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ROLE OF DNA TOPOLOGY IN TRANSCRIPTION OF COLIPHAGE λ *IN VIVO*

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SUMMARY

Aim.

To define the role played by DNA template topology in provision of λ early gene functions *in vivo*.

Experimental System.

Kaiser Hogness transformation assay involving (a) host bacteria; (b) helper phage and (c) transfecting λ DNA.

Theory.

In the above system, transfecting whole λ DNA, which possesses two intact cohesive ends, will permit a circular template for λ transcription. Sheared λ DNA, consisting of half molecules which have only one cohesive end per fragment, is denied the possibility of circularity and can permit only linear templates *in vivo*. In order to measure gene function from the transfecting DNA, rather than simply gene presence, experimental conditions must be manipulated such that the transfecting DNA is transcribed before it recombines with the helper phage. This was accomplished by use, initially at least, of a *rec*$^-$ host, a *red*$^-$ helper phage, and *red*$^+$ DNA. Since the only source of recombination function is the wild type *red* gene located on the transfecting DNA, this DNA must be transcribed before recombination can occur. Using this technique, the ability of a half molecule to serve as a template for both *red* genes and the *N* gene was assayed. Assessment of template activity for gene *N* was based on the fact that *red* transcription is dependent on *N* product stimulation. λ*sus* NN helper phage, which is phenotypically *red*$^-$, is used to produce competent *rec*A *su*$^-$ host

cells which are then infected with half or whole DNA. The only source of N product is the gene copy on the transfecting DNA which, if transcribed and translated, will 'turn on' red gene transcription and wild type recombinants will be produced. In theory then, the measurement of wild type recombinants is indicative of transcription of the red or N genes located on the transfecting DNA template.

Conclusions.

1. Although red genes can be efficiently transcribed from a linear half molecule, gene N requires a whole molecule for its transcription. This is interpreted to reflect a need for circularity in expression of λN gene.

2. A half DNA molecule entering a gam^{+} cellular environment retains template activity. A half molecule entering a gam^{-} cellular environment is degraded before transcription can occur. The assay for gene N involves entry of the half molecule to a gam^{-} environment, resulting in degradation of the template. λgam gene product protects the half molecule from nuclease attack. A circular template is required in a gam^{-} environment as protection against nuclease degradation.

3. Linear half molecules are efficiently transcribed for both N and red genes in host cells mutant in recB. Therefore degradation of half molecules is due to recBC exonuclease activity. In vivo, recBC nuclease attacks only linear, not circular, molecules.

4. E. coli recA product affords no protection from the recBC exonuclease for the half molecule. In vivo the only host

or helper gene function controlling recBC activity on half molecules is the λgam gene product.

5. Under conditions where helper phage and transfecting DNA are coimmune but CI product is absent, tof product from the helper phage represses transcription of the transfecting DNA, inhibiting expression of the resident λred genes. This "coimmune inhibition" effect is observed at maximal levels in the assay for redB function, suggesting that considerably higher levels of redB product, as compared to levels of redX product, are required for a successful red mediated recombination event between helper phage and transfecting DNA.

6. The role of template topology in λmRNA synthesis is an indirect, but very essential one. Although the linear molecule will suffice as template for transcription by RNA polymerase, circular topology is required for protection of the template from recBC nuclease activity. This topological protection would be of maximum importance for λRNA synthesis occurring immediately after infection, since the phage DNA enters a cell containing active recBC nuclease. Gene N and the N controlled gam gene must be transcribed and translated to yield gam product which can then inhibit the recBC activity. Thus, circular topology ensures survival of the template until gam product protection is established.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the text.

Linda M. Pilarski.

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SECTION I

INTRODUCTION

The experiments to be described in this thesis were designed to elucidate the role DNA template topology plays in early λmRNA transcription. I have studied two types of template, DNA molecules capable of circular topology, and DNA molecules restricted to linear topology. The circular template might be regarded as that normally occurring in a λ infected cell, while a linear template is less likely to occur early in the vegetative cycle, under normal infection conditions. This idea is supported by the demonstrations that circular λDNA is required for λDNA replication (Schnös and Inman, 1970), that it is a major product of *de novo* λDNA synthesis (Carter, Shaw and Smith, 1970), and finally, that it appears to be necessary for late gene transcription. I have asked the following question : is the use of circular topology for early λmRNA transcription simply fortuitous, deriving from the fact that circular templates are necessary for DNA replication, or is the use of a circular template an essential feature of λmRNA synthesis, relating either to transcriptional control or to template survival in a hostile cellular environment?

I would like to divide this introduction into two main sections; firstly, a discussion of the role of circular DNA in the λ lytic cycle; and secondly, a theoretical discussion of the assay system used to study the role of template topology in transcription of λ *red* and N genes.

I. The role of circular DNA in the λ lytic cycle.

DNA derives its capacity to assume a circular form

from its cohesive ends. These single stranded 5' ends are 12 nucleotides long (sequenced by Wu and Taylor, 1971), and are complementary to each other, thereby allowing cohesion to form a hydrogen bonded circle. This can then be converted to a covalent circular form by E. coli ligase (Gellert, 1967). On infection of a sensitive or immune host, parental λDNA assumes a covalent circular form within 5 minutes after infection (Young and Sinsheimer, 1964; Bode and Kaiser, 1965). This covalent circular structure can be easily distinguished from hydrogen bonded circles or linear λDNA by sedimentation in sucrose gradients.

One might ask how these complementary cohesive ends are generated during the λ replication cycle. Mousset and Thomas (1969) have described a λ system, ter, which produces two staggered nicks in λDNA at an A-R join, yielding two single stranded ends. ter is a late function which may be enzymatically active only when it forms an integral part of the head protein packaging apparatus (Wake, Kaiser, and Inman, 1972) and thus may cut DNA concatamers at the A-R join as the DNA is packaged into the phage head.

Kaiser has suggested that the widespread occurrence of circular DNA is due to the fact that a circular structure is required for replication of DNA since circles can exhibit diverse patterns of replication, while linear forms either cannot replicate at all or replicate only once (Kaiser, 1971). An exception to this rule is phage T7 DNA which replicates as a linear

molecule (Dressler et al., 1972). The work presented in this thesis suggests that at least for λDNA, circularity plays an additional essential role in protection of the genome from exonuclease attack, until a λ system to inhibit the exonuclease is established (gam, Unger and Clark, 1972). This in turn raises the possibility that all organisms whose life cycle involves a linear DNA stage will have evolved, of necessity, a system to protect their genetic complement from the ravages of cell exonucleases. In effect, a circular DNA species may have survival value in two ways, first as a protection against exonuclease attack, and secondly, as a more efficient template for DNA replication. Circles appear to be involved in several aspects of the lambda vegetative cycle; I will now discuss the role of circles in (a) λDNA replication, (b) late gene mRNA transcription, and (c) early mRNA transcription.

(a) Role of Circular DNA Topology in λDNA replication.

Circular λDNA plays a prominent role as a replicating form in both early and late phases of λDNA replication, and as a *de novo* product of λDNA synthesis. Electron micrographs of circular replicating λDNA molecules have been published by Ogawa *et al.* (1968); Carter *et al.* (1969), and Schnös and Inman (1970). Schnös and Inman (1970) have also demonstrated that λ replication proceeds in both directions around the circular template. These electron micrographs are the most convincing proof that circular molecules are utilized as a template for λDNA replication during the early phase of replication. Similar

evidence involving circular DNA in the λDNA replication process at early times comes from zone sedimentation profiles of replicating DNA. Infecting parental DNA becomes associated with the host cell membrane, and during the first 20 minutes of the infection process replication is limited to those membrane genomes. (Salivar and Sinsheimer, 1969). Analysis of these parental DNA forms revealed that no linear λDNA was bound to the membrane, the majority species being circular forms, suggesting that only circular λDNA species are able to replicate early in the vegetative cycle (Salivar and Gardinier, 1970).

Replicating λDNA species have been examined in detail by several groups of scientists (Young and Sinsheimer, 1967; Salzman and Weissbach, 1967; Carter _et al_., 1970; Mackinlay and Kaiser, 1969; and Skalka _et al_., 1972). The general conclusion is that λDNA replication occurs in two stages, an early phase in which mainly circular species are synthesized from an _E. coli_ type replicating circular template (Schnös and Inman, 1970; Cairns, 1963); and a second phase whose main product is linear concatenated molecules which eventually are packaged as monomeric units. These concatanated forms appear to be synthesized by a rolling circle mechanism (Kiger and Sinsheimer, 1969). After fractionating replicating λDNA on benzoylated-napthoylated DEAE cellulose columns, and then doing sedimentation analysis of the fractions, Kiger and Sinsheimer (1969) concluded that the replicating form which yielded concatenates consisted (after denaturation) of three pieces of DNA: a single monomer length

circle, a longer linear DNA molecule (possibly a dimer in length), and a monomer length linear DNA molecule. They suggested that concatamer synthesis is initiated on an open circle of λDNA.

After infection, λDNA replicates semi-conservatively producing covalent circular structures one monomer unit in length (Young and Sinsheimer, 1967). Carter et al., (1969) demonstrated that by 15 minutes after infection a pool of closed circles are formed which comprised 30% of the total early DNA synthesized during that time period. Their technique was to pulse label for 2 min beginning at 5 minutes after infection and look at the changing distribution of this label as DNA synthesis progressed. At 7 minutes after infection only a small proportion of the label sedimented in the region for closed circles. By 14.5 minutes, the label had accumulated in the closed circular fraction as measured by both neutral and alkaline sucrose gradients. This circular DNA pool, made early in the infectious cycle, remained constant at 20 phage equivalents per bacterium during the latent period. The closed circles were not degraded and label present in closed circles never appeared in progeny phage particles (Carter et al., 1969; Carter and Smith, 1970).

Models for synthesis of late λDNA forms generally depend on a nicked circular template from which newly synthesized linear DNA peels off - i.e. the rolling circle model (Gilbert and Dressler, 1968; Kiger and Sinsheimer, 1969). Since parental λDNA assumes a closed circular form after infection,

a nicking enzyme is necessary for conversion of closed to open circles. Schuster and Weissbach (1969) and Boyce *et al.*, (1969) have both demonstrated the presence of an endonuclease, which causes loss of closed circular species, coded for by the phage genome. A contradiction existed between these two sets of experiments as to whether genes *O* and *P*, or gene *N*, were responsible for the nicking action. Thirion (1971) has resolved this contradiction and demonstrated that accumulation of closed circular species is prevented solely by the *O* and *P* gene products. Loss of the *O* or *P* gene function by mutation also prevents λDNA synthesis (Joyner *et al.*, 1966; Ogawa and Tomizawa, 1968).

Circumstantial evidence that a circular template is required for λDNA replication derives from the fact that all autonomously replicating λ plasmids studied exist in a closed circular form. λ*dv*, a plasmid derived from λ*vir*, has been shown by Matsubara and Kaiser (1968) to be a circular structure. In *Salmonella typhosa* - *Escherichia coli* hybrids, λ prophage exists as an independently replicating plasmid rather than as part of the bacterial replicon. λdg prophage in these conditions assumes a covalently closed circular form, as demonstrated by alkaline sucrose gradient analysis of lysogenic *E. coli* - *S. typhosa* hybrids (Falkow and Baron, 1970).

(b) Role of the Circular Template in λ Late Gene Expression

The main evidence implicating circular molecules as the template for late genes *S*, *R*, and *A*-*J* stems from the work of Herskowitz and Signer (1970) who examined a set of lysogens

carrying deletions extending into the resident prophage. Mapping studies using this family of prophage deletions revealed the existence of a site between Q and S necessary for expression of late gene R, located on the right half of the vegetative map, and of late genes F and K, located on the left half of the vegetative map. They suggest that the only region where RNA polymerase can initiate late gene transcription is between Q and S, implying that during the λ infectious cycle, the cohesive ends must be joined at the time late genes are transcribed so as to link left half genes A-J to the initiation site on the right half of λDNA.

In addition, to having right and left halves of the genome linked by joining of cohesive ends, a template for late gene transcription might be expected to have three other important characteristics if maximal synthesis of late proteins is to occur. Firstly, it should be detectable in the cell at the time late proteins begin to appear. Secondly, multiple copies of the template should exist, since most late proteins must be synthesized in stoichiometric amounts. And thirdly, the template should be stable throughout the time period covered by late protein synthesis.

Two possible template forms could serve as late gene template. The closed circles synthesized during the first 15 minutes of infection are an obvious candidate; however the end joined concatamers made late in λ infection also meet the requirement for linkage of right and left halves of the λ genome.

The concatamers seem an unlikely possibility since they are only present in significant numbers in the cell at times much later than after the beginning of late protein synthesis. In addition any multimer DNA units being actively packaged into phage heads are unlikely to be available for transcription. Carter and Smith (1970) have shown that 15% of the total λDNA synthesized is in the closed circular form which is never packaged into mature phage, and the timing of closed circle synthesis coincides very neatly with the beginning of late protein synthesis. The circles begin to appear by 7 minutes after infection and reach their peak between 15-25 minutes after infection (Carter et al., 1969). McMacken et al., (1970) found detectable levels of tail antigen occurring at 18-24 minutes after infection. E gene protein appears between 15-20 minutes after infection (Hendrix, 1970), and λ lysozyme between 20-25 minutes after infection (McMacken et al., 1970). The template for late gene synthesis must be stable since both tail antigen and lysozyme continue to be made throughout the latent period (35-40 minutes after infection).

All three of the above mentioned conditions, 1- the presence of the template within the cell at about 15 minutes after infection, 2- a stable template present throughout the latent period, and 3- multiple copies of the template, are met by the closed circular DNA species described by Carter et al. and by many others. The closed circles are stable and present at 20 phage equivalents per bacterium by 15 minutes after infection, at which time late proteins begin to appear. It is

likely therefore that closed circular DNA molecules serve as templates for late gene transcription.

(c) Circumstantial evidence for the role of circular DNA as a template for early gene transcription.

After heat induction of a superinfected temperature sensitive λ lysogen, the superinfecting λDNA becomes associated with the host cell membrane. Most of this membrane bound DNA is in the covalent circular form. Essentially 100% of the superinfecting DNA is bound to the membrane within 5 minutes after release of repression (Hallick et al., 1969). Other workers further investigating this phenomonen have discovered that membrane association involves a binding site located on the right half of λDNA (Sakaki et al., 1971); more specifically, the binding of λDNA to the membrane requires active transcription of the x region of λDNA in an immune host (Sakakibara & Tomizawa, 1970; Nishimoto & Matsubara, 1971), or transcription of the x or N-int operon in a sensitive host (Kolber & Sly, 1971). All three sets of authors conclude that active λ transcription is a prerequisite for membrane binding. One can correlate the results of Hallick et al., that covalent circles bind to the membrane rapidly after release of repression, with the studies showing the requirement for transcription in membrane binding and conclude that covalent circular templates are being transcribed *in vivo* at early times. More specifically, early operons N-int and xOP are being transcribed from a covalent circular template.

A second line of evidence that RNA polymerase binds

to a covalent circular template for early mRNA initiation comes from the experiments of Hayward and Green (1969). RNA polymerase - λDNA complexes were allowed to form in vivo; these complexes were then purified and, on analysis, found to consist of RNA polymerase molecules bound to a covalent circular λDNA molecule. This RNA polymerase which had bound to the template in vivo, was then allowed to transcribe λmRNA in vitro. Deletion hybridization studies of the in vitro synthesized mRNA indicated that 48% of the RNA was derived from the x and N operons. Since the isolated complexes consisted of covalent circular DNA plus RNA polymerase, bound in vivo, one can conclude that, in vivo, circular DNA serves as a template for transcription initiated at promotors for the N and x operons.

Although these experiments indicate that a closed circular template is likely to be used for mRNA synthesis in vivo, there is no evidence to show whether this is an essential feature of λmRNA synthesis. I have designed experiments to investigate the role of the circle as template for transcription, by investigating the ability of linear and circular DNA molecules to provide gene function, in vivo, in a transformation assay.

II. The Transformation Assay.

A transformation assay was developed by Kaiser and Hogness in 1960 as a means of assay for the presence of a given gene in a purified sample of λDNA. The technique involves preinfection of the host bacteria with helper phage to yield a cell competent to be infected with purified DNA. For DNA to be

infective, it must possess a free cohesive end which is complementary to a cohesive end of the helper phage; for instance lambdoid phages can help and be helped by all members of the lambdoid class, however non-lambdoid phages P2 and 186, which help each other, cannot help or be helped by the lambdoid phages (Kaiser and Wu, 1968) (Mandel, 1967) (Mandel & Berg, 1968). Since the assay depends absolutely on the nature and presence of a free cohesive end on the transfecting DNA (Kaiser & Wu, 1968) (Strack & Kaiser, 1965), it has proved an extremely useful tool for analyzing the properties of intracellular replicating λDNA (Dove & Weigle, 1965) (Mackinlay & Kaiser, 1969) and for studies on the nature of cohesive ends (Kaiser & Inman, 1965) (Strack & Kaiser, 1966) (Wu & Kaiser, 1968).

As a background to appreciating the value of the transformation assay, which has been used extensively in this thesis, I will briefly discuss three of its applications; 1- in the study of λDNA replication and cohesive ends, 2- as a means of precise physical mapping of λ genes, and 3- to assess the role of DNA topology in various λ functions.

The requirement for a cohesive end in the transformation assay was convincingly demonstrated by shearing λDNA into halves and sixths. Kaiser and Inman (1965) looked at ability of a DNA preparation to donate $\underline{A}^+\underline{B}^+$, $\underline{imm}^\lambda$, and $\underline{Q}^+\underline{R}^+$ markers by recombination with a defective helper phage. If the DNA was sheared such that only half molecules were present, $\underline{A}^+\underline{B}^+$ and $\underline{imm}^\lambda$ $\underline{Q}^+\underline{R}^+$ markers could be rescued; however shearing into sixths resulted in a

loss of ability to recover the imm$^{\lambda}$ marker. Since this marker would be separated from a cohesive end in sixth molecules, while both $A^{+}B^{+}$ and $Q^{+}R^{+}$ would remain on fragments possessing cohesive ends, the authors concluded that a free cohesive end was essential for successful donation of genetic markers to a helper phage. Exposure of sheared half or sixth molecule preparations to annealing conditions coheres the ends and results in the loss of ability to donate any of the markers. Alteration of cohesive ends by DNA polymerase or exonuclease III enzymic activity also alters infectivity. If cohesive ends were rendered double stranded by DNA polymerase "filling in", infectivity was lost. Restoration of a single stranded end by exonuclease III treatment restored infectivity (Strack & Kaiser, 1966).

Transformation studies on intracellular λDNA forms have demonstrated that infective DNA appears late in the latent period shortly before progeny phage particles can be detected (Dove & Weigle, 1965), suggesting that cohesive ends are generated late in the infectious cycle. Initially, DNA lacking cohesive ends is synthesized (probably the circular DNA detected by Carter et al., 1969). At late times, when cohesive ends normally appear, λ head mutants fail to yield infective DNA species, suggesting that generation of cohesive ends is dependent on a proper sequence of head assembly (Mackinlay & Kaiser, 1969). More recent studies have used the transformation assay to analyze this noninfective DNA accumulation in λ head mutants and the inability to produce cohesive ends. Although these mutant extracts con-

tain the end cutting activity, it appears to be non-functional <u>in vivo</u> and unable to cut single stranded ends from newly synthesized λDNA. This suggests that end cutting function is active only as part of the head protein assembly and the non-infective accumulation of DNA corresponds to multi unit concatamers (Wake et al., 1972).

