consists of one bubble. An electron micrograph is shown in Appendix 4 (Plate XX). The left double-stranded segment, K, the left end of the λ vector, NM540 up to the *shnIII*λ3 restriction target, is 46.2 ± 1.2 (s.d.; n = 19)%λ in length. This result compares well with the value of 47.3 ± 1.3 (s.d.; n = 15)%λ determined for the same stretch of DNA in the heteroduplex NM540/λ*tna imm*$^{\lambda}$*cI*857 *nin*$^{+}$ spread under the same conditions and using the same standard (pSC101). The bubble that follows consists of the *trpABC*^{1} and *trpABC*^{r} bacterial DNA fragments. Because they were inserted in opposite orientations in the phage vector, they would not hybridize in the heteroduplex. These two strands should be the same length because they were generated in the same way and therefore, I treated them equally in determining the size of the *trpABC* fragment. The size of this fragment is 11.1 ± 0.4 (s.d.; n = 38)%λ. The size of the right hand stretch of double-stranded DNA, L', is 32.4 ± 0.9 (s.d.; n = 19)%λ. The sum of the left and right double-stranded segments gives the total length of the λ vector, NM540 as 78.6 ± 1.5 (s.d.; n = 19)%λ. The size of the λ vector, NM540 determined by restriction and gel electrophoresis is 79%λ (Murray and Murray, 1975). I have concluded from examining this heteroduplex and the heteroduplex previously discussed (NM540/λ*tna imm*$^{\lambda}$*cI*857 *nin*$^{+}$) that in a heteroduplex between a λ*trpABC* transducing phage and a λ*tna* transducing phage, the longer single strand in the nonhomology bubble composed of these two bacterial segments will be the *tna* fragment and the shorter single strand will be the *trpABC* fragment. I can also conclude that the *trpABC*^{1} and *trpABC*^{r}

fragments are indeed inserted in opposite orientations in the λ vector, NM540.

The length of the trpABC fragment was also determined from the heteroduplexes made between λ and λtrpABC^1 and between λ and λtrpABC^r; these were spread under the standard conditions and analyzed as above. Two populations of heteroduplex molecules were present in each spreading (Figs. 10, 11). Electron micrographs of these heteroduplexes are shown in Appendix 4 Plates XXI, XXII. The lengths of the segments are given in Table 10. From this heteroduplex I expected to see two single-stranded loops in the left arm. These loops, b_1 and b_1', correspond to the length of DNA between the srIλ1 and srIλ2 restriction sites and the length of the trpABC^1 or trpABC^r bacterial fragments, respectively. This structure corresponds to "2" in Figure 11. These two loops are separated by a short length of double-stranded DNA corresponding to the distance between the srIλ2 restriction site and the shnIIIλ3 restriction site. The other structure seen, corresponding to "3" in Figure 11 , had a single nonhomology bubble extending from the srIλ1 restriction site to the shnIIIλ3 restriction site; in other words, the small double-stranded segment separating the srIλ2 and shnIIIλ3 restriction site was denatured. This is most likely because this small segment is in the region of lowest G + C content and the T_m could be close to the spreading temperature, in 50% formamide. This interpretation that this segment has a T_m close to the spreading temperature under the conditions used is justified because the a's are equal in length and the L's are equal in length in the two heteroduplexes and; the length of b_2 is about equal to the sum of b_1' and b_3 and the length of b_2' is about equal to the sum of the lengths of b_1 and b_3. The T_m of this double-stranded segment, b_3, must be close to the spreading

TABLE 10

LENGTHS OF DNA SEGMENTS IN THE λ/λtrpABC HETERODUPLEXES (%λ)[i]

	ii	iii	iv	v	vi
a	44.5	43.6 ± 3.4 (5)	43.3 ± 2.7 (15)	41.6 ± 1.6 (3)	44.9 ± 1.2 (3)
b_1	9.8		8.8 ± 0.5 (13)		8.8 ± 0.5 (4)
b_3	2.7		2.6 ± 0.2 (14)		2.7 ± 0.3 (3)
b_2'	12.5	10.4 ± 0.8 (5)		11.6 ± 0.9 (4)	
b_1'			10.2 ± 0.6 (13)		10.4 ± 0.7 (4)
b_2		12.9 ± 1.1 (5)		13.1 ± 0.7 (4)	
L		14.0 ± 1.0 (5)	14.2 ± 0.7 (13)	13.6 ± 0.8 (4)	14.9 ± 0.5 (4)

All values quoted consist of the mean ± standard deviation. The number comprising the mean is in parentheses following the standard deviation.

i See Figure 10 and Figure 11.

ii Values were determined by agarose gel electrophoresis of endonuclease digests (Murray and Murray, 1975).

iii Values were determined from λ/λtrpABC^{l} heteroduplex data.

iv Values were determined from λ/λtrpABC^{l} heteroduplex data.

v Values were determined from λ/λtrpABC^{r} heteroduplex data.

vi Values were determined from λ/λtrpABC^{r} heteroduplex data.

temperature so that it is not always renatured at this temperature.

From these heteroduplexes I have deduced the lengths of the other DNA segments. The length of "a" represents the length from the left end of the λ^+ DNA molecule to the srIλ1 restriction site. The length of b_1 represents the length of DNA between the srIλ1 and srIλ2 restriction sites. The length of b_3 represents the length of DNA between the srIλ2 and shnIIIλ3 restriction sites. Therefore, I have concluded that the srIλ1, srIλ2 and shnIIIλ3 restriction targets are at 43.3 $\pm$ 3.1 (s.d.; n = 26), 52.2 $\pm$ 2.4 (s.d.; n = 16) and 54.7 $\pm$ 2.4 (s.d.; n = 16)%, respectively from the left end of the λ DNA molecule. The positions of these restriction targets, determined by gel electrophoresis of DNA restriction fragments are: 44.3, 54.2 and 56.6%, respectively from the left end of the λ DNA molecule. From these heteroduplexes I can deduce the length of "K": 54.7 - 52.2 + 43.3 = 45.8%λ. The length of "K" was determined to be 47.3 $\pm$ 1.3 (s.d.; n = 15)%λ from the NM540/λtna imm$^{\lambda}$ cI857 nin^{+} heteroduplex and 46.2 $\pm$ 1.2 (s.d.; n = 19)%λ from the λtrpABC^{1}/λtrpABC^{r} heteroduplex.

ARE ANY REGIONS OF THE trpABC AND tna GENES HOMOLOGOUS?

To answer this question, four heteroduplexes were made and analyzed. Two heteroduplexes spread under normal conditions are discussed below. Two heteroduplexes spread under slightly modified conditions are discussed in the next section.

A heteroduplex was made between λtrpABC^{1} and λtna imm$^{\lambda}$ cI857 nin^{+} and spread under the standard conditions. The heteroduplex is shown in Figs. 10, 11; the lengths of its segments are given in Table11. An electron micrograph is shown in Appendix 4 Plate XXIII. For determining the size of each single- and double-stranded segment, internal standards were used (imm$^{\lambda}$ + imm^{21} + nin = 17.0 $\pm$ 0.1 (s.d.; n = 15)%λ

TABLE 11

THE LENGTHS OF DOUBLE- AND SINGLE-STRANDED DNA SEGMENTS IN THE λ*trpABC*/λ*tna* *imm*$^{\lambda}$ *cI857* *nin*$^{+}$ HETERODUPLEX[a]

	K'	*tna*'	*trpABC*'	L"
b	44.6 ± 1.9 (9)	11.4 ± 1.2 (11)	10.3 ± 0.8 (11)	13.6 ± 0.8 (11)
c	46.2 ± 1.8 (10)	11.2 ± 0.5 (11)	10.2 ± 0.6 (11)	14.3 ± 0.5 (9)
d	44.2 ± 2.7 (13)	10.6 ± 1.2 (16)	9.6 ± 1.2 (16)	14.0 ± 0.8 (16)
e	44.9 ± 2.5 (19)	10.8 ± 1.1 (20)	9.5 ± 1.3 (20)	13.9 ± 0.8 (20)

All values quoted consist of the mean ± standard deviation. The number comprising the mean is in parentheses following the standard deviation.

[a] See Figure 10 and Figure 11.

[b] Data from λ*trpABC*^{l}/λ*tna* *imm*$^{\lambda}$ *cI857* *nin*$^{+}$ heteroduplex spread at 20°C.

[c] Data from λ*trpABC*^{r}/λ*tna* *imm*$^{\lambda}$ *cI857* *nin*$^{+}$ heteroduplex spread at 20°C.

[d] Data from λ*trpABC*^{l}/λ*tna* *imm*$^{\lambda}$ *cI857* *nin*$^{+}$ heteroduplex spread at 4°C.

[e] Data from λ*trpABC*^{r}/λ*tna* *imm*$^{\lambda}$ *cI857* *nin*$^{+}$ heteroduplex spread at 4°C.

as the single-stranded standard and M + N = 14.3 ± 0.5 (s.d.; n = 15)%λ as the double-stranded standard). The total number of heteroduplexes analyzed was 11 but a few segments were tangled and could not be measured. The heteroduplex begins with a double-stranded segment, K', 44.6 ± 1.9 (s.d.; n = 9)%λ in length. Following this is a non-homology bubble in which the longer strand, tna, is from the tna fragment of the λtna transducing phage DNA. (I have concluded from the previous experiments that the longer single strand of the trpABC/tna bubble in a heteroduplex between these two fragments will be tna). If homology exists between these two segments, with trpABC in this orientation, then the tna fragment will be shorter than the length of the tna fragment in the NM540/λtna imm^{λ} cI857 nin^{+} heteroduplex. In this heteroduplex, the shorter of the single strands in the nonhomology bubble ($trpABC^{1}$) is 10.2 ± 0.8 (s.d.; n = 11)%λ. The tna fragment is 11.4 ± 1.2 (s.d.; n = 11)%λ. The double-stranded segment that follows is 13.6 ± 0.8 (s.d.; n = 11)%λ.

A heteroduplex was also made between λ$trpABC^{r}$ and λtna imm^{λ} cI857 nin^{+} and analyzed as above. The general features are the same as in the previous heteroduplex and the lengths of its segments are given in Table 11. An electron micrograph is shown in Appendix 4 Plate XIX. From these two heteroduplexes I have concluded that there is greater than 21% nonhomology (assuming 50% G + C content in this region; see Appendix 1) between the tna and trpB genes or elsewhere between the tna and trpABC fragments, except possibly at the ends, which will be discussed later. These conclusions are substantiated in two ways. First of all, the lengths of the tna and trpABC fragments are about equal to the lengths of these fragments previously determined (See previous sections). Secondly, gene trpB lies between genes trpA and trpC in the trpABC fragment (See Figure 12).

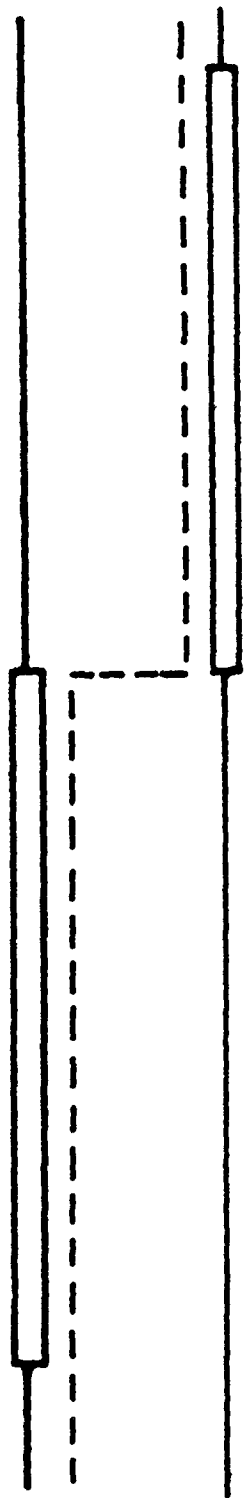


Figure 12. The trpABC Restriction Fragment. The vertical dashed lines indicate the location of the HindIII restriction site, within the trpB gene.

and therefore it is not possible for homology to exist between trpB and tna in view of the configurations of the heteroduplex. If homology existed at the ends of these genes, gene trpB would not be involved. At this point, I decided to try to spread the heteroduplexes under less denaturing conditions to reveal lower homology.

ATTEMPTS TO SPREAD UNDER LESS DENATURING CONDITIONS

The denaturing capacity can be altered by varying the formamide concentration or the ionic strength of the solution. By appropriately adjusting the formamide and Tris concentrations, conditions that were nearly iso-denaturing were maintained.

A heteroduplex was spread under the standard conditions but with the concentrations of formamide in the hyperphase and hypophase lowered to 30 and 5%, respectively. The T_m of the DNA in the hyperphase was 62.9°C. Thus, if the DNA remained single-stranded in this spreading, there must have been more than 31% mismatch, i.e. 1 in 3.3 bases. The DNA was well spread on the grid but the single strands were very difficult to distinguish from the double-stranded DNA.

I tried using other combinations of formamide concentration and ionic strength to further reduce the denaturing capacity but with little success. I tried spreading a hyperphase solution of 17.8% formamide, 0.1 M Tris, 0.01 M EDTA onto a hypophase solution of 0% formamide, 0.0167 M Tris, 0.001 M EDTA ($T_m$hyperphase = $T_m$hypophase = 70.8°C). The DNA in this experiment was not well spread out and flower-like structures were present in the double-stranded regions. The single-stranded regions were often tangled.

Perhaps the formamide concentration was too low and/or the ionic strength too high in these latter experiments causing the DNA to aggregate and form these flower-like structures and tangled regions

during spreading. The purpose of having formamide present in the solutions during renaturation and spreading is to prevent intrachain hydrogen-bond formation. The low formamide concentration may not have been sufficient to do this. The action of the increased ionic strength may have been to decrease the repulsion between the charged phosphate groups reducing the extension of the DNA molecule.

Lowering the temperature at which a heteroduplex is spread increases the percent mismatch required to prevent renaturation. This means that spreading at 4°C under standard conditions may allow one to detect regions of homology, which were not detected at 20°C. This denaturing capacity is very close to that with low formamide concentrations in the hyperphase and hypophase (30 and 5%, respectively), as described in the previous section. The spreading done at 4°C under standard conditions gave DNA that was well spread out and the single strands and double strands were distinguishable.

A heteroduplex was made between λ*trpABC*^{1} and λ*tna imm*$^{\lambda}$ *cI*857 *nin*$^{+}$ under the standard conditions but was spread at 4°C instead of at the standard 20°C. By spreading at 4°C, 33% mismatch is required to prevent renaturation of DNA of 50% G + C content. Under standard spreading conditions 21% mismatch is required to prevent renaturation of DNA of 50% G + C content. This heteroduplex was analyzed as above and the lengths of its segments are given in Table 11. An electron micrograph is shown in Appendix 4 Plate XXIV. The visibility of the molecules, particularly in the single-stranded regions was much less than at 20°C.

A heteroduplex made between λ*trpABC*^{r} and λ*tna imm*$^{\lambda}$ *cI*857 *nin*$^{+}$ was also spread at 4°C and analyzed as above. The lengths of its segments are given in Table 11. An electron micrograph is shown in Appendix 4 (Plate XXV).

My conclusions from these two experiments is that there is more than 33% nonhomology (assuming 50% G+C content in the DNA) between the trpB and tna genes or elsewhere between the trpABC and tna DNA fragments, except possibly at the ends, which will be discussed later.

Preliminary spreadings between λtrpABC and λtna imm$^{\lambda}$ cI857 nin^{+} DNA's at 4^{o}C with 30% formamide spread onto 5% formamide (all other conditions standard) to give less denaturing conditions indicated that there is more than 42% nonhomology between the trpB and tna genes. The visibility of the DNA under these spreading conditions was much lower than that in previous experiments (See Appendix 4, Plates XXVI, XXVII); this could be because of the low concentration of formamide present. It was shown in my previous experiments that even at room temperature the visibility of the DNA decreases as the formamide concentration decreases. The lengths of homologous and nonhomologous regions within the heteroduplexes between the trp/tna transducing phages discussed in this section are summarized in Table 12.

IS THERE HOMOLOGY AT THE ENDS OF THE trpABC AND tna FRAGMENTS?

The segments of interest here are the inserted bacterial fragments trpABC^{l}, trpABC^{r} and tna and the double-stranded segments on each side of these single strands. The lengths determined for the trpABC and tna fragments with the trpABC inserted in each orientation differed slightly. I therefore decided to analyze the lengths of the relevant segments statistically to determine if there was any significant difference.

First of all, I will discuss the lengths of the trpABC fragments in both orientations and the tna fragment from the spreadings λtrpABC^{l}/λtna imm$^{\lambda}$ cI857 nin^{+} and λtrpABC^{r}/λtna imm$^{\lambda}$ cI857 nin^{+} spread at 20^{o}C. I will refer to the tna fragment from the former spreading as tna^{l} and from the latter spreading as tna^{r}, even though

TABLE 12

LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN HETERODUPLEXES BETWEEN trp/tna TRANSDUCING PHAGES

λ*tna* *imm*$^{\lambda}$ *nin*$^{+}$/NM540	47.3	12.0 0		14.1	8.0 3.6		3.8	5.4 0		10.5		
λ*trpABC*^{l}/λ*trpABC*^{r}	46.2	11.1 11.1		32.4								
λ/λ*trpABC*^{l} (a)	43.3	8.8 0		2.6	0 10.2		14.2	7.7 3.7		3.9	5.5 0	10.4
λ/λ*trpABC*^{l} (b)	43.6	10.4 12.9		14.0	8.0 3.7		3.8	5.4 0		10.5		
λ/λ*trpABC*^{r} (a)	44.9	8.8 0		2.8	0 10.4		14.9	7.9 3.6		3.9	5.5 0	10.4
λ/λ*trpABC*^{r} (b)	41.6	11.6 13.1		13.6	7.7 3.6		4.0	5.7 0		10.3		
λ*tna* *imm*$^{\lambda}$ *nin*$^{+}$/λ*trpABC*^{l}	44.6	11.4 10.2		13.6	7.7 3.5		3.9	5.6 0.0		10.5		
λ*tna* *imm*$^{\lambda}$ *nin*$^{+}$/λ*trpABC*^{r}	46.2	11.3 10.2		14.3	7.9 3.5		4.0	5.6 0		10.3		

TABLE 12 (CONTINUED)

λtna imm$^{\lambda}$ nin^{+}/λtrpABC^{l}	(c)	44.2	10.6	9.6	14.0	8.0	3.6	3.9	5.3	0	10.4
λtna imm$^{\lambda}$ nin^{+}/λtrpABC^{r}	(c)	44.9	10.8	9.5	13.9	7.9	3.6	3.9	5.4	0	10.4

All heteroduplexes were spread under standard conditions (50% formamide spread onto 15% formamide).

Two populations of heteroduplex molecules were present in the λ/λtrpABC^{l} and λ/λtrpABC^{r} spreadings: (a) corresponds to structure 1 in Figure 11 (b) corresponds to structure 2 in Figure 11.

(c) These heteroduplexes were spread at 4°C instead of 20°C. See Table 11.

the tna fragment is in the same orientation in both heteroduplexes. Unless the trpABC or tna fragments contain inverted repeated sequences we would expect the homology to exist between either trpABC^{l} and tna or between trpABC^{r} and tna. If this is the case, we would expect to see a difference in length between the trpABC fragments and between the tna fragments in these two spreadings.

The lengths of the trpABC^{l} and trpABC^{r} fragments in the 20°C spreadings were determined to be 10.3 ± 0.8 (s.d.; n = 11) and 10.2 ± 0.6 (s.d.; n = 11)%λ, respectively (See Table 12). There appears to be no significant difference between the lengths of these fragments. The pooled estimate of the variance (pev) is 0.50. The standard error of the difference between the means (sedm) is thus 0.30, and the estimated deviate, t, is 0.30. Referring to a "t" table, with 20 degrees of freedom, as high a value as 0.30 occurs by chance with greater than 50% probability if the samples are from the same population. Therefore, I have concluded that there is no difference between the lengths of the trpABC^{l} and the trpABC^{r} fragments in these two spreadings.

The lengths of the tna^{l} and tna^{r} fragments from the 20°C spreadings were determined to be 11.4 ± 1.2 (s.d.; n = 11) and 11.2 ± 0.5 (s.d.; n = 11)%λ. Is there any significant difference between the lengths of these two fragments? The pev is 0.81, the sedm is 0.38 and t is 0.45. With 20 degrees of freedom, as high a value as 0.45 occurs by chance with greater than 50% probability. I have concluded that there is no difference between the lengths.

So, I have determined that there is no difference in the lengths of the trpABC^{l} and trpABC^{r} fragments at 20°C. There is no difference between the lengths of the tna^{l} and tna^{r} fragments. In other words, there appears to be no homology between the trpABC and tna fragments

under the conditions of these spreadings. Thus, there is more than 21% homology between these two fragments.

Is there any significant difference between the lengths of the trpABC^{l} and trpABC^{r} fragments from the 4°C spreadings? The lengths of these fragments were determined to be 9.6 ± 1.2 (s.d.; n = 16) and 9.5 ± 1.3 (s.d.; n = 20)%λ respectively. It is obvious that there is no significant difference between these two values, however, a t test was done. The pev is 1.54, the sedm is 0.52 and the t is 0.04. With 34 degrees of freedom the probability of as high a value as 0.04, if the two samples are from the same population, is greater than 50%.

The lengths of the tna^{l} and tna^{r} fragments from these 4°C spreadings were determined to be 10.6 ± 1.2 (s.d.; n = 16) and 10.8 ± 1.1 (s.d.; n = 20)%λ. Are these two means significantly different? The pev is 1.23, the sedm is 0.37 and the t is 0.46. With 34 degrees of freedom, as large a difference as has been observed would occur by chance with more than 50% probability in two samples from the same population. Thus, these two means are not significantly different. There appears to be no homology between the trpABC and tna fragments even at 4°C and therefore there must be greater than 33% mismatch in their nucleotide sequences.

In view of the above conclusions, I would expect the double-stranded segments on either side of the trpABC/tna nonhomology bubble to be the same in all heteroduplexes between transducing phages carrying the trpABC and tna fragments. These were therefore also tested statistically and no significant differences were found.

From Table 11 it is clear that the lengths of the trpABC fragments in the two orientations at 4°C differ from the lengths at 20°C by significantly more than they differ from each other at either

temperature. The same is true for the tna fragments. Statistical analysis as above, however, indicates that there is no reason to consider that this is more than the result of chance sampling. The decrease in length also does not correlate with an increase in length of the flanking double-stranded regions K' and L" (See Figure 11).

DISCUSSION

Although no homology was detected between the *trp* and *tna* genes under the conditions used in this research, which means that there was less than 58% homology, these genes are **nevertheless** functionally similar. Also, there are regions among the DNA's of lambdoid phages, which have less than 58% homology and yet, are believed to be functionally identical and may have evolved from a common ancestral sequence. The λ and 424 DNA's in the region extending from about 45% to about 95% are no more than 58% homologous (Highton and Beattie, unpublished results). This region presumably include analogous functionaly regions in the two phage DNA's, for example, the integration, excision, recombination, regulation, DNA replication and host cell lysis genes. That the lambdoid phage DNA's consist of analogous functional units has been discussed by Dove (1971), Simon *et al* (1971) and Fiandt *et al* (1971). The fact that homologous segments are positioned about equidistantly from the left ends of the DNA molecules indicates that in some phages some stretches of genes are in roughly the same position.

It has been shown independently in several laboratories that under the appropriate selection pressure segments of the chromosome can be duplicated (See Introduction). However, in the cases reported so far, the segments that have been duplicated are quite small. Such a duplication followed by translocation of one of the copies would explain the origin of pairs of such identical genes as *argI* and *argF* and *tufA* and *tufB* and others. Such duplications of small DNA segments could have been very important in the evolution of the *E. coli* chromosome.

It is possible that my results are compatible with the proposal that the present *E. coli* chromosome evolved via duplication of an

ancestral chromosome. If the trpB and tna genes have evolved by duplication of an ancestral chromosome followed by mutation of the duplicated genes then my results indicate that these genes have diverged to such an extent that less than 58% homology remains between them. That is, at least 1 in 2.4 bases of the ancestral sequence must have mutated in either the trpB or the tna gene. There are ϕX174 and G4 genes with as much as 50% nonhomology, which presumably came from a common ancestor and are presumably still doing the same job (Godson, 1974; Godson, Fiddes, Barrell and Sanger, 1978).

If an electron microscope technique could be devised, which would detect less than 58% homology between two DNA sequences, this could be used to further analyze the DNA sequences of the trpB and tna genes. Others pairs of functionally and spatially related genes would also be examined for DNA sequence homology. The results might reveal if chromosome duplication occurred in the evolution of the E. coli chromosome. However because the trpB and tna genes share so little homology, other pairs of biochemically and spatially related genes may not share DNA sequence homology.

I declare that I have composed this thesis myself, and that the work described in it is my own.

Rhonda Jan Myers

APPENDIX 1

The denaturing capacity of a solution is dependent on the concentration of formamide present, the ionic strength (measured in terms of the concentration of Na^+,$[Na^+]$) and the temperature. The T_m is defined as the midpoint of transition from double-stranded DNA to melted or single-stranded DNA. The T_m is dependent on the concentration of formamide, the ionic strength, the G + C content of the DNA and the percent mismatch between the two DNA sequences.

The amount of non-homology seen in a heteroduplex in the electron microscope under the particular conditions used depends on the temperature at which the DNA is spread. So, if under a given concentration of formamide and a given ionic strength a region of DNA is non-homologous, we can say that that region has a T_m less than the temperature at which the DNA was spread. By decreasing the denaturing capacity we are, in effect, decreasing the amount of homology required to form a double-stranded structure.

There is a linear dependence of T_m on log $[Na^+]$ expressed by the following equation:

$$T_m = 176 - (2.6 - x_o)\ (36 - 7.04 \log [Na^+])$$

where x_o is the fractional G + C content of the DNA and $[Na^+]$ represents the molar concentration of sodium ions (Frank-Kamenetski, 1971). The G + C content of λ and <u>E. coli</u> DNA's is about 50 %. I have assumed that the G + C content of the <u>E. coli trp</u>ABC and <u>tna</u> genes is 50 %.

There is a linear dependence of the T_m on both the percent mismatch between the two DNA's and the percent formamide in solution. On average, the T_m is reduced by 1.4 ^oC for each percent mismatch and 0.65 ^oC for each percent formamide (Davis and Hyman, 1971). These relationships allow the determination of the percent mismatch

between the DNA's in a heteroduplex. For example, in a solution of 0.06 M $[Na^+]$ (equivalent to 0.1 M Tris as in the standard hyperphase of my spreadings) the T_m of DNA with 50 % G + C content when no formamide is present is 82.4 °C. If the solution contains 50 % formamide the T_m is reduced to 49.9 °C. If the DNA is single-stranded in this medium when spread at 20 °C (i.e. T_m - 29.9 °C) the T_m of the heteroduplex must be less than 20 °C, and at least 29.9/1.4 or 21.4 % mismatch must be present between the two DNA's. If these conditions are modified and the DNA is spread at 4 °C instead of 20 °C (i.e. T_m - 45.9 °C), 32.8 % mismatch is required for the DNA's to be denatured.

APPENDIX 2

A. DEFINITIONS OF STATISTICAL TERMS

Arithmetic mean: $\sum_{i=1}^{n} x_i/n = \bar{X}$ where n = number of observations

Standard deviation: s (a measure of the spread of the set of observations)

Variance: s^2

Standard error: $s/\sqrt{n}$ (an indication of the accuracy of the mean of n observations)

Pooled estimate of the variance (pev): $$\frac{(s_1^2)\ (n_1-1) + (s_2^2)\ (n_2-1)}{(n_1-1) + (n_2-1)}$$

Standard error of the difference between the means (sedm):

$$\sqrt{(pev)(1/n_1 + 1/n_2)}$$

Estimate deviate (t): $$\frac{Mean_1 - Mean_2}{sedm}$$

B. STATISTICAL ANALYSES

It is usually true to say that about two thirds of a set of observations differ from the mean by less than the standard deviation. To test the difference between two means we assume that the means are from the same population and therefore the distribution of the difference has a zero mean. This distribution is used to calculate the probability of getting as large a difference as that observed. If this probability is small, we can assume that there is a difference between the means. When the number of observations is small, the variances will be inaccurately determined and in testing the difference between means a correction is made. Because I will assume that the variances of two samples are equal, the

correction to be made depends upon the degrees of freedom of the pooled variance. In my experiments the numbers of observations is small (less than 100) and the fact that the variances are not accurately estimated is taken into account by using a "t" Table. A "t" Table gives the "t" values corresponding to given probabilities and degrees of freedom. The values of the normal deviate-the difference of the mean expressed in terms of the standard error of the difference between the means-when the estimated variance is used are called "t" values.

APPENDIX 3

TABLE 13

SCALED LENGTHS OF HOMOLGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE 434/424 HETERODUPLEX MOLECULES

4446,7	38.7	0.4 0.4	4.2	48.8 43.6		4.1[a]		0.1 1.8		0.8
4449	36.8	0.8 0.8	3.7	52.2 48.3	3.0	0.4 0.4	0.2	0.1 2.5		0.7
4452,3	36.5	0.7 0.7	3.4	48.4 44.0	2.7	0.3 0.3	0.2	0.2 2.2		0.8
4456	36.3	0.8 0.8	3.6	49.0 46.3	2.9	0.3 0.3	0.2	0.1 2.3		0.6
4457	36.1	0.6 0.6	4.0	44.9 41.7		3.6[a]		0.1 2.4		1.0
4459	38.0	0.8 0.8	3.6	55.0 50.0		3.4[a]		0.2 3.1		0.6
4463,4	37.5	0.4 0.4	3.6	53.1 50.5		3.9[a]		0.1 2.3		0.8
4467	36.4[a]			58.4 53.8		3.5[a]		0.1 2.4		0.9
4470,1	43.3[a]			56.5 52.1		3.8[a]		0.2 2.5		0.6
4474,5	41.7[a]			53.6 50.5	3.2	0.3 0.3	0.1	0.1 2.6		1.0
4477,8	37.3	0.4 0.4	4.0	52.2 48.4	3.0	0.4 0.4	0.2	0.1 2.4		1.0
6033-35	36.0	0.3 0.3	3.9	49.8 43.2		3.7[a]		0.1 2.3		0.8
6129-31		39.1[a]		51.7 46.1	3.0	0.1 0.1	0.2	0.1 3.0		0.8

TABLE 13 (Continued)

6132-4	36.6	0.2 0.2	3.9	53.5 44.8	2.9	0.1 0.1	0.1	3.1	0.6
6135-8	37.5	0.3 0.3	3.8	52.2 47.4	3.1	0.1 0.1	0.2	2.3	0.8
6142-4	37.2	0.3 0.3	3.8	49.0 43.3	3.2	0.5 0.5	0.1	2.6	0.6
6152-5	37.0	0.5 0.5	4.0	49.8 42.7	3.1	0.2 0.2	0.1	2.2	0.8
6156-8		41.2[a]		49.9 44.4			4.8		
6159-61		43.0[a]		45.9 42.0	3.0	0.2 0.2	0.1	2.3	0.8
6162-4	37.0	0.3 0.3	4.2	49.2 44.7	2.8	0.1 0.1	0.1	2.4	0.8
6165-8	36.8	0.6 0.6	3.8	51.6 44.8		3.2[a]		2.4	0.8
6169-72	37.3	0.7 0.7	3.9	54.9 49.8		3.6[a]		1.7	0.8
6173-6		40.4[a]		53.0 44.4	2.8	0.3 0.3	0.2	2.9	0.7
6177-9	34.4	0.3 0.3	3.8	54.2 48.1	2.9	0.2 0.2	0.2	2.5	0.7
6185-7	36.4	0.5 0.5	4.0	46.6 41.7	3.0	0.1 0.1	0.2	2.2	0.8
6189-92	37.0	0.7 0.7	3.8	51.8 45.9	2.9	0.2 0.2	0.2	2.3	0.8
6193-5	35.6	0.3 0.3	3.9	50.8 42.4	3.2	0.3 0.3	0.1	2.2	0.7

TABLE 13 (Continued)

6204-8	37.4	0.5 0.5	4.0	50.8 45.3	3.3	0.1 0.1	0.2	0.1 2.5	0.9	

[a] In several molecules the small nonhomology bubble was not present and this region appeared homologous.

Numbers on the left represent the molecule number. Numerical values represent scaled lengths of homologous and nonhomologous regions within the individual 434/424 heteroduplex molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded bubbles are represented by a pair of values--the top one belongs to 434, the bottom one belongs to 424.

The standards used to scale the measurements within each molecule are:

Molecule	Average Length M13 (inches)	Average Length pSC101 (inches)	No. Comprising Average M13	No. Comprising Average pSC101
4446-4478	See Results	See Results		
6033-5	7.8	12.3	13	4
6129-31	7.7	12.4	5	3
6132-4	7.6	12.5	5	2
6135-8	7.6	12.4	7	3
6142-4	7.6	12.3	4	3
6152-5	7.9	12.5	4	2
6156-8	8.0	12.3	6	3
6159-61	7.8	12.5	6	3
6162-4	7.8	12.5	6	2
6165-8	8.2	12.7	8	2
6169-72	8.1	12.2	4	2
6173-6	8.1	12.7	8	3
6177-9	8.4	12.8	3	2
6185-7	8.3	12.6	4	2
6189-92	8.4	12.6	4	2
6193-5	8.2	12.3	4	3
6204-8	7.9	12.0	6	4

TABLE 14

SCALED LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE 434/21 HETERODUPLEX MOLECULES

	15.9		0.5		0.5		2.7		17.6		7.1		14.3		1.0	
4784-6		16.9		2.8		0.7		0.3		8.6		7.3		4.6		
	16.7		0.5		0.5		1.2		21.4		5.1		2.7		2.4	
	15.9				0.6				18.2		6.5		13.6		0.9	
4856		21.1[a]						0.3		7.4		7.2		3.9		
	16.7				0.6				22.5		4.1		2.1		2.1	
	15.9				0.2		2.0		15.4		6.8		12.2		0.9	
4788-90		17.6[a]				0.7		0.5		7.2		5.7		3.9		
	16.7				0.2		0.9		20.1		4.9		3.4		2.1	
	15.9				0.1		2.6		17.5		6.3		12.2		0.9	
4792-4		20.7[a]				0.6		0.6		7.4		8.0		3.9		
	16.7				0.1		1.0		20.1		4.3		3.3		2.1	
	15.9				0.2		2.0		13.6		6.0		11.7		0.9	
4768		19.5[a]		0.2				0.6		7.0		6.7		3.5		
	16.7				0.2		0.8		17.4		4.4		2.6		2.1	
	15.9						2.5		15.0		6.4		14.4		0.9	
4745		22.2[a]						0.7		7.6		8.5		3.8		
	16.7						0.9		20.3		4.9		2.4		2.1	
	15.9				1.8				17.7		6.2		14.6		1.0	
4774		23.3[a]						0.6		8.1		8.2		2.0		
	16.7				1.7				19.3		5.6		2.8		2.4	
	15.9			0.7			2.3		15.0		6.5		11.0		1.3	
4832		20.6[a]			0.7			0.6		7.4		8.0		5.2		
	16.7			0.7			0.9		18.8		3.9		3.2		3.2	

TABLE 14 (Continued)

	15.9			0.6		2.6		15.8		6.2		11.9		0.9				
4763		20.9[a]			0.7		0.7		7.6		8.6		4.1					
	16.7			0.6		0.9		20.0		4.4		2.6		2.2				

[a] In several molecules the small nonhomology bubble was not present and this region appeared homologous. Numbers on the left represent the molecule number. Numerical values represent scaled lengths of homologous and nonhomologous regions within the individual 434/21 heteroduplex molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded regions are represented by a pair of values--the top number belongs to 434, the bottom number belongs to 21.

The standards used to scale the measurements within each molecule are homoduplexes:

Molecule	Length of Standard (inches)	Standard Assignment
4784-6	56.1	434
4856	64.3	434
4788-90	61.2	434
4792-4	62.2	434
4768	66.1	434
4745	52.5	21
4774	50.4	21
4832	56.5	21
4763	52.4	21

TABLE 15

SCALED LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE 424/21 HETERODUPLEX MOLECULES

7094,5	15.9		22.6	2.1		0.8	45.7		3.7[a]	2.8
	16.7			1.1			39.2			2.3
7083	15.9		22.0	2.0		1.0	47.7		4.0[a]	3.6
	16.7			0.9			43.2			3.0
7096,7	15.9		22.2	1.8		0.7	44.1		3.0[a]	3.0
	16.7			0.8			40.7			2.5
7098,9	15.9		24.7	3.0		0.7	46.4		3.6[a]	3.1
	16.7			1.1			43.3			2.6
7109,10	15.9		19.0	2.2		1.0	43.7		3.7[a]	2.8
	16.7			1.3			40.7			2.3
7127,8	15.9		21.7	1.6		0.4	36.4	2.6	0.4	0.2 2.8
	16.7			0.9			33.6		0.4	2.3
3902[b]	15.9		22.2				40.9		7.8	
	16.7						30.0			
9616[b]	15.9		21.3				49.4		2.6	3.2
	16.7						43.9			3.1
3786[b]	15.9		22.2				48.4		2.7	3.5
	16.7						40.9			3.0
3783[b]	15.9		22.6	2.1		1.4	45.8		3.2	2.8
	16.7			1.0			40.3			2.0

[a] In several molecules the small nonhomology bubble was not present and this region appeared homologous.

[b] Homoduplexes were used as standards.

Numbers on the left represent the molecule number. Numerical values

TABLE 15 (Continued)

represent scaled lengths of homologous and nonhomologous regions within the individual 424/21 molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded regions are represented by a pair of values--the top number belongs to 424, the bottom number belongs to 21.

The standards used to scale the measurements within each molecule are:

Molecule	Average Length M13 (inches)	Average Length pSC101 (inches)	No. Comprising Average M13	No. Comprising Average pSC101
7094,5	6.5	12.4	4	2
7083	6.7	11.9	4	1
7096,7	6.3	12.0	3	1
7098,9	6.4	12.0	2	1
7109,10	6.3	12.7	4	2
7127,8	8.1	12.7	1	2

Molecule	Length of Standard (inches)	Standard Assignment
3902	44.0	21
9616	48.4	21
3786	58.2	424
3783	52.5	21

TABLE 16

SCALED LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE PA2/424 HETERODUPLEX MOLECULES

6212-5	43.6	1.6 2.4		2.5		46.7 43.4	6.7
6224-6	43.2	1.6 2.5	0.5	0.2 0.1	1.6	43.4 39.7	6.4
6229-32	43.0	1.2 2.6		2.9		44.2 41.5	6.7
6233-5	42.7	1.4 2.3	0.8	0.1 0.1	1.5	44.7 41.2	6.7
6236-9	45.0	1.6 2.4		2.4		45.8 42.9	6.9
6240-3	44.4	1.5 2.2	0.8	0.1 0.1	1.6	43.7 39.1	7.0
6244-7	42.5	1.5 2.1		2.7		45.5 41.8	6.7
6248-50	44.8	1.7 2.3	0.8	0.2 0.2	1.7	42.4 38.9	7.0
6254-7	43.2	1.5 2.3	0.6	0.1 0.1	1.6	43.8 40.1	6.8
6258-61	41.9	1.5 2.6	0.7	0.2 0.1	1.6	42.8 40.0	6.9
6265-8	42.3	1.4 2.4	0.7	0.1 0.1	1.5	45.2 42.9	6.0
6269	43.2	1.5 2.2	0.6	0.3 0.3	1.5	42.2 39.8	6.3
6271-3	41.9	1.3 2.3	0.8	0.1 0.1	1.6	42.9 39.1	6.4

TABLE 16 (Continued)

Numbers on the left represent the molecule number. Numerical values represent scaled lengths of homologous and nonhomologous regions within the individual PA2/424 heteroduplex molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded regions are represented by a pair of values--the top number belongs to PA2, the bottom number belongs to 424.

The standards used to scale the measurements within each molecule are:

Molecule	Average Length M13 (inches)	Average Length pSC101 (inches)	No. Comprising Average	
			M13	pSC101
6212-5	8.3	12.0	6	1
6224-6	7.8	12.2	6	2
6229-32	8.2	12.2	7	3
6233-5	8.9	12.6	6	2
6236-9	8.4	12.3	9	4
6240-3	8.6	12.1	5	6
6244-7	8.6	12.5	7	4
6248-50	8.4	12.0	6	3
6254-7	8.8	12.6	7	3
6258-61	8.7	12.3	4	2
6265-8	8.8	12.5	11	3
6269	8.9	12.6	10	2
6271-3	9.2	13.3	13	2

TABLE 17

SCALED LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE λ/424 HETERODUPLEX MOLECULES

		0.2		1.1		0.3		0.7		0.3		47.5		
5535,6	36.5		0.4		0.3		2.5		0.4		1.9		4.4	
		0.5		1.2		0.3		1.3		0.4		42.0		
		0.2		1.2		0.4		0.2		0.3		47.7		
5782,3	38.0		0.4		0.1		3.3		0.2		2.5		4.9	
		0.4		1.4		0.5		0.8		0.4		42.3		
		0.4		1.2		0.6		0.3		49.0				
5788,9	36.6		0.9		3.4		0.8		1.5		4.9			
		0.4		1.6		1.5		0.3		43.0				
				1.9				0.7		0.2		48.4		
5793,4	36.1						2.7		0.7		1.5		4.6	
				1.9				1.0		0.2		43.3		
		0.2		0.9		0.1		0.1		0.8		0.2		46.3
5806,7	38.4		0.2		0.2		2.4		0.2		0.7		2.2	5.0
		0.5		1.1		0.1		0.2		1.4		0.2		38.6
		0.4		0.2						0.9		0.5		46.4
5812,3	37.3		0.2				4.4				0.5		1.5	4.4
		0.8		0.4						1.4		0.6		40.1
		0.2		1.2				0.5		1.7				46.4
5814	39.0		0.3				2.8		2.8		0.3			5.1
		0.4		1.5				1.5		2.5				41.1
		0.2		0.9		0.3				0.8		0.2		47.4
5819	38.7		0.8		0.2		2.7				0.7		1.3	5.1
		0.3		1.1		0.4				1.4		0.4		41.6
				1.7				0.1		0.5				45.7
5827,8	36.1						2.2		0.4			2.6		4.8
				1.8				0.1		1.0				38.5
		0.2		0.9						0.6		0.1		48.0
5833,4	38.0		0.2				3.3				0.5		1.6	4.7
		0.2		1.2						1.4		0.1		43.0
		0.2		0.8		0.1		0.6		0.2				47.3
5838,9	38.3		0.3		0.2		3.2		0.4			1.7		4.7
		0.4		1.7		0.1		0.9		0.4				42.3
		0.5		0.3						0.6				49.3
5849,50	38.2		0.6				4.0					2.8		5.2
		0.7		0.5						1.4				41.9
		0.2		1.9						0.8				50.4
5855	36.5		0.4				3.1					2.6		4.6
		0.6		2.3						1.1				44.3

TABLE 17 (Continued)

		0.4		0.9				3.4					46.7	
5860,1	36.8		0.2				0.5				1.8			4.8
		0.4		1.9				4.4					41.6	
		0.2		1.5						1.0		0.2	47.2	
5867,8	36.2		0.3				2.6				0.3		1.5	4.9
		0.3		2.1						1.4		0.2	41.9	
		0.1		1.1		0.2		0.4		0.2			46.6	
5869,70	36.9		0.4		0.1		1.7		1.6			1.2		4.6
		0.2		1.2		0.3		0.8		0.2			41.4	
		0.3		0.8						0.8			48.9	
5873-5	37.1		0.3				3.2					2.2		5.1
		0.4		1.1						1.3			44.4	
		0.2		1.7		0.2		0.5					45.7	
5876	36.2		0.2		1.4		0.2				3.3			5.0
		0.6		1.9		0.3		0.9					41.0	

Numbers on the left represent the molecule number. Numerical values represent scaled lengths of homologous and nonhomologous regions within the individual λ/424 molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded regions are represented by a pair of values--the top number belongs to λ, the bottom number belongs to 424.

The standards used to scale the measurements within each molecule are:

Molecule	Average Length M13 (inches)	Average Length Homoduplexes (inches)	No. Comprising Average		Homoduplex Assignment
			M13	Homoduplex	
5535,6	8.2	62.8	10	2	424
5782,3	8.3	67.6	13	2	λ
5788,9	8.0	66.8	12	2	λ
5793,4	8.2	65.0	6	1	424
5806,7	8.2	66.2	11	2	λ
5812,3	8.2	66.2	11	2	λ
5814	7.6	61.0	2	1	424
5819	7.8	64.5	17	2	λ

TABLE 17 (Continued)

Molecule	Average Length M13 (inches)	Average Length Homoduplexes (inches)	No. Comprising Average		Homoduplex Assignment
			M13	Homoduplex	
5827,8	8.2	65.1	5	2	424
5833,4	7.7	64.2	11	2	λ
5838,9	7.6	63.2	7	2	λ
5849,50	8.1	63.2	12	1	424
5855	8.4	66.0	11	1	424
5860,1	8.4	64.3	9	2	424
5867,8	8.4	64.3	9	2	424
5869,70	8.3	63.5	13	1	424
5873-5	8.3	63.5	13	1	424
5876	8.2	63.8	7	1	424

TABLE 18

SCALED LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE PA2/21 HETERODUPLEX MOLECULES

6297	15.9		1.7		21.7		7.5		12.6		3.8	
		22.5		1.0		5.5		3.0		3.1		
	16.7		1.0		20.7		7.5		4.5		3.2	
6300-2	15.9		2.0		21.5		7.8		12.4		2.6	
		22.6		0.9		5.7		3.0		3.8		
	16.7		0.9		19.6		7.8		4.1		2.2	
6311-3	15.9		1.6		21.2		7.4		13.1		2.9	
		22.1		0.7		6.1		3.0		3.8		
	16.7		0.8		18.5		7.4		5.5		2.4	
6314-7	15.9		2.4		20.2		7.2		13.5		2.9	
		22.1		0.8		6.2		2.7		3.5		
	16.7		1.0		20.2		7.2		5.1		2.4	
6318-21	15.9		1.6		22.0		9.2		13.0		2.8	
		22.6		0.6		3.8		3.8		3.8		
	16.7		0.9		18.7		9.2		4.4		2.3	
6328-30	15.9		1.8		22.8		7.5		13.0		2.7	
		21.5		0.8		5.8		2.6		3.6		
	16.7		0.8		19.3		7.5		4.9		2.2	
6337-40	15.9		1.8		20.3		7.2		13.3		3.1	
		21.3		0.8		5.8		2.8		3.5		
	16.7		1.0		18.6		7.2		5.0		2.6	

Numbers on the left represent the molecule number. Numerical values represent scaled lengths of homologous and nonhomologous regions within the individual PA2/21 heteroduplex molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded regions are represented by a pair of values--the top number belongs to PA2, the bottom number belongs to 21.

The standards used to scale the measurements within each molecule are:

Molecule	Average Length M13 (inches)	Average Length pSC101 (inches)	No. Comprising Avg. M13	pSC101
6297	8.2	12.2	10	4
6300-2	8.0	12.2	6	3
6311-3	8.1	12.0	7	3
6314-7	7.5	12.0	6	3
6318-21	7.7	11.9	6	5
6328-30	7.8	11.7	10	5
6337-40	7.6	11.7	14	5

TABLE 19

SCALED LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE PA2/434 HETERODUPLEX MOLECULES

		0.8		19.2		7.1		9.4		6.1		2.0	
7285,6	37.5		3.4		8.8		3.2		0.3		3.2		0.8
		0.8		13.6		9.7		7.8		10.3		0.2	
		15.6		3.5		8.2		9.0		5.2		3.0	
7297,8	37.4		0.8		8.6		2.4		0.2		3.0		0.8
		8.5		2.9		10.1		8.1		9.0		0.7	
		0.5		18.7		3.2		27.0		0.4		2.1	
7322,3	36.5		0.5		0.3		5.3		2.5		0.2		0.8
		0.5		13.3		2.7		35.2		0.4		0.2	

Numbers on the left represent the molecule number. Numerical values represent scaled lengths of homologous and nonhomologous regions within the individual PA2/434 heteroduplex molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded regions are represented by a pair of values--the top number belongs to PA2, the bottom number belongs to 434.