In addition to its valuable contributions in the field of physical structure of the λDNA molcule, the transformation assay has been productively used to physically map λ genes on the λ chromosome. Early studies on distribution of genetic markers on sheared half molecule preparations were convincing demonstrations of the colinearity of physical and genetic maps of λ (Kaiser, 1962) (Radding & Kaiser, 1963) (Hogness & Simmons, 1964). Development of extremely sensitive methods for separation of populations of fragments with right or left cohesive ends, had made very sophisticated gene mapping studies possible. Egan and Hogness (1972) did density gradient separation of their sheared DNA preparations to resolve right and left half peaks. Application of greater shearing speeds yields molecules consisting of a family of sizes; a graduated series of molecules bearing a right cohesive end can be prepared by zone sedimentation. Egan and Hogness then looked at the distribution of a given λ gene within this family of fragments. With this technique they were able to very accurately map 6 amber mutations of λ genes located on the right half molecules. The principal advantage to using the transformation assay for mapping studies is the ability to map

point mutations, in a given λ gene, fairly precisely on the λ chromosome.

A third application of the transformation technique has been used to determine effects of DNA topology on various λ functions. Cohn (1972) analysed the ability of λ half molecules to serve as a substrate for int catalysed site specific recombination and discovered that successful int recombination was possible between a helper phage DNA and a half molecule only in conjunction with a generalised recombination event (i.e. rec or red promoted). He concludes that int activity requires two circular DNA substrates if viable recombinants are to result. Cohn's results suffer from the fact that his efficiency of infection for half molecules as compared to wholes is never more than 12% and in some hosts drops to as low as only 2% for wild type right halves and red^{+} helper phage. With this reservation however, the results are convincing. Similar studies have been conducted by Weisberg, Gottesman, and Little (pers. Com.) who also concluded that integrase requires circular DNA substrates.

A very productive approach to studying the parameters of a biological function, is to force the organism to use a substrate not normally available *in vivo*, and then analyse its response. I have used the transformation assay as a means of analyzing the effects of altered template topology on early λmRNA transcription. Since purified DNA is biologically active in this system, the purified DNA can be physically altered and then presented to a competent cell as the only source of a given gene

function. It is important to note that the assay measures gene function not simply gene presence. The system was challenged with a linear DNA template in conditions where no viable phage particles could be produced unless the linear transfecting DNA was transcribed and translated.

As a source of a template capable of circularization, I used whole λDNA molecules, which possess two intact cohesive ends. As a template that will, of necessity, remain linear *in vivo*, I used λDNA sheared into half molecules which have only one cohesive end and thus no potential to circularize. There was no necessity to separate right and left DNA halves since the functions to be assayed were located solely on the right half of the λ genome. In addition, the multiplicity of DNA infection per cell was such that the majority of cells received only one fragment of DNA, with only those receiving a right half fragment scoring in the assay.

In order to implement the approach stated above, two conditions must be met. First, the linear half molecule must be transcribed before any recombination events can incorporate it into a whole, and therefore circular, template. The second requirement of the system is that the helper phage be unable to plaque on the selective indicator unless gene function has been provided by the half molecule. Assaying for λ*red* function fulfils both conditions, ensuring transcription of the infecting DNA before recombination can occur by making the linear DNA the only source of recombination function. Then no recombination

will occur until the *red* genes of the half molecule have been transcribed and translated. In this way, I can assay ability of the half molecule to serve as a template for early λ genes (*red*), and measure the efficiency with which this occurs, by scoring helper phage-transfecting DNA recombinants catalysed by *red* function encoded on the transfecting linear DNA template. Helper phage were constructed which were *red*$^-$ either by deletion of the *red* genes or through point mutation in *red*B. Host bacteria were *rec*A to remove bacterial recombination systems. Since Cohn (1972) has demonstrated that viable half molecule-helper phage recombinants are not generated by *int* product alone, I have assumed that recombinants were produced either by *red* product alone, or by *int* and *red* products acting in concert. The second condition, an inability of the helper phage to plaque on the indicator, was met by constructing genetically marked helper phage. Helper phage were marked with either *sus*R mutations or *sus*N mutations and the selective indicator was *su*$^-$. Only wild type recombinants will plate on the *su*$^-$ indicator, the only source of a wild type *R* or *N* gene being the gene copy on the transfecting DNA molecule.

Two early genes were tested for ability to be transcribed from a linear template, genes *N* and *red*. Briefly, the assay for *red* gene is as described above. A *rec*A bacterium made competent with λ*red*$^-$ *sus*R helper phage is infected with half DNA molecules. If the half is transcribed for its resident *red* genes, the resulting *red* product will catalyze a recombination event

between helper and half molecule to yield a wild type R^{+} recombinant which will plaque on su^{-} indicator.

The assay for N gene is less direct, based on N dependent red transcription. A recA su^{-} bacterium is made competent by λsusN helper phage. This helper is phenotypically red^{-}. The transfecting DNA is red^{+} N^{+}; therefore if the N gene on the half molecule is transcribed, the resulting N product will "turn on" transcription of the red genes located on either helper or half molecule. This red product will then catalyze recombination between helper and half to yield a λN^{+} recombinant which will plate on su^{-} indicator. In summary, unless the N gene on the half molecule is transcribed, no wild type recombinants will ensue.

This thesis will be divided into three main sections.

1. Experiments which show that red gene function can be rescued from a half molecule and, in contrast, that N gene function cannot be rescued from a half molecule.

2. An analysis of the factors influencing the inability of the half molecule to act as template for gene N, concluding that lack of activity is due to preferential degradation of the half molecule by recBC nuclease in the absence of λgam product, which is under N gene control.

3. A study of the influence of redX, redB, and tof products on recombination of half molecules in rec^{+},

recA, and *recArecB* host cells.

SECTION II

MATERIALS

(a) Media and buffers

H1	0.1M KPO_4 pH7.0, 0.015M $(NH_4)_2SO_4$, 0.001M $MgSO_4$, 1.8×10^{-6}M $FeSO_4 \cdot 7H_2O$. Supplemented with glucose or Maltose and strain dependent growth requirements.
TB	1.0% Difco Tryptone, 0.5% NaCl (Kaiser, 1965).
LB	1% Difco Bactotryptone, 0.5% Bacto yeast extract, 1% NaCl.
Soft Agar	TB + 0.7% Difco Bactoagar.
Plates	TB + 1.5% Difco Bactoagar. H1 + 1.5% Difco agar + 0.2% glucose.
TM(λdil)	.01M $MgSO_4$, .01M Tris pH 7.1.
TE	.01M Tris pH 8.0, .001 Na_2EDTA.
Mg-PO_4	.01M $MgSO_4$, .01M KPO_4 pH7.0.
TCM	.01M Tris pH7.1, .01M $MgCl_2$, .01M $CaCl_2$.
I medium	.01M Tris pH7.1, 6×10^{-5}M $MgCl_2$, .006% glucose, 6×10^{-4}M KPO_4 pH7.0, 5×10^{-4}M $(NH_4)_2SO_4$, 4×10^{-10}M $FeSO_4 \cdot 7H_2O$.

All chemicals in these buffers were of analytical reagent grade. Buffers were prepared from sterile glass distilled water to which was added appropriate amounts of sterile stock solutions, also

made with glass distilled water and autoclaved at 20 psi for 25 minutes.

Chemicals

$CHCl_3$ - Analar A.R.

CsCl - British Drug House A.R.

NNG - N-methyl, N-nitroso guanidine, Aldrich Chemicals

Phenol- Redistilled and stored at -15℃ away from light. Mallinkrodt A.R.

DNase - Sigma Grade B.
A stock solution at 30 μgm/ml in 10^{-2}M Tris pH7.1 was prepared and stored at freezer temperature.

Trimethoprim - Calbiochem Grade B.
This was dissolved in deionized H_2O acidified with 1NHCl until solution occurred. A stock solution remained active over a 30 day period when kept refrigerated.

Thymine - Made up at 2.5 mg/ml stock; sterilised by filtration and stored at refrigerator temperature. Sigma.

Streptomycin Sulfate - Drug Houses of Australia, Adelaide, S.A.
Made from sterile vials in sterile H_2O at 100 mg/ml and stored at refrigerator temperature.

Dialysis Tubing - Size 18/32 Visking Seamless cellulose

tubing, Union Carbide.

U.V. irradiation - General Electric 15 watt lamp at a distance of 50 cm.

(b) Bacterial Strains (E. coli K12 derivatives)

Strain	Genotype	Source	Obtained from
152	recA56 su^-	Meselson Ref: Gottesman & Yarmolinsky (1968)	D. Hogness
W3350	rec^+ su^-	Campbell (1961)	D. Hogness
R^-A9605	rec^+ suIII	Yanofsky & Ito(1966)	A.D. Kaiser
1200	$rec^+ su_{II} Thi^- Endo_I RNase^-_I$	Hoffman-Berling	H. Echols
JC5495	recA13recB21su^- $thr^- leu^- pro^- arg^- his^- thi^- mal^-$	Willetts & Clark (1969)	J. Clark
DM22	recB21 su^-	J. Clark	J. Clark
E835	recA recB21 su^-	This work	
152 (imm^{434})	recA56 su^-	This work	
152 (CI_{857})	recA56 su^-	This work	

Bacterial Strains Cont'd.

Strain	Genotype	Source	Obtained from
C600	su_{II} thr^- leu^- thi^-	Appleyard (1954)	D. Hogness
QR48	recA13 su_{II}-	Signer & Weil (1968)	H. Echols
MS501	su^- λcrp	J. Shapiro	H. Echols
C600(imm^{λ} $susQ_{21}$)	su_{II}	H. Echols	J. Davison
C600(imm^{λ} $susQ_{21}$)(imm^{434} $susR_{60}$)	su_{II}	This work	
C600(imm^{434} $susR_{60}$)	su_{II}		D. Hogness
W3350(imm^{434} $susR_{54}R_{60}$)	su^- str^r		D. Hogness
C600(imm^{434} $susA_{11}B_1$)	su_{II}	This work	
W3350(imm^{434} $susA_{11}B_1$)	su^- str^r	This work	
W3101(λCI_{857})($\lambda dgCI_{857}$)	λ^r	J. Davison	J. Davison
C600 (P2)	su^+	W.H. Woods	W.H. Woods

Bacterial Strains Cont'd.

Strain	Genotype	Source	Obtained from
W3350 (P2)	su^-	C.A. Bradley	C.A. Bradley
E836	152 thy A	This work	
JC5412	recB Hfr	Willetts & Clark(1969)	N. Willetts

(c) Bacteriophage Strains – λ derivatives

Genotype	Source	Use in this work
CI$_{857}$susN$_{7}$N$_{53}$	J.B. Egan	Helper phage
imm^{434}CI susN$_{7}$N$_{53}$	This work	Helper phage
imm^{434}bio72gam$_{3}$susN$_{7}$N$_{53}$	This work	Helper phage
CI$_{857}$susR$_{54}$R$_{60}$	This work	Helper phage
CI$_{857}$redB$_{114}$susR$_{60}$	This work	Helper phage
CI$_{857}$redB$_{114}$int$_{6}$susR$_{60}$	This work	Helper phage
imm^{434}CIredB$_{114}$susR$_{54}$R$_{60}$	This work	Helper phage
CI$_{857}$redB$_{270}$susR$_{54}$R$_{60}$	This work	Helper phage
imm^{434}CIredB$_{113}$susR$_{54}$R$_{60}$	This work	Helper phage
CI$_{857}$redB$_{113}$susR$_{60}$	This work	Helper phage
CI$_{857}$redXB$_{3}$susR$_{60}$	J.B. Egan	Helper phage
CI$_{857}$redX$_{504}$susR$_{60}$	This work	Helper phage
CI$_{857}$bio 7-20 susR$_{60}$	This work	Helper phage
CI$_{857}$bio 7-20 redXB$_{3}$susR$_{54}$R$_{60}$	This work	Helper phage
CI$_{857}$bio 72 susR$_{60}$	This work	Helper phage
imm^{434}CI bio 72 susR$_{54}$R$_{60}$	This work	Helper phage

Bacteriophage Strains Cont'd.

Genotype	Source	Use in this work
$imm^{434}CI bio72\ sus\ gam_{14} susR_{54}R_{60}$	This work	Helper phage
$imm^{434} bio72\ susR5$	H. Echols	Helper phage
$CI_{857} bio\ 11\ susR_{60}$	This work	Helper phage
$CI_{857} bio\ 1\ susR_{60}$	This work	Helper phage
$imm^{434}CI\ bio\ 1\ susR_{60}$	This work	Helper phage
$imm^{434}CI\ bio\ 10\ susR_{54}R_{60}$	This work	Helper phage
$imm^{434}CI\ susA_{11}B_{1}$	Kaiser & Inman(1965)	Helper phage
$imm^{434}CI\ sus\ gam_{210} susR_{54}R_{60}$	This work	Helper phage
$imm^{434}CI\ gam_{5} susR_{54}R_{60}$	This work	Helper phage
$CI_{857} redB_{114}$	H. Echols	Source of purified λDNA; construction of Helper phage
CI_{857}	D. Hogness	Source of purified DNA
$imm^{21}C_{ts}$	L.Reichhardt	Source of purified DNA
$V_1V_2V_3$ (λvir)	Jacob & Wollman(1954)	" " " " " "

Bacteriophage Strains Cont'd.

Genotype	Source	Use in this work
CI_{857}<u>sus</u>P_{228}	R.Thomas(1970)	Source of purified DNA
CI_{857}<u>red</u>B_{114}<u>sus</u>P_3	This work	" " " " " "
CI_{857}<u>bio</u> 72 <u>sus</u>P_{228}	This work	" " " " " "
CI_{857}<u>sus</u>J_6	H. Echols	" " " " " "
$\lambda dg_{A-J}CI_{857}$	J. Davison	" " " " " "
<u>imm</u>^{434}<u>sus</u>$R_{54}R_{60}$	D. Hogness	Construction of Helper phage
C^+<u>sus</u> <u>red</u>B_{270}	E. Signer	" " " " " "
C^+<u>red</u>B_{113}	H. Echols	" " " " " "
C^+<u>red</u>A_{329}	H. Echols	" " " " " "
CI_{857}<u>red</u>B_{114}<u>int</u>$_6$	H. Echols	" " " " " "
CI_{857}<u>bio</u> 7-20 <u>red</u>$_3$	H. Echols	" " " " " "
C^+<u>bio</u> 7-20	A.D. Kaiser	" " " " " "
C^+<u>bio</u> 72	A.D. Kaiser	Construction of Helper phage and Phage for DNA
C^+ <u>bio</u> 11	A.D. Kaiser	Construction of Helper phage

Bacteriophage Strains Cont'd.

Genotype	Source	Use in this work
CI_{857} *bio* 1	H. Echols	Construction of Helper phage
imm^{434} CI *bio* 10	H. Echols	" " " " " "
C^{+} *bio* 72 *sus* *gam* 14	This work	" " " " " "
C^{+} *bio* 72 *gam*$_{3}$	This work	" " " " " "
CI_{2047}*sus*P_{3}	R. Thomas	Construction of Phage for DNA
CI_{857}*sus*$A_{11}B_{1}$	W. Dove	Construction of *imm*^{434}sus$A_{11}B_{1}$
imm^{434}CIII$_{co2}$	H. Echols	" " " " " " " "
imm$^{\lambda}$ *gam*$_{5}$	J. Zissler (1971a)	Construction of Helper phage
imm$^{\lambda}$ *sus* *gam*$_{210}$	"	" " " " " "

Legend - All λ *red* mutants are described in Schulman et al.,(1970)

All λ *bio* mutants are described in Manly et al., (1969)

SECTION III

METHODS

METHODS

1. Construction of Bacterial Strains.

(a) Construction of Bacterial lysogens :

The bacterial strain to be lysogenized was grown in TB to an $A_{600} = 2.0$ (or an overnight culture was used). The cells were chilled, then pelleted at 6000 rpm for 10 minutes, resuspended in an equal volume of $Mg-PO_4$, and incubated at 39°C for 15 minutes; infection was at a multiplicity of approximately 10 (cell titer was assumed to be about 1.5×10^9 cells/ml) and was allowed to proceed for 20 minutes at 39°C. This incubation mixture was diluted 10^{-2} in TB, incubated for 5 minutes at 39°C, and plated for survivors on TB plates by the spreading technique. The lysogens among the survivors were selected by stabbing single colonies into both a master TB plate and TB plate containing 10^9 coimmune phage (either λ_imm_^{434}CI or λC_{71}). Colonies resistant to homoimmune superinfection were taken from the master plate and purified through three single colony isolations. Final isolates were characterised by four tests:

(a) immunity to homoimmune superinfection.

(b) sensitivity to λ_vir_ infection.

(c) ability to complement a heteroimmune defective phage.

(d) ability to release phage by spontaneous induction.

(b) Construction of a recA recB su$^-$ host (E835) by Conguga-tion Techniques :

Strain 152 (recA su$^-$str^r) was made thy A by tri-methoprim (TMP) selection as follows: 152, grown over-night in H1 + 0.2% glucose + 50 μgm/ml thymine + 20 μgm/ml trimethoprim and allowed to grow at 37° until cell growth could be observed (about 72 hours) (Stacey & Simson, 1965). These cells were plated on minimal H1 + glucose plates, and H1 + glucose + 50 μgm/ml thy-mine plates; nearly 100% were thy$^-$. Single colonies were purified through three single colony isolations on minimal agar. The final isolate showed no growth in the absence of thymine or on 5 μgm/ml of thymine but grew well if 50 μgm/ml thymine was added to the growth medium. For conjugation, 152 thyA was used as a recipient strain. Hfr recB21str^s (JC 5412) was ob-tained from N. Willetts for use as donor strain (Willetts & Clark, 1969).

Donor and recipient were grown overnight in LB at 37°C, diluted 1:100 in LB and grown for 2 hours at 37°C. For mating, a ratio of 1 donor cell per 10 recipients was used. Donor-recipient mix was swirled gently for 5 minutes at 37°C, allowed to remain stationary for 15′ at 37°C, and then vigorously interrupted for 15 sec. (Woods & Egan, 1972). str^r thy$^+$ recombinants were selected by plating for single colonies on minimal

H1 + glucose + streptomycin 200 μgm/ml. *rec*A *rec*B mutants among the *str*^{r} *thy*$^+$ recombinants were detected by testing isolates for (a) sensitivity to 150 ergs/$_{mm}2$ of UV irradiation, characteristic of *rec*A, and (b) sensitivity to λ*spi* infection, characteristic of *rec*B. Those colonies which were killed by both 150 ergs/$_{mm}2$ of U.V. and λ*spi* were further purified. One of these was more extensively tested and proved to have characteristics of a double *rec*A *rec*B recombinant. It was purified through three single colony isolations, and given strain number E835.