The standards used to scale the measurements within each molecule are:

Molecule	Average Length M13 (inches)	Average Length pSC101 (inches)	No. of Molecules Comprising Avg. M13	pSC101
7285,6	7.3	12.0	3	3
7297,8	7.4	12.5	5	2
7322,3	7.5	12.4	5	2

TABLE 20

SCALED LENGTHS OF HOMOLOGOUS AND NONHOMOLOGOUS REGIONS WITHIN THE λ/PA2 HETERODUPLEX MOLECULES

		0.4		1.8		0.3		3.0		0.2		14.5		5.6		3.8		2.7		0.2		1.7		0.2		1.3		7.1
7256	38.4		0.2		0.1		2.8		0.7		1.4		6.4		2.2		0.2		1.7		0.4		0.2		0.2		0.2	5.6
		0.4		1.8		0.3		1.0		0.2		17.2		3.4		2.1		2.5		1.8		2.5		1.1		1.3	7.1
		0.5		0.9				2.0						13.1				1.8				12.1				10.5	
7232-4	34.1		0.4				3.4					2.6					0.2		6.0					3.2			5.6
		0.5		1.8				1.0						13.3				2.5				8.0				13.0	
		0.8		1.9				2.4		0.7				17.4								12.0				12.7	
7235-7	37.1		0.2				2.8				0.7		1.5								6.1				2.8			4.8
		0.8		2.7				1.0		0.7				18.0								7.0				13.7	
		0.4		1.7		0.4								13.6			2.2			2.2			10.5			10.9	
7250,1	36.0		0.4				6.0		0.9							0.2			5.9			0.3			3.0		5.0
		0.4		2.2		0.4								13.6			2.2			1.8			5.6			14.7	
		0.8		2.0				3.3						15.0						10.5			0.9			11.7	
7252,3	37.9		0.2				2.8				2.7							5.9			0.2			3.2			5.6
		0.8		2.5				0.9						16.5						7.2			0.9			15.8	
		0.8		1.7				2.6						12.2		2.3						10.8				11.3	
7254,5	37.4		0.2				2.5				2.6					0.2		5.7						2.8			5.2
		0.8		2.6				0.9						13.2		2.3						8.3				14.2	
				0.2										12.9		1.9						12.7				11.8	
7258,9		39.62						7.8								0.2		5.8						3.2			4.9
				0.2										15.0		1.9						8.1				15.2	
		0.6		2.6				1.3						15.2								11.2				10.1	
7260,1	38.4		0.3				3.0				1.8							6.0						2.9			5.0
		0.6		3.0				0.3						15.2								7.9				14.0	

TABLE 20 (Continued)

		0.5		2.2		2.4			13.7		1.9			13.0		4.2		6.8						
7262-4	37.4		0.8		2.8		3.0			0.2		6.0			2.7		0.2		5.2					
		0.5		2.6		0.6			15.4		1.9			8.2		11.0		3.4						
			1.8			2.7				15.8				12.4				10.4						
7265	36.6				2.5			2.4				5.8				3.0			4.7					
			1.8			1.1				15.8				6.9				13.4						
			1.9			0.6				14.2		9.0		0.2				8.4						
7266,7	39.1				5.0		1.2				9.0		1.4		1.8				4.9					
			1.9			0.3				15.2		8.6		0.2				11.6						
			1.6			1.7				13.9				11.5				10.9						
7268-70	39.3				3.4			2.6			7.1					2.9			5.0					
			1.6			0.3				15.4				7.6				13.3						
		0.2		1.1		0.7		2.3			11.9			11.0				10.7						
7271,2	38.0		0.5		0.2		3.0			2.5		5.0				2.9			4.9					
		0.2		1.6		0.6		1.0			14.6			7.4				14.9						
		0.7		2.2				2.3			15.5			12.1				10.3						
7273-5	36.9		0.2				3.0			2.4		5.5				2.8			4.9					
		0.7		2.4				1.0			16.0			7.9				13.9						

Numbers on the left represent the molecule number. Numerical values represent scaled lengths of homologous and nonhomologous regions within the individual λ/PA2 heteroduplex molecules (See λ Results). These lengths are in %λ. Duplex regions are represented by single values, single-stranded regions are represented by a pair of values--the top number belongs to λ, the bottom number belongs to PA2.

TABLE 20 (Continued)

The standards used to scale the measurements within each molecule are:

Molecule	Average Length M13 (inches)	Average Length pSC101 (inches)	No. of Molecules Comprising Average M13	pSC101
7256	7.0	11.8	4	4
7232-4	7.2	11.8	8	2
7235-7	7.4	12.1	7	2
7250,1	7.7	12.5	5	2
7252,3	7.3	11.6	4	2
7254,5	7.7	12.0	7	3
7258,9	7.2	11.9	5	2
7260,1	7.8	11.9	4	3
7262-4	7.3	12.0	7	4
7265	7.6	12.6	4	1
7266,7	7.6	12.0	4	2
7268-70	8.2	11.8	8	2
7271,2	7.6	12.1	6	6
7273-5	7.6	12.1	9	2

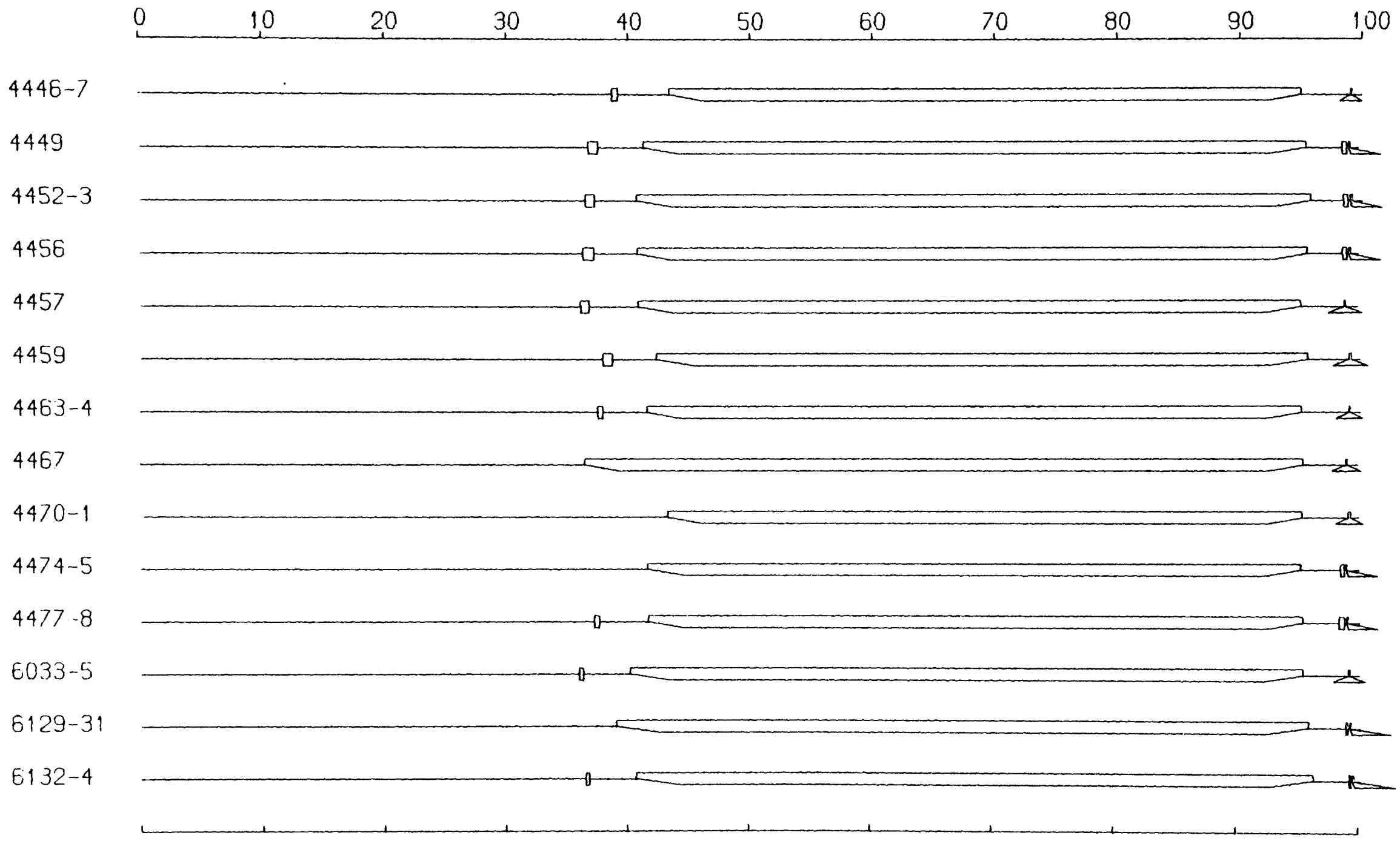


Figure 13. SCALED 434/424 HETERODUPLEX MOLECULES.

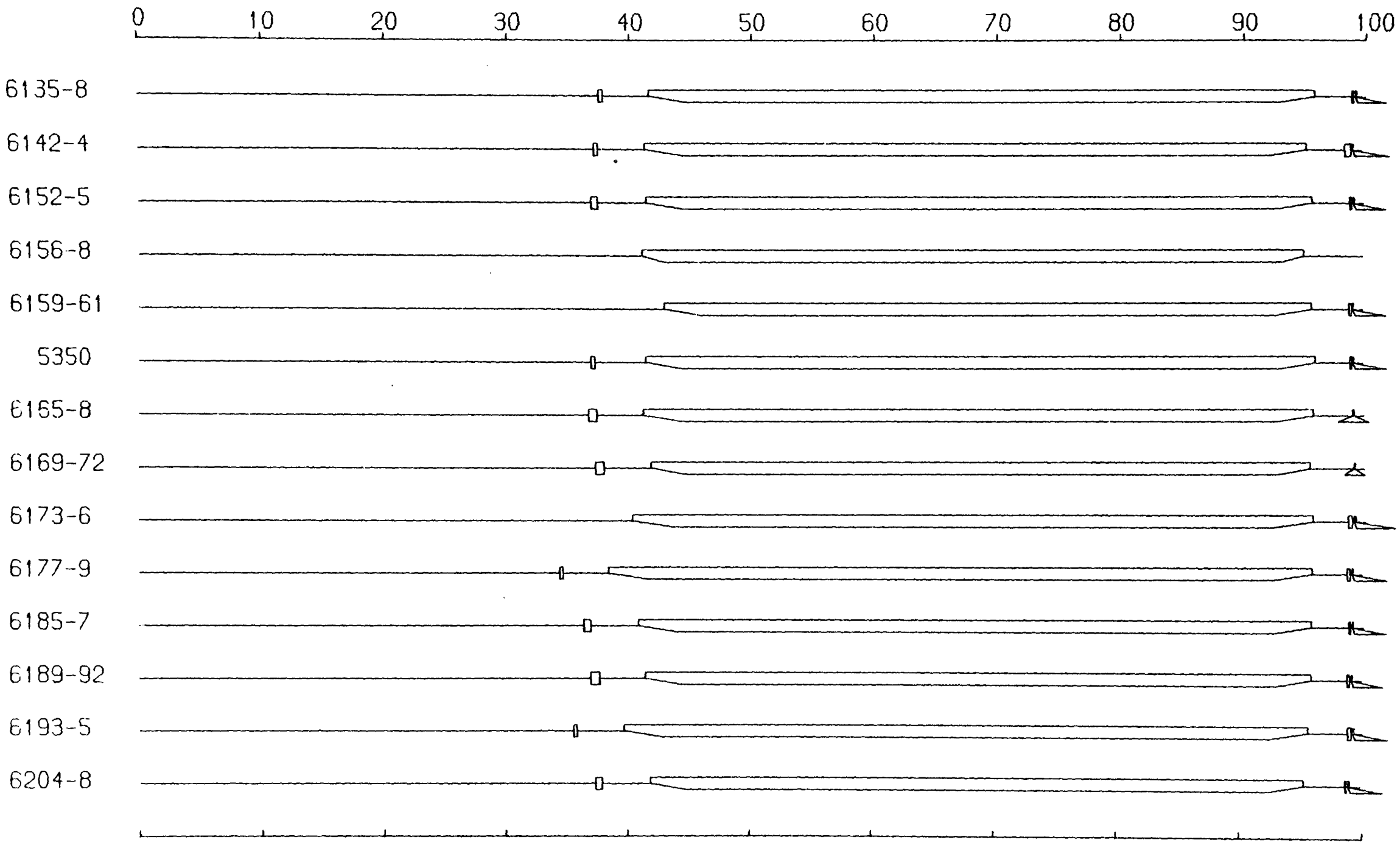


Figure 13. SCALED 434/424 HETERODUPLEX MOLECULES .

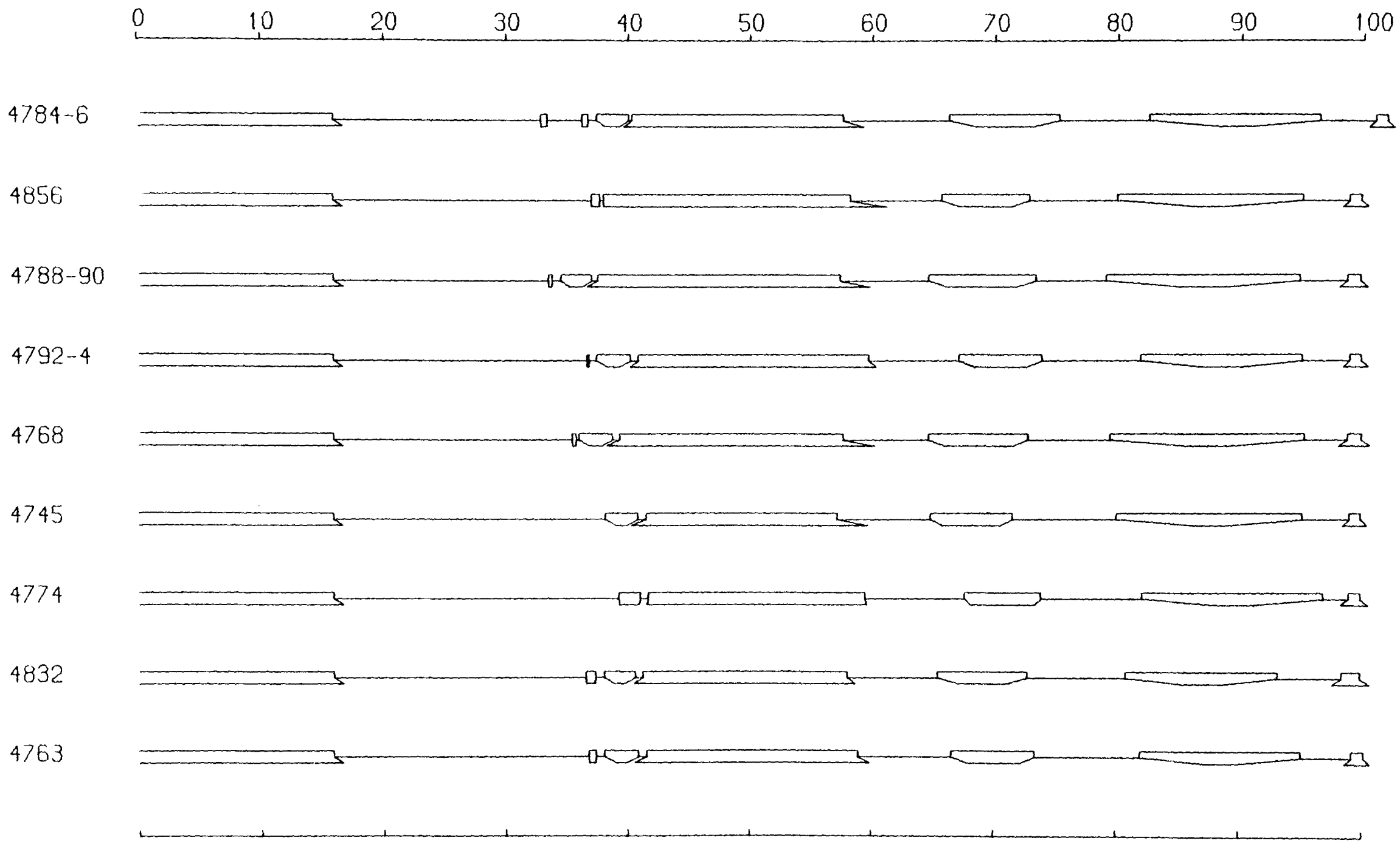


Figure 14. SCALED 434/21 HETERODUPLEX MOLECULES.

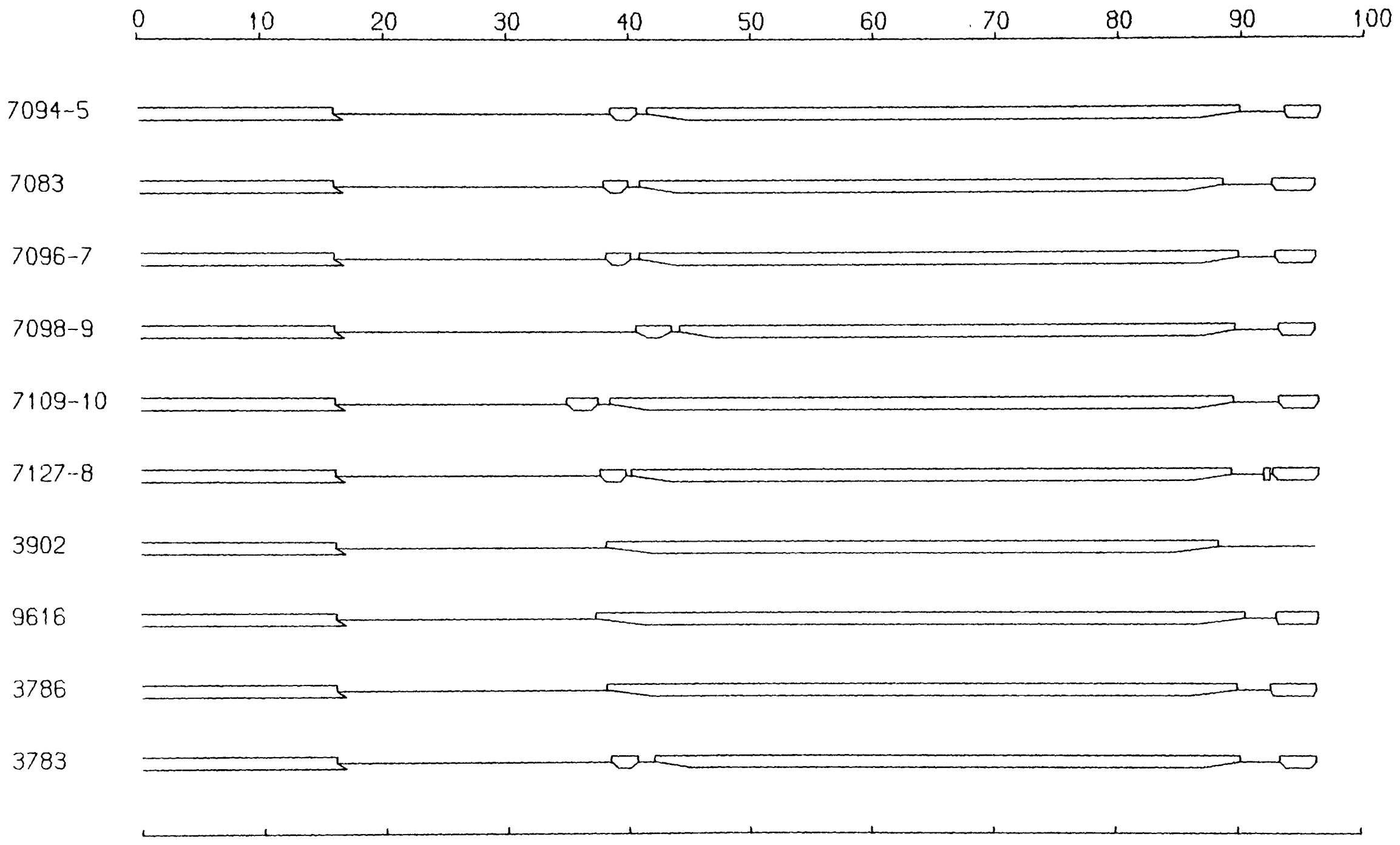


Figure 15. SCALED 424/21 HETERODUPLEX MOLECULES.

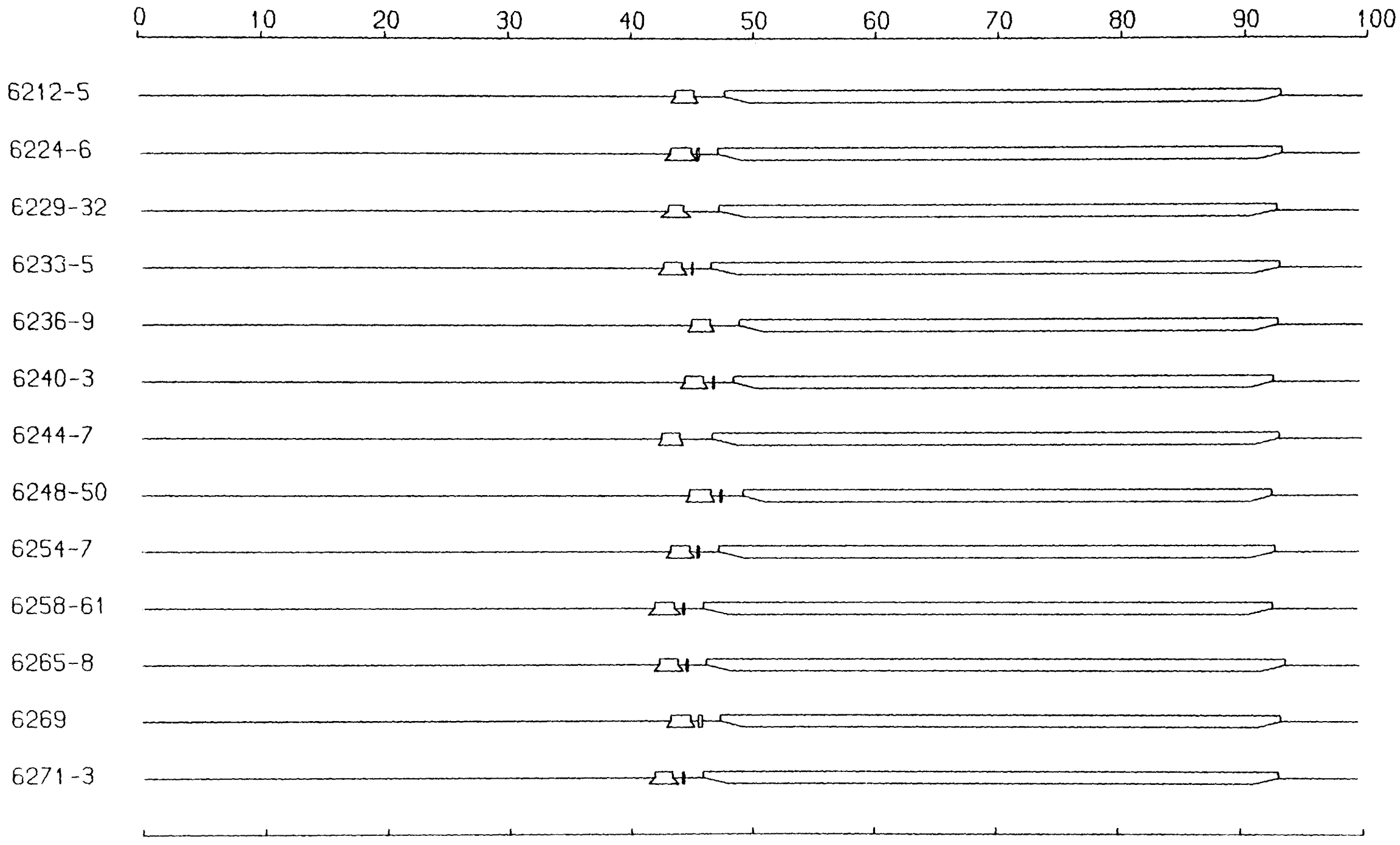


Figure 16. SCALED PA2/424 HETERODUPLEX MOLECULES.

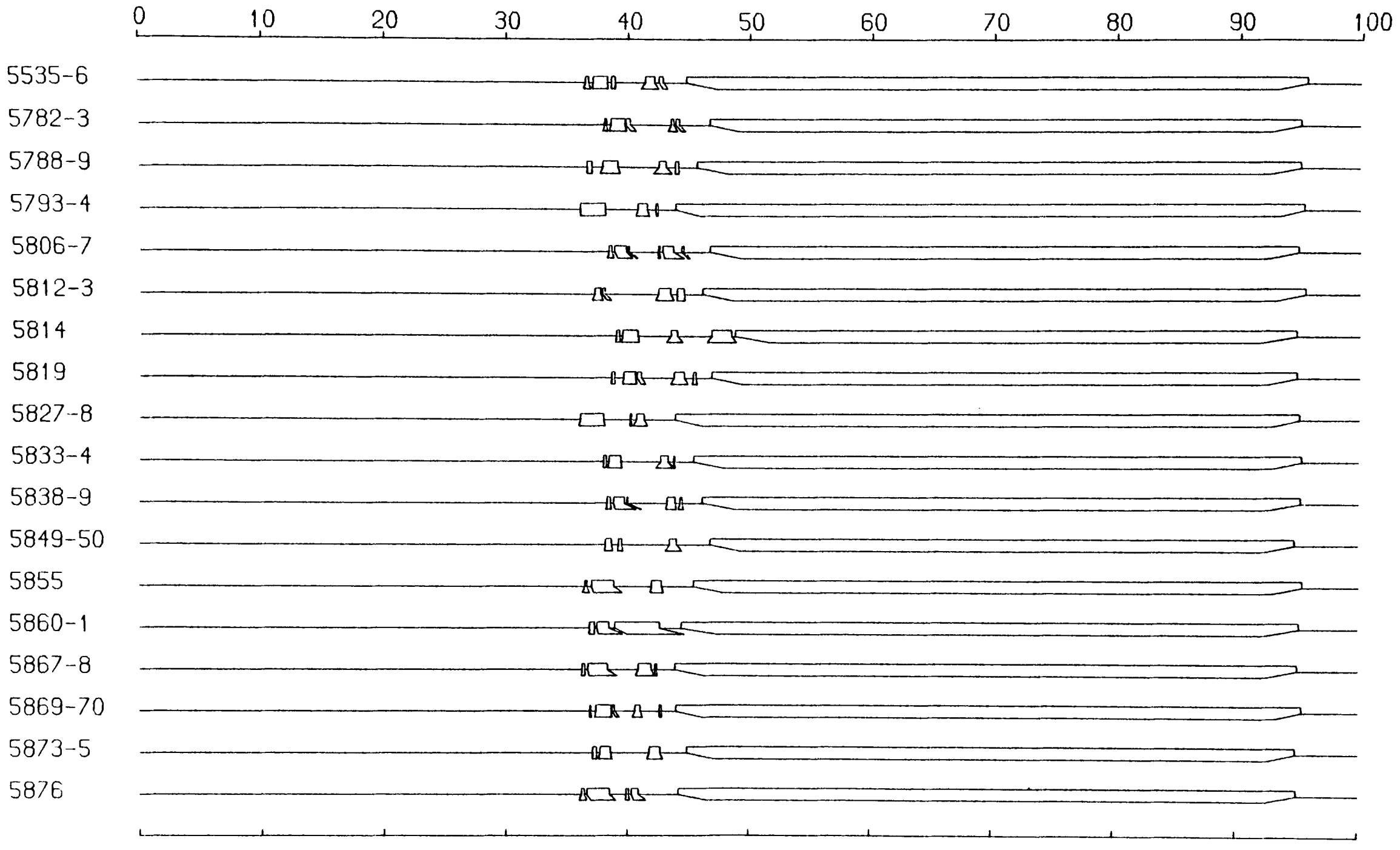


Figure 17. SCALED λ/424 HETERODUPLEX MOLECULES.

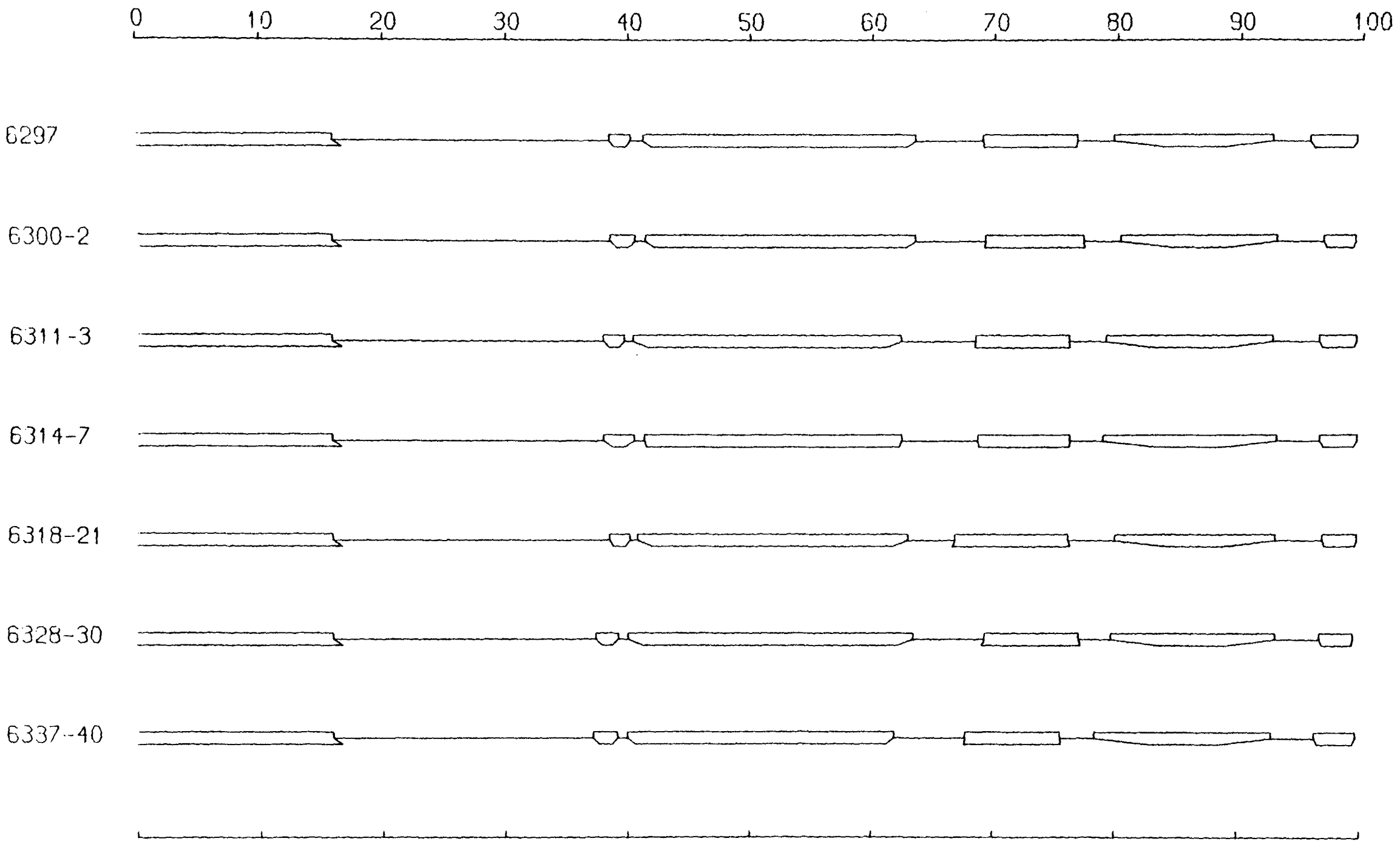


Figure 18. SCALED PA2/21 HETERODUPLEX MOLECULES.

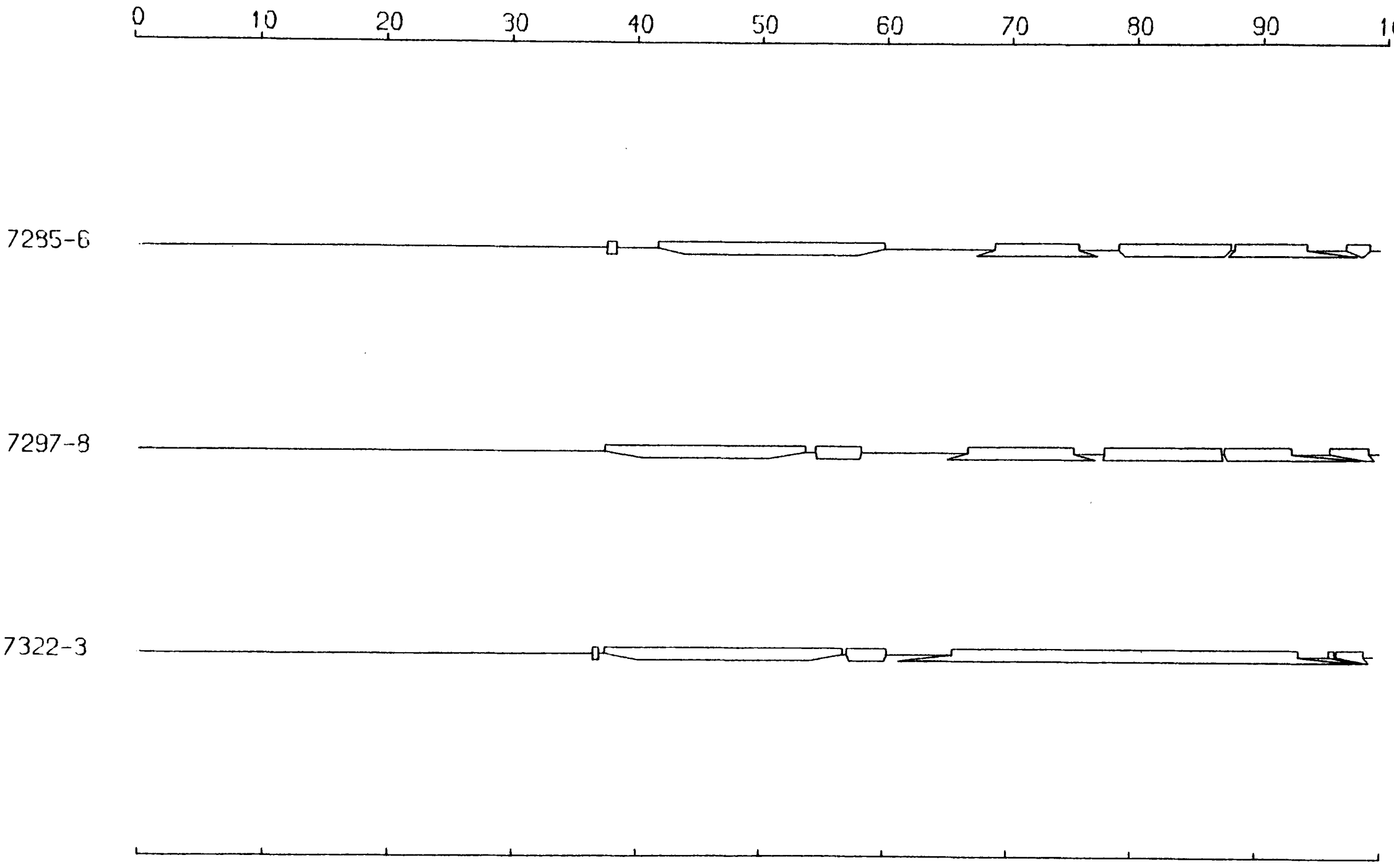


Figure 19. SCALED PA2/434 HETERODUPLEX MOLECULES.

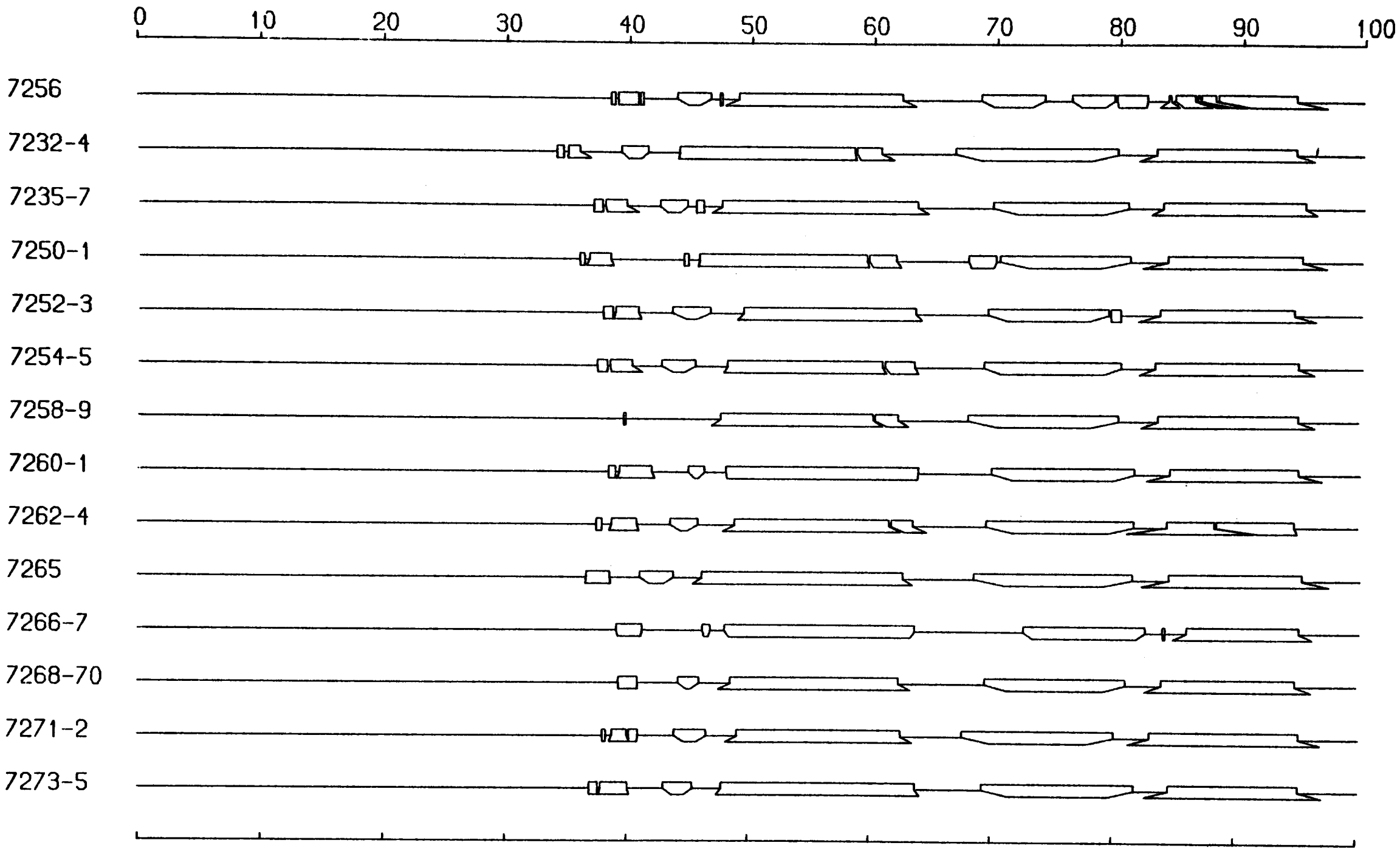
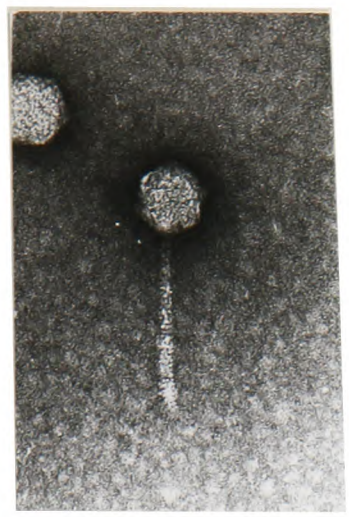


Figure 20. SCALED λ/PA2 HETERODUPLEX MOLECULES.

APPENDIX 4

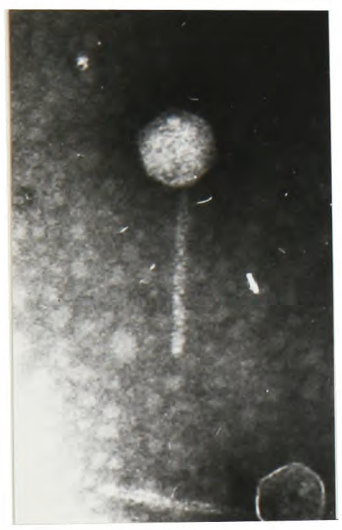
λ (UA)



PA2 (UA)

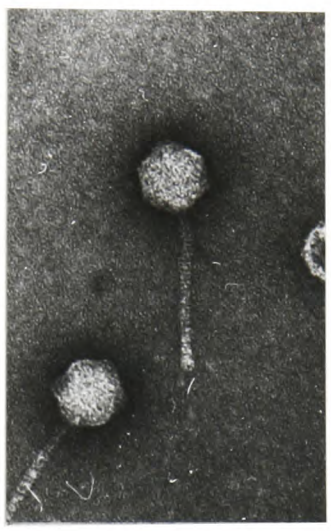
434 (UA)

Ø80 (UA)

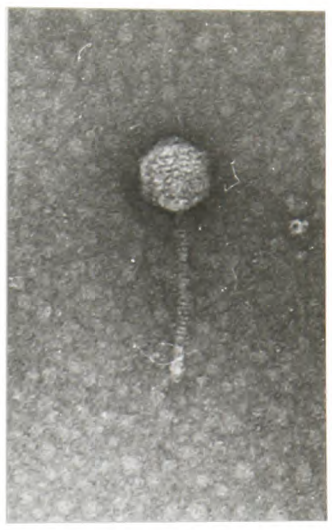


21 (NaPT)

21 (NaPT)



21 (UA)



21 (UA)