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	no U.V.	U.V. + 150 ergs/mm^2	% survival
E835	1.8x10^9 viable cells/ml	1.9x10^3 viable cells/ml	10^{-4}

	Plating efficiency of λbio 10 imm^{434}CI	
W3350	1.0	(*rec*$^+$)
152	$< 10^{-7}$	(*rec*A)
JC5495	1.0	(*rec*A *rec*B)
E835	1.0	

======

2. Assay of bacteria and bacteriophage

(a) Bacteria. Cultures to be assayed were diluted in H1 and aliquots spread on a TB plate with an alcohol sterilized glass spreader. Plates were then incubated overnight at 30°C or 37°C.

(b) Assay of phage.

Phage stocks were diluted in TM. Indicator bacteria were grown to log phase (A_{600} 0.8 - 1.5) and chilled. TB soft agar was dispensed into tubes (3 ml/tube) and held at 47°C. 0.2 ml. of indicator bacteria and 0.1 of phage dilution were added to the soft agar containing tubes and the whole mixture poured onto a TB agar plate. Plates were then incubated overnight at 30°C or 37°C.

3. Construction and characterization of bacteriophage strains :

(a) Recombination Technique:

C600 was grown to A_{600} = 1.5, pelleted, and resuspended in 2x volume of $Mg-PO_4$. The two parental phage, both at a multiplicity of 10, were mixed together and the C600 host added to the phage mixture. Incubation of this mix was at 39° for 20 minutes. To promote recombination, the mixture was pipetted into a petri dish and U.V. irradiated for 50 seconds, with a 15 watt lamp at a distance of 50 cm., while being shaken on a rotary platform. The recombination mixture was diluted 10 fold with TB, further diluted 10^{-2} into TB, shaken at 37° for 90 minutes, chilled and chloroformed. Progeny phage were plated at final dilutions of 10^{-3} and 10^{-4} on appropriate selective indicators. All indicators used were fresh log phase cultures grown in TB.

(b) Selection Techniques:

1. Selection of susR recombinants - Progeny phage were plated on su^+ indicator on thick TB agar in glass petri dishes (about 50 ml of TB agar per plate) and incubated overnight at 37°. Plates were then inverted over chloroform for 20 minutes at 37°, removed to room temperature and exposed to air for at least 60 minutes. λsusR mutants are characterized by an absence of halo around the plaque and stand out as sharp distinctly outlined plaques in comparison to the fuzzy, haloed wild type plaques. These non-haloed plaques can then be stabbed into various indicator plates for further characterization.

λsusR mutations were further characterized by complementation tests to determine presence of $R_{54}R_{60}$ or a single R_{60}. Phage were diluted to 10^7 phage/ml. in λdil and spotted onto a nonpermissive W3350 indicator lawn. Known stocks of susR_{54}, susR_{60} and sus$R_{54}R_{60}$ were also diluted to 10^7/ml and spots of these known mutants were superimposed on the spots of phage to be tested. An area of lysis indicated a positive backcross.

2. Use of the fec phenotype as a selection technique.

A great many of the phage constructed were of the red^- susR genotype. A powerful selection technique was used for crosses of the character λred^- c^+x λbio10 imm^{434} CI sus$R_{54}R_{60}$ where the λred^- is fec^+ and λbio10 is fec^-.

Progeny of this type cross were plated on QR48 which does not plate the *bio*10 parent. By combining this QR48 indicator with the $CHCl_3$ technique for *sus*R mutants it is possible to efficiently select the red^- *sus*R recombinant in one plating step. Observation of clear or turbid plaques even allows selection of immunity marker in this step. λ*sus*N_7N_{53} imm^{434}CI was constructed from *bio*10 imm^{434}CI x *sus*$N_7N_{53}CI_{857}$ by selecting clear fec^+ recombinants on QR48 at 30°C.

3. With some crosses performed, no initial selection was possible so progeny phage were plated on C600 and large numbers of plaques were tested for the appropriate genotype by stabbing into various indicator plates. Stabbing was done with sterile round toothpicks and careful alignment of indicator plates to be stabbed, i.e., indicators on which no growth was expected were stabbed first, then the C600 master plate, and lastly lysogenic indicators.

4. Selection of red^- *sus*P or *sus*R recombinants by stabbing into a mixed indicator of MS501 : W3350 at a ratio of 1/10.

This technique was very useful for selection of red^-*sus*P, but only marginally so for red^-*sus*R, and is a modification of a technique described by Echols & Gingery (1968) . Phage which are red^+*sus*P

produce an area of lysis around the stab, while *red*$^-$ phage do not. This is due to rescue of a λ marker from the λ cryptic in MS501 by recombination, and lysis of the surrounding W3350 by the resultant wild type progeny.

5. Other markers which were determined by the stabbing technique were:

(a) *sus*$^{+/-}$ (b) *imm* (c) *fec*$^{+/-}$ (d) *int*$^{+/-}$

(a) *sus*$^{+/-}$ was determined by stabbing into W3350.

(b) *imm*^{434} " " " " " C600 (*imm*^{434}*sus*R_{60})

imm$^{\lambda}$ " " " " into C600 (*imm*$^{\lambda}$ *sus*Q_{21})

(c) *fec*$^{+/-}$ " " " " into 152 or QR48.

(d) *int*$^{+/-}$ " " " " into EMBO agar (Gottesman & Yarmolinsky, 1968) seeded with 10^9 coimmune phage. *int*$^-$ yielded a dark pink colony of bacteria while *int*$^+$ yielded a white colony.

(c) Mutagenesis to produce *gam*$^-$ and *sus* *gam*.

Techniques used were essentially those of Zissler et al., (1971a). The host strain, 536, was grown to A_{600}=0.5 in TB, made 10^{-2}M $MgSO_4$ and infected with λ*bio*72 at a multiplicity of 10. This mixture was incubated for 20 minutes at 37°C, diluted 10^{-1} into H1 pH6.0 containing 20 μgm/ml NNG, and shaken at 37°C

for 30 minutes. It was pelleted, washed in TB, resuspended in the same volume of TB, shaken for 2½ hours to ensure lysis, chilled and chloroformed.

gam$^-$ mutants were selected by plating on su$^-$(P2), which allows growth of bio72 gam$^-$ but not bio72 gam$^+$. The total burst, assayed on W3350, was 1.5 x 10^7 of which 0.6% was of the bio72 gam$^-$ phenotype.

λbio72 sus gam$^-$ were characterized as phage which would not grow on 152 but would grow on QR48.

λbio72 gam$^-$ (absolute defective) were characterized as those which will grow on neither 152 nor QR48.

λbio72 sus gam_{14} and bio72 gam_3 were further characterized, and found to map between bio11 and bio10.

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TABLE I

% gam$^+$ recombinants

	λ bio11	λ bio1	λ bio10
bio72 sus gam_{14}	0.46%	0.24%	$< 10^{-5}$%
bio72 gam_3	0.8%	-	$< 10^{-5}$%

Cross performed in 536 as described for C600 above. Total progeny were assayed on 536 and gam$^+$ recombinants assayed on 152. Recombination frequency (fec$^+$ x 2) between 2 gam$^-$ mutants was in the 0.2% range. bio72 sus gam_{14} has an efficiency of plating on 152 of 10^{-7}, bio72gam_3 has $EOP_{152} = 10^{-7}$. These two mutants were purified through three single plaque isolations.

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(d) All recombinant phage were purified through three single plaque isolations after initial selective screening and then plate stocks were prepared from a single plaque. These stocks were given more rigorous tests of their genetic characteristics as shown in the Table below which lists all phage constructed for this thesis and their characterization.

(e) Characteristics of λ recombinants constructed for use in the experiments reported in this thesis.

TABLE II

TABLE II

Genotype	sus$^{+/-}$ EOP on W3350(su^{-})	fec$^{+/-}$ EOP on QR48(d)	red$^{+/-}$ % recombination in 152 (f)	imm EOP on Lysogen(a)	int stab on EMB
$imm^{434}CIsusN_{7}N_{53}$	10^{-8}	1.0	-	10^{-7}	-
$imm^{434}CIbio72gam_{3}susN_{7}N_{53}$	$< 10^{-7}$	10^{-6}	(c)	$< 10^{-9}$	-
$CI_{857}\ sus\ R_{54}R_{60}$	10^{-8}	1.0	(g)	ts	white
$imm^{434}CIsus\ gam_{210}susR_{54}R_{60}$	$< 10^{-7}$	1.0	(g)	$< 10^{-7}$	-
$imm^{434}CIgam_{5}susR_{54}R_{60}$	$< 10^{-7}$	1.0	(g)	$< 10^{-7}$	-
$CI_{857}red_{114}int_{6}susR_{60}$	10^{-6}	-	.016%(2)	ts	pink
$imm^{434}CI\ red_{114}susR_{54}R_{60}$	$< 10^{-7}$	1.0	.002%(2)	$< 10^{-7}$	pink
$CI_{857}red_{270}susR_{54}R_{60}$	$< 10^{-7}$	-	.002%(e) (2)	ts	-
$imm^{434}CI\ red_{113}susR_{54}R_{60}$	$< 10^{-7}$	1.0	.006%(2)	$< 10^{-7}$	-
$CI_{857}red_{113}susR_{60}$	10^{-6}	1.0	6×10^{-4}(2)	ts	-
$CI_{857}red_{504}susR_{60}$	10^{-6}	1.0	.06% (1)	ts	-

TABLE II Cont'd.

Genotype	$sus^{+/-}$ EOP on W3350(su^{-})	$fec^{+/-}$ EOP on QR48(d)	$red^{+/-}$ % recombination in 152 (f)	imm EOP on Lysogen(a)	int stab on EMB
CI_{857}bio7-20 susR_{60}	10^{-6}	1.0	-	ts	pink
CI_{857}bio7-20red$_3$sus$R_{54}R_{60}$	$< 10^{-7}$	-	.04% (2)	ts	pink
CI_{857}bio72 susR_{60}	10^{-6}	-	.045% (3)	ts	-
imm^{434}CIbio72 sus$R_{54}R_{60}$	$< 10^{-7}$	1.0(b)	.12% (1)	$< 10^{-7}$	-
imm^{434}CIbio72 sus gam$_{14}$ sus$R_{54}R_{60}$	$< 10^{-7}$	1.0(b)	.2% (1)	$< 10^{-7}$	-
CI_{857}bio11 susR_{60}	2×10^{-6}	$< 10^{-7}$	(c)	ts	-
CI_{857}bio1 susR_{60}	10^{-6}	$< 10^{-7}$	.02% (2)	ts	pink
imm^{434}CI bio1 susR_{60}	10^{-6}	$< 10^{-7}$	(c)	$< 10^{-7}$	-
imm^{434}CI bio10 sus$R_{54}R_{60}$	10^{-6}	$< 10^{-7}$	.06% (2)	$< 10^{-7}$	-
CI_{857}red$_{114}$sus P_3	10^{-6}	-	.04% (4)	ts	white
CI_{857}bio72 sus P_{228}	$< 10^{-7}$	-	.054%(5)	ts	pink

TABLE II Cont'd.

Genotype	sus$^{+/-}$ EOP on W3350(su$^-$)	fec$^{+/-}$ EOP on QR48(d)	red$^{+/-}$ % recombination in 152 (f)	imm EOP on Lysogen(a)	int stab on EMB
imm^{434}susA$_{11}$B$_1$	$< 10^{-7}$	-	-	$< 10^{-7}$	-

(a) Phage were tested on C600 (imm^{434}susR$_{60}$), or tested for CI$_{857}$ phenotype - Turbid at 30°C and clear at 37°C (= ts).

(b) gam$^-$ character of bio72 sus gam susR had to be tested by inability to recombine with bio72 sus gam$_{14}$ to produce su$^+$ fec$^+$ recombinants. % recombination was 10^{-5}% indicating that the phage does carry the gam$_{14}$ marker. In contrast bio72 gam$^+$ susR gave 1.8% recombination with bio72 sus gam$_{14}$.

(c) red$^-$ character of these strains was tested only in the transformation assay by the inability to recombine with either bio72 susP$_{228}$ or red$_{114}$susP$_3$ DNA, in host 152. No recombination was detected at the level of the assay.

(d) Although no plaques were observed at the lowest phage dilutions assayed, extensive killing of the QR48 lawn was apparent.

(e) To ascertain that the red$_{270}$ mutation was really sus, this cross was also done in QR48 in which the recombination frequency rose to 3.2%.

Table II Cont'd.

(f) % recombination $= \left(\frac{\underline{sus}^{+} \ (\underline{Fec}^{+}) \text{ recombinants}}{\text{total progeny}} \right) (2)(100)$

1- % recombination with bio72 $CI_{857}susP_{228}$ to yield sus^{+} recombinants.

2- % recombination with red_{114} $CI_{857}susP_{3}$ to yield sus^{+} recombinants.

3- % recombination with biol CI_{857} to yield fec^{+} sus^{+} recombinants.

4- % recombination with $int_{6}red_{114}CI_{857}susR_{5}$ (From H. Echols) to yield sus^{+} recombinants.

5- % recombination with bio72 $CI_{857}susR_{60}$ to yield sus^{+} recombinants.

(g) These strains were red^{+} in the transformation assay as determined by their ability to rescue bio72 susP DNA in 152. gam^{-} character was tested by ability to produce λspi recombinants when crossed with λbio72.

$gam_{210}imm^{434}susR$ x bio72 0.022% λspi (Selected on su^{+} (P2)).

$gam_{5}imm^{434}susR$ x bio72 0.017% λspi (selected on su^{+} (P2)).

4. Preparation and Purification of λ stocks

(a) Plate stocks - Initial stocks of each virus were prepared from a single plaque. The plaque was picked from a plate with a pasteur pipette, added to 0.2 ml of C600, allowed to elute and adsorb for 20 minutes at 39°C, added to 3 ml. TB soft agar and poured on a TB plate. Incubation was at 37° for 4-5 hours, after which the plates were removed to the cold room (4°C), flooded with 5 ml λdil and allowed to elute overnight. The λdil was then decanted and assayed. Titers were generally 1-5 x 10^{10}pfu/ml.

(b) Large scale stocks - 1 liter.
C600 was grown up at 37°C in TB (500 ml/21 flask) to A_{600}=0.5, made 10^{-2}M $MgSO_4$, and infected with phage at a multiplicity of 0.1. Adsorption occurred for 20' at 37° with no shaking. Then agitation was resumed and the culture allowed to continue to lysis. Good lysis occurred within 3 hours for most strains although some required 5-6 hours. The culture was chilled, $CHCl_3$ added, and allowed to sit overnight at 4°C. Titer = 1-5 x 10^{10}pfu/ml.

Bacterial debris was removed by centrifugation at 10,000 rpm for 20 minutes. The supernatant was decanted and re-centrifuged to pellet phage at 8000 rpm for 16 hours at 4°C. This supernatant was discarded and the pellet gently resuspended in 5 ml of λdil. Titer = 1-5 x 10^{12}pfu/ml.

(c) CsCl purification of λ stocks. The volume of the pelleted stock was accurately measured and the amount of solid CsCl necessary to yield a final density of 1.5 was calculated, according to the following formula:-

1) $(D_{\lambda}V_{\lambda}) + (D_{CsCl}\ V_{CsCl}) = 1.5\ (V_{\lambda} + V_{CsCl})$.

Where D = Density. gm cm^{-3}; V = volume mls.

$D_{\lambda} = 1.0$ $D_{CsCl} = 3.98$ V_{λ} is measured

Solve for V_{CsCl}

2) Then the weight of solid CsCl to be added is calculated as follows:-

gm CsCl = (V_{CsCl}) (3.98)

Solid CsCl was added to the phage stock, allowed to dissolve, and the final density checked by weighing 0.1 ml of the solution. A density of from 1.49 to 1.52 produced a phage band near the center of the tube.

The CsCl-phage solution was pipetted into a nitrocellulose Beckman centrifuge tube and the remainder of the tube was filled with mineral oil.

Centrifugation was in a Spinco Model L ultracentrifuge in the Ti50 rotor for 16 hours at 30,000 rpm at 4°C. The resulting opaque phage band was collected by puncturing the bottom of the tube and collecting fractions. The phage fraction was then dialyzed in size 18/32 dialysis tubing against three 1 liter changes of TM. The final phage preparation had a titer of from 2-8 x 10^{12}pfu/ml.

(d) $\lambda dgCI_{857}$ was prepared by heat induction of W3101 (λCI_{857}) ($\lambda dgCI_{857}$). 1 liter of lysogen (500 ml/2l flask) was grown to A_{600}=0.9 at 30°C, heat induced for 15 minutes at 45°C, and allowed to grow at 37° until lysis (about 2 hours). This lysed culture was then treated in the fashion already described for infections. The phage were banded in density 1.45 CsCl and the 2 bands fractionated. The top band (λdg) was repurified through a second 1.45 CsCl density gradient, collected, dialysed, and assayed. The preparation contained 0.8% λ^+; λdg was assayed by plating on C600 ($\underline{imm}^{434}R_{60}$).

5. Preparation of λDNA

DNA was prepared from CsCl purified high titer phage preparations. The phage stock was diluted to 2-3 x 10^{12}pfu/ml with TE buffer. To this was added an equal volume of redistilled phenol previously equilibrated with TE at 0°. The mixture of phage and phenol was gently shaken in ice slush for 5 minutes, pipetted into a glass centrifuge tube and centrifuged at 4000 rpm for 15 minutes in a refrigerated centrifuge. The aqueous layer was removed with a "u" shaped pasteur pipette and re-extracted with fresh phenol twice more using the technique described above. The final aqueous layer was placed in size 18/32 dialysis tubing (boiled with .005 M Na_2EDTA, washed, and stored in sterile glass distilled water) and dialyzed at 4°C against 4 one liter changes of TE. Disposable plastic gloves were worn throughout this procedure to prevent contamination of the DNA with

nucleases. Concentration of DNA was determined by reading the optical density at A_{260}. $A_{260/280}$ ratios were used to determine the purity of the DNA. This ratio was 1.8 or higher for all DNA preparations used. DNA was diluted to A_{260}=1.0 with TE for use in the transformation assay.

6. Preparation of λDNA halves.

Shearing of λCI_{857} and λvir DNA was done with a Virtis 23 homogenizer and macroblades. Number of revolutions per minutes of the blades and shaft was calibrated using an IEC Stroboscope Model ST66R. The homogenizer was found to maintain an even speed if allowed to run overnight before use.

The λDNA sample was diluted to an A_{260} = 0.5 in a total of 25 ml of TE at 4°C. Shearing was done in a fluted Virtis 50 ml bottle surrounded with ice slush. Initially the DNA was stirred at 1200 rpm to separate cohered ends and then the speed was raised to 1400 rpm to actually shear the DNA. Shearing proceeded for 180 minutes with aliquots taken every 30 minutes to monitor the procedure. Speed of the shaft was continually monitored with the Stroboscope.

Loss of imm^{λ} susAB linkage in the transformation assay indicated loss of whole λDNA molecules and appearance of λ half molecules. Characterization of half molecules will be described in section 8. Loss of whole molecules began at 120' of shearing and was complete by 180' such that at the limits of detection in the assay used, no whole molecules remained. The assay for

halves is described in Methods, part 8.