Plates I-VIII Negatively stained phages

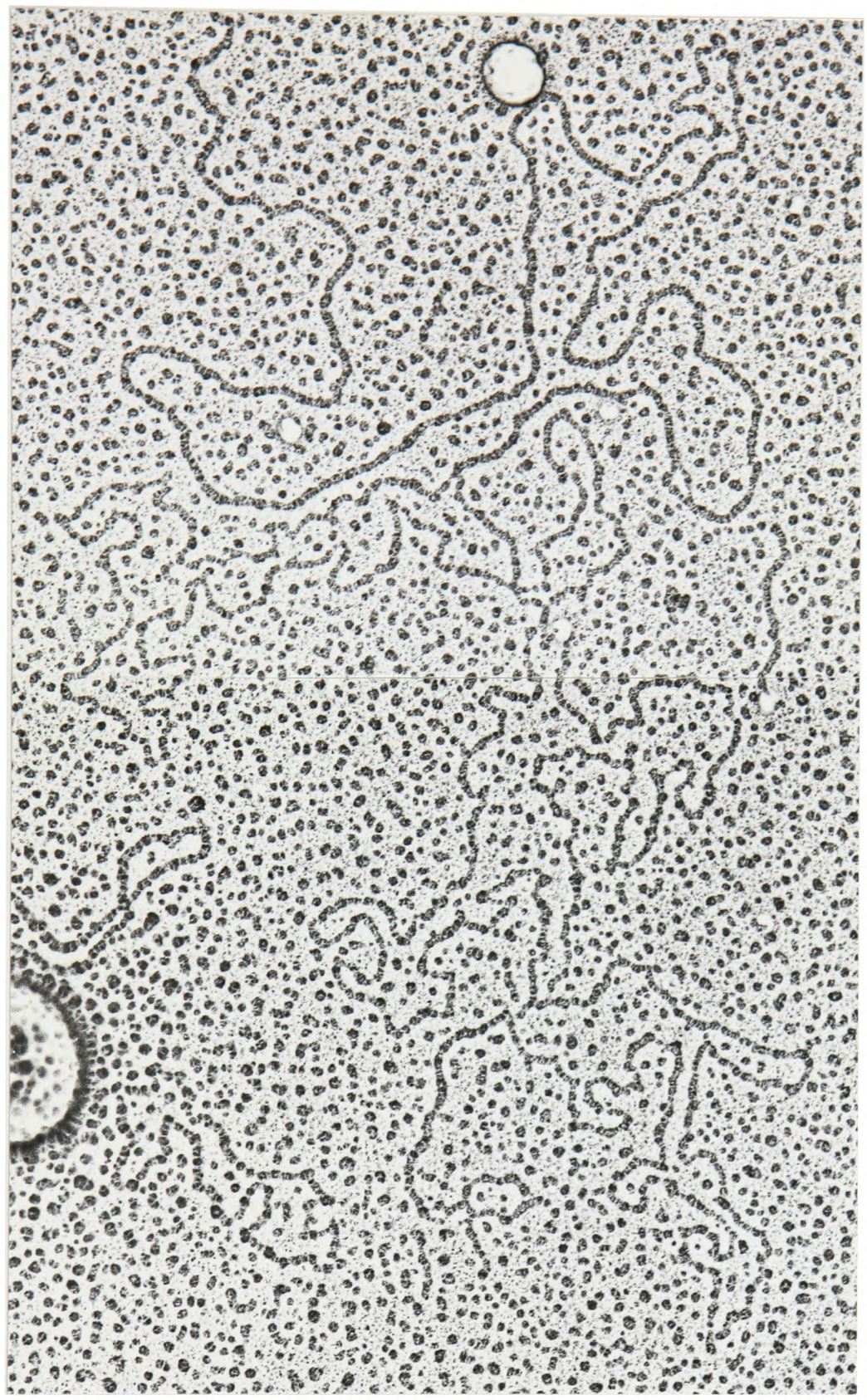


PLATE IX 434/424

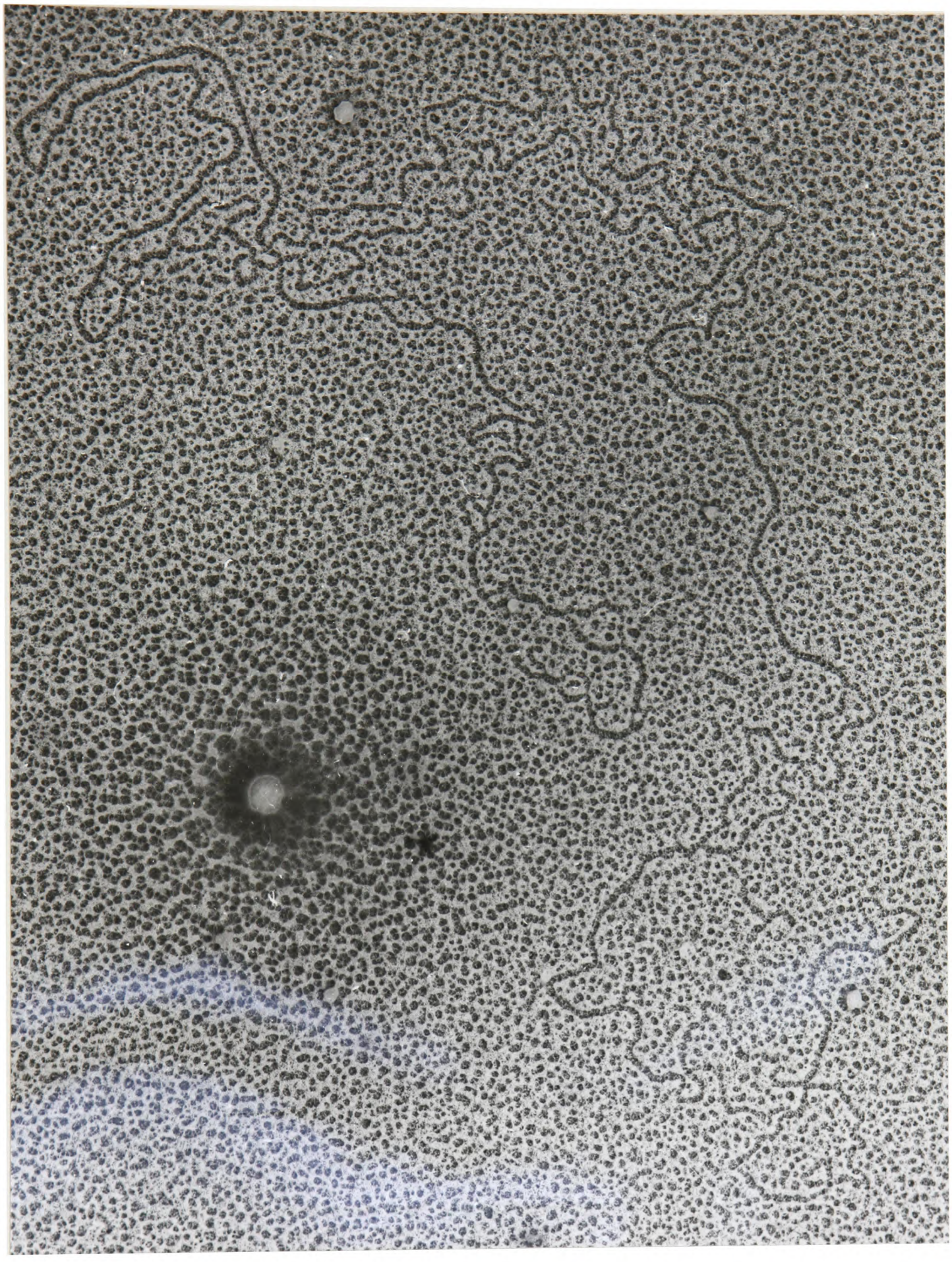


PLATE X 434/21

PLATE XI 424/21

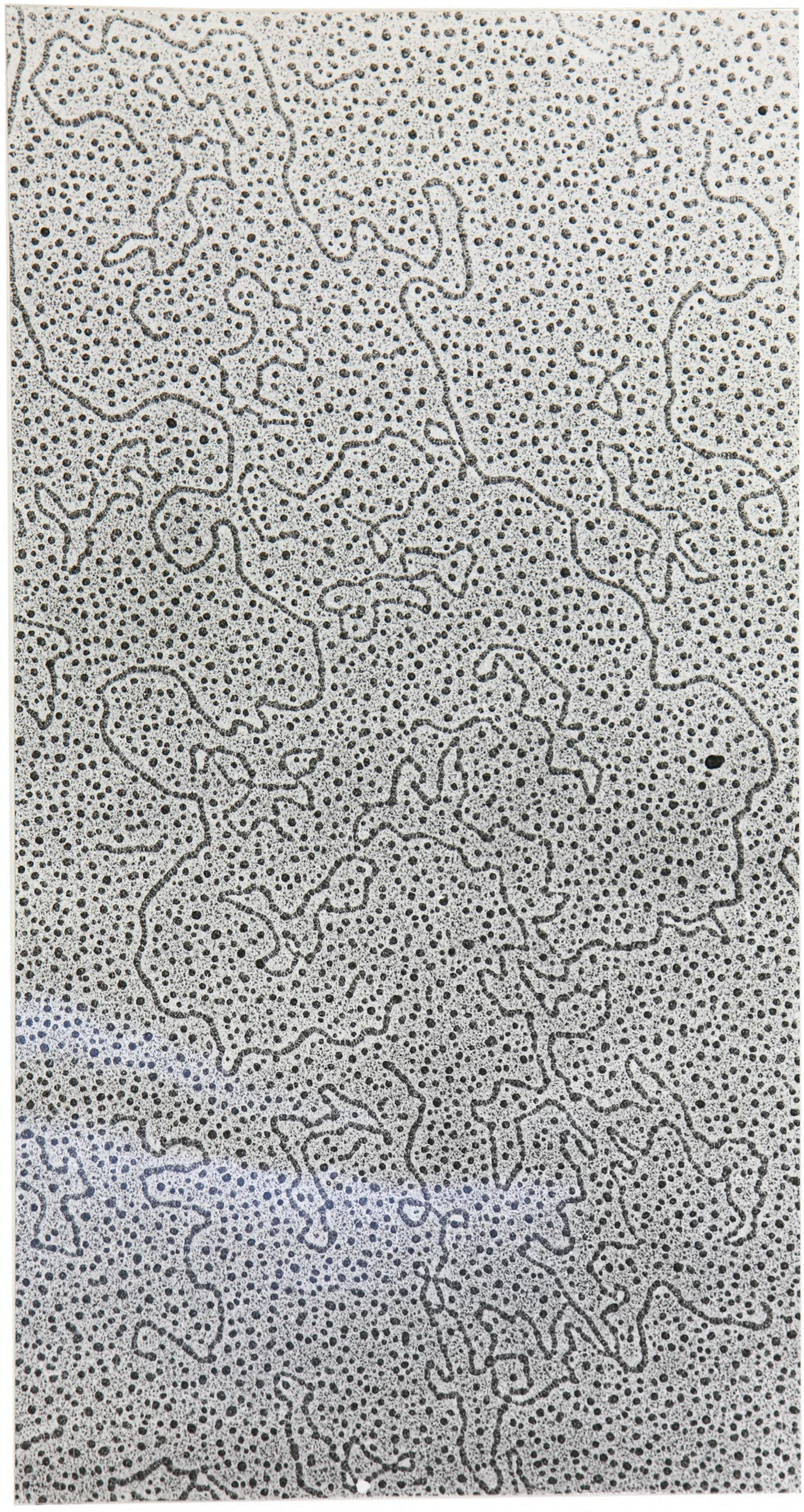


PLATE XII PA2/424

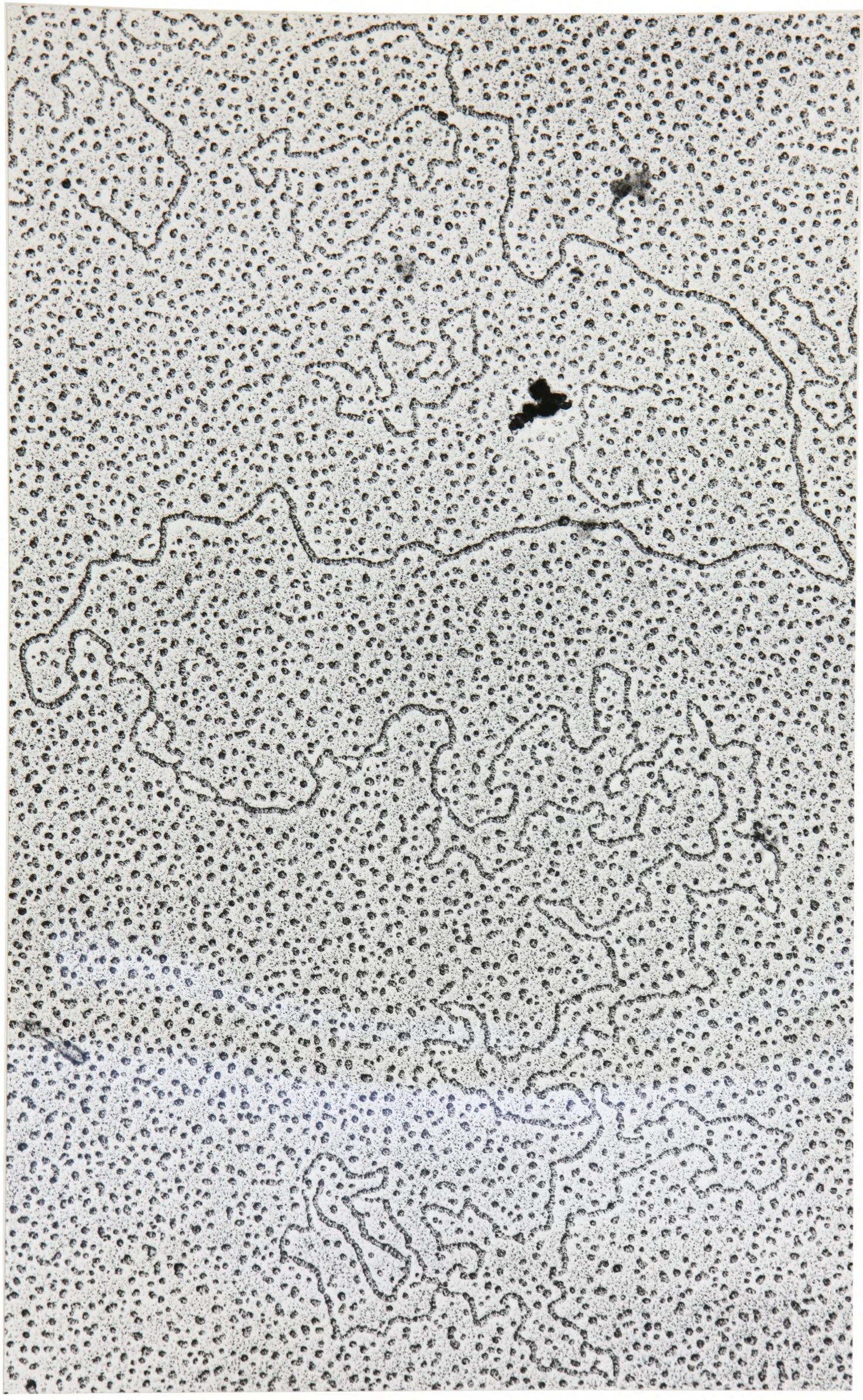


PLATE XIII $\lambda/424$

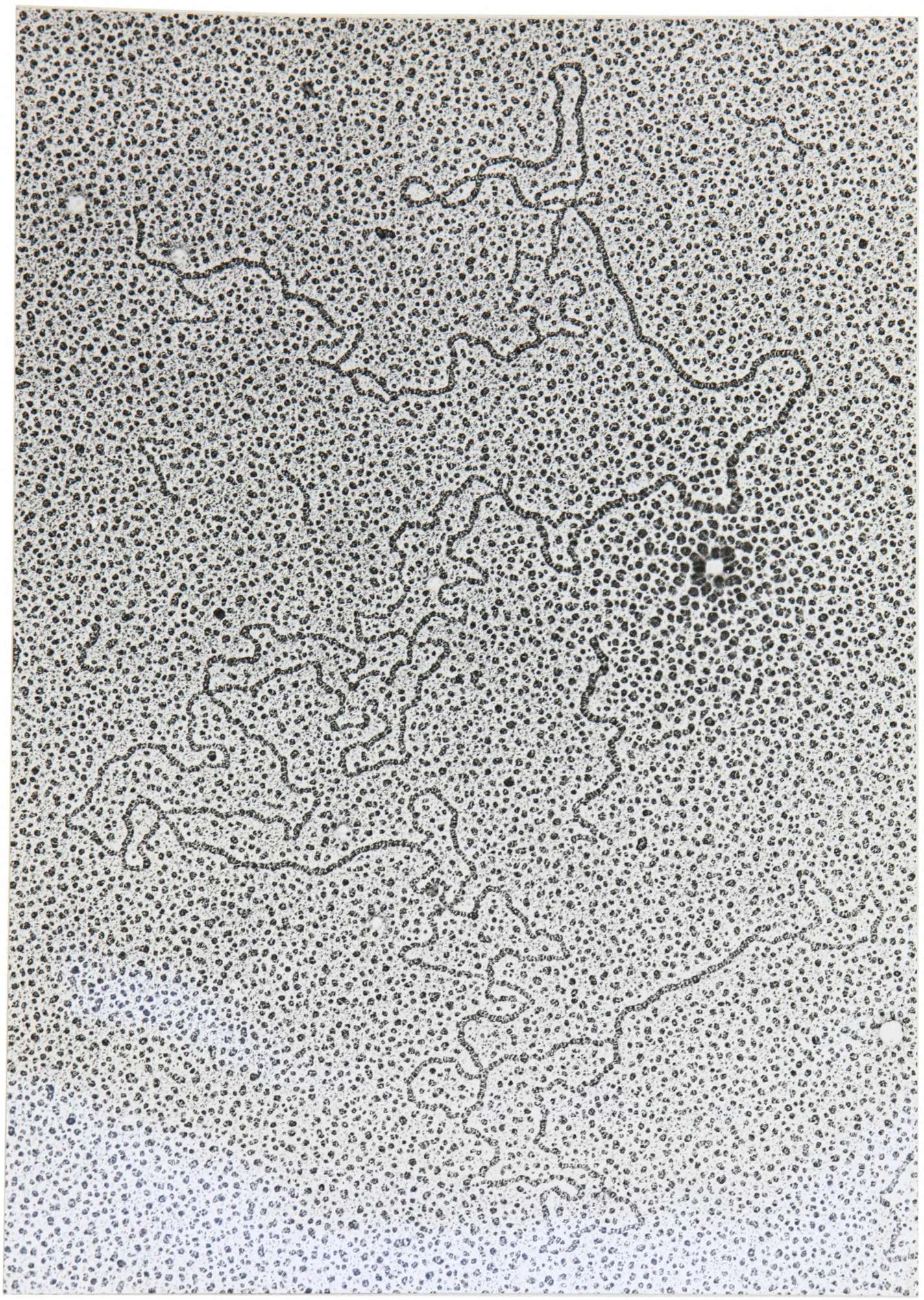


PLATE XIV PA2/21

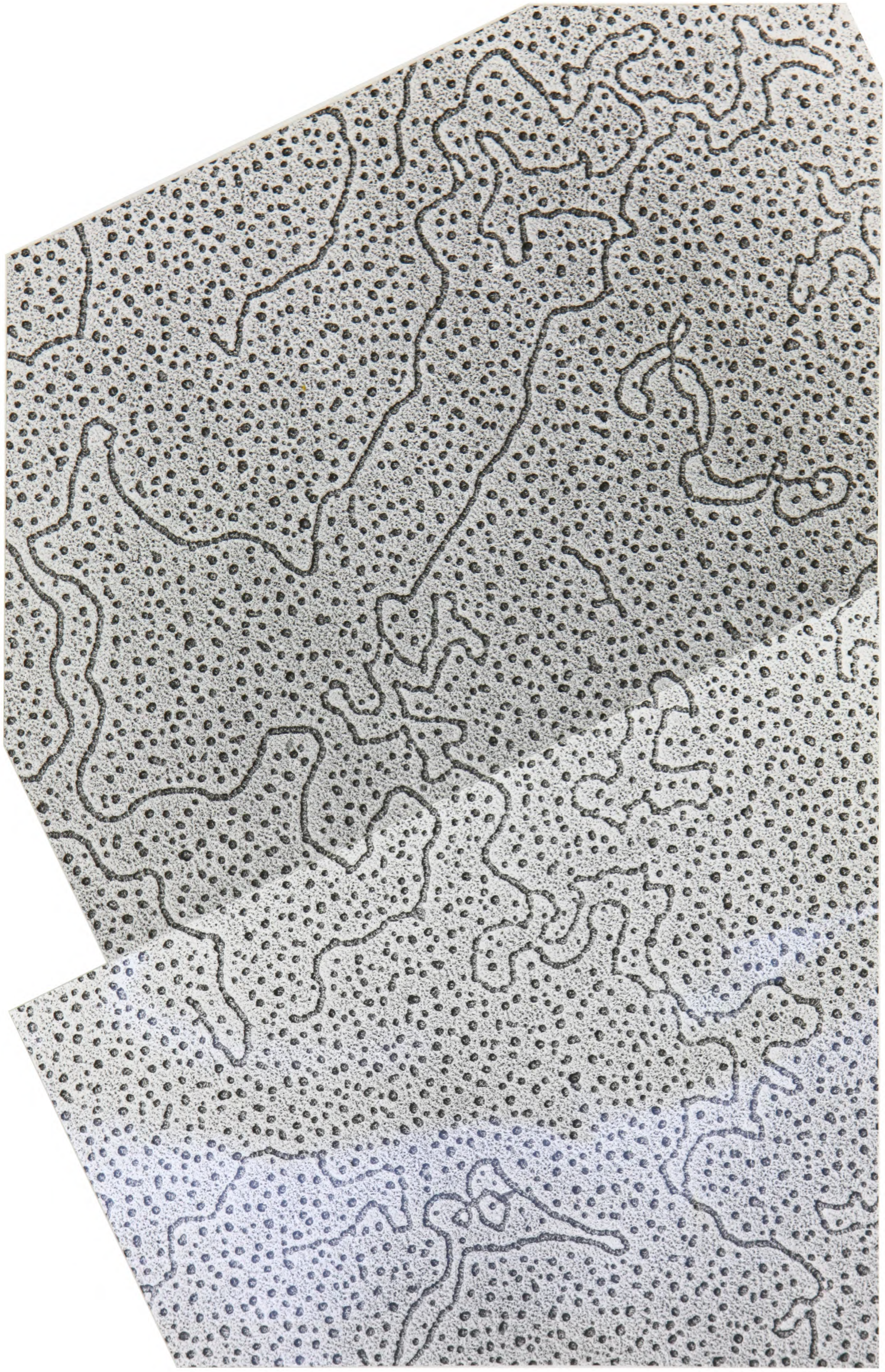


PLATE XV PA2/434

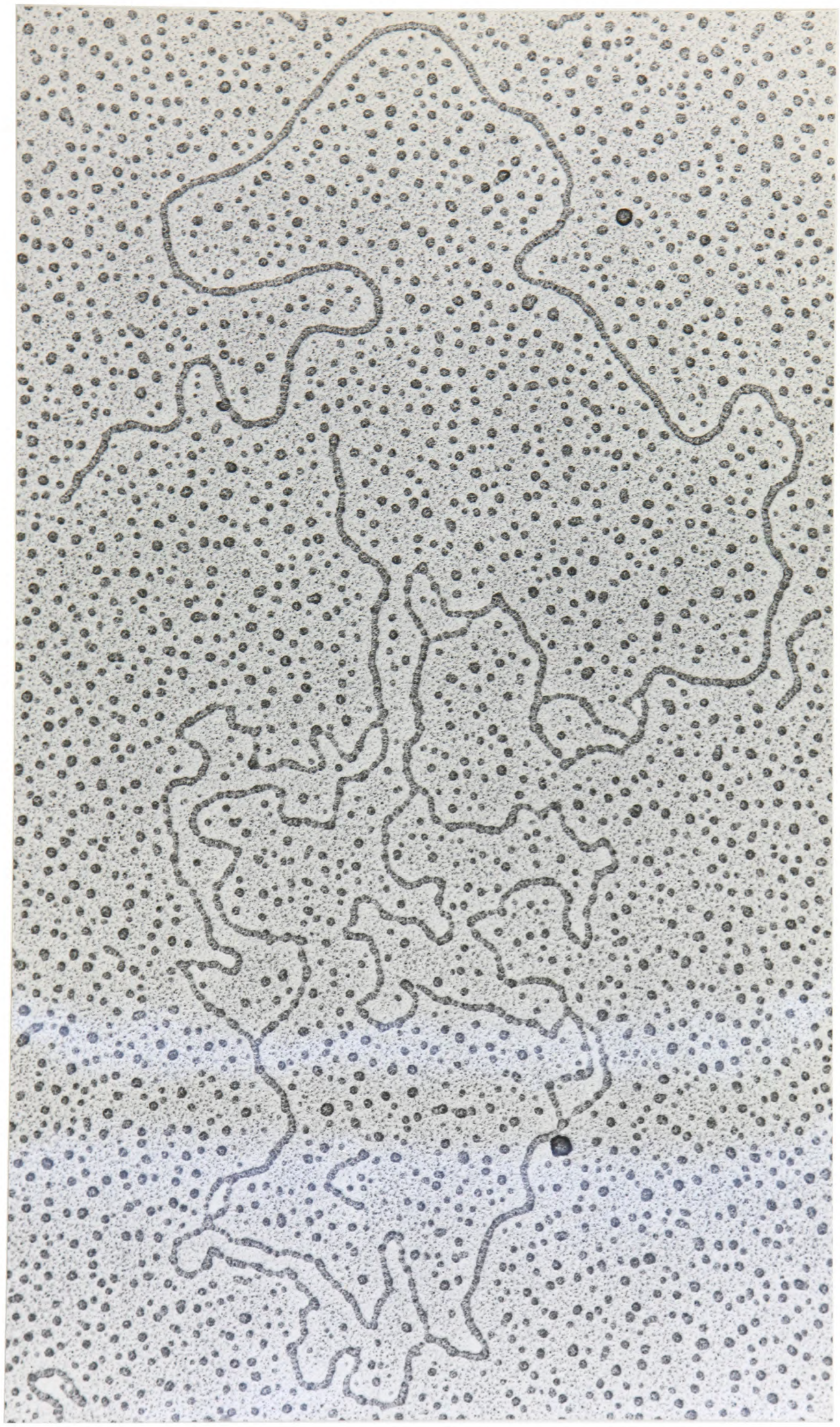


PLATE XVI λ/PA2

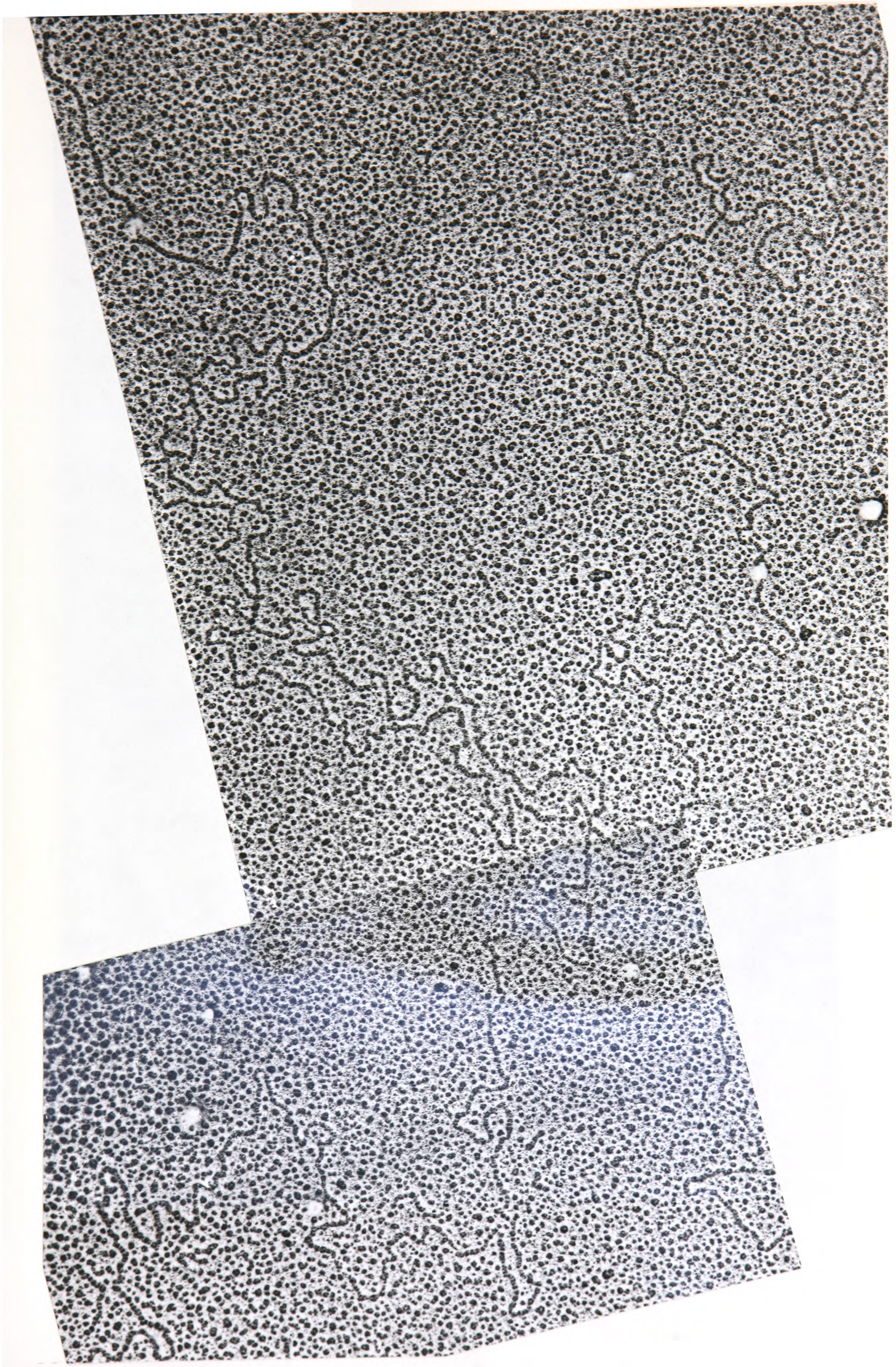


PLATE XVII 434/Ø80

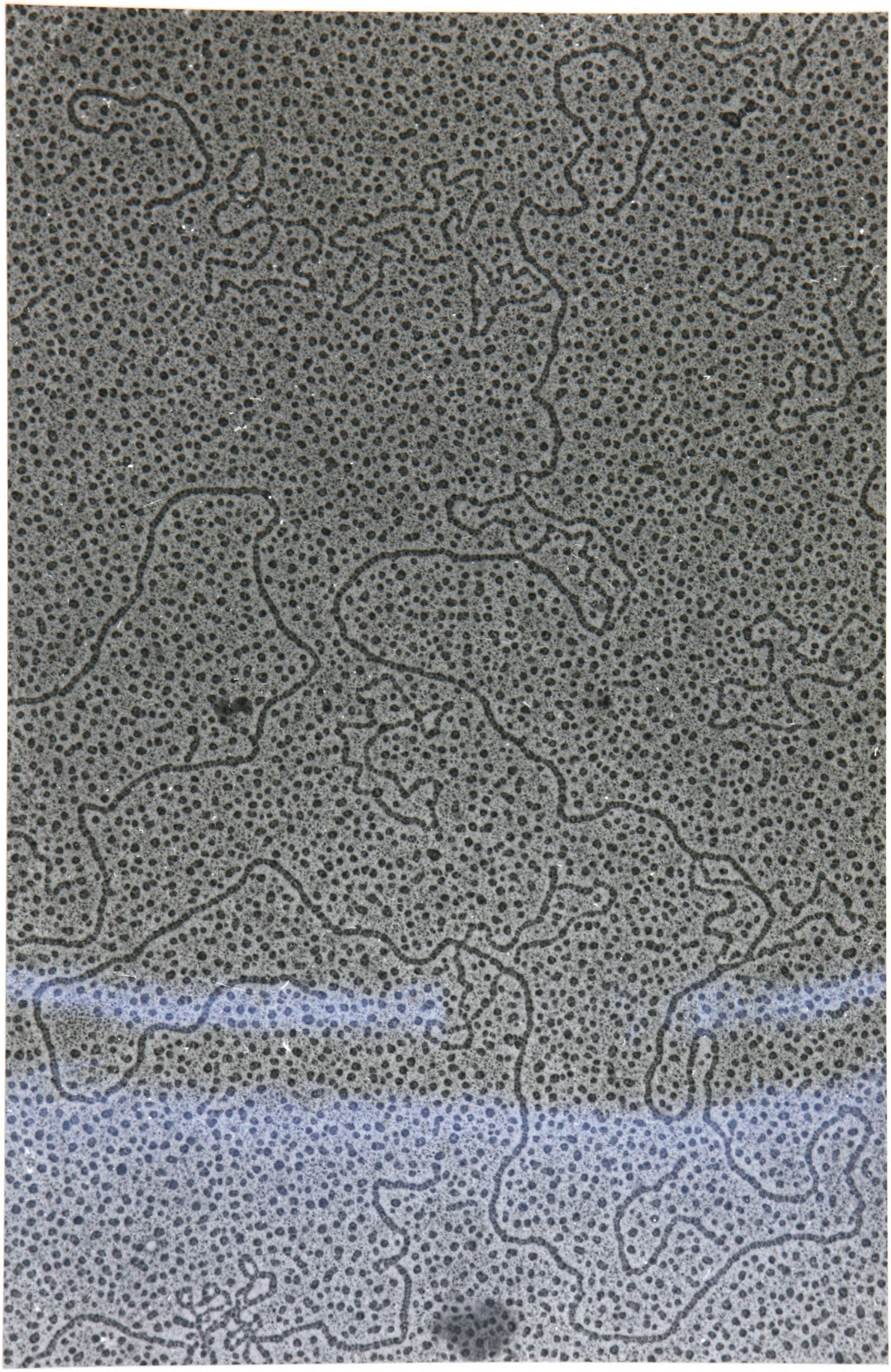


PLATE XVIII NM540/λ<u>tna</u> <u>imm</u>$^{\lambda}$ <u>c</u>I857 <u>nin</u>$^{+}$

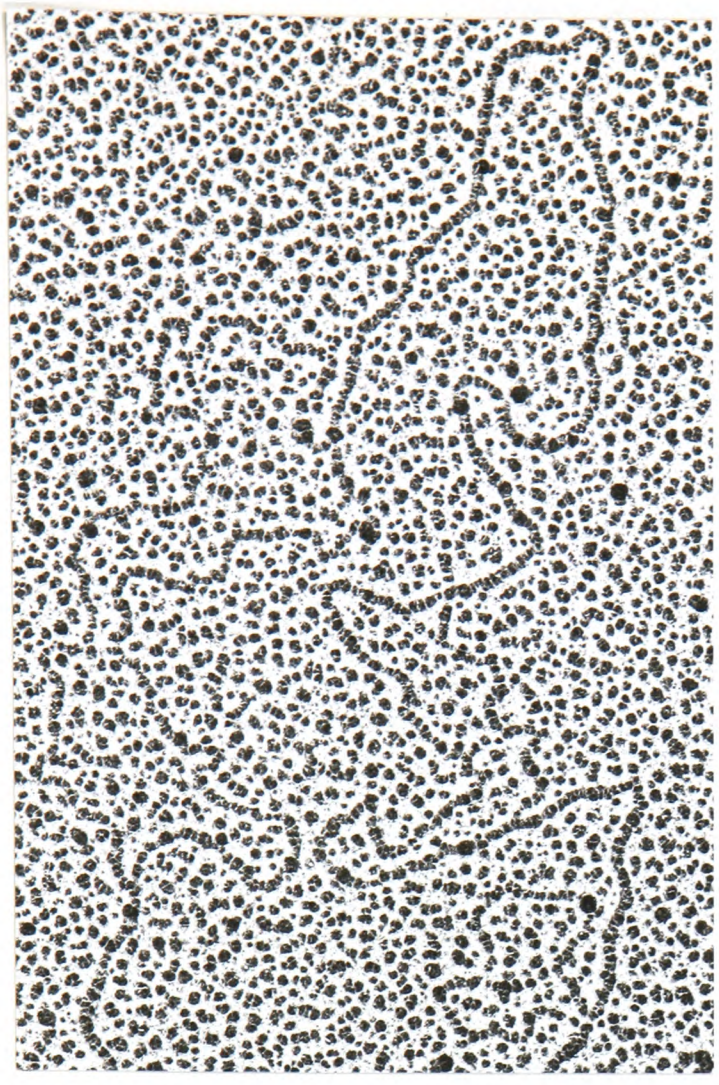


PLATE XIX

λ<u>trpABC</u>^{r}/λ<u>tna imm</u>$^{\lambda}$ <u>c</u>I857 <u>nin</u>$^{+}$

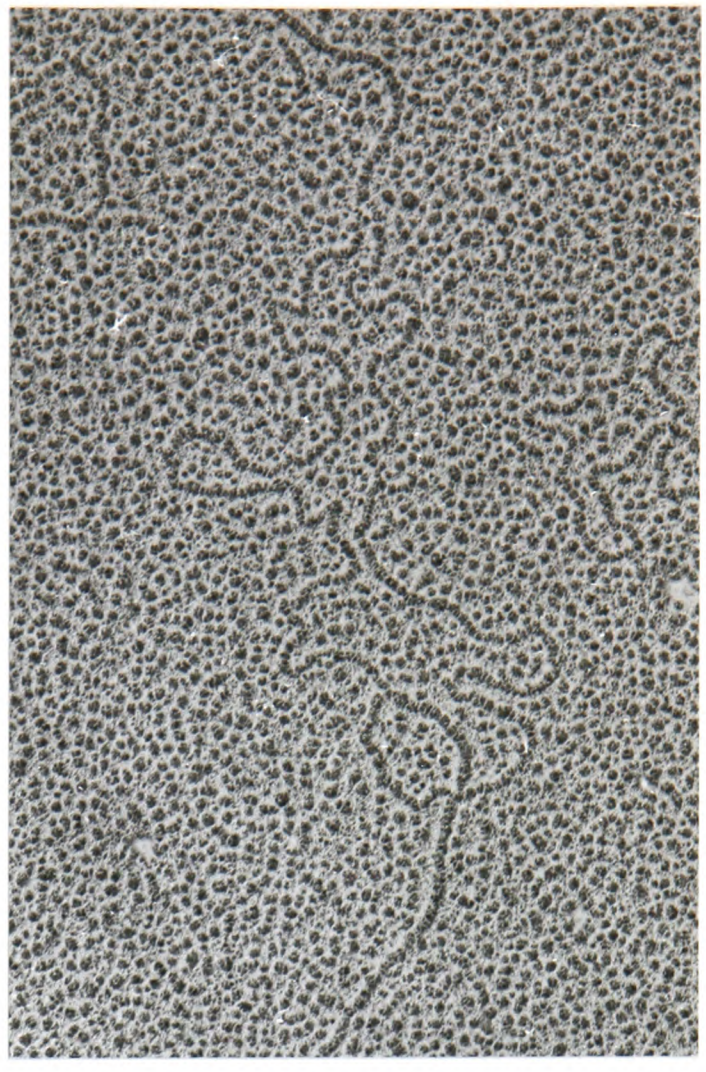


PLATE XX

λ<u>trpABC</u>^{1}/λ<u>trpABC</u>^{r}

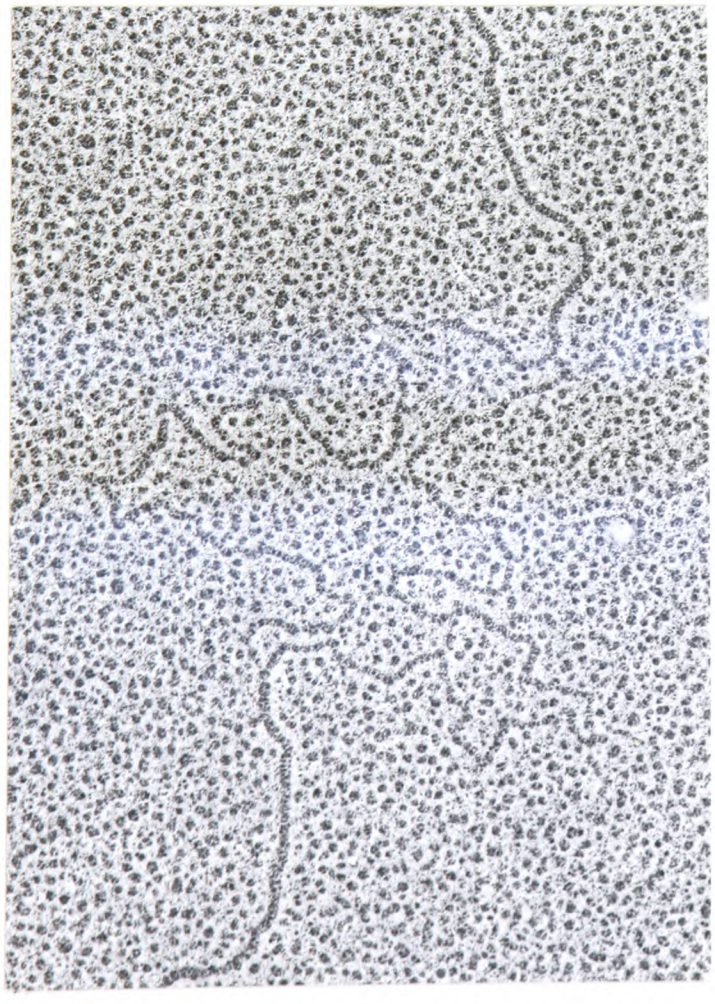


PLATE XXI

λ/λ<u>trpABC</u>^{r}

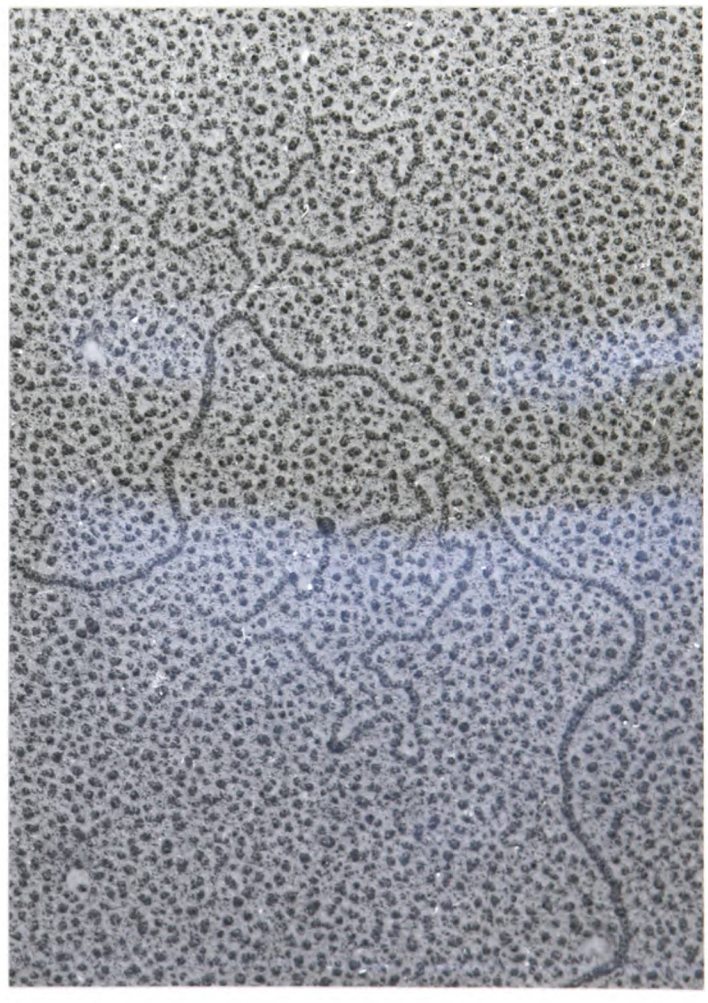


PLATE XXII

λ/λ<u>trpABC</u>^{r}

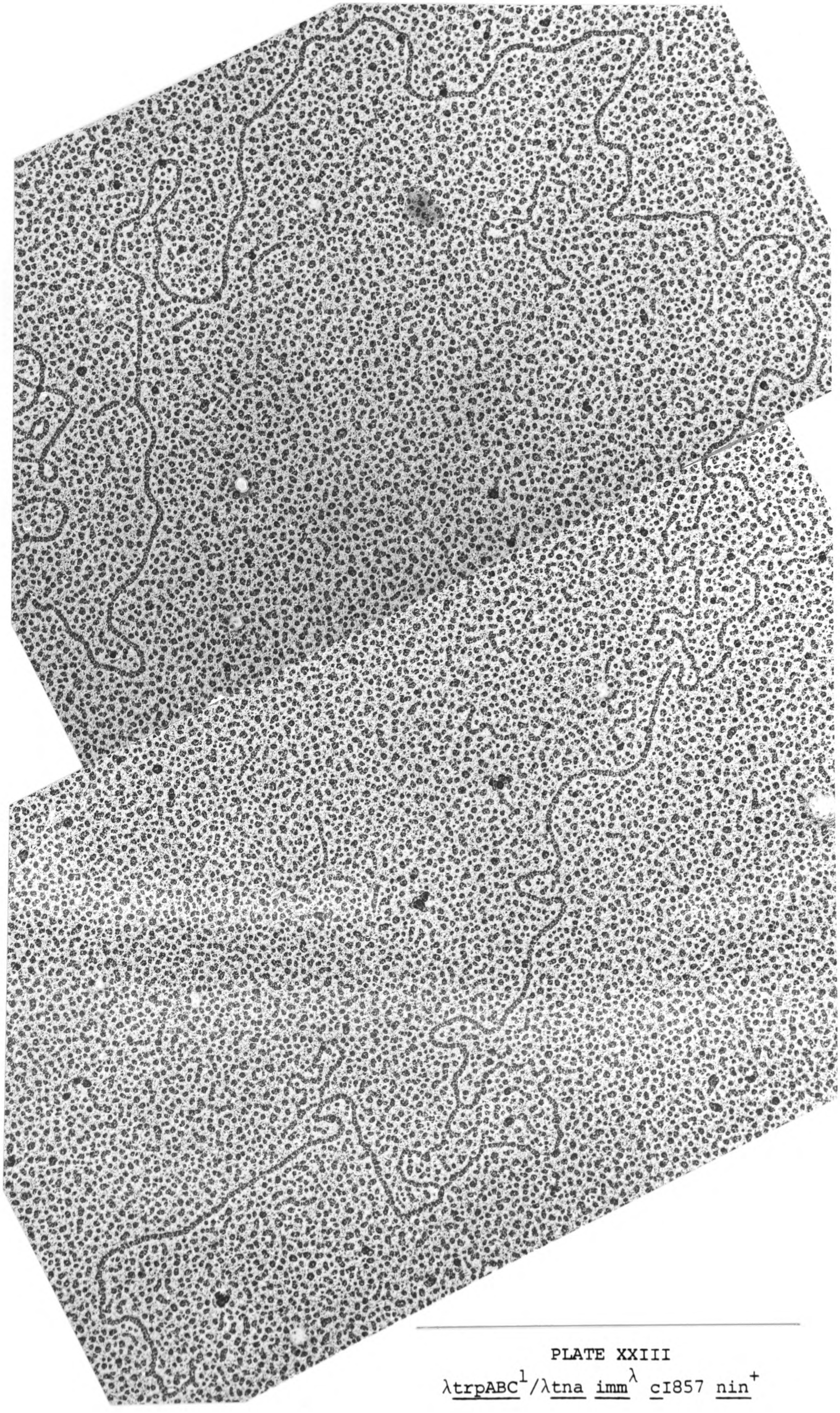


PLATE XXIII

λ<u>trpABC</u>^{1}/λ<u>tna</u> <u>imm</u>$^{\lambda}$ <u>c</u>I857 <u>nin</u>$^{+}$

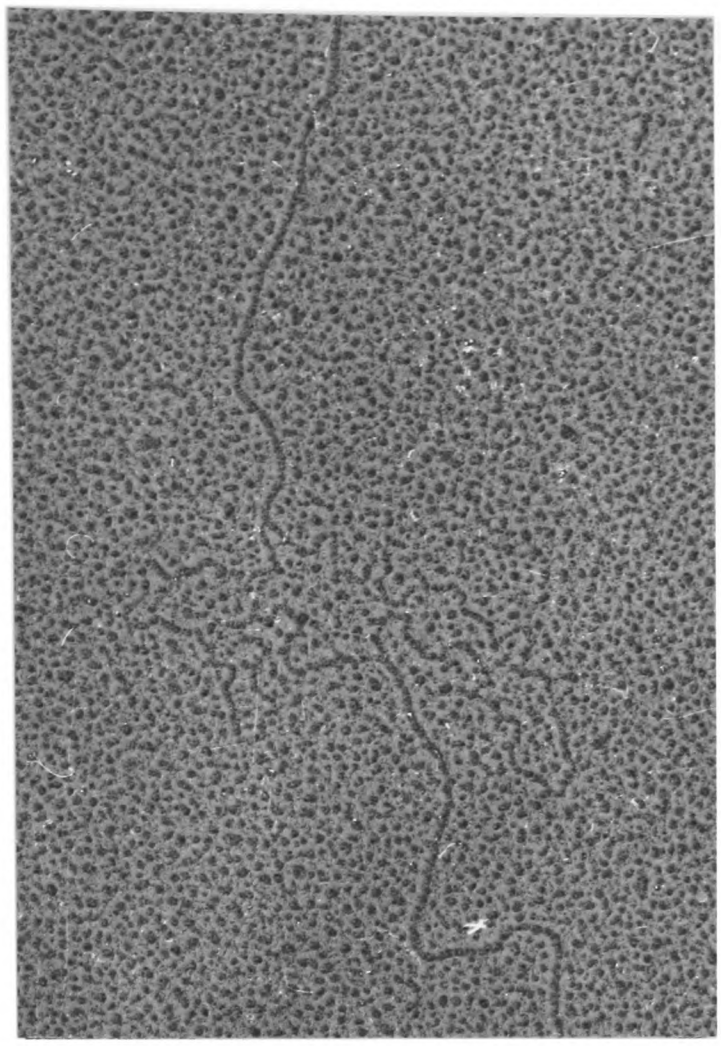


PLATE XXIV

λ<u>trpABC</u>^{l}/λ<u>tna imm</u>$^{\lambda}$ <u>c</u>I857 <u>nin</u>$^{+}$

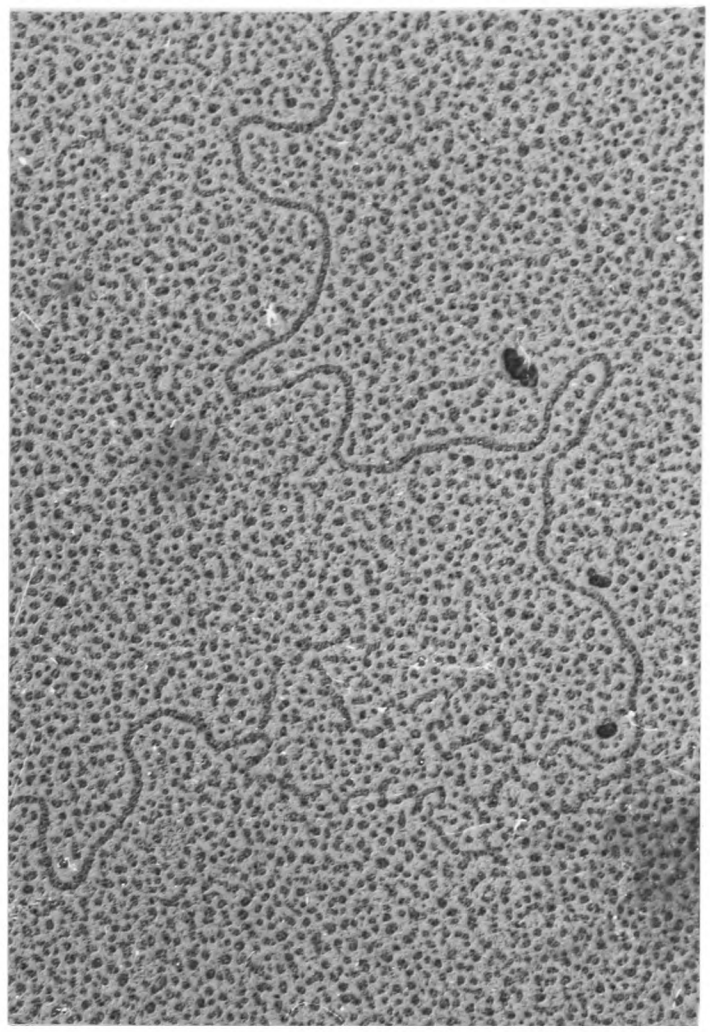


PLATE XXV

λ<u>trpABC</u>^{r}/λ<u>tna imm</u>$^{\lambda}$ <u>c</u>I857 <u>nin</u>$^{+}$

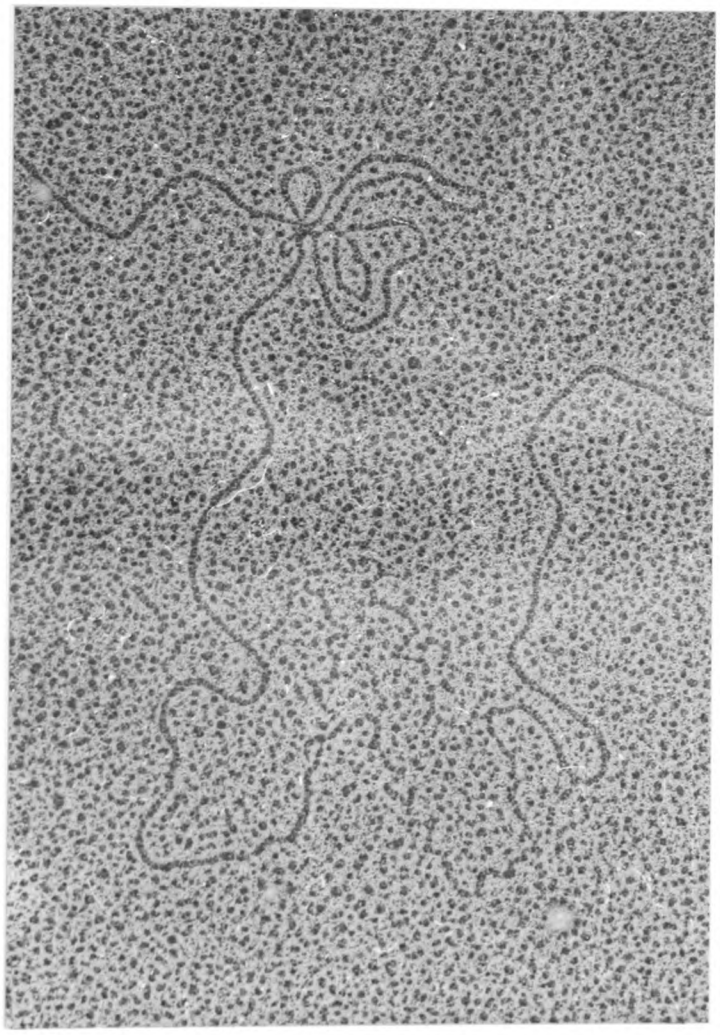


PLATE XXVI

λ<u>trpABC</u>^{l}/λ<u>tna imm</u>$^{\lambda}$ <u>c</u>I857 <u>nin</u>$^{+}$

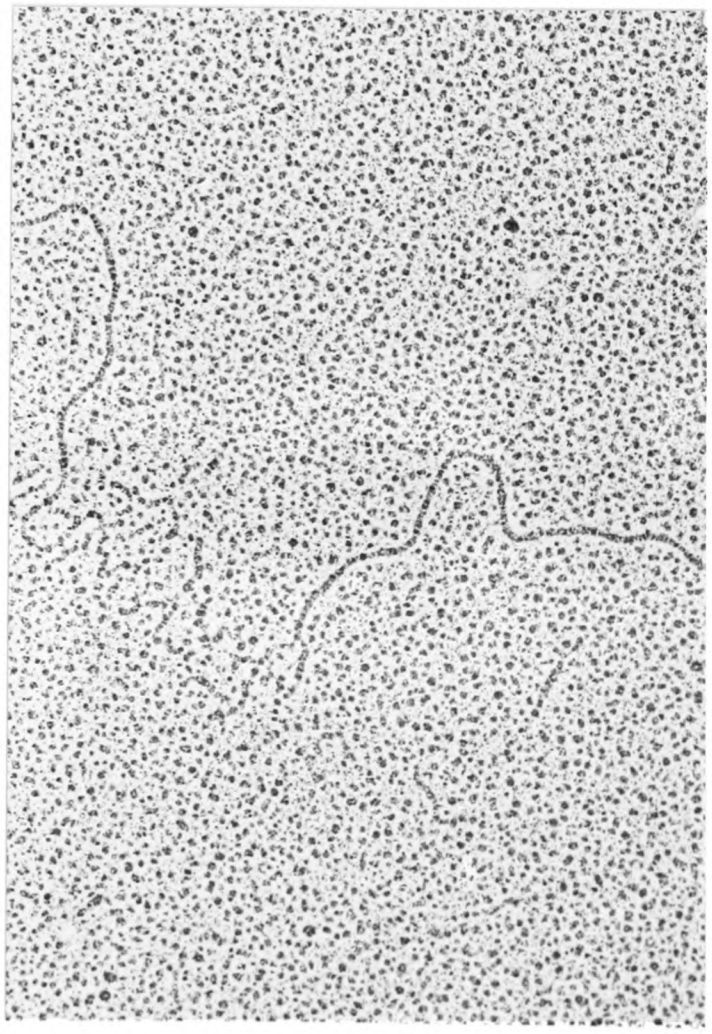


PLATE XXVII

λ<u>trpABC</u>^{r}/λ<u>tna imm</u>$^{\lambda}$ <u>c</u>I857 <u>nin</u>$^{+}$

BIBLIOGRAPHY

Adams, M. H. (1959). In Bacteriophages, Interscience, New York.