λ<u>imm</u>^{21} halves were prepared using a variation of the above techniques. Virtis microblades and an acid washed 10 ml beaker were used as shearing apparatus. <u>imm</u>^{21} DNA was diluted to 8.0 mls. of A_{260}= 0.05. Shearing proceeded for 30 minutes at 3600 rpm.

7. Transformation Assay

The technique used was that of Egan & Hogness (1972) with minor modifications. Host bacteria were grown up overnight at 37°C in H1 + 0.06 % maltose which halts cell growth at approximately A_{600}= 0.8. Additional Maltose to yield 0.18% maltose was added and cells were shaken at 37°C to A_{600}=1.7. Cells were chilled, pelleted at 6000 rpm for 10 minutes, washed in an equal volume of I medium, and resuspended in ½ volume of I medium. Host cells were pipetted into a sterile flask (size = 10 x volume of cells), shaken at 37°C for 10 minutes, and chilled on ice for 5 minutes. This was assayed for viable cell count.

Helper phage were added to I medium in sterile 50 ml flasks, mixed, and an aliquot immediately pipetted into λdil for assay of titer. The phage titer at this point was calculated to be 2 x 10^{10}/ml and always confirmed by assay. An equal volume of host bacteria (2 x 10^{9}/ml in I med.) was added to the phage - I medium, gently mixed, allowed to remain stationary at 0°C for 15 minutes, shaken very gently at 37°C for 10 minutes, and quickly chilled in ice slush for 5 minutes.

From this point onwards, helper infected cells were pipetted gently with prechilled pipettes, into pre-chilled glassware. Cells were pelleted at 6000 rpm for 10 minutes at 4°C, re-suspended in cold TCM to yield 2 x 10^9 cells/ml and allowed to remain at 4°C for 120 minutes before infection with DNA.

Competent cells (0.1 ml) were pipetted into small test tubes. DNA, diluted in TCM to $A_{260}=10^{-4}$, was added in 0.1 ml volumes, the mixture gently shaken, and incubated for 25 minutes at 39.5°C. Incubation of mixtures was at 30 second intervals to allow timed plating of the mixture precisely 25 minutes later. At 25 minutes, 0.8 ml of 39.5° TB was added to the incubation mixture, gently mixed, and 0.1 ml was pipetted into a tube containing 3 ml soft agar plus 0.2 ml indicator bacteria, and poured onto a TB agar plate. Although experiments performed to determine ability of the indicator to yield plaques after prolonged incubation in agar at 47° showed no variability over a 90 minute period, experiments were arranged so that indicator remained at 47°C for a maximum of 2-3 minutes. Plates were incubated overnight at 37°C.

The following conditions were found to produce optimum efficiency of Transformation: Unacceptable variability in cleanliness of general lab glassware necessitated use of a new set of tubes, flasks, buffer bottles, and centrifuge tubes which were used only for transformation and which were extensively hand rinsed in glass distilled water after washing. These were all sterilized before use. Glass distilled water for TCM and I

medium was tested in comparison to deionized glass distilled water, and found to produce equal efficiencies of infection. Only glass distilled water was used throughout this work and this was autoclaved in specially washed glass bottles with teflon cap liners.

Because this assay is the basic technique for all my experimental results, I have characterized it as described in the following sections:

(a) Analysis of various stages of the assay as related to efficiency of half and whole DNA transcription.

(b) Physiological state of competent cells.

(c) Uptake of DNA by competent cells.

(d) Influence of <u>red</u> and <u>rec</u> functions.

(e) Indicators and standard control.

(a) <u>Analysis of various stages of the transformation assay as related to whole and half molecule transcription</u>.

The standard transformation assay conditions as perfected by Kaiser, Hogness and others were designed for experiments in which marker resue, rather than rescue of gene function, was being assayed. It was important to be aware of any variations in level of transcription of transfecting DNA which might be caused by altering certain stages of the assay procedure. Three such stages were analysed; (a) growth phase at which host cells were harvested, (b) length of

37°C incubation periods, in I medium before and after helper phage infection (period 1 and period 2), and (c) length of the 0°C period in TCM buffer before addition of DNA. As variations in these three stages produced no highly significant changes in transcription of half molecules, standard assay conditions, as described above, were used throughout the series of experiments presented in this thesis.

1. Effect of growth phase at which cells were harvested. Cells were grown in H1 + maltose $A_{600} = 0.85$, considered to be early log phase and A_{600}1.7, $A_{600} = 2.1$, considered to be late log phase. Standard conditions involve growth to $A_{600} = 1.7$.

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TABLE A Effect of Host Cell Growth Phase

Host 152	Helper	λ^+ Halves	susP$_{228}$ wholes	susJ$_6$ wholes	λ^+ wholes	bio72 susP$_{228}$ wholes
		sus$^+$ infectious centres/10^{-6} A_{260} units of DNA				
1. A_{600}=0.85	λbio72susR	1738	1542	1471	1638	22
2. A_{600}=1.7	λbio72susR	1047	519	767	911	8
3. A_{600}=2.1	λbio72susR	454	190	580	704	<1

Assay was as described earlier - cells that were concentrated to approximately 2 x 10^9 cells/ml in I med. in each line. Helper was bio72 imm^{434}CIsusR$_{54}$R$_{60}$ indicator was W3350. bio72 susP$_{228}$ whole DNA is simply a measure of residual recombination in a totally rec/red$^-$ system.

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Early log phage host cells produce the highest efficiency of transcription of all the <u>red</u>$^{+}$ DNA samples. The most dramatic effect is seen on <u>sus</u>P_{228} DNA which is reduced 8-fold in A_{600} = 2.1 host cells. In general, A_{600}=2.1 host cells are the least efficiently transformed. Whole <u>sus</u>P_{228} DNA reacts more drastically to alterations in growth phase than do λ^{+} wholes, <u>sus</u>J wholes and halves.

2. Effect of variation in periods 1 and 2 at 37°C.

Period 1 occurs after resuspension of harvested host cells in I medium. The standard time of incubation is 10 minutes.

Period 2 occurs after infection of host with helper phage, in I medium. Again, the standard length of time at 37°C is 10 minutes.

Both these periods are effectively starvation periods since I medium contains low salt and only 0.006% glucose.

TABLE B Effect of 37°C incubations in I medium.

	Host 152	Helper	min. at 37°C Period 1	min. at 37°C Period 2	λ^+ Halves	susP$_{228}$ Wholes	λ^+ Wholes
					sus^+ infectious centers/ 10^{-6} A_{260} units of DNA		
1.	A_{600}=1.7	λbio72susR	10	10	1152	424	1460
2.	"	"	5	5	415	935	1243
3.	"	"	5	15	865	271	605
4.	"	"	15	5	772	992	1572
5.	"	"	15	15	537	125	413

Conditions were as described in methods 7a except for variations in periods 1 and 2. Helper was bio72 imm^{434} CIsus$R_{54}R_{60}$. Indicator was W3350.

======

Efficiency of transcription of halves was highest when standard incubation times were used. As with the host cell growth phase experiment, whole susP$_{228}$ exhibits a strikingly different pattern of efficiency from that for whole λ^+ and half λ^+ DNA, although the 15' period 1 + 15' period 2 gives poor results for all DNA samples. In general, incubation periods giving highest efficiencies are the points at which period 1 and period 2 = 20 minutes (lines 1, 3, 4) for half molecules. For susP$_{228}$ whole DNA, highest efficiencies are correlated with

shortened periods in period 2 (lines 2 and 5).

3. Effect of variation development of competence at 0°C in TCM.

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TABLE C 0°C TCM incubation time.

	Host	Helper	minutes at 0° in TCM	λ^+ Halves	λ^+ Wholes
1.	152	λbio72susR	1	1180	1352
2.	"	"	30	1363	1119
3.	"	"	60	1399	897
4.	"	"	120	1152	1460
5.	"	"	180	1098	1136

Conditions were as already described except for variations in 0° incubation. Helper was bio72 imm^{434}CI $susR_{54}R_{60}$. Indicator was W3350.

======

No significant variation was observed in efficiency of transcription with increasing time at 0°C. The 120 minute time point was chosen for convenience. These competent cells exhibited full competence almost immediately after resuspension in TCM.

A few comments are in order concerning the divergent patterns between transcription of half DNA and whole

susP_{228} DNA in response to variations in treatment of competent cells (Tables A and B). Whole *sus*P_{228} DNA is recombined into wild type molecules most efficiently in young cells which have been subjected to minimal times of semi-starvation. It is possible that although transcription of this whole DNA is at a level equal to that observed for the λ^+ halves, the actual measurement of that transcription, in terms of *P-R* recombination is less efficient in older cells. Recombination between *P* and *R* must of necessity be *red* catalyzed while half molecule recombination is possibly *int* and *red* catalyzed. If *red* product activity were more strongly affected by cellular conditions than was *int* product activity the observed results could occur. This idea is supported by the fact that whole λ^+ DNA which requires neither *int* nor *red* recombination for plaque forming ability, shows a greater correlation with λ^+ halves, although λ^+ halves and λ^+ wholes do exhibit different patterns of behaviour. In order to keep the above sorts of uncorrelated variations as minimal as possible, I decided to use, as my whole DNA control, a DNA which would be influenced by the same factors as λ halves. The closest approximation to such a DNA is λ*sus*J wholes. The region involved in recombination, *J-R*, is essentially the same as that involved in half molecule recombination and importantly, it too can be affected by both *int* and *red* activities.

(b) Physiological state of competent cells.

1- Ability to support a phage lytic cycle.

152 or 9605 host cells were infected with helper phage in standard transformation assay conditions except that instead of a 25' infection period with DNA, TCM buffer was substituted. Unadsorbed phage were removed with λ antiserum and infectious centers assayed. These infectious centers were destroyed if they were pretreated with chloroform before plating. Infective centers were assayed on C600. A viable cell count was performed, in the absence of helper phage, to determine total cell number. 55% or more of the competent cells are capable of hosting a lytic cycle except in the case of recA recB strain JC5495 in which viability, as measured in ability to support a burst, is reduced 10 fold compared to cell number as calculated from optical density of the resuspended cells.

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TABLE D-1

Host	Helper	
152	none	1.2×10^8 colony formers/0.1 ml
152	λCI_{857}	1.1×10^8 infectious centers/0.1 ml
R^-A9605	none	3.8×10^8 colony formers/0.1 ml
R^-A9605	$\lambda imm^{434}CIsusN_7N_{53}$	2.1×10^8 infectious centers/0.1 ml
JC5495	λCI_{857}	0.2×10^8 infectious centers/0.1 ml

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2- Test of helper phage leak in competent cells.

Transformation assay conditions described above were used, except that competent cells were incubated for 25' with TCM instead of DNA and plated on C600 indicator. Initially it was intended to assay R and N gene function directly from the transfecting DNA but these experiments showed that the level of helper phage leak provided far too high a background for this to be possible.

TABLE D-2

	Host	Helper	No. of colony formers or infective centers on C600	% leak(a)
	152	None	1.2×10^8	-
recA	152	λCI_{857}sus N_7N_{53}	1.9×10^5	0.16
	152	λCI_{857}sus $R_{54}R_{60}$	2.2×10^5	0.18
	DM22	None	0.8×10^8	-
recB	DM22	λCI_{857}sus N_7N_{53}	2.5×10^6	3.1
	DM22	λCI_{857}sus $R_{54}R_{60}$	2.0×10^6	2.5
	E835	None	1.8×10^8	-
recArecB	E835	λCI_{857}sus N_7N_{53}	2.6×10^6	1.47
	E835	λimm^{434}CIsusN_7N_{53}	6.6×10^7	36.7
recArecB	JC5495	λCI_{857}sus N_7N_{53}	9.8×10^6	48 (b)
	W3350	None	2.0×10^8	-
rec$^+$	W3350	λCI_{857}sus N_7N_{53}	1.6×10^6	0.8
	W3350	λimm^{434}CIsusN_7N_{53}	5.2×10^7	26.0

(a) $\% = \frac{\text{infectious centers}}{\text{colony formers}} \times 100$

(b) $\% = \frac{\text{infectious centers of } N_7N_{53}}{\text{infectious centers of } CI_{857}} \times 100$

(from Table D-1)

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(c) Uptake of DNA by competent cells.

1- The multiplicity of infection by DNA molecules was

varied and the efficiency of transformation found to be linear in the range of the DNA concentration used, to the extent that a ten fold increase in DNA yielded 10 times as many plaque forming units.

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TABLE E

Host	Helper	A_{260} Units of Half DNA added to competent cells	moi	Infectious Centers per A_{260} unit of DNA
W3350	λCI_{857}susR$_{54}$R$_{60}$	10^{-5}	0.10	1.25×10^{9}
		10^{-6}	0.01	1.20×10^{9}

The concentration of DNA chosen (10^{-5}) ensures that the majority of competent cells infected with one DNA molecule are unlikely to receive a second, and allows a significant number of plaques on each plate. Indicator bacteria was W3350.

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All transformation assay results will be expressed as infectious centers per $10^{-6} A_{260}$ unit of DNA. A value of 1000 equals 1×10^{9} pfu/A_{260} unit of DNA. One A_{260} unit of DNA was considered to equal 5×10^{11} DNA molecules. Dilutions were arranged such that 500-1000 plaques were produced per plate for the standard λCI_{857}susR control transformation in each set of assays.

2- Rate of DNA uptake by competent cells.

The rate of uptake was measured (a) by treating with

DNase for 2' at 39°C after the stated period of infection and (b) by plating immediately after stated period of infection. The difference between these two sets of results represents DNA which is adsorbed to competent cells but has not completely penetrated the cells.

======

TABLE F

Host	Helper	DNA	Time of Infection	+DNase	-DNase
				sus^+ Infectious Centers/ $10^{-6}A_{260}$ units of DNA	
152	$\lambda CI_{857}\underline{sus}R_{54}R_{60}$	CI_{857} Halves	1'	65	370
			10'	626	1364
			15'	662	1100
			25'	716	1008

Techniques used were standard ones described at the beginning of this section. Indication was W3350.

======

Maximal adsorption of λDNA occurs within the 25 minute incubation period of this assay, in absence of DNase treatment. These were the standard conditions for all transformations reported in this thesis unless stated otherwise. No DNase treatment was used in the standard assay.

(d) Influence of *red* and *rec* functions.

λ*red* and *E. coli rec* functions were found to be unnecessary

for transformation by wild type λDNA. This agrees with results of Cohn (1972).

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TABLE G

Host	Helper	sus^{+} infectious centers/$10^{-6}A_{260}$ units of DNA: $red_{114}CI_{857}$ wholes	CI_{857} wholes
recA 152	$\lambda int_{6}red_{114}CI_{857}susR_{60}$	650	600
recA 152	$\lambda CI_{857}susR_{54}R_{60}$	1480	720

Transformation assay conditions as described at the beginning of this section. Indicator was W3350.

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(e) Indicators and Standard Control

All indicators used in the transformation assay were always checked by spotting known phage mutants (10^{7}pfu/ml stock) on a lawn of the indicator. Tester phage included λ^{+}, $imm^{434}CI$, $CI_{857}susR_{54}R_{60}$, $CI_{857}susN_{7}N_{53}$, λbiol CI_{857}, λvir and $imm^{21}Cts$.

Each transformation assay always included an internal control on the host bacteria and DNA dilutions, which also served as a standard by which assays performed at widely varying time intervals could be compared. $\lambda CI_{857}susR_{54}R_{60}$ was used as a control helper phage each time a transformation assay was performed and was infected with all DNA preparations used in that experiment to assess their activity.

8. Characteristics of λ half and λdg molecules.

(a) Extent of shearing into halves.

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TABLE H

	Host	Helper	DNA	Infective centers/10^{-6} A_{260} units of DNA		
				Right half	Left half	Wholes
1.	R^{-}A9605	imm^{434}CIsus$A_{11}B_{1}$	CI_{857} wholes	164	211	141
2.	"	"	CI_{857} halves	465	169	<1
3.	"	"	λvir wholes	960	1050	514
4.	"	"	λvir halves	236	175	<1
5.	"	"	λimm^{21}Cts wholes	1000	1025	980
6.	"	"	λimm^{21}Cts halves	1280	434	<1
7.	"	"	λdgCI_{857} wholes	199	<1	<1

	Host	Helper	DNA	Infective centers/10^{-4} A_{260} unit of DNA		
				Right half	Left half	Wholes
8.	"	"	λ CI_{857} halves	31700	4000	21

Transformation was as described in Section 7. Right halves were assayed on C600 (imm^{434}sus$A_{11}B_{1}$), left halves on W3350, and

Cont'd.

wholes on W3350 ($\underline{imm}^{434}\underline{sus}A_{11}B_1$). For the experiment in line 8, $10^{-3}A_{260}$ units of half molecules were added to 1×10^8 cells to determine extent of whole molecule contamination.

======

All preparations of half molecules were free of whole molecule contamination within the range of DNA concentration used in the standard assay. By increasing the amount of sheared DNA added to competent cells, it was possible to determine the efficiency of the shearing procedure (line 8, Table H). Whole molecules constitute only 2×10^{-4}% of the preparation when compared to the level of right halves (line 2). The experiments in lines 1-7 demonstrate the location of the imm marker on the right half preparations and also on the λdg wholes.

(b) Rescue of R^{+} marker from infecting λDNA halves and wholes.

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TABLE J

Host	Helper	DNA Genotype	sus^{+} Infectious centers/10^{-6} A_{260} units of DNA	
			Halves	Wholes
152	$\lambda CI_{857}susR_{54}R_{60}$	λCI_{857}	1184	1108
		λvir	1724	1000
		$\lambda imm^{21}C_{ts}$	1244	-
		λdg	-	405

The transformation assay was as described in Part 7. Indicator was W3350.

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In contrast to results on transformation by half molecules reported by others (Cohn 1972; Elseth & Simmons, 1967;

J. Little Pers. Comm.) who observe that halves transform at about 10% the efficiency of whole λDNA, I consistently observed halves transformation, in the presence of CI_{857}susR helper phage, in the range of 58-135% of the whole λDNA level (example in Table J) in all genotypes of host bacteria utilised for the experiments in this thesis. One can conclude that essentially 100% of the λ^+ halves possess the R^+ marker.

9. Recombination systems involved in rescue of half and whole DNA

(a) Analysis of half molecule recombinants.

TABLE K

Host	Helper	Infectious Centers/10^{-6} A_{260} units of DNA			
		λCI_{857} halves		$\lambda dgCI_{857}$ wholes	
		R^+	fec^+R^+	R^+	fec^+R^+
DM22	λbio10imm^{434}CIsusR R_{54} $_{60}$	1786	946	2002	1660
JC5495	"	327	267	-	-
152	"	-	-	1100	1000

Transformation assay as in section 7. R^+ indicator was W3350, fec^+R^+ indicator was 152.