Adhya, S., Gottesman, M. and De Crombrugghe, B. (1974). Proc. Nat. Acad. Sci. U. S. A. 71:2534-2538.

Adhya, S., Sen, A. and Mitra, S. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, New York, pp. 743-746.

Ahmed, A. (1975). Molec. Gen. Genet. 136:243-253.

Allet, B. and Bukhari, A. I. (1975). J. Molec. Biol. 92:529-540.

Allet, B., Jeppesen, P. G. N., Katagiri, K. J. and Delius, H. (1973). Nature 241:120-122.

Alt, F. W., Kellms, R. E., Bertino, J. R. and Schimke, R. T. (1978). J. Biolog. Chem. 253:1357-1373.

Anderson, R. P. and Roth, I. R. (1977). Ann. Rev. Microbiol. 31: 473-505.

Anderson, R. P. and Roth, I. R. (1978). J. Molec. Biol. 119:147-166.

Appleyard, R. K. (1954). Genetics 39:440-452.

Arber, W. (1960). Virology 11:273-288.

Ausubel, F. M. (1974). Nature 247:152-154.

Baker, R. and Tessman, E. S. (1967). Proc. Nat. Acad. Sci. U. S. A. 58:1438-1445.

Baldwin, R. L. (1971). In Procedures in Nucleic Acid Research Vol. II, edited by G. L. Cantoni and D. R. Davies, Harper and Row, New York.

Baldwin, R. L., Barrard, P., Fritsch, A., Goldthwait, D. and Jacob, F. (1966). J. Molec. Biol. 17:343-357.

Beckendorf, S. K. (1973). J. Molec. Biol. 73:37-53.

Benbow, R. M., Hutchison, C. A., Fabricant, J. D. and Sinsheimer, R. L. (1971). J. Virol. 7:549-558.

Bertani, G. and Bertani, L. E. (1974). Proc. Nat. Acad. Sci. U. S. A. 71:315-319.

Bertani, L. E. and Bertani, G. (1971). Advan. Genet. 16:199-237.

Bertrand, K., Korn, L., Lee, F., Platt, T., Squires, C. L., Squires, C. and Yanofsky, C. (1975). Science 189:22-26.

Bode, V. C. and Kaiser, A. D. (1965). Virology 25:111-121.

Boice, L. (1969). J. Virol. 4:47-49.

Boice, L., Eiserling, F. A. and Romig, W. R. (1969). Biochem. Biophys. Res. Comm. 34:398-403.

Bolton, E. T. and McCarthy, B. J. (1962). Proc. Nat. Acad. Sci. U. S. A. 48:1390-1397.

Borck, K., Beggs, J. D., Brammar, W. J., Hopkins, A. S. and Murray, N. E. (1976). Molec. Gen. Genet. 146:199-207.

Borrias, W. E., Hagenaar, M., Van Den Brekel, R., Kuhlemeijer, C. and Weisbeek, P. J. (1979). J. Virol. 31:288-298.

Botstein, D., Chan, R. K. and Waddell, C. H. (1972). Virology 49: 268-282.

Botstein, D. and Herskowitz, I. (1974). Nature 251:584-589.

Brammar, W. J., Dunbar, S. and Muir, S. (1977). Personal communication.

Brooks, K. (1965). Virology 26: 489-499.

Britten, R. J. and Kohne, D. E. (1968). Science 161:529-540.

Brown, D. D. and David, I. B. (1968). Science 160:272-280.

Brunovskis, I., Hyman, R. W. and Summer, W. C. (1973). J. Virology 11:306-313.

Buchwald, M., Murialdo, H. and Siminovitch, L. (1970). Virology 42: 390-400.

Buchwald, M. and Siminovitch, L. (1969). Virology 38:1-7.

Buchwald, M., Steed-Glaister, P. and Siminovitch, L. (1970). Virology 42:375-389.

Burman, L. G., Park, J. T., Lindström, E. B. and Boman, H. G. (1970). J. Bacteriol. 116:123-130.

Burnet, F. M. (1927). Brit. J. Exp. Pathol. 8:121-134.

Butler, B. and Echols, H. (1970). Virology 40:212-222.

Calef, E. and Neubauer, Z. (1968). Cold Spring Harbor Symp. Quant. Biol. 33:765-767.

Campbell, A. (1961). Virology 14:22-32.

Campbell, A. (1962). Advan. Genet. 11:101-145.

Campbell, A. (1962). Genetics 48:409-421.

Campbell, A. (1965). Virology 27:340-345.

Campbell, A. (1969). In Episomes; Harper and Row, Publishers; New York, Evanston and London.

Campbell, A. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, New York, pp. 13-44.

Campbell, A. (1972). In Evolution of Genetic Systems, edited by H. H. Smith, Brookhaven Symposia in Biology, 23:534-562.

Campbell, A. (1977). Comprehensive Virology 8:259-328.

Campbell, A. and del Campillo-Campbell, A. (1963). J. Bacteriol. 85: 1202-1207.

Campbell, A. Heffernan, L., Hu, S.-L. and Szybalski, W. (1977). In DNA Insertion Elements, Plasmids and Episomes, edited by A. I. Bukhari, J. A. Shapiro, and S. L. Adhya, Cold Spring Harbor Laboratory, New York, pp. 375-379.

Carlton, B. C. and Yanofsky, C. (1962). J. Biolog. Chem. 231:1531-1534.

Casjens, S. Hohn, T. and Kaiser, A. D. (1970). Virology 42:496-507.

Casjens, S. and Hendrix, R. (1974). J. Molec. Biol. 88:535-545.

Casjens, S. and King, J. (1975). Ann. Rev. Biochem. 44:555-611.

Cavanaugh, D. C. and Quan, S. F. (1953). Amer. J. Clinical Pathol. 23:619-620.

Chan, R. K. and Botstein, D. (1972). Virology 49:257-267.

Chattoraj, D. K. and Inman, R. B. (1974). Proc. Nat. Acad. Sci. U. S. A. 71:311-314.

Chow, L. T., Boice, L. and Davidson, N. (1972). J. Molec. Biol. 68: 391-400.

Chow, L. T. and Davidson, N. (1973). J. Molec. Biol. 75:257-264.

Chow, L. T., Davidson, N. and Berg, P. (1974). J. Molec. Biol. 86: 69-89.

Chung, S. and Echols, H. (1977). Virology 79:312-319.

Clewell, D. D., Yagi, Y. and Bauer, B. (1975). Proc. Nat. Acad. Sci. U. S. A. 72:1720-1724.

Cohen, G. and Jacob, F. (1959). Compt. Rend. Acad. Sci. (Paris) 248: 3490-3492.

Compton, J. L. and Sinsheimer, R. L. (1977). J. Molec. Biol. 109: 217-234.

Court, D., Adhya, S., Nash, H. and Enquist, L. (1977). In DNA Insertion Elements, Plasmids and Episomes, edited by A. Bukhari, J. Shapiro and S. Adhya, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 389-394.

Court, D., Green, L. and Echols, H. (1975). Virology 63:484-491.

Court, D. and Sato, K. (1969). Virology 39:348-352.

Couturier, M. and Dambly, C. (1970). Compt. Rend. Acad. Sci. (Paris) 270:428-430.

Cowie, D. B. (1964). Carnegie Inst. Wash. Year Book 63:380-387.

Cowie, D. B. (1974). J. Franklin Inst. 298:307-319.

Cowie, D. B., Avery, R. J. and Champe, S. P. (1971). Virology 45: 30-37.

Cowie, D. B. and Hershey, A. D. (1965). Proc. Nat. Acad. Sci. U. S. A. 53:57-62.

Cowie, D. B. and McCarthy, B. J. (1963). Proc. Nat. Acad. Sci. U. S. A. 50:537-543,

Cowie, D. B. and Szafranski, P. (1967). Biophys. J. 7:567-584.

Cunin, R., Elseviers, D. and Glansdorff, N. (1970). Molec. Gen. Genet. 108:154-157.

Davidson, N. and Szybalski, W. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 45-82.

Davies, R. W., Schreier, P. H. and Büchel, D. E. (1977). Nature (London) 270:757-760.

Davis, R. W. and Davidson, N. (1968). Proc. Nat. Acad. Sci. U. S. A. 60:243-250.

Davis, R. W. and Hyman, R. W. (1971). J. Molec. Biol. 62:287-301.

Davis, R. W. and Parkinson, J. S. (1971). J. Molec. Biol. 56:403-423.

Davis, R. W., Simon, M. and Davidson, N. (1971). In Methods in Enzymology, edited by L. Grossman and K. Moldave, Academic Press, New York and London, Vol. XXI:413-428.

Dawson, P., Skalka, A. and Simon, L. D. (1975). J. Molec. Biol. 93: 167-180.

Deeb, S. S. (1970). J. Virol. 5:27-31.

Deeb, S. S., Okamoto, K. and Hall, B. D. (1967). Virology 31:289-295.

Delbrück, M. (1946). Biolog. Rev. Cambridge Phil. Soc. 21:30-40.

Delbrück, M. (1946). Harvey Lect. 41:161-187.

Delbrück, M. and Bailey, W. T. (1946). Cold Spring Harbor Symp. Quant. Biol. 11:33-37.

Demerec, M. and Fano, U. (1945). Genetics 30:119-136.

Dettori, R., Maccacaro, G. A. and Piccinin, G. L. (1961). G. Microbiol. 9:141-150.

Doerfler, W. and Hogness, D. S. (1968). J. Molec. Biol. 33:635-659.

Doi, R. H. and Spiegelman, S. (1962). Science 138:1270-1272.

Dove, W. F. (1966). J. Molec. Biol. 19:187-201.

Dove, W. F. (1968). Ann. Rev. Genet. 2:305-340.

Dove, W. F. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 297-312.

Dove, W. F., Inokuchi, H. and Stevens, W. F. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 747-771.

Echols, H. (1970). J. Molec. Biol. 47:575-583.

Echols, H. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 247-270.

Echols, H. and Gingery, R. (1968). J. Molec. Biol. 34:239-249.

Echols, H., Gingery, R. and Moore, L. (1968). J. Molec. Biol. 34: 251-260.

Echols, H. and Green, L. (1971). Proc. Nat. Acad. Sci. U. S. A. 68: 2190-2194.

Echols, H., Green, L., Oppenheim, A. B., Oppenheim, A. and Honigman, A. (1973). J. Molec. Biol. 80:203-216.

Echols, H. and Murialdo, H. (1978). Microbiol. Reviews 42:577-591.

Edlund, T., Grundström, T. and Normark, S. (1979). Molec. Gen. Genet. 173:115-125.

Eisen, H., Brachet, P., Pereira da Silva, L. and Jacob, R. (1970). Proc. Nat. Acad. Sci. U. S. A. 66:855-862.

Eisen, H. A., Fuerst, C. R., Siminovitch, L., Thomas, R., Lambert, L., Pereira da Silva, L. and Jacob, F. (1966). Virology 30:224-241.

Elseviers, D., Cunin, R. and Glansdorff, N. (1969). FEBS Letters 3: 18-20.

Emmons, S. W., MacCosham, V. and Baldwin, R. L. (1975). J. Molec. Biol. 91:133-146.

Emmons, S. W., MacCosham, V. and Baldwin, R. L. (1975). J. Molec. Biol. 95:83-89.

Emmons, S. W. and Thomas, J. O. (1975). J. Molec. Biol. 91:147-152.

Endo, H., Ayabe, K., Amako, K. and Takeya, K. (1965). Virology 25: 469-471.

Enquist, L. W. and Skalka, A. (1973). J. Molec. Biol. 75:185-212.

Enquist, L. W. and Weisberg, R. A. (1977). J. Molec. Biol. 111:97-120.

Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, E., Chevalley, K., Edgar, R. S., Susman, M., Denhardt, G. H. and Leilausis, A. (1963). Cold Spring Harbor Symp Quant. Biol. 28:375-392.

Falkow, S., Haapala, D. K. and Silver, R. P. (1969). In Bacterial Episomes and Plasmids, a Ciba Foundation Symposium, edited by G. E. W. Wolstenholme and M. O'Connor, Little, Brown and Co., Boston, pp. 136-158.

Fiandt, M., Hradecna, Z., Lozeron, H. A. and Szybalski, W. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 329-354.

Fiandt, M. and Szybalski, W. (1973). Abstracts of Ann. Meetings of Amer. Soc. of Microbiol. p. 218.

Fiandt, M., Szybalski, W. and Malamy, M. H. (1972). Molec. Gen. Genet. 119:223-231.

Fiddes, J. C. and Godson, G. N. (1978). J. Virology 89:322-326.

Fiddes, J. C. and Godson, G. N. (1979). J. Molec. Biol. 133:19-43.

Filer, D. and Furano, A. V. (1980). J. Biolog. Chem. 255:728-734.

Fischer-Fantuzzi, L. (1967). Virology 32:18-32.

Folk, W. R. and Berg, P. (1971). J. Mol. Biol. 58:595-610.

Folkmanis, A., Maltzman, W., Mellon, P., Skalka, A. and Echols, H. (1977). Virology 81:352-362.

Frampton, E. W. and Brinkley, B. R. (1965). J. Bacteriol. 90:446-452.

Frank-Kamenetski, M. D. (1971). Biopolymers 10:2623-2624.

Franklin, N. C. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 621-638.

Franklin, N. C. (1974). J. Molec. Biol. 89:33-48.

Franklin, N. C. and Dove, W. (1969). Genet. Res. 14:151-157.

Franklin, N. C., Dove, W. F. and Yanofsky, C. (1965). Biochem. Biophys. Res. Comm. 18:910-923.

Fraser, O. and Williams, R. C. (1953). J. Bacteriol. 65:167-170.

Freedman, R. and Brenner, S. (1972). J. Molec. Biol. 69:409-419.

Fuerst, C. R. (1966). Virology 30:581-583.

Furano, A. V. (1975). Proc. Nat. Acad. Sci. U. S. A. 72:4780-4784.

Furano, A. V. (1977). J. Biolog. Chem. 252:2154-2157.

Furano, A. V. (1978). Proc. Nat. Acad. Sci. U. S. A. 75:3104-3108.

Geiser, M. and Gordon, J. (1978a). Experientia 34:940.

Geiser, M. and Gordon, J. (1978b). Proc. Nat. Acad. Sci. U. S. A. 75:1140-1144.

Gelderman, A. H., Lincoln, T. L., Cowie, D. B. and Roberts, R. B. (1966). Proc. Nat. Acad. Sci. U. S. A. 55:289-297.

Gellert, M. (1967). Proc. Nat. Acad. Sci. U. S. A. 57:148-155.

Harris, A.W., Mount, D.W.A., Fuerst, C.R. and Siminovitch, L. (1967). Virology 32: 553-569.

Gemski, P., Baron, L. S. and Yamamato, N. (1972). Proc. Nat. Acad. Sci. U. S. A. 69:3110-3114.

Georgopoulos, C. P. and Herskowitz, I. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp.553-564.

Gilbert, W. (1978). Nature 271:501.

Gingery, R. and Echols, H. (1967). Proc. Nat. Acad. Sci. U. S. A. 58:1507-1514.

Glansdorff, N. and Sand, G. (1968). Genetics 60:257-268.

Glansdorff, N., Sand, G. and Verhoef, C. (1967). Mutation Res. 4: 743-751.

Godson, G. N. (1973). J. Molec. Biol. 77:467-477.

Godson, G. N. (1974). Virology 58:272-289.

Godson, G. N. (1975). Virology 63:320-335.

Godson, G. N. (1978). In The Single-Stranded DNA Phages, edited by D. T. Denhardt, D. Dressler and D. S. Ray, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 103-112.

Godson, G. N., Barrell, B. G., Staden, R. and Fiddes, J. C. (1978). Nature 276:236-247.

Godson, G. N., Fiddes, J. C., Barrell, B. G. and Sanger, F. (1978). In The Single-Stranded DNA Phages, edited by D. T. Denhardt, D. Dressler and D. S. Ray, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 51-86.

Godson, G. N. and Roberts, R. J. (1976). Virology 73:561-567.

Goldberg, A. R. and Howe, M. (1969). Virology 38:200-202.

Goldberg, M. E., Creighton, T. E., Baldwin, R. L. and Yanofsky, C. (1966). J. Molec. Biol. 21:71-82.

Gorini, L., Gundersen, W. and Burger, M. (1961). Cold Spring Harbor Symp. Quant. Biol. 26:173-182.

Gottesman, M. E. and Weisberg, R. A. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 113-138.

Gottesman, M. E. and Yarmolinsky, M. B. (1968). Cold Spring Harbor Symp. Quant. Biol. 33:735-747.

Gottesman, M. E. and Yarmolinsky, M. B. (1968). J. Molec. Biol. 31: 487-505.

Gough, M. (1968). J. Virology 2:992-998.

Gough, M. and Levine, M. (1968). Genetics 58:161-169.

Green, M. H. (1963). Proc. Nat. Acad. Sci. U. S. A. 50:1177-1184.

Greer, H. (1975). Virology 66:589-604.

Grindley, J. N. and Godson, G. N. (1978). J. Virology 27:745-753.

Grosveld, F. G., Ojamaa, K. M. and Spencer, J. (1976). Virology 71: 312-324.

Guarneros, G. and Echols, H. (1970). J. Molec. Biol. 47:565-574.

Guerry, P. and Falkow, S. (1971). J. Bacteriol. 107:372-374.

Hall, B. D. and Spiegelman, S. (1961). Proc. Nat. Acad. Sci. U. S. A. 47:137-146.

Hayes, S. and Szybalski, W. (1973). Molec. Gen. Genet. 126:275-290.

Heinemann, S. F. and Spiegelman, W. G. (1970a). Cold Spring Harbor Symp. Quant. Biol. 35:315-318.

Heinemann, S. F. and Spiegelman, W. G. (1970b). Proc. Nat. Acad. Sci. U. S. A. 67:1122-1129.

Heinemann, S. F., Spiegelman, W. G., Brachet, P., Pereira da Silva, L. and Eisen, H. (1970). Cold Spring Harbor Symp. Quant. Biol. 35:325-330.

Henderson, E. J. and Zalkin, H. (1971). J. Biolog. Chem. 246:6891-6898.

Henderson, E. J., Zalkin, H. and Hwang, L. H. (1970). J. Biolog. Chem. 245:1424-1431.

Hendrix, R. W. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 355-370.

Hershey, A. D. (1964). Carnegie Inst. Wash. Year Book 63:581-583.

Hershey, A. D. (1969). Carnegie Inst. Wash. Year Book 68:655-661.

Hershey, A. D. (1971). Carnegie Inst. Wash. Year Book 70:3-5.

Hershey, A. D. and Burgi, E. (1965). Proc. Nat. Acad. Sci. U. S. A. 53:325-328.

Hershey, A. D., Burgi, E. and Ingraham, L. (1963). Proc. Nat. Acad. Sci. U. S. A. 49:748-755.

Hershey, A. D. and Dove, W. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 3-11.

Herskowitz, I. and Signer, E. (1970). J. Molec. Biol. 47:545-556.

Highton, P. and Whitfield, M. (1975). Virology 63:438-446.

Hill, C. W. and Echols, H. (1966). J. Molec. Biol. 19:38-51.

Hill, C. W., Foulds, J., Soll, L. and Berg, P. (1969). J. Molec. Biol. 39:563-581.

Hirsch, H.-J., Starlinger, P. and Brachet, P. (1972). Molec. Gen. Genet. 119:191-206.

Hopkins, A. S., Murray, N. E. and Brammar, W. J. (1976). J. Molec. Biol. 107:549-569.

Hopkins, N. (1970). Virology 40:223-229.

Hopkins, N. and Ptashne, M. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 571-574.

Hopwood, D. (1967). Bacteriolog. Rev. 31:373-403.

Horowitz, N. H. (1965). In Evolving Genes and Proteins, edited by V. Bryson and H. J. Vogel, Academic Press, New York, pp. 15-23.

Howard, B. D. (1967). Science 158:1588-1589.

Hradecna, Z. and Szybalski, W. (1967). Virology 32:633-643.

Hyman, R. W., Brunovskis, I. and Summers, W. C. (1973). J. Molec. Biol. 77:189-196.

Hyman, R. W., Brunovskis, I. and Summers, W. C. (1974). Virology 57: 189-206.

Ikeda, H. and Tomizawa, J. I. (1968). Cold Spring Harbor Symp. Quant. Biol. 33:791-798.

Imamoto, F., Morikawa, N. and Sato, K. (1965). J. Molec. Biol. 13: 169-182.

Inman, R. B. (1967). J. Molec. Biol. 28:103-116.

Inokuchi, H. and Ozeki, H. (1970). Virology 41:701-710.

Isaacs, L. N., Echols, H. and Sly, W. S. (1965). J. Molec. Biol. 13: 963-967.

Ito, J., Cox, E. C. and Yanofsky, C. (1969). J. Bacteriol. 97:725-733.

Ito, J. and Yanofsky, C. (1966). J. Biolog. Chem 241:4112-4114.

Ito, J. and Yanofsky, C. (1969). J. Bacteriol. 97:734-742.

Jackson, E. N. and Yanofsky, C. (1973). J. Bacteriol. 116:33-40.

Jackson, E. N. and Yanofsky, C. (1974). J. Bacteriol. 117:502-508.

Jacob, F. (1955). Virology 1:207-220.

Jacob, F. and Fuerst, C. R. (1958). J. Gen. Microbiol. 18:518-526.

Jacob, F. and Wollman, E. L. (1954). Ann. Inst. Pasteur (Paris) 87: 653-673.