At least 53-81% of the halves and 82-91% of the λdg wholes could contribute fec^+ and so must terminate at att or to the left of att, since the helper and infecting DNA species are nonhomologous between att to CIII for bio10. A more important conclusion to be drawn from these results concerns

the type of recombination which is responsible for rescue of halves. One might expect that physically, recombination between λbio helper and a right half, in the region to the left of the heterologous bio DNA addition, would be a relatively minor event compared to recombination to the right of the bio addition where both half and helper are homologous. The fact that recombination occurring in the region to the left of the nonhomology is the major class suggests that int mediated recombination may play a role in rescue of halves and almost certainly is important in rescue of λdg. Since other workers have presented evidence that integrase produces viable half molecule-helper recombinants only in conjunction with red mediated recombination (Cohn, 1972) (Weisberg et al., pers. comm.), one must postulate a second red mediated event to the right of $susR_{60}$ in my experiments. A less complicated explanation of the preponderance of $fec^{+}sus^{+}$ half molecule recombinants would be that all these recombinants are produced by red product activity. One could postulate that red product acts preferentially at any free DNA end yielding a majority of events in the region to the left of att, the internal terminus of the right half molecule. Purified λ exonuclease, the redX gene product, has a very strong affinity for free DNA ends as compared to internal nicks (Radding & Carter, 1971) and degrades only DNA with free ends in vitro Carter & Radding, 1971) which would support this idea.

(b) Analysis of whole molecule recombinants.

TABLE L - Recombination with whole DNA: int and/or red promoted events.

Host	Helper	Infectious Centers/$10^{-6}A_{260}$ units of DNA					
		susJ wholes		susP wholes		λ^+ wholes	
		sus^+	fec^+sus^+	sus^+	fec^+sus^+	sus^+	fec^+sus^+
152	λbio10susR	335	183	110	2	247	187
JC5495	λbio10susR	-	-	158	17	513	412
DM22	λbio10susR	1246	784	1100	75	1578	1500

Transformation was as described. Helper was $imm^{434}CIsusR_{54}R_{60}$. Indicator for sus^+ = W3350. Indicator for fec^+sus^+ = 152. DNA was CI_{857}.

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(1) λbio10susR x susJ DNA wholes 55-85% fec^+sus^+ recombinants.

(2) λbio10susR x susP DNA wholes 1.8-10.7% fec^+sus^+ recombinants.

(3) λbio10susR x λ^+ wholes 76-95% fec^+sus^+ recombinants.

(4) λbio10susR x λ^+ halves 53-81% fec^+sus^+ recombinants.

λsusJ wholes correspond most closely to the recombination pattern set by λ^+ halves. Since λ^+ wholes require no recombination events for plaque forming ability, they provide a measure of the plating efficiency of transformed complexes on the two indicator strains used. The fact that λ^+ wholes, susJ wholes, and λ^+ halves all show essentially the same plating characteristics, suggests that in actual fact 100% of the susJ and λ^+ halves recombinants are fec^+sus^+.

λ*sus*J whole DNA therefore seems the best whole DNA control with which to compare activity of halves since the region of *sus*J DNA affected by recombination events corresponds very closely to the recombination region for half molecules.

SECTION IV.

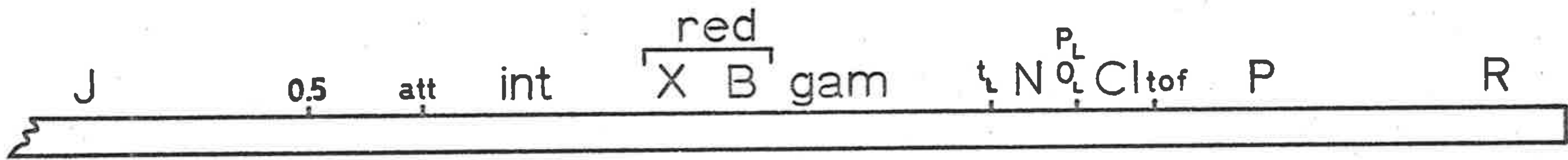
RESULTS and DISCUSSION

Rescue of Gene Function from Half DNA Templates

CHAPTER I

Rescue of _red_ and _N_ gene function from a half molecule.

FIGURE 1 - Genetic and physical map of the right half of the λDNA molecule showing the extent of deletions used in this study (according to Davidson and Szybalski, 1971).
Drawing courtesy of Dr. E.M. Pilarski.


red
J
0.5
att
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X
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t_L
N
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O_L
CI
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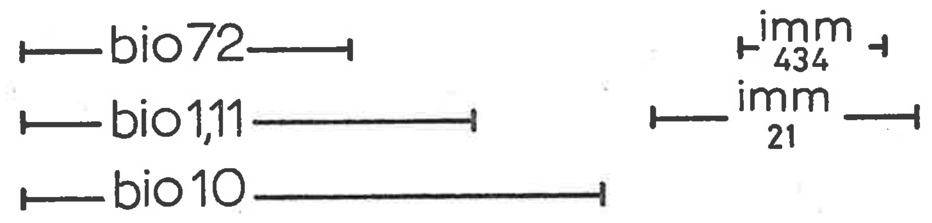
R


bio72
bio1,11
bio10
imm 434
imm 21

(a) Can red be expressed from a half molecule?

The transformation system consisted of three main elements; the host cell, the helper phage, and the transfecting DNA. The host cell was defective in bacterial recombination (*rec*A), and the helper phage was defective in the phage mediated recombination system either through deletion of the *red* region or point mutation in one of the *red* genes. In such a system the only source of recombination function was via transcription of the wild type *red* gene located on the transfecting DNA molecule. Expression of these *red* genes was monitored by measuring recombination between the helper phage DNA and the transfecting DNA. Thus transfecting whole DNA carried the *sus*J marker, and recombination with the *sus*R helper phage DNA was assayed by scoring infectious centers plaquing on su^- indicator bacteria. Recombination in the case of transfecting half DNA was similarly assayed, but without the need of marking the transfecting DNA.

Control experiments included (1) the use of whole wild type DNA to establish the transformability of a particular helper - host combination, as no recombination was necessary for plaque activity on the su^- indicator bacteria; (2) the use of whole *red sus*P DNA to detect any residual recombination in the system other than that due to the host *rec* and the phage *red* system.

Two types of red deficient helpers, deletion mutants and point mutants, were used in Table 1; i.e. bio72 which has a deletion extending from att to exo, or $redB_{113}$ and $redB_{114}$ which are point mutations in redB (Signer et al., 1968). There was essentially no residual recombination in the system indicating that production of wild type recombinants must be due to activity from the red^{+} gene on the infecting DNA. That the red^{-}susP DNAs are capable of transformation is demonstrated in line 4 where the helper phage is red^{+}. The wild type recombinants produced in the susP DNA transfection give an indication of red promoted recombination in the P-R interval, catalyzed by red gene product from the transfecting DNA. By comparison, the level of wild type recombinants with susJ DNA indicates combined int and red promoted recombination occurring within the J to R interval, spanning the region within which recombination must occur between the half DNA molecule and helper phage. Both half molecules and susJ whole DNA generally gave better transformation than did susP DNA, suggesting that both int and red may participate in the rescue of half molecules. However, Cohn (1972) has shown that int product in the absence of red or rec recombination is unable to catalyze a recombination event between helper and half DNA, so the rescue of halves cannot be due to the action of int product alone. Since a combination of both int and red systems are likely to act on the half, the whole susJ transfection was considered the better approximation of the situation occurring with half molecules.

TABLE 1 - Ability to rescue red function from whole and half molecules of λDNA in a recA su⁻ host.

Infectious Centers containing sus^+ recombinants/ $10^{-6}A_{260}$ units of DNA

Helper	λ^+ halves	susJ wholes	susP wholes	λ^+ wholes	redBsusP wholes	bio72susP wholes
1. λbio72susR	733	1110	327	681	-	10
2. $\lambda redB_{114}susR$	1775	1608	1130	1348	10	804
3. $\lambda redB_{113}susR$	1728	2952	1048	1944	1	-
4. $\lambda red^+ susR$	977	864	392	996	203	315

Host was 152. All DNA preparations carried the CI_{857} marker. DNA genotypes were as follows: $susJ_6$, $susP_{228}$, $redB_{114}susP_3$, $bio72susP_{228}$. Indicator bacteria were W3350. The line 2 - $bio72susP_{228}$ DNA result is due to complementation between the redB helper and the redX DNA confirming that red_{113} is a redB mutation. λimm^{434} helpers were more efficient than imm^{λ} DNA. All above helpers were $imm^{434}CIsusR_{54}R_{60}$ except the line 4 control which was $CI_{857}susR_{54}R_{60}$.

When halves were actually tested for their ability to provide *red* function, the following results were obtained. Comparison of rescue of halves with rescue of whole *sus*J DNA for each *red*$^-$ helper indicates that both *red*X and *red*B gene functions are efficiently rescued from a linear molecule template since the *bio*72 helper measures rescue of *red*X function (64% as compared to *sus*J wholes, Table 1, line 1) and the *red*B helpers measure rescue of *red*B function (56-110% as compared to *sus*J wholes Table 1, lines 2 and 3). I conclude that half molecules are able to serve as a template for λ *red* genes.

(b) Can N function be expressed from a half molecule?

As detailed in methods section 7e, a direct assay for *N* product was impossible in this system, since in the absence of transfecting DNA, 0.18% of the helper infected competent cells (λ*sus*NN-*rec*A*su*$^-$) leaked sufficiently to yield an infectious center on *su*$^+$ indicator bacteria. I therefore made use of the facts that *N* product must be present before *red* genes can be transcribed (Radding & Echols, 1968; Kumar et al., 1969), and that *red* activity can be assayed by the appearance of infectious centers on *su*$^-$ indicator bacteria, which avoids the problem of leaky competent cells scoring in the assay. Furthermore, the *red* genes lie immediately to the left of the *N* gene on the λ chromosome; this means that both *N* and *red* will be located on the same half molecule (Figure 1). Thus in a competent bacterium consisting of *rec*A *su*$^-$

host bacteria and λ*susN* helper phage the display of phage recombination reflects the expression of the wild type *N* gene located on the infecting DNA molecule.

In contrast to the behaviour of the *red* genes, gene *N* cannot be efficiently expressed from a half molecule (Table 2, lines 1 and 2). Although the rescue of halves was 56-110% , as compared to *susJ* wholes, with the *red*⁻ helpers (Table 1), this figure was reduced to only 1-2% for the *susN* helper in *recA su*⁻. It is very likely that even this minimal level is due not to a function from the half DNA, but to helper phage leak. Since 0.18% of the λ*susN* competent cells leak sufficiently to produce a complete lytic cycle, it is not difficult to imagine that adequate levels of *N* product would be available to turn on *red* function. Radding & Echols (1968) have demonstrated that only 10% of normal *N* product levels are sufficient to turn on full λ exonuclease (*redX*) production.

As described in the previous section, use of *susP* DNA measures levels of *red* promoted recombination in the λ*susN* competent host, and use of *susJ* DNA indicates the amount of recombination between genes *N* and *J* which approximates the region of the half molecule where recombination must occur. As before, *int* promoted recombination is unlikely to catalyze this event alone, so recombination of the half molecule must be due to *red* product, or a combination of *red* and *int* products turned on by the *N* gene of the infecting DNA. Thus,

TABLE 2 - Ability to rescue N function from whole and half molecules of λDNA.

	Host	Helper	Infectious centers/$10^{-6}A_{260}$ units of DNA				
			λ^+ halves	susJ wholes	λdgA-J wholes	λ^+ wholes	susP wholes
1.	recAsu$^-$	λCI_{857}susNN	8	800	916	780	241
2.	recAsu$^-$	λimm^{434}CIsusNN	19	1236	1948	880	540
3.	rec$^+$su$^+$-1	λCI_{857}susNN	1055	548	235	971	871
4.	rec$^+$su$^+$-1	λimm^{434}CIsusNN	1128	1063	-	699	674
5.	rec$^+$su$^+$-2	λCI_{857}susNN	692	740	-	1120	-

All DNA preparations carried the CI_{857} marker. Indicator was W3350. recAsu$^-$ = 152, rec$^+$su$^+$-1 = R$^-$A9605, rec$^+$su$^+$-2 = 1200. Full genotype of DNA was: susJ_6, susP_{228}. Both helper phage were susN_7N_{53}.

although rescue of N function occurs for whole molecules, it does not occur for half molecules in the recAsu$^-$ host. I conclude therefore, that this inability to rescue N function from halves is indicative of the inability of a half molecule to serve as a template for N gene.

Since the outstanding feature unique to a whole λDNA molecule compared to a half molecule is the ability of a whole molecule to circularise, I postulated that if two cohesive ends could be attached to a right half molecule, it might now be able to provide N gene function. The closest approximation to such a molecule is λdg_{A-J} DNA, which is effectively a right half molecule with two cohesive ends, all of the left half genes A to att being deleted. The λsusN competent cell was infected with λdg_{A-J} DNA and N function from the transfecting DNA assayed as before. This type of "half molecule" template allowed 114-158% rescue of N gene function (as compared to susJ, Table 2, lines 1 and 2), indicating that a right half molecule which can circularize is an effective source of N function. This experiment also excludes the possibility, admittedly unlikely from present interpretation of the mutants available, that gene N needs some function from the left half of the λ molecule in order to be effective.

Alternative explanations might be envisaged to explain the inability of the half molecule to provide N gene function.

These possibilities are considered below:

(i) It was possible that a physical block existed on the half molecule, preventing recombination between gene N and the broken DNA end. In the rec^+su^+ host, where the nonsense $susN_7N_{53}$ mutations are translated and active N product produced by the helper phage, both halves and wholes are efficiently recombined into a wild type recombinant. This indicates that, given the presence of N product, no physical block exists to prevent recombination between helper and half molecule, and secondly, that essentially 100% of the half molecules possess gene N. (Table 2, lines 3 and 4 compared with control Table 1, line 4). Since these were important points, and since the $recAsu^-$ and rec^+su^+ strains were not isogenic, I performed this experiment in a second rec^+su^+ host (Table 2, line 5) and obtained the same result. The recombination observed between susN helper phage and transfecting half molecules is not due to the host rec^+ system, since essentially no recombination was observed in a rec^+su^- host (4-6%, see table 14, line 1). The recombination observed in the rec^+su^+ hosts therefore, is dependent upon an N activated recombination system.

(ii) Two λ control elements act to turn off the N operon, therefore it was necessary to assess the influence of these (tof and CI products) on the half molecules,

as compared to whole molecules, since a preferential action on halves, although intuitively unlikely, could nevertheless produce the result observed.

Pero (1970) has shown that the early turn off gene, tof, maps within the imm^{434} immunity region, as does its site of action on the l strand of λDNA. In addition, she has demonstrated that λimm^{434} and λimm^{λ} have different, non-interchangeable, tof products. If helper phage tof^{λ} product were causing turn off of l strand transcription on the imm^{λ} half molecules in λsusN competent cells, then use of λimm^{434} susN helper phage should eliminate the problem since tof^{434} will not act on imm^{λ} halves. Rescue of halves by λimm^{434} susN helper in $recAsu^{-}$ is still only 1.6% indicating that tof product activity is not responsible for poor rescue of halves (compare Table 2, lines 1 and 2).

A second negative control factor of N gene transcription is the CI repressor which directly represses both N and red. Luzatti (1970) has shown that N product fosters red transcription only in the absence of active CI repressor. Any influence the CI repressor might have in our system was minimized since both the helper phage and the infecting DNA preparations generally carried either unconditional CI mutations or the CI_{857} marker to render the repressor inactive at the incubation temperature of the assay (39.5°C).

Nevertheless, to further exclude the possibility, I investigated λ half molecules carrying the <u>vir</u> marker and thereby insensitive to the repressor (Ptashne & Hopkins, 1968).

Rescue of λ<u>vir</u> halves at 2.9% (Table 3, line 1) was as inefficient as rescue of λCI_{857} halves indicating that preferential repression of halves in the λ<u>sus</u>N competent cells does not occur, even though the λ<u>vir</u> halves recombine well in an $\underline{N}^{+}\underline{red}^{+}$ competent cell (line 2). I conclude that effect of <u>C</u>I repression is not significant in this system.

(iii) In comparing rescue of halves with $\lambda\underline{red}^{-}\underline{N}^{+}$ helpers (Table 1) and λ<u>sus</u> <u>N</u> helpers (Table 2,) it was possible that the different result in these two systems was due to a combination of the position of the marker rescued and exonucleolytic activity at the double stranded broken terminus of the half molecule. In the $\lambda\underline{red}^{-}$ experiments gene <u>R</u> which is 5% from the right end, is much further from the broken left terminus than gene <u>N</u>, 27% from the right end (Egan & Hogness, 1972). One could envisage an exonuclease active at the internal terminus of the half molecule such that gene <u>N</u> was lost before gene <u>R</u>. The results recorded in Table 4 show that the recovery in the $\underline{rec}A\underline{su}^{-}$ host of the $\underline{imm}^{\lambda}$ marker (25% from the right end) from half molecules is 60-100% the frequency of

TABLE 3 - Effect of CI repressor on rescue of halves.

	Host	Helper	Infective centers/10^{-6} A_{260} units of DNA		
			λvir halves	λvir wholes	susJ wholes
1.	152	λCI_{857}susN_7N_{53}	13	1398	443
2.	152	λCI_{857}sus$R_{54}R_{60}$	1170	1092	550

susJ DNA carried the CI_{857} marker. Indicator was W3350.

TABLE 4 - Frequency of imm^{λ} marker rescue compared to R marker rescue in the recAsu^{-} - λred system.

Helper	DNA	Number of infectious centers containing recombinants/$10^{-6}A_{260}$ units of DNA		
		imm^{λ}	R	imm^{λ}/R
1. $\lambda red_{113}imm^{434}CIsusR_{54}R_{60}$	wholes	1288	1200	1.1
2.	halves	1176	1214	1.0
3. $\lambda bio72imm^{434}susR_{5}$	wholes	1100	1350	0.89
4.	halves	674	1200	0.61

The host bacterium was recAsu^{-} (Meselson 152) and the DNA whole and half molecule preparations were from λCI_{857}. The rescue of the R gene was assayed on W3350, and the rescue of imm^{λ} was assayed on W3350 ($\lambda imm^{434}susR_{54}R_{60}$).

recovery of the R marker, making it impossible that the difference in recovery of recombinants from half molecules between the red and the N experiments was simply due to the position of the marker involved.

(c) DISCUSSION

Two fundamental facts summarize the experiments described in this chapter.

1. The differing response of the recAsu⁻ λsusN system toward the whole and the half molecule - the ability to recover N function from a whole λ molecule but not a half molecule;

2. The differing responses of the recAsu⁻ - λ red system and the recAsu⁻ - λsusN system toward the same infecting half molecule - the ability to recover red function from a half molecule but the inability to recover N function. As a corollary, one can state that although N function cannot be recovered from a half molecule, N function can act on a half molecule to foster red transcription. In a formal sense, N function plus half molecule is equivalent to a whole molecule.

In order to reconcile the differing responses summarized above, one must first consider the factors which impinge upon transfecting whole and half molecules; these fall into two main classes, genetic control factors and physical-structural

properties. Secondly, one must ask if any of these operate preferentially on half molecules, as compared with whole molecules, such that template activity is altered in cells which lack N, and N controlled products. In addition, any explanation of the preferential inactivation of halves in λsus N helper infected recAsu⁻ host cells must take into account the efficient template activity of halves in the λred⁻N⁺ helper infected recAsu⁻ host. In this discussion, I will deal first with the situation occurring in the λsus N helper infected host, concluding that circularity of the template is essential for gene expression in a cell lacking N product; and secondly, I will correlate this conclusion with the situation existing for half molecules in a cell containing N product (i.e. the λred⁻N⁺ helper infected host).

The effects of both tof product and CI repressor have been assessed (Table 2, lines 1 and 2; Table 3) for the λsus N competent cells infected with half DNA and found to exert no preferential repression of half as compared to whole λDNA, as discussed earlier. In addition, marker rescue experiments showed that inability to rescue N gene was not due simply to more rapid degradation of the N gene as compared to the R gene which is rescued in the λred experiments (Table 4).

Since genetic control elements do not account for the discrepancy in template activity of whole and half

molecules, it is reasonable to consider topological dissimilarities of these two types of template. The most obvious features of the whole molecule are its two single stranded 5' ends which are homologous, allowing cohesion to yield a circular molecule. The half molecules used in these experiments possess only one cohesive end, the internal terminus being a broken, possibly double stranded end. These halves are thus unable to circularize and can provide only a linear template, while a whole molecule permits either a linear or a circular template. To test the relevance of this definition of half molecules as linear templates in relation to the inability to provide N gene function, λdg DNA was tested for template activity of N gene. This DNA is derived from a deletion-addition mutant which has lost the left half of λDNA and is effectively a right half with two cohesive ends. This λdg DNA is able to circularize and its template activity is identical to that observed for λ*sus*J whole DNA (Table 2, lines 1 and 2).

I conclude that in a host cell lacking N product, a circular template is essential for gene expression in a *rec*A mutant host.

Why is a circular template necessary for the provision of λ gene function in cells lacking N product?

Circularity of the DNA might provide essential protection against hostile cell nucleases while linear DNA loses template potential due to its digestion. An exonuclease

activity does exist in rec$^+$ and recA cells coded for by the recBC loci, which at least *in vitro* is very destructive to linear but not circular DNA (Barbour & Clark, 1970; Oishi, 1969; Goldmark & Linn, 1970). This enzyme appears to be hyperactive in recA mutant cells. Hyperactivity of the recBC nuclease, directed against a linear DNA molecule, is shown in the following experiments. Dubneau and Maas (1969) have demonstrated breakdown of lac z gene expression when the gene is transferred to a recA recipient as part of the linear chromosomal DNA (Hfr transfer), and persistence of function if the gene is transferred, as part of a circular F' factor. In addition, Itoh and Tomizawa (1971) have demonstrated loss of the λ marker when a λhC_{ts} genome is transferred chromosomally to a recA (λCts) recipient which is subsequently induced by heat. Loss of ability to produce λh^+ infective centers occurs very rapidly in a recA (λCts) recipient but remains high for at least 120 minutes in a rec$^+$ (λCts) recipient, suggesting physical destruction of the repressed λh^+ located on the linear chromosomal fragment in a recA cell. Transfer of the λh^+ marker as part of an episome resulted in persistence of the marker even in the recA recipient. As before, the genetic marker is lost when it is located on a linear DNA molecule, but survives when located on a circular DNA molecule.

The above results are relevant to my experiments since these experiments involved rescue of gene function from a

linear molecule in a *rec*A host. However, they do not explain the apparently contradictory fact that while gene *N* is not rescued from a linear molecule, *red* genes are very effectively rescued from that same linear molecule. A linear piece of DNA survives in a λred^-N^+ helper infected *rec*A host but that same piece of DNA is inactive in a λ*sus N* helper infected *rec*A host. Precedents exist for both these results. In agreement with the λred^-N^+ results, Weisberg, Gottesman and Little (pers. comm.) have shown that linear λDNA in a *rec*A cell survives long enough to provide measurable *int* product activity; this occurs in cells containing an int^-N^+ helper phage. On the other hand, Terzi (1968) has reported selective breakdown of linear DNA fragments in a λ*sus N* infected *rec*Asu^- host in an assay for rescue of λ genes from a host-restricted genome. Restriction enzymes of the host endonucleolytically cut the genome of an unmodified phage yielding a linear template, a very relevant fact as regards comparisons of the transformation assay and Terzi's system. Although his experimental technique is quite different from the transformation assay, Terzi's results are very similar in that although *N* gene cannot be rescued, genes *O*, *P* and *R* are very effectively rescued from restricted DNA.

These two very different systems, the transformation assay and Terzi's restriction system, have two main elements in common, namely the use of a λ*sus* phage needing gene function for rescue, and λDNA fragments as a source of the gene

function to be rescued. In both systems, gene function could be rescued when either helper phage, in the transformation assay, or unrestricted phage, in Terzi's system, were $\underline{N}^{+}$, but not if these rescuing phage were susN. It was possible that in the absence of N product itself or of an N controlled λ gene product, λDNA fragments were preferentially degraded and thus prevented from serving as a template for mRNA transcription. One could postulate that in the λred⁻N⁺ helper infected cell, the relevant exonuclease activity is inhibited, possibly by a λ coded gene product which protects linear λDNA and which is absent in a λsus N helper infected cell. In other words, half molecules are intact and thus transcribed in λred⁻N⁺ competent cells; they are prevented from activity as templates in λsusN competent cells by exonuclease degradation before transcription can begin.

If such degradation is occurring in my system, as would seem likely from the evidence just cited, then the extent of degradation of half molecules in a host cell lacking N product can be determined by infecting λsusN competent cells with half DNA molecules and, at various times after infection, adding N and red products to the cell. If halves remain undegraded inside the cell, introduction of these products should allow recombination of a half molecule marker into a whole λDNA recombinant. These experiments are described in the following chapter.

A second approach to the problem of preferential

degradation of half molecules is use of deletion and point mutant helper phage to determine if a λ gene product (other than the *N* gene product itself), under *N* control, exists which allows survival of half molecules in the λ*red*$^-$*N*$^+$ helper infected cell. A very likely candidate for this role is the λ*gam* gene which is under *N* control and which has been shown to inactivate the *rec*BC exonuclease *in vitro* (Unger & Clark, 1972). Use of host cell mutations in *rec*B, which lack the exonuclease activity should elucidate the role of this host enzyme in the degradation of halves. The experiments using this approach are described in Chapter 3.

CHAPTER TWO

Half molecules in the λ*sus*N Competent Cell

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Rescue of half DNA markers from a λ*sus*N competent cell

Numerous attempts were made to demonstrate the persistence of markers from the half molecule inside the λ*sus* *N* competent cell, since it was possible that the physiology of the λ*sus* *N* infected *recA**su*$^-$ cell was drastically different from the λ*red*$^-$*N*$^+$ infected cell such that in it the half molecule was rapidly and preferentially degraded. Three techniques of marker rescue were attempted: (a) Superinfection of transformed cells by free phage (b) superinfection of transformed cells by purified λDNA and (c) transformation of a lysogenic competent cell and induction of the prophage after infection by λDNA halves.

The theory of these experiments was as follows; if the half molecule were present in undegraded form and simply unable to be transcribed then introduction into the infected cell of both *N* and *red* products should allow rescue of markers on the half molecule by recombination. The above three techniques were attempts to introduce these products into the half molecule infected cell.

(a) Superinfection by λCI_{857}*sus*$R'_{54}R_{60}$ phage at moi = 10

This technique utilizes standard transformation assay conditions up to the point of infection of competent cells by λDNA. λ*sus*N competent cells were prepared, incubated with λ*vir* DNA wholes or halves for 10 minutes at 39°C, and then free phage were added at a moi = 10. This means that if half molecules remain undegraded, introduction of *N* and *red* products by the superinfecting phage should catalyze

recombination and rescue of the λvir marker from halves as a recombinant whole molecule.

As shown in Table 5, although superinfection causes about 10 fold increase in rescue of the λvir marker from halves in the λsus N cell, the ratio of halves to wholes remains low (from 4.3% to 6.7%) (Table 5, line 4, +/- superinfection) and this increase is not observed if infected cells are treated with DNase before superinfection. This suggests that the increase is due to a stimulatory effect of superinfection on DNA uptake by competent cells, since it is observed with both helpers.

By 10 minutes after infection with λDNA, uptake has reached its maximum level (Methods, Table F, section 7c) under normal or DNase treated conditions of infection.

Since the λvir marker is not effectively rescued when N and red products are introduced, one could conclude that it is not available for rescue. However the proportion of competent cells productively infected with DNA constitute only 0.01% of the total cell population making it difficult to determine if all cells infected with DNA were also superinfected with free phage. In addition, the possibility exists that transformed cells may have special properties which render them resistant to free phage infection. Although not conclusive these results support the idea that halves may be preferentially degraded in the λsusN host.

TABLE 5 - Rescue of λvir marker from transformed cells by superinfection.

	Helper	DNA	Infectious centers/$10^{-6} A_{260}$ units of DNA		
			No super-infection (c)	Superinfection moi = 10 (a)	DNase + Super-infection (b)
1.	$\lambda CI_{857}susR_{54}R_{60}$	λvir wholes	2100	1400	600
2.	"	λvir halves	588	760	240
3.	$\lambda CI_{857}susN_7N_{53}$	λvir wholes	350	1800	530
4.	"	λvir halves	15	119	2

Host was 152 recAsu$^-$

(a) Helper infected cells were infected with DNA at T = 0', superinfecting phage added at T = 10', and complexes were plated at T = 25'.

(b) DNA infection at T = 0', 3.0 μg DNase added at T = 10', superinfecting phage added at T = 12' and complexes plated at T = 25'.

(c) Transformation conditions as described in section 7 of methods, complexes were plated at T = 25', Indicator was C600 (imm^{λ} $susQ_{21}$). Superinfecting phage was CsCl purified $CI_{857}susR_{54}R_{60}$ diluted in TCM before addition to transformed cells in 0.1 ml volumes. DNase was added in 0.1 ml volumes (30 μgm/ml in λ dil).

(b) Introduction of N and red products by superinfection with purified λDNA at high multiplicity.

The conditions of assay were essentially the same as those described in (a) but purified λCI_{857} DNA was used as the superinfecting agent. If only certain classes of the helper infected host cell population are capable of being infected by λDNA then superinfection of λ half molecule infected competent cells by whole λDNA should ensure that those same cells which receive a half molecule will also receive an $N^+ red^+$ genome on superinfection. Competent cells were infected with λvir half molecules for 10 minutes and then superinfected with λCI_{857} DNA, moi = 10, and λvir recombinants were scored on lysogenic indicator.

Treatment of transformed complexes with high multiplicities of λDNA causes loss of plaque forming ability since the efficiency of DNA infection fell by over 80% for both wholes and halves, making this technique unsuitable for measurement of rescue of half molecule markers in the λsus N helper infected cell.

(c) Introduction of N and red products by prophage induction.

152 (λCI_{857}) host cells were made competent at 30°C by infection with λsus N helper phage, and then transformed by whole and half λDNA molecules. Since every host cell contains an N^+ red^+ prophage, on induction at 39°C, every host cell should receive N and red products. Competent cells

TABLE 6 - Superinfection of transformed cells by high moi of λCI_{857}DNA

	Host	Helper	DNA	Infective centers/$10^{-6}A_{260}$ units of DNA: No superinfecting DNA	Infective centers/$10^{-6}A_{260}$ units of DNA: CI_{857} superinfection at moi = 10
1.	152	λCI^{-}_{857} $\underline{sus}R_{54}R_{60}$	λvir wholes	440	90
2.	"	"	λvir halves	942	15

At 10' after infection with DNA, 0.1 ml of $10^{-2}A_{260}$/ml CI_{857} wholes were added and incubation continued to 25'. Indicator was C600 (λimm^{λ} $\underline{sus}Q_{21}$).

were infected with λ*vir* half DNA, and simultaneously incubated at 39°C to induce the prophage. Control experiments at 30°C provide a measure of half molecule rescue in the absence of added *N* and *red* products. Helper phage carry the imm^{434}*CI* region so as to remain derepressed in the lysogenic host, and λDNA carries the *vir* marker which is insensitive to the λ*CI* repressor.

At 39°C plating conditions, induced transformed cells are unable to support a lytic cycle directed by the infecting DNA (column 5), however overnight plating at a reduced temperature of 37°C allows transformation by whole λ*vir* DNA at normal efficiency (column 6). Halves in the 37°C experiment were rescued at 0.54%; this could not be due to re-establishment of repression since mock transformation experiments, using buffer instead of helper phage and DNA infection, indicated that essentially 100% of competent cells induced at 37°C. Table 7 records the results of an experiment designed to rescue half molecule markers if they remained intact within the transformed cell. Transformed complexes were plated on (imm^{λ}) (imm^{434}) indicator so that only λ*vir* recombinants could form a plaque. Column 4 records a control incubation at 30°C; little prophage induction occurs at this temperature and rescue of halves should be identical to that in the non-lysogenic competent host. This is in fact the case - rescue of halves is only 0.07% the level of wholes. In conclusion then, even though introduction of *N* and *red* products by prophage

TABLE 7 - Induction of prophage in transformed cells.

	Host	Helper	DNA	Infectious centers/10^{-6} A_{260} units of DNA		
				30° - 30° (a)	39° - 39° (b)	39° - 37° (c)
1.	152(CI_{857})	λimm^{434}CIsusNN	λvir wholes	2612	< 1	2400
2.	"	"	λvir halves	2	< 1	13

Competent cells were prepared by techniques described in methods Section 7 except that all incubations were at 30°C until the time of DNA infection itself. The indicator was C600 (λimm$^{\lambda}$ susQ_{21}) (λimm^{434} susR_{6C}). For mock transformed helper (no helper or DNA, only buffer) colony formers at 30°C = 1.7 x 10^8/ml; infective centers at 39°C (c) = 1.6 x 10^8/ml on W3350 indicator; infective centers at 30°C = 1.05 x 10^4/ml.

(a) Infection with DNA was at 30°C for 25 minutes. Overnight plating for infective centers was also at 30°C.

(b) Infection by DNA (25 minutes) and overnight plating were both at 39°C.

(c) Infection by DNA (or mock infection by buffer) was at 39°C for 25 minutes, overnight plating at 37°C.

induction, and infection by λ halves occur nearly simultaneously, little rescue of the vir marker results, suggesting that degradation of halves occurs immediately upon entry to a λsus N, recAsu$^-$ host.

DISCUSSION

Since half molecule markers cannot be rescued from a λsus N competent cell under any of the above rescue conditions, the simplest conclusion is that they are no longer available for rescue (i.e. they have been degraded). This in turn suggests that in the λsus N competent cell, circular topology provides essential protection against exonuclease degradation. A half molecule cannot be transcribed because the template no longer exists in intact form within the λsus N competent cell. Degradation of linear half molecules must occur very rapidly since in part c, where N and red products are introduced by prophage induction, repression ceases very rapidly and one would expect full levels of red product by 3-5 minutes after induction at 39°C, since high levels of l strand mRNA can be detected at this time (Kumar et al., 1969).

The inability to provide evidence that half molecules survive for any length of time in the λsus N cell leads one to the conclusion that λN^+ helpers code for a protective device which ensures survival and transcription of linear molecules. This conclusion is further discussed and experimentally supported in Chapter 3.

CHAPTER 3.

Influence of λ*gam* product and
E. coli recBC nuclease in template
activity of half molecules.

INTRODUCTION

The objective of the experiments reported here was to explore the roles played by the λgam product and the E. coli recBC exonuclease in provision of λ gene function from linear DNA templates. In chapters one and two, I discussed the inability to rescue N gene function from a half molecule of DNA using a Kaiser Hogness transformation assay system (Pilarski & Egan, 1972; Kaiser & Hogness, 1960). This system included λsusN_7N_{53} helper phage in a recAsu$^-$ host infected with half molecules of λDNA. N gene function from half molecules was assayed by measuring production of recombinants catalyzed by N dependent red function, the only source of active N product being the N gene on the infecting half molecule. No recombinants were produced, indicating an inability of the half molecule to serve as a template for N gene product. In this chapter, I demonstrate that the lack of template activity is due to degradation of the half molecules before transcription can occur, and that if degradation is prevented, N gene is efficiently transcribed from half molecules.

Both phage and host gene functions participate in the inactivation versus protection phenomenon of half molecules in both rec$^+$ and recA hosts. I will first consider the relevant features of the host rec system and then discuss the λgam gene effect as related to the fec and spi phenotypes.

The major E. coli recombination system consists of three genes, recA, recB and recC. recB and recC code for an exonuclease activity composed of two non-identical polypeptide chains

(Clark, 1971). This exonuclease has been extensively purified and found to possess four enzymatic activities *in vitro* - ATP dependent single stranded and double stranded exonuclease activities, ATP independent endonuclease activity, and an ATP ase activity (Goldmark & Linn, 1972; Barbour & Clark, 1970). *E. coli recA* mutants are characterized by extreme U.V. sensitivity resulting in extensive degradation of cell DNA after short U.V. treatments ("reckless" phenotype). By comparison, *rec*B or *rec*C mutants are far less sensitive than *rec*A to U.V. treatment ("cautious" phenotype). Double (*rec*A *rec*B), or triple (*rec*A *rec*B *rec*C) mutants behave like single *rec*B or *rec*C mutants, suggesting that the "reckless" phenotype of *rec*A mutants is due to *rec*BC nuclease activity which is hyperactive in the absence of *rec*A product (Willets & Clark, 1969). No known enzyme activity has been attributed to the *rec*A gene product, and the mechanism by which it reduces *rec*BC nuclease activity *in vivo* is unknown. The reduction in *rec*BC activity by *rec*A product has also been observed in *in vitro* lysates of *rec*A^{+} as compared to *rec*A^{-} cells (Clark, 1971; Hout et al., 1970).

Certain λ mutants are unable to plaque on a *rec*A host cell although they grow normally on *rec*B and *rec*A *rec*B hosts. This growth pattern has been termed the *fec* phenotype (Manly et al., 1969; Zissler et al., 1971a). These mutants fall into two classes; λ*bio* deletion - addition mutants which extend from *att* to beyond the *red*B gene, and secondly, double point mutants defective in *red*X or *red*B, and in *gam*. λ*gam* gene was postulated to exist in

the region of the biol endpoint, and on isolation and mapping of gam point mutants, it was found that biol was deleted into the gam gene (Fig. 1)(Zissler et al., 1971a). λgam gene is positively controlled by gene N (Franklin, 1971; Unger et al., 1972), and is a nonessential function since λgam plates normally on rec^+ and recA hosts (Zissler et al., 1971a).

Lack of gam product is pivotal for the spi phenotype as well as the fec phenotype (Zissler et al., 1971b). Like fec, the spi phenotype was first demonstrated as a property of extended λbio deletion-addition phage, and refers to the ability of a λ phage to grow on a P2 lysogen (spi = sensitive to P2 interference). λspi^+ does not grow on P2 lysogens, on which λspi plates normally (Lindahl et al., 1970). Zissler et al., (1971b) have shown that reconstruction of point mutations to yield the spi phenotype requires mutations in three of four λ nonessential genes, del, redX or redB, and gam. The role of λspi^+ products in P2 lysogens is suggested by consideration of two observations; 1- a P2 gene product, old^+, kills recB mutant hosts and 2- λspi mutants do not grow on a recA $recB^+$ host but plate normally on a recA recB host (Sironi et al., 1971). Sironi and his co-workers have postulated that λspi^+ products convert a $recB^+$ host to a recB phenocopy. If this occurs in a P2 lysogen, since old product is constituitively produced, the P2 old product will then kill the phenotypically recB host. The λspi mutants do not convert the rec^+ host to a recB phenocopy, and thus are able to grow normally on a P2 lysogen. Since λspi cannot yield a recB phenocopy in a recA host,

it may be attacked by the recB product and thus can grow only in recA mutants which are also recB, or rec^+ cells where recA product control exists. λspi^+ can "turn off" the recBC nuclease whereas λspi cannot.