Jacob, F. and Wollman, E. L. (1956). Ann. Inst. Pasteur (Paris) 91: 486-510.

Jacob, F. and Wollman, E. L. (1961). In Sexuality and the Genetics of Bacteria, Academic Press Inc., New York.

Jacobson, G. R. and Rosenbusch, J. P. (1976). Nature 261:23-26.

Jacoby, G. A. (1971). J. Bacteriol. 108:645-651.

Jacoby, G. A. and Gorini, L. (1969). J. Molec. Biol. 39:73-87.

Jaskunas, S. R., Lindahl, L., Nomura, M. and Burgess, R. R. (1975). Nature 257:458-472.

Jeng, Y., Gelfand, D., Hayashi, M., Schleser, R. and Tessman, E. S. (1970). J. Molec. Biol. 49:521-526.

Johnson, A., Meyer, B. J. and Ptashne, M. (1978). Proc. Nat. Acad. Sci. U. S. A. 75:1783-1787.

Jordan, E. (1964). J. Molec. Biol. 10:341-344.

Joyner, A., Isaacs, L. N., Echols, H. and Sly, W. S. (1966). J. Molec. Biol. 19:174-186.

Kaiser, A. D. (1957). Virology 3:42-61.

Kaiser, A. D. (1966). J. Gen. Physiol. 49:171-178.

Kaiser, A. D. and Jacob, F. (1957). Virology 4:509-521.

Kaiser, A. D. and Masuda, T. (1970). J. Molec. Biol. 47:557-564.

Kaiser, D. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 195-210.

Katsura, I. and Kuhl, P. W. (1974). J. Supramol. Structure 2:239-253.

Katsura, I. and Tsugita, A. (1977). Virology 76:129-145.

Katzir, N., Oppenheim, M., Belford, M. and Oppenheim, A. B. (1976). Virology 74:324-331.

Kayajanian, G. (1968). Virology 36:30-41.

Keegstra, W., Godson, G. N., Weisbeek, P. J. and Jansz, H. S. (1979). Virology 93:527-536.

Kellenberger, E. and Edgar, R. S. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 271-295.

Kellenberger, G., Zichichi, M. L. and Weigle, J. (1961). J. Molec. Biol. 3:399-408.

Kiger, J. and Green, M. H. (1964). Virology 22:602-607.

Kikuchi, A. and Gorini, L. (1975). Nature 256:621-624.

Kim, J.-S. and Davidson, N. (1974). Virology 57:93-111.

Kleinschmidt, A. K. (1968). In Methods in Enzymology, edited by S. P. Colowick and N. O. Kaplan, Academic Press Inc., New York, Vol. XIIB, 361-377.

Klotz, L. C. and Zimm, B. H. (1972). J. Molec. Biol. 72:779-800.

Kourilsky, P., Bourguignon, M.-F., Bouquet, M. and Gros, F. (1970). Cold Spring Harbor Symp. Quant. Biol. 35:305-314.

Kourilsky, P., Marcaud, L., Sheldrick, P., Luzzati, D. and Gros, F. (1968). Proc. Nat. Acad. Sci. U. S. A. 61:1013-1020.

Kumar, S., Bøvre, K., Guha, A., Hradecna, Z., Maher, S. V. M. and Szybalski, W. (1969). Nature 221:823-825.

Landy, A. and Ross, W. (1977). Science 197:1147-1160.

Lederberg, E. M. (1951). Genetics 36:560.

Lederberg, E. M. and Lederberg, J. (1953). Genetics 38:51-64.

Lee, A. S. and Sinsheimer, R. L. (1974). Proc. Nat. Acad. Sci. U. S. A. 71:2882-2886.

Lee, C. S. and Davidson, N. (1970). Virology 40:102-117.

Legrain, C., Halleux, P., Stalon, V. and Glansdorff, N. (1972). Eur. J. Biochem. 27:93-102.

Leidke-Kulke, M. and Kaiser, A. D. (1967). Virology 32:465-481.

Lennox, E. S. (1968). Virology 1:190-206.

Lewis, E. B. (1961). Cold Spring Harbor Symp. Quant. Biol. 16:159-174.

Lucad-Lenards, J. and Lipmann, F. (1971). Ann. Rev. Biochem. 40: 409-448.

Lunan, K. D. and Sinsheimer, R. L. (1956). Virology 2:455-462.

Luzzati, D. (1970). J. Molec. Biol. 49:515-519.

Lwoff, A. and Gutmann, A. (1950). Ann. Inst. Pasteur (Paris) 78:711-739.

Lwoff, A., Siminovitch, D. and Kjeldgaard, N. (1950). Compt. Rend. Acad. Sci. (Paris) 231:190-191.

Maas, W. K. (1961). Cold Spring Harbor Symp. Quant. Biol. 26:183-191.

Maas, W. K. and Clark, A. J. (1964). J. Molec. Biol. 8:365-370.

MacHattie, L. A. and Thomas, C. A. Jr. (1970). In Handbook of Biochemistry Selected Data for Molecular Biology, Second Edition, edited by H. A. Sorber, The Chemical Rubber Company, Cleveland, Ohio.

Malamy, M. H., Fiandt, M. and Szybalski, W. (1972). Molec. Gen. Genet. 119:207-222.

Mandel, M. (1967). Molec. Gen. Genet. 98:88-96.

Mandel, M. and Berg, P. (1968a). Proc. Nat. Acad. Sci. U. S. A. 60:265-268.

Mandel, M. and Berg, P. (1968b). J. Molec. Biol. 38:137-139.

Maniatis, T., Ptashne, M., Backman, K., Kleid, D., Flashman, S., Jeffrey, A. and Maurer, R. (1975). Cell 5:109-113.

Masters, M. and Pardee, A. B. (1965). Proc. Nat. Acad. Sci. U. S. A. 54:64-70.

Matsubara, K. and Kaiser, A. D. (1968). Cold Spring Harbor Symp. Quant. Biol. 33:769-775.

Matsushiro, A. (1963). Virology 19:475-482.

McCarthy, B. J. and Bolton, E. T. (1963). Proc. Nat. Acad. Sci. U. S. A. 50:156-164.

McMacken, R., Mantei, N., Butler, B., Joyner, A. and Echols, H. (1970). J. Molec. Biol. 49:639-655.

Meselson, M. (1964). J. Molec. Biol. 9:734-745.

Mizobuchi, K. and Saito, H. (1975). In Proc. of the 1975 Molec. Biol. Meetings of Japan, Dec. 9-Dec. 11, 1975, Tokyo, Japan, pp 60-62.

Morse, M. L. (1967). Genetics 56:331-340.

Morse, M., Lederberg, E. and Lederberg, J. (1956a). Genetics 41: 142-156.

Morse, M., Lederberg, E. and Lederberg, J. (1956b). Genetics 41: 758-779.

Mount, D. W. A., Harris, A. W., Fuerst, C. R. and Siminovitch, L. (1968). Virology 35:134-149.

Muller-Hill, B., Crapo, L. and Gilbert, W. (1968). Proc. Nat. Acad. Sci. U. S. A. 59:1259-1264.

Murialdo, H. and Siminovitch, L. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 711-724.

Murialdo, H. and Siminovitch, L. (1972a). Virology 48:785-823.

Murialdo, H. and Siminovitch, L. (1972b). Virology 48:824-835.

Murray, K. and Murray, N. E. (1975). J. Molec. Biol. 98:551-564.

Murray, K., Murray, N. E. and Bertani, G. (1975). Nature 254:262-265.

Murray, N. E., De Ritis, P. M. and Foster, L. A. (1973). Molec. Gen. Genet. 120:261-281.

Murray, N. E. and Murray, K. (1974). Nature 251:476-481.

Naylor, H. B. and Burgi, E. (1956). Virology 2:577-593.

Neubauer, Z. and Calef, E. (1970). J. Molec. Biol. 51:1-14.

Niwa, O., Yamagishi, H. and Ozeki, H. (1978). Molec. Gen. Genet. 159: 259-268.

Normark, S. and Burman, L. G. (1977). J. Bacteriol. 132:1-7.

Normark, S., Edlund, T., Grundström, T., Bergström, S. and Wolf-Watz, H. (1977). J. Bacteriol. 132:912-922.

Oda, K.-I., Sakakibara, Y. and Tomizawa, J.-I. (1969). Virology 39: 901-918.

Ozeki, H. and Ikeda, H. (1968). Ann. Rev. Genet. 2:245-278.

Parkinson, J. S. (1968). Genetics 59:311-325.

Parkinson, J. S. (1971). J. Molec. Biol. 56:385-401.

Parkinson, J. S. and Davis, R. W. (1971). J. Molec. Biol. 56:425-428.

Parkinson, J. S. and Huskey, R. J. (1971). J. Molec. Biol. 56:369-384.

Pero, J. (1970). Virology 40:65-71.

Pero, J. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp.559-608.

Pirrotta, V. (1975). Nature 254:114-117.

Pirrotta, V., Ptashne, M. (1969). Nature 222:541-544.

Protass, J. J., Korn, D. (1966). Proc. Nat. Acad. Sci. U. S. A. 55: 1089-1095.

Ptashne, M. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 221-237.

Ptashne, M. and Hopkins, N. (1968). Proc. Nat. Acad. Sci. U. S. A. 60:1282-1287.

Radding, C. M. and Carter, D. M. (1971). J. Biolog. Chem. 246:2513-2518.

Radding, C. M. and Echols, H. (1968). Proc. Nat. Acad. Sci. U. S. A. 60:707-712.

Radding, C. M. and Shreffler, D. C. (1966). J. Molec. Biol. 18:251-261.

Radding, C. M., Szpirer, J. and Thomas, R. (1967). Proc. Nat. Acad. Sci. U. S. A. 57:277-283.

Ray, P. and Murialdo, H. (1975). Virology 64:247-263.

Reader, R. W. and Siminovitch, L. (1971). Virology 43:607-622.

Reichardt, L. and Kaiser, A. D. (1971). Proc. Nat. Acad. Sci. U. S. A. 68:2185-2189.

Reichardt, L. F. (1975). J. Molec. Biol. 93:267-288.

Rhoades, M., MacHattie, L. A. and Thomas, C. A. (1968). J. Molec. Biol. 37:21-40.

Riley, M., Solomon, L. and Zipkas, D. (1978). J. Molec. Evolution 11: 47-56.

Roberts, J. W. (1969). Nature 224:1168-1174.

Roberts, J. W. (1970). Cold Spring Harbor Symp. Quant. Biol. 35: 121-126.

Roberts, J. W. (1975). Proc. Nat. Acad. Sci. U.S.A. 72:3300-3304.

Roberts, J. W. and Roberts, C. W. (1974). Proc. Nat. Acad. Sci. U. S. A. 72:147-151.

Rock, S., Moillo, A., Lundqvist, B., Ljungquist, E., Inman, R. B., Bertani, G. and Afzelius, B. (1974). J. Gen. Virol. 22:425-429.

Rothman, J. F. (1965). J. Molec. Biol. 12:892-912.

Russell, R. L., Abelson, J. N., Landy, A., Gefter, M. L., Brenner, S. and Smith, J. D. (1970). J. Molec. Biol. 47:1-13.

Rutberg, L. (1969). J. Virol. 3:38-44.

Sanderson, K. E. (1972). Bacteriological Rev. 36:558-586.

Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., Hutchison, C. A. III, Slocombe, P. M. and Smith, M. (1977). Nature 265:687-695.

Sanger, F., Coulson, A. R., Friedmann, T., Air, G. M., Barrell, B. G., Brown, N. L., Fiddes, J. C., Hutchison, C. A. III, Slocombe, P. M. and Smith, M. (1978). J. Molec. Biol. 125:225-246.

Sanger, F., Nicklen, S. and Coulson, A. R. (1977). Proc. Nat. Acad. Sci. U. S. A. 74:5463-5467.

Sato, K. and Campbell, A. (1970). Virology 41:474-487.

Sato, K., Nishimune, Y., Sato, M., Numick, R., Matsushiro, A., Inokuchi, H. and Ozeki, H. (1968). Virology 34:637-649.

Scherer, G., Hobom, G. and Kössel, H. (1977). Nature 265:117-121.

Schildkraut, C. L., Wierzchowski, K. L., Marmur, J., Green, D. M. and Doty, P. (1962). Virology 18:43-55.

Schimke, R. T., Alt, F. W., Kellems, R. E., Kaufman, R. J. and Bertino, J. R. (1977). Cold Spring Harbor Symp. Quant. Biol. 42:649-657.

Schimke, R. T., Kaufman, R. J., Alt, F. W. and Kellems, R. F. (1978). Science 202:1051-1055.

Schnaitman, C. A., Smith, D. and de Salas, M. F. (1975). J. Virol. 15:1121-1130.

Schnos, M. and Inman, R. B. (1970). J. Molec. Biol. 51:61-73.

Scott, J. R. (1969). Virology 36:564-574.

Scott, J. R. (1970). Virology 40:144-151.

Scott, J. R. (1971). Virology 41:66-71.

Sens, D., Natter, W. and James, E. (1977). Cell 10:275-285.

Shapiro, J., MacHattie, L., Eron, L., Ihler, G., Ippen, K. and Beckwith, J. (1969). Nature 224:768-774.

Sharp, P. A., Cohen, S. N. and Davidson, N. (1973). J. Molec. Biol. 75:235-255.

Sharp, P. A., Hsu, M.-T. and Davidson, N. (1972). J. Molec. Biol. 71:499-501.

Shaw, J. E., Bingham, H., Fuerst, C. R. and Pearson, M. L. (1977). Virology 83:180-194.

Shimada, K. and Campbell, A. (1974a). Proc. Nat. Acad. Sci. U. S. A. 71:237-241.

Shimada, K. and Campbell, A. (1974b). Virology 60:157-165.

Shulman, M. and Gottesman, M. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 477-487.

Shulman, M. and Gottesman, M. E. (1973). J. Molec. Biol. 81:461-482.

Shulman, M., Hallick, L. M., Echols, H. and Signer, E. R. (1970). J. Molec. Biol. 52:501-520.

Signer, E. R. (1964). Virology 22:650-651.

Signer, E. R. (1969). Nature 223:158-160.

Signer, E. R. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 139-174.

Signer, E. R., Beckwith, J. R. and Brenner, S. (1965). J. Molec. Biol. 14:153-166.

Signer, E. R., Manly, K. F. and Brunstetter, M. A. (1969). Virology 39:137-141.

Signer, E. R. and Weil, J. (1968a). J. Molec. Biol. 34:261-271.

Signer, E. R. and Weil, J. (1968b). Cold Spring Harbor Symp. Quant. Biol. 33:711-719.

Simon, M., Davis, R. W. and Davidson, N. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 413-428.

Sinsheimer, R. L. (1960). In The Nucleic Acids, edited by E. Chargaff and J. N. Davidson, Academic Press, New York, Vol III, pp. 187-244.

Skalka, A. (1966). Proc. Nat. Acad. Sci. U. S. A. 55:1190-1195.

Skalka, A. (1969). J. Virology 3:150-156.

Skalka, A. (1975). In Mechanisms in Recombination (1974 Gatlinburg Symposium), edited by R. F. Grell, Plenum Publishing Co., New York, pp. 421-432.

Skalka, A., Burgi, E. and Hershey, A. D. (1968). J. Molec. Biol. 34:1-16.

Skalka, A., Butler, B. and Echols, H. (1967). Proc. Nat. Acad. Sci. U. S. A. 58:576-583.

Skalka, A. and Hanson, P. (1972). J. Virology 9:583-593.

Skalka, A., Poonian, M. and Bartl, P. (1972). J. Molec. Biol. 64: 541-550.

Smith, H. O. and Nathans, D. (1973). J. Molec. Biol. 81:419-423.

Smith, O. H. (1967). Genetics 57:95-105.

Smith, O. H. and Yanofsky, C. (1962). In Methods in Enzymology, edited by S. P. Colwick and N. O. Kaplan, Academic Press, New York, Vol. 5, pp. 794-806.

Sparrow, S. H. and Nauman, A. F. (1976). Science 192:524-529.

Spencer, J. H., Rassart, E., Kaptein, J. S., Harbers, K., Grosveld, F. G. and Goodchild, B. (1978). In The Single Stranded DNA Phages, edited by D. T. Denhardt, D. Dressler and D. S. Ray, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 87-101.

Spiegelman, W. G., Reichardt, L. F., Yaniv, M., Heinemann, S. F., Kaiser, A. D. and Eisen, H. (1972). Proc. Nat. Acad. Sci. U. S. A. 69:3156-3160.

Spizizen, J. (1958). Proc. Nat. Acad. Sci. U. S. A. 44:1072-1078.

Stevens, W. F., Adhya, S. and Szybalski, W. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 515-533.

Streisinger, G. and Bruce, V. (1960). Genetics 45:1289-1296.

Streisinger, G., Emrich, J. and Stahl, M. M. (1967). Proc. Nat. Acad. Sci. U. S. A. 57:292-295.

Studier, F. W. (1969). Virology 39:562-574.

Sussman, R. and Jacob, F. (1962). Compt. Rend. Acad. Sci. (Paris) 254:1517-1519.

Szpirer, J. and Brachet, P. (1970). Molec. Gen. Genet. 108:78-92.

Szpirer, J., Thomas, R. and Radding, C. M. (1969). Virology 37: 585-596.

Szybalski, W., Bovre, K., Fiandt, M., Guha, A., Hradecna, Z., Kuman, S., Lozeron, H. A., Maher, V. M., Nijkamp, H. J. J., Summers, W. C. and Taylor, K. (1969). J. Cell. Physiol. 74:33-70.

Szybalski, W. and Szybalski, E. H. (1974). In Viruses, Evolution and Cancer, edited by E. Kurstak and K. Maramorosch, Academic Press, New York, pp. 563-582.

Takahashi, S. (1975). J. Molec. Biol. 94:385-396.

Takeda, Y., Folkmanis, A. and Echols, H. (1977). J. Biolog. Chem. 252:6177-6183.

Takeda, Y., Matsubara, K. and Ogata, K. (1975). Virology 65:374-384.

Takeda, Y., Morishita, T. and Yura, T. (1970). Virology 41:348-355.

Taylor, A. (1971). Nature New Biol. (London). 234:144-145.

Taylor, A. L. and Trotter, C. D. (1972). Bacteriol. Rev. 36:504-524.

Tessman, E. S. (1965). Virology 25:303-321.

Tessman, E. S. and Schleser, R. (1963). Virology 19:239-240.

Thomas, C. A. Jr. (1964). Cold Spring Harbor Symp. Quant. Biol. 28: 395-396.

Thomas, C. A. and MacHattie, L. A. (1967). Ann. Rev. Biochem. 36: 485-518.

Thomas, C. A. and Rubenstein, I. (1964). Biophys. J. 4:93-106.

Thomas, R. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 211-220.

Thomas, R. C. (1964). J. Molec. Biol. 8:247-253.

Thomas, R. C. (1970). J. Molec. Biol. 49:393-404.

Tomizawa, J. and Ogawa, T. (1968). Cold Spring Harbor Symp. Quant. Biol. 33:533-551.

Tonegawa, S. and Hayashi, M. (1968). Proc. Nat. Acad. Sci. U. S. A. 61:1320-1327.

Touissaint, A. (1969). Molec. Gen. Genet. 106:89-92.

Unger, R. C. and Clark, A. J. (1972). J. Molec. Biol. 70:539-548.

Unger, R. C., Echols, H. and Clark, A. J. (1972). J. Molec. Biol. 70:531-537.

Wallace, D. C. and Morowitz, H. J. (1973). Chromosoma (Berlin) 40: 121-126.

Walz, A. and Pirrotta, V. (1975). Nature 254:118-121.

Wang, J. C. and Kaiser, A. D. (1973). Nature New Biol. 241:16-17.

Watanabe, T. and Okada, M. (1964). J. Bacteriol. 87:727-736.

Weil, J. and Signer, E. R. (1968). J. Molec. Biol. 34:273-279.

Westmoreland, B. C., Szybalski, W. and Ris, H. (1969). Science 163: 1343-1348.

Wilson, D. and Crawford, I. P. (1965). J. Biolog. Chem. 240:4801-4808.

Wing, J. P. (1968). Virology 36:271-276.

Wollman, E. L. (1963). Compt. Rend. Acad. Sci. (Paris) 257:4225-4226.

Wu, R. (1970). J. Molec. Biol. 51:501-521.

Wu, R. and Taylor, E. (1971). J. Molec. Biol. 57:491-511.

Wulff, D. L. (1976). Genetics 82:401-416.

Wyatt, G. R. and Cohen, S. S. (1953). Biochem. J. 12:774-782.

Yamagishi, H. (1970). J. Molec. Biol. 49:603-608.

Yamagishi, H., Nakamura, K. and Ozeki, H. (1965). Biochem. Biophys. Res. Comm. 20:727-732.

Yanofsky, C. (1971). J. Amer. Med. Assoc. 218:1026-1035.

Yanofsky, C. and Ito, J. (1966). J. Molec. Biol. 21:313-334.

Yanofsky, C. and Lennox, E. S. (1959). Virology 8:425-447.

Yarmolinsky, M. B. (1971). In The Bacteriophage Lambda, edited by A. D. Hershey, Cold Spring Harbor Symposium Laboratory, Cold Spring Harbor, New York, pp. 97-111.

Yudkin, M. D. (1976). J. Gen. Microbiol. 92:125-132.

Yudkin, M. D. (1977). J. Bacteriol. 130:57-61.

Zaharia, O. and Soru, E. (1971). Eur. J. Biochem. 18:28-34.

Zichichi, M. L. and Kellenberger, G. (1963). Virology 19:450-460.

Zipkas, D. and Riley, M. (1975). Proc. Nat. Acad. Sci. U. S. A. 72: 1354-1358.

Zissler, J. (1967). Virology 31:189.

ABBREVIATIONS USED FOR JOURNALS

Advan. Genet.	Advances in Genetics
Amer. J. Clinical Pathol.	American Journal of Clinical Pathology
Ann. Inst. Pasteur (Paris)	Annales de 1' Institut Pasteur (Paris)
Ann. Rev. Biochem.	Annual Reviews of Biochemistry
Ann. Rev. Genet.	Annual Reviews of Genetics
Ann. Rev. Microbiol.	Annual Reviews of Microbiology
Bacteriolog. Rev.	Bacteriological Reviews
Biochim. Biophys. Acta	Biochimica et Biophysica Acta
Biochem. Biophys. Res. Comm.	Biochemical and Biophysical Research Communications
Biolog. Rev.	Biological Reviews
Biolog. Rev. Cambridge Phil. Soc.	Biological Reviews of the Cambridge Philosophical Society
Biochem J.	Biochemical Journal
Biophys. J.	Biophysical Journal
Brit. J. Exp. Pathol.	British Journal of Experimental Pathology
Carnegie Inst. Wash. Year Book	Carnegie Institute of Washington Year Book
Cold Spring Harbor Symp. Quant. Biol	Cold Spring Harbor Symposia for Quantitative Biology
Compt. Rend. Acad. Sci. (Paris)	Comptes Rendues de 1' Academie des Sciences (Paris)
Eur. J. Biochem.	European Journal of Biochemistry
G. Microbiol.	Giornale Di Microbiologia (Milan)
Genet. Res.	Genetical Research
Harvey Lect.	Harvey Lectures
J. Bacteriol.	Journal of Bacteriology
J. Biolog. Chem.	Journal of Biological Chemistry

ABBREVIATIONS USED FOR JOURNALS (Continued)

J. Cell. Physiol.	Journal of Cellular Physiology
J. Franklin Inst.	Journal of the Franklin Institute
J. Gen. Microbiol.	Journal of General Microbiology
J. Gen. Physiol.	Journal of General Physiology
J. Gen. Virol.	Journal of General Virology
J. Molec. Biol.	Journal of Molecular Biology
J. Molec. Evolution	Journal of Molecular Evolution
J. Supramol. Structure	Journal of Supramolecular Structure
J. Virol.	Journal of Virology
Microbiol. Reviews	Microbiological Reviews
Mol. Gen. Genet.	Molecular and General Genetics
Mutation Res.	Mutation Research
Nature New Biol.	Nature New Biology (London)
Proc. of the 1975 Mol. Biol. Meet. of Japan	Proceedings of the 1975 Molecular Biology Meeting of Japan
Proc. Nat. Acad. Sci. U.S.A.	Proceedings of the National Academy of Sciences (U.S.A.)