A direct demonstration of role in the fec and spi phenotypes played by λgam gene has been provided by Unger & Clark (1972). *In vitro* experiments which measure the effect of gam product on recBC nuclease activity have confirmed the postulates of Sironi et al., (1971). Infection of a rec^+ host cell by λ^+ results in loss of ATP dependent recBC exonuclease activities as well as the endonuclease activity; conversely, λbio10 (which is λspi) infection allows full levels of recBC nuclease activity. Further studies showed that single point mutations in λgam gene produced the same effect - full activity of recBC nuclease after λgam infection, and complete loss of activity after λgam^+ infection; in addition λgam^+ was dominant to λgam in mixed infection. *In vitro*, addition of λgam^+ lysates to purified recBC nuclease also results in inactivation of the nuclease (Unger & Clark, 1972). Unger, Echols and Clark (1972), in related studies, have demonstrated the inactivating effect of λgam product on recBC catalyzed recombination between two red^- phage; recombination is reduced to approximately 10% of the level observed for λbio10 ($red^- gam^-$) in a rec^+ host. In summary, *in vivo*, λ phage requires both red and gam products for growth in a recA host; however only gam product is required for loss of recBC nuclease activity as measured *in vitro*.

If the inability of half molecules to provide N function were due to a combination of recBC nuclease activity and lack of λgam product, the following predictions could be made:

1. Since λsusN in an su⁻ host is phenotypically red⁻gam⁻ (Unger & Clark, 1972) it should exhibit the fec phenotype in a recAsu⁻ host and this may be responsible for the inability of λsusN to rescue halves. If this is true, λbio deletion-addition phage which are fec⁻ should also fail to yield recombinants when used as helper for half molecules of λDNA, even though these λbio phage are N⁺.

2. If the fec effect is due to lack of gam product which then allows uncontrolled degradation of halves, then helper phage which are red⁺gam⁻ should behave in similar fashion to susN helper phage, in a recAsu⁻ host. That is, they should be unable to protect half molecules, denying them participation in recombination events.

3. If recBC exonuclease is degrading halves and thereby preventing their transcription in recA hosts, then a recB mutant host should allow efficient transcription of the N gene on a half molecule.

The following experiments verify all of the above predictions and demonstrate convincingly that gam gene product is required for protection of linear molecules in a recA host cell. In addition, it is shown that recBC exonuclease activity acts preferentially on half molecules of λDNA in recA hosts. All experiments were done in vivo using the Kaiser Hogness transformation assay system.

RESULTS

(a) Effect of *fec* phenotype in the transformation assay.

The λ*sus*N mutation, because of its pleiotropic effect, is *red*$^-$*gam*$^-$ phenotypically and thus may exhibit the *fec* phenotype in a *recA su*$^-$ host. To test this postulate, I constructed λ*bio*1 and λ*bio*10 helper phage (Fig. 1) which are "*fec*-less" in a *recA* host, and measured the ability of these helpers to elicit *red* function from half and whole λDNA templates. These helpers are *N*$^+$ and therefore the transformation will be occurring in the presence of *N* product, contrasting with the λ*sus*N helper which produces no functional *N* product.

Transformation by the λ*bio* phage is presented in Table 8. Comparison of the λ*sus*N result (line 1) with λ*bio*1 and λ*bio*10 (lines 2 and 3) indicates that their behaviour is virtually identical. None of these helpers permit efficient transcription of half molecules even though transformation by various whole DNA's is unimpaired. λ*sus*N, *bio*1 and *bio*10 allow only 3.2%, 4.2% and 3% recombination of halves as compared to *sus*J wholes, while *bio*72 permits 66% rescue of halves through recombination mediated by the *red* gene located on the half molecule. λ*bio*72 (line 4; Table 8) which is *fec*$^+$ allows efficient transcription of both wholes and halves indicating that the gene function required in lines 1-3 (Table 8) is not *N* gene but rather some function encoded in the region between the *bio*72 and *bio*1 end points.

TABLE 8 - Ability of *fec* helper phage to rescue *red* function from whole and half molecules of λDNA.

	Host	Helper	λ$^+$ halves	susJ wholes	susP wholes	bio72 susP wholes	% rescue of halves
			sus$^+$ infectious centers/10^{-6} A_{260} units of DNA				
1.	*recAsu*$^-$	λ*susN*	40	1236	440	-	3.2
2.	"	λ*bio*1*susR*	22	526	230	< 1	4.2
3.	"	λ*bio*10*susR*	10	335	110	3	3.0
4.	"	λ*bio*72*susR*	733	1110	327	10	66

Host was 152. Indicator was W3350. All DNA preparations carried the CI_{857} marker. Complete helper phage genotypes as follows: λ*sus*N_7N_{53}*imm*^{434}CI, λ*bio*1*imm*^{434}CI*sus*R_{60}, λ*bio*10*imm*^{434}CI*sus*$R_{54}R_{60}$, λ*bio*72*imm*^{434}CI*sus*$R_{54}R_{60}$. *sus*P DNA was P_{228}. *sus*J was J_6.

$$\% \text{ rescue of halves} = \frac{\lambda^+ \text{ halves}}{susJ \text{ wholes}} \times 100$$

*sus*J wholes were considered the better approximation of the situation occurring with half molecules since recombination can occur over the same region of DNA (*J*-*N* for the *sus*N helper and *J*-*R* for the *bio* helpers) and would involve both *int* and *red* systems. *sus*P DNA indicates the extent of *red* mediated recombination between *P* and *R*, and *bio*72*sus*P DNA measures residual recombination in the system when all known general recombination systems are mutant - the helper is *red*$^-$, the DNA is *red*$^-$ and the host is *rec*$^-$. Since this *red*$^-$ DNA

Cont'd.

yields no recombinants in lines 2-4, the levels of recombination observed are mediated by the _red_ genes of the DNA for _bio_ helpers.

Variability in Assay

	% rescue of halves
Line 1	1.5, 1.6, 3.2, 4.3, 6.9
Line 2	0.6, 4.2
Line 3	3.0, 4.0
Line 4	41, 59, 60, 64, 66, 110, 135

Data presented in the table was chosen from the midpoint of the range of variability whenever possible, in this and future tables.

(b) Gene function responsible for inability of λsusN and λbio helpers to permit gene function from halves.

Two known genes are located between the bio72 and bio1 end points - redB and gam. Helper phage mutant in redB were constructed, and bio72 gam point mutants were prepared by NNG mutagenesis. Helpers carrying a redB point mutation efficiently (65%) rescue both half and whole molecules of λDNA; however, bio72 gam helper phage mimic the behaviour of λsusN helpers in that they are unable to rescue half molecules efficiently (11.6%) (lines 1 & 2, Table 9). The behaviour of the bio72 gam mutant suggests that loss of gam product is responsible for the lack of transcription of half molecules in the λsusN, λbio1 and λbio10 experiments. The ability of the λbio72 gam$^-$ helper to rescue halves varied from 2 - 23% as compared to susJ wholes. This is higher than the values observed in the λsusN and λbio cases but could be due to leak of the sus gam mutation (see Appendix, Part 1). The λbio72 sus gam helper is not inherently defective in ability to recombine with the half molecule since in a rec$^+$su$^+$ host, in which the gam mutation is suppressed, recombination is normal (line 4, Table 9). [This is not due to the host rec$^+$ system, as will be discussed later (Table 14)]. The bio72 gam$^+$ helper (line 3, Table 9) allows efficient transcription and recombination of halves.

Since λbio72 gam is defective in both red and gam genes, λred$^+$ gam$^-$ helper phage were constructed to elucidate the

TABLE 9 - Effect of redB and gam mutations on rescue of λ half molecules.

	Host	Helper	sus^+ infectious centers/$10^{-6}A_{260}$ units of DNA: λ^+ halves	susJ wholes	redB susP wholes	% rescue of halves
1.	$recAsu^-$	λredBsusR	1728	2664	12	65
2.	$recAsu^-$	λbio72sus gam susR(a)	111	955	9	11.6
3.	$recAsu^-$	λbio72susR	885	1472	28	60.
4.	rec^+su^+	λbio72sus gam susR	1070	944	-	114

Hosts were 152 and R^-A9605. Indicator was W3350. Helper phage were $imm^{434}CIredB_{114}susR_{54}R_{60}$ and $bio72sus\ gam_{14}imm^{434}$ $CIsusR_{54}R_{60}$. DNA carried the CI_{857} marker. susJ was J_6. For complete set of results see Appendix at end of thesis (Part 2).

Column 5, line 1 - $red_{114}CI_{857}susP_3$ ‖ % rescue = $\frac{\lambda^+ \text{ halves}}{\text{susJ wholes}} \times 100$

line 2 - $bio72CI_{857}susP_{228}$

(a) The values presented in Table 11, line 2 are artificial - they represent an average of 5 separate transformations with this helper under identical assay conditions. The actual values varied widely (2-23%), a situation not observed for other helpers in the $recAsu^-$ host. The experimental results from which this average was calculated are recorded in the Appendix, Part 1 (Table 24) along with a partial analysis of the factors influencing this variability.

Cont'd.

Variability in Assay

	% rescue of halves
Line 1	65.[illegible], 69
Line 2	2, 7.2, 12.4, 13.6, 23
Line 3	41, 59, 60.[illegible], 64, 66, 110, 135
Line 4	114.[illegible]

role played by the gam product in rescue of half molecules. λsus gam$_{210}$ helper, which is red$^+$, exhibited the low rescue of half molecules (9.7%) seen in bio72 gam helpers, indicating that the red$^+$ genes exert no influence in ability of a helper to rescue halves in a recAsu$^-$ host. This helper is able to recombine with halves when the gam point mutation is suppressed (Table 10, line 3). λgam$_5$, which displays 21% recombination with halves, has been described by Zissler et al., (1971a) as yielding an extremely "leaky" fec phenotype when combined with bio72; the 21% rescue of halves could be due to higher levels of active gam product synthesized by this mutant. λred$^+$ gam helpers produced variable levels of half molecule rescue similar to those observed for the bio72 gam helper (see legend to Table 10). In conclusion, a defect in λgam gene alone results in inability to rescue halves by recombination, even though the λgam$_{210}$ helper is red$^+$ both genotypically and phenotypically (i.e. high recombination with red$^-$susP whole DNA, Table 10, Column 6, line 1), and the only function required from the half molecule is that it be available for recombination.

(c) Ability of helper to rescue halves in the presence of CI repressor.

Theoretically, a helper phage entering a lysogenic host containing a coimmune prophage will be immediately repressed and should be phenotypically gam$^-$; one would expect gam to be under direct negative control by CI repressor. Under

TABLE 10 - Effect of λgam helpers on rescue of halves.

	Host	Helper	sus⁺ infectious centers/$10^{-6}A_{260}$ units of DNA				% rescue of halves
			λ^+ halves	susJ wholes	susP wholes	bio72 susP wholes	
1.	recA su$^-$	λsus gam_{210} susR	305	3128	1140	1048	9.7
2.	recA su$^-$	λgam_5 susR	603	2880	1164	620	20.9
3.	rec$^+$ su$^+$	λsus gam_{210} susR	1119	2360	-	-	47

Transformation assay was as described in methods. Helper phage were imm^{434}CIsus$R_{54}R_{60}$.

$$\% \text{ rescue} = \frac{\lambda^+ \text{ halves}}{\text{susJ wholes}} \times 100$$

Variability in assay

	% rescue of halves
Line 1	1.8, 9.7, 12.5
Line 2	3.4, 20.9, 24.2

The low level of rescue of half molecules, and the variability in this level were not unique for λCI_{857} halves. Similar results were obtained when λimm^{21} and λvir halves were tested in the assay. See Table 29, Appendix Part 2.

these circumstances, gam$^+$ helper phage, which normally allow transcription of halves, should now give a negative result similar to that observed for λsusN helper in a recAsu$^-$ host. This prediction is supported by the results in Table 11. λCI_{857}susN helper (line 1; Table 11) provides a control, demonstrating that rescue of halves is comparable to that in a non-lysogen, and that spontaneous induction of the prophage does not produce "psuedo" rescue of halves.

In the experiment of line 2, Table 11, helper phage functions are repressed by the λimm^{434} prophage repressor but the infecting DNA is heteroimmune and hence unaffected by this repressor. If the DNA is available for transcription, red product should be made and imm^{λ} recombinants will result which can be plated on selective indicator. Comparison of lines 2 and 3, (Table 11), the λimm^{434} helper in the lysogenic and non-lysogenic host cells, verifies the above prediction. λimm^{434}bio72 helper, which in the non-lysogen shows 66% rescue of half molecules (line 3; Table 11), exhibits a 13-fold drop in rescue of halves when transformation occurs in the lysogenic host (line 2; Table 11). This reduction in transcription of halves for red functions when helper phage functions are repressed suggests that a λ gene product under CI repressor control acts to protect half molecules and allow their transcription. This result is in agreement with a repressor control mechanism for the gam gene.

TABLE 11 - Effect of repressed helper on rescue of λ halves.

	Host	Helper	sus^+ infectious centers/ $10^{-6}A_{260}$ units of DNA: λ halves	sus^+ infectious centers/ $10^{-6}A_{260}$ units of DNA: susJ wholes	% rescue of halves
1.	recAsu$^-$(imm^{434})	λCI_{857}susN	7	276 (a)	2.5
2.	recAsu$^-$(imm^{434})	λimm^{434}bio72susR	23	441	5.2
3.	recAsu$^-$	λimm^{434}bio72susR	865	1449	66.[illegible]

Hosts were 152 (imm^{434}) and 152. Helper phage were susN$_7$N$_{53}$ and susR$_5$. DNA carried the CI_{857} marker. Indicator was W3350 (imm^{434}susR$_{54}$R$_{60}$).

$$\% \text{ rescue} = \frac{\lambda^+ \text{ halves}}{\text{susJ wholes}} \times 100$$

(a) λ^+ whole DNA was used in this line.

(d) Effect of the recBC exonuclease on rescue of half molecules.

The experiments so far reported were all performed in a host possessing an active recBC product. Several lines of evidence suggest the involvement of the recBC exonuclease in the process inactivating λ half DNA molecules. First, λfec phage, which cannot plate on a recA mutant, plate normally on a recB mutant host and, by analogy, it might be expected that λfec helpers would efficiently rescue half molecules of λDNA in a recB host. Secondly, I have shown that λgam product is required for efficient transcription of halves, which can be correlated with Unger & Clark's (1972) observation that gam product inhibits recBC exonuclease activity. One could predict that halves would be efficiently transcribed in a recB mutant host for both λfec and λsusN helpers, the necessity for gam product protection having been removed.

Both recB and recA recB mutants were used as host bacteria (Table 12) with no significant differences between them, although rescue of halves does seem to be higher in the recB as compared to the recA recB host (Table 12, lines 1-3 vs lines 4-10). λsusN, λbio and λgam helpers all allow extensive rescue of halves (at 57-143%) which indicates that the linear half molecule remains intact long enough to permit red product activity. Residual recombination in both host types occurs at a low level comparable with that observed for the recA host. Therefore the recombination

TABLE 12 - Rescue of halves in recB and recA recB hosts.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA				
			λ^+ halves	susJ wholes	bio72 susP wholes (a)		% rescue of halves
1.	$recBsu^-$	λsusNN	1713	1245	421		138
2.	"	λbio1susR	2170	2080	95		104
3.	"	λbio10susR	1786	1246	< 1		143
4.	$recArecBsu^+$	λbio10susR	327	513	6		64
5.	$recArecBsu^-$	λsusNN	1893	2077	271	(a)	91.
6.	"	λbio1susR	1212	2112	76		57.
7.	"	λbio10susR	1632	1816	23		90.
8.	"	λbio72gam_{14}susR	1024	1815	< 1		56.
9.	"	λgam_{210}susR	2912	3176	-		91.
10.	"	λgam_5susR	1976	2704	-		73

$recBsu^-$ = DM22; $recArecBsu^+$ = JC5475; $recArecBsu^-$ = E835.
Helper phage are imm^{434}CI except for line 1 which is CI_{857}.
Assay was as described in Methods - 7. Indicator was W3350.

(a) The recombination observed here is due to N product from the transfecting DNA activating red genes of the helper phage.

observed is due to red function from the infecting λDNA molecules (Table 12, Column 5, lines 2-4, 6-8) and indicative of transcription of the infecting DNA. These experiments also indicate that no physical block to recombination with halves exists for helpers used in Table 12.

Elimination of the recB product activity allows survival of the half molecules regardless of the helper phage genotype. This suggests that the recBC exonuclease preferentially degrades half molecules, thereby preventing their transcription in recA host cells.

(e) Degradation of halves in a rec^+ host.

Since Clark (1971) has suggested that the recA product limits or inhibits the activity of the recBC nuclease, it might be expected that half molecules in a rec^+ host would have a better chance of survival than in a recA host in which the recBC nuclease is virtually uncontrolled. Various helper phage which were unable to elicit function from half molecules in the recA host were tested in a rec^+ host. The basic requirement in these experiments is that the half molecule persist in the cell long enough for recombination to occur; this recombination may be catalyzed by host rec^+ enzymes, or by λred enzymes (from either transfecting DNA or helper phage, depending on helper phage genotype). Recombination with halves will depend upon protection of the halves by the $recA^+$ product in all experiments where the helper phage is gam^-.

If the helper phage is red^-gam^+, halves are efficiently rescued at 60% of susJ wholes in the rec^+ host (Table 13, line 3). However if the helper phage is gam^-, halves are very poorly rescued (4-6%) indicating degradation of halves even in red^+, rec^+ conditions (Table 13, line 5). This result is identical to that observed for the recA host and suggests that the $recA^+$ product does not exert a limiting effect on recBC nuclease activity, at least as far as degradation of half molecules is concerned. Possibly the nuclease level in a rec^+ host is more than sufficient to decimate the half molecules and the excess activity in the recA host is superfluous.

The gam helper phage, $bio72gam_{14}$ and gam_{210}, both exhibit a lowered ability to rescue halves in the rec^+ as compared to the recA host. In the recA host, both rescue halves at a variable level which can rise as high as 20% for the gam_{14} mutant; however in a rec^+ host this level is non-variable at about 4%. Assuming that the 20% value represents gam product from "leak" translation of the gam^- gene on the helper phage, and also that this "leak" should be comparable for both $recAsu^-$ and rec^+su^- (these hosts are isogenic), this suggests that recA product may antagonize λgam product inactivation of the recBC nuclease. Furthermore, functional recA product does not truncate the action of recBC nuclease on half molecules as might have been expected. The behaviour of the gam mutants suggests that possibly more gam product is required in a rec^+ host than in a recA host.

TABLE 13 - Rescue of halves in a rec^+ host.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA: λ^+ halves	susJ wholes	% rescue of halves (a)
1.	rec^+su^-	λsusN	100	1833	6.0
2.	"	λbio72sus gam_{14}susR	69	1573	4.4
3.	"	λbio72susR	1066	1744	60
4.	"	λbio1susR	52	1294	4.0
5.	"	λsus gam_{210}susR	120	2644	4.5

rec^+su^- = W3350. All helpers carried the imm^{434}CI marker, susN helpers were N_7N_{53}, susR helpers were R_{60} or $R_{54}R_{60}$. The DNA was λCI_{857}. Transformation assay was as described in Materials & Methods. Indicator was W3350.

(a) $\% \text{ rescue} = \frac{\lambda^+ \text{ halves}}{\text{susJ wholes}} \times 100$

Variability in assay of half molecule rescue

	% rescue of halves
Line 1	0.15, 4.2, 4.6, 6.0, 7.3
Line 2	2.5, 3.1, 3.6, 4.4
Line 3	60.
Line 4	2.1, 4.0
Line 5	0.4, 4.5

The degradation of half molecules, in the absence of λ*gam* product, by the *rec*BC exonuclease, can be observed as clearly in the *rec*$^{+}$ as in the *rec*A host. Therefore, although the phenomenon was initially observed in an abnormal situation, the *rec*A host, it is still a valid assay of the difficulties which beseige a linear template since identical observations can be made in a *rec*$^{+}$ host, which possesses a cellular environment of the type which an infecting phage might normally encounter during selective evolution. In conclusion, the low rescue of half molecules by gam_{210} helper phage in the *rec*$^{+}$ host confirms that *gam* gene product alone protects halves, and the presence of active *red* and *rec*A product provides no protective activity in this assay system. Inactivation of the *rec*BC nuclease activity is accomplished solely by the activity of *gam* gene product.

DISCUSSION

First, I would like to review the experimental observations made in this chapter, and then discuss their significance.

I have demonstrated that a λ gene product, under direct control of CI repressor and positively controlled by gene N, located between the bio72 and biol end points, is essential for survival of linear half molecules of λDNA in a rec^+ or recA host. Isolation of gam^- point mutants, which cannot ensure survival of the half molecule in the rec^+ and recA hosts, identifies the factor responsible for maintaining the integrity of the half molecules as gam gene product. In a recB or recA recB mutant host, gam gene product is no longer required for half molecule integrity since half molecules are efficiently transcribed and must therefore be intact.

It is important to note the divergent behaviour exhibited by whole molecules as compared to half molecules, since my conclusions are derived from comparisons of the activity of these two template types. Whole molecules are efficiently transcribed with both gam and gam^+ helpers in rec^+, recA, and recB hosts. The interaction of gam product and recBC nuclease is observed only in the case of transfecting half molecules. On infection whole molecules of λDNA are transcribed for gam ensuring that at later stages of phage development gam product is present. The half molecule may be degraded immediately upon entry to a λgam helper infected recA host since although it is gam^+, the protective effect of this resident gam gene is never observed and it is assumed that no

transcription or translation of gam occurs. In a rec$^+$ host, the half molecule is still very vulnerable to exonuclease attack in the absence of gam product. One must conclude that, at least in the case of a half molecule, recA product control of the recBC nuclease has essentially no protective activity. In these assay conditions recA product and λ red products do not cooperate in protection of half molecules; this is accomplished solely by λgam gene product.

Two main conclusions can be drawn from this work. Firstly, the λgam gene product, although non-essential for normal λ infection, is essential for protection of half molecules of λDNA in both rec$^+$ and recA host cells. In the absence of gam product, halves are not transcribed for red or N genes. Whole molecules are efficiently transcribed in both the presence and absence of gam product. And, secondly, the recBC nuclease preferentially degrades half molecules of λDNA in the absence of λgam product. In a recB mutant host, halves are transcribed irrespective of the helper phage phenotype; therefore gam product protection of halves is not required if recB product is inactive. One must also conclude, at least for the red$^+$gam$^-$ helper phage in rec$^+$ or recA hosts, that λ red product is unable to repair the damage caused by recBC nuclease acting on the half molecule.

In order to determine the factors responsible for this preferential activity of recBC nuclease on halves, it is necessary to consider physical differences between half and whole molecules. The major difference between whole and half molecules is the

ability of whole molecules to circularize via cohesion of the "sticky ends". Half molecules of necessity remain linear since they possess only one cohesive end. In addition, whole molecules have two single stranded ends, while half molecules possess one single stranded end with the internal terminus very possibly a double stranded break. Either or both of these factors - a free single stranded end and a free double stranded end - may be responsible for the preferential degradation of halves exhibited in λgam-helper infected recA and rec$^+$ cells. Unger & Clark (1972) have noted that the double stranded exonuclease activity possessed by the recBC product is particularly destructive, and that λgam product may have evolved as protection for λDNA during multiplication or maturation steps which may involve double stranded DNA ends. Both genetic and biochemical techniques have demonstrated the inactivating effect of λgam product on the recBC exonuclease (Unger, Echols & Clark, 1972; Unger & Clark, 1972).

Enquist and Skalka (1972) have demonstrated the involvement of λgam product in the production of concatameric DNA forms during λDNA replication (similar results for λspi, Hobom & Hobom, 1972). In the absence of λgam product, recBC nuclease blocks the transition from early to late modes of λDNA replication possibly through attack at a free end of DNA, or an extended single stranded region, such that concatamers do not appear as products of the replication process. This abnormal DNA replication in the absence of gam product is seen in rec$^+$ as well as recA hosts, in agreement with my results. The low burst sizes exhibited by λgam mutants (37%

of the λ*gam*$^+$ burst, Enquist & Skalka, 1972) in the *rec*$^+$ host are no doubt due to the less efficient mode of replication necessitated by lack of *gam* product. By exerting a protective effect on λDNA at stages in the replication cycle where free DNA ends exist, *gam* product enhances survival of λ phage, yielding higher numbers of progeny phage. To summarize, the results of Enquist & Skalka agree with my results at several key points; (a) λ*gam* product protects DNA forms with free ends from *rec*BC nuclease attack. In both systems, only circular forms survive in the absence of *gam* product, (b) *rec*A product and/or λ*red* product cannot substitute for λ*gam* product in the protection of λDNA species with free ends, and (c) the need for λ*gam* product is obviated by removal of *rec*BC nuclease (i.e. as in *rec*B mutants).

Some interesting insights into the *in vivo* functioning of the *rec*BC nuclease activity are possible in our system. Goldmark & Linn (1972) have extensively purified the *rec*BC product and studied its *in vitro* properties; it possesses ATP dependent single stranded and double stranded exonuclease activities, and a single stranded endonuclease activity. It is important to note that they were unable to detect any endonuclease activity on covalently closed double stranded circular DNA even at enzyme concentrations 10-fold higher than that needed to nick an equivalent number of single stranded DNA circles. Injected phage λDNA assumes a covalent circular form immediately after infection (Salzman & Weissbach, 1967) and I have extrapolated this fact to purified infecting whole λDNA. Using the transformation assay as an

in vivo assay for *rec*BC activity, (with λ*gam* helper so as to leave *rec*BC activity unimpaired (Table 2, lines 1-3)) whole λDNA is unaffected while linear half DNA is degraded. This indicates that, *in vivo*, *rec*BC nuclease does not nick and degrade covalent circular whole λDNA, but does extensively and rapidly degrade linear λDNA (halves), in agreement with the *in vitro* observations of Goldmark and Linn.

In searching for an evolutionary motivation to develop an enzyme activity which acts preferentially on linear molecules of DNA, I hypothesized that such a nuclease is essential for removal of irrelevant DNA pieces which could otherwise uselessly expend cell energy on their transcription and translation, for example, fragments of DNA "left over" from recombination processes. The term "irrelevant" could be redefined as "linear"; selective pressure would then ensure that "relevant" DNA molecules assume the covalent circular form as a protective measure. Itoh and Tomizawa (1971) note that no report of abortive conjugation has appeared in the literature and suggest that inactivation of transferred fragments of the Hfr chromosome in zygotes may be responsible for the lack of abortive conjugation. Transferred, unrecombined fragments of Hfr DNA would be linear pieces and therefore very susceptible to *rec*BC nuclease activity.

The evidence appears to involve the *rec*BC nuclease in cell sanitary engineering projects such as disposal of unrecombined linear DNA fragments, since such removal is extremely rapid in *rec*A mutants (Dubnau & Maas, 1969; Itoh & Tomizawa, 1970). This idea

is further supported by the fact that all known autonomous replicons within the bacterial cell are covalent circular structures (i.e. col, R factors; λ and Pl plasmids; and the E. coli genome itself, Clewell & Helenski, 1971). One could postulate that all organisms whose life cycles involve a linear DNA stage will have evolved a protective mechanism to ensure survival of this linear form. Both λ and phage T4 have phage coded systems for inhibiting recBC nuclease activity; these exert their effect at about 5 minutes after infection (Unger & Clark, 1972; Tanner & Oishi, 1971). However, it seems obvious that for many phage, protection of linear DNA will be required at two stages; first, immediately after infection when linear parental DNA is injected, and secondly, during later DNA replication stages. I will briefly consider two such phages, P22 and T4, which inject their DNA in a form which cannot immediately circularize. Phage P22 is terminally redundant and in order to circularize, it requires a recombination event between these ends. This is catalyzed by the P22 erf product or, in its absence, the rec^+ system of the S. typhimurium host (Yamagami & Yamamoto, 1970; Botstein & Matz, 1970; Woodworth-Gutai et al., 1972). In this case a functioning recBC type nuclease may actually enhance the ability of the phage DNA to circularize by degrading terminal redundancies to yield "cohesive" ends. At later stages in the P22 cycle, a phage function inhibits a Salmonella typhimurium exonuclease activity, possibly analagous to λgam activity (Israel et al., 1972). Phage T4 also injects its DNA in linear form; T4 is circularly permuted and has glucosylated hydroxy methyl cytosine containing DNA which has been shown to

protect the DNA from host restriction DNases (Cohen, Chap. 2, 1968). This may not be true for recBC nuclease activity since Oishi (1969) has demonstrated degradation of native T4 DNA by recBC nuclease in vitro; in vivo T4 may have a nuclease inhibiting activity, possibly associated with tail or internal proteins, which inactivates recBC nuclease until the T4 "gam" type system becomes functional. This idea is supported by the fact that T4 ghosts will inhibit host syntheses, indicating that T4 structural proteins may have enzymatic activity (Vallee, Cornett, & Bernstein, 1972). Similar protective systems may be employed by other T phages, such as T7 which injects its DNA in linear form and replicates as a linear form (Dressler et al., 1972).

A second important observation to emerge from this investigation is the fact that in wild type conditions - a rec^+ host and λgam^+ helper - linear molecules of λDNA serve as efficient templates for transcription of λ genes in vivo. Only in certain circumstances, a rec^+ or recA mutant host with λgam helper phage, is a circular template required as protection against exonuclease degradation. This suggests that DNA topology plays no direct role in early λmRNA transcription in vivo. Similar conclusions can be drawn from the work of Blattner & Dahlberg (1972) who obtained highly specific initiation of very early RNA synthesis, identical to that obtained in vivo, using only whole λDNA and purified RNA polymerase. This suggests that in vitro circular topology is not an essential co-factor for specific initiation of λmRNA synthesis, nor are any additional protein co-factors necessary. The relationship between circles and transcription will be discussed

further in Section V.

In summary, I have reached the following conclusions:

(a) λ*gam* product has an inactivating effect on the *E. coli* *rec*BC product *in vivo*.

(b) In the absence of *gam* product, linear half molecules are preferentially degraded in *rec*$^{+}$ and *rec*A cells.

(c) The *rec*BC nuclease is responsible for this degradation of half molecules but has no effect on whole molecules which can circularize.

(d) Linear λDNA serves as a very efficient template for both *red* and *N* genes *in vivo*, in conditions where *rec*BC nuclease is inactive.

CHAPTER 4.

COIMMUNE INHIBITION OF HALF MOLECULES.

INTRODUCTION

The *red* system of λ was identified by isolation of λ mutants unable to catalyze generalised recombination (Echols & Gingery, 1967; Signer & Weil, 1968). These mutants were found to fall into three main groups based on complementation behaviour, *redX*, *redB*, and *redXB* (Shulman et al., 1970). Group X can be further subdivided into three classes of mutations but biochemical studies by Radding (1970) suggest that these are all part of the same gene and represent intragenic complementation. Similar conclusions can be drawn from mapping studies (Shulman et al., 1970).

Correlations between temperature sensitive *redX* mutants and production of temperature sensitive λ exonuclease provides strong evidence that *redX* is the structural gene for exonuclease. λ*redB* mutants make altered levels of β precipitin and in some cases also alter λ exonuclease levels. It seems probable that *redB* mutants define the structural gene for β protein (Signer et al., 1968; Shulman et al., 1970).

λ*red*$^-$ phage can be obtained by deletion of the *red* genes as well as by point mutation. λ*bio* deletion addition mutants which are *red*$^-$ also fall into two complementation groups, *redX* which lacks λ exonuclease, and *redXB* which lacks both exonuclease and β precipitin as well as other λ genes, most notably *gam* (Manly et al., 1969; Shulman et al., 1970). Both *red* point mutants and *red* deletion mutants have been used in the experiments to be presented.

Genes of the *red* system, *redX* and *redB*, as well as *gam*, are subject to three λ regulatory systems; positive regulation by

gene N, and negative regulation by genes CI and tof. In a normal λ induction, CI repression of the red genes is lifted, synthesis of N product yields active transcription and translation of red genes early in the lytic cycle, and at later times red mRNA synthesis is severely depressed by negative activity of tof product. Only small amounts of N product are needed for complete turn on of exonuclease synthesis, and N product control is epistatic to negative CI repressor control (Thomas, 1970; Luzzati, 1970; Radding & Echols, 1968; Kumar et al., 1969; Pero, 1970). The gene responsible for turn off of λ exonuclease was identified as tof by Pero (1970) (otherwise known as cro-Eisen et al., 1970, or fed-Franklin, 1971). In the absence of tof product, production of λ exonuclease continues to occur at a linear rate beyond the point at which, in the presence of tof product, synthesis levels off (Pero, 1970). A similar behaviour, in the presence and absence of tof product, has been observed for l strand mRNA synthesis after induction (Kumar et al., 1970). The tof structural gene and the site of action of tof product are both located within the region defined by the imm^{434} substitution (Fig. 1). Furthermore, tof product activity is immunity specific; 434 and λtof products are not interchangeable (Pero, 1970, 1971).

λred gene mRNA is synthesized on the l strand of λDNA as part of a large polycistronic mRNA molecule, defined as l_3, and presumed to include the entire CIII - att region (Kcurilsky et al., 1969; Oda et al., 1969). Therefore I am assuming that all controls which apply to the red genes will also act in identical

fashion on gam gene synthesis since gam lies between redB and the control sites. Transcription of the CIII - att operon requires both active N product and prior transcription of the DNA immediately to the right (i.e. N gene itself); thus mutations which prevent transcription of N gene, such as the sex mutation, prevent transcription of the CIII operon even in the presence of added N product (Franklin, 1971). Convincing evidence that an N product dependent transcriptional "restart" site is located between gene N and CIII has been presented by Franklin (1971). Deletion of this N target site yields an operon no longer dependent on N product and directly controlled by CI repressor (Franklin, 1971). Transcription of the red genes depends on protein - DNA interactions at three sites on the l strand; the CI operator site (O_L) defined by the V_2 mutation which reduces sensitivity to CI repressor (Ptashne & Hopkins, 1968), the l strand promotor site (P_L) at least partially defined by sex (Roberts, 1969; Blattner & Dahlberg, 1972; Blattner et al., 1972), and the N dependent "restart" site between N and CIII (t_L). I will briefly discuss the interactions known to occur at these three sites.

The terminator site between N and CIII interacts with N product and possibly with RNA polymerase as well since evidence exists of direct N product-β subunit of RNA polymerase interaction (Georgeopolus, 1972; Pironio & Ghysen, 1972). The promotor site, P_L, must by definition bind RNA polymerase to allow initiation of mRNA transcription. Protein-DNA interactions at the operator site, O_L, fall into two categories, (a) CI repressor binding and

(b) tof product binding. λV_2 mutants, carrying a mutation in O_L, do not bind λ repressor efficiently *in vitro*, and are able to constitutively synthesize N operon proteins in the presence of CI repressor (Ptashne & Hopkins, 1968). However Ordal (1971), using a considerably more sensitive assay for repressor binding, presented evidence that λV_2 binds repressor at the same high level as does λ^+. λV_2 must therefore retain considerable sensitivity to the repressor. Sly et al. (1971) have shown that λV_2 is able to produce λ exonuclease in an immune host although at much lower levels than in a sensitive host. In the presence of an ind^- repressor, λvirL (equivalent to λV_2) does not synthesize λ exonuclease (Sakakibara et al., 1972) again suggesting that λV_2 retains considerable sensitivity to CI repressor. In a sensitive host, λV_2 overproduces λ exonuclease , suggesting that the V_2 mutation lowers sensitivity of the N operon to tof product (Sly et al., 1971) and may be the tof target site for the l strand transcription. One can conclude that the V_2 mutation has reduced sensitivity to the CI repressor and tof product, suggesting that V_2^+ may be the target site for both CI and tof products.

The experiments in this chapter deal with the ways various genetic alterations in the helper phage impinge upon the ability of that helper phage to rescue gene function from a half molecule. Helper phage mutant in redB exhibit a very much reduced ability to rescue function from a coimmune half molecule even though rescue of function from a heteroimmune half molecule is unimpaired. As will be documented later, the CI repressor is not involved.

In contrast, a *red*X mutant helper phage rescues *red*X function from coimmune halves at an intermediate level as compared to heteroimmune half molecule rescue. These effects of *red*X and *red*B helper phage are observed in *rec*A, *rec*AB, and rec^+ hosts. The reduced ability of some helper phage to elicit gene function from a coimmune half molecule will be termed coimmune inhibition. The gene function responsible for this effect may be *tof*.

RESULTS

(a) Rescue of redB function from half molecules.

Early experiments attempting to rescue red function from half molecules were puzzling, since, using imm^{λ} helpers and imm^{λ} halves, redB helper phage were unable to rescue halves while redX (bio72) helper phage rescued halves more efficiently (Table 14). Further experiments using redB helper phage showed that imm^{434}redB helpers gave very high levels of imm^{λ} half molecule rescue. The behaviour of these redB helper phage was investigated in recA, rec^{+}, and recArecB hosts. Any effects due to CI repression were minimized by use of the CI_{857} mutation in all imm^{λ} helper phage and DNA preparations, imm^{434}CI helpers, and $imm^{21}C_{ts}$ DNA, with assay temperature at 39.5°C, to ensure inactivation of temperature sensitive repressors.

Because of this coimmune effect, I have used $imm^{21}C_{ts}$ halves as the standard of comparison for rescue of function from coimmune halves, since except for immunity effects imm^{21} halves should react to helper phage genotype and host cell genotype in identical fashion to imm^{λ} halves. Under conditions where both imm^{21} and imm^{λ} halves are heteroimmune (i.e. with an imm^{434} helper) this is true. If imm^{λ} halves are rescued efficiently, imm^{21} halves are rescued equally well, if imm^{λ} halves are not rescued, neither are imm^{21} halves (Table 15). The results presented were obtained in a recA host; however a similar correspondence in imm^{λ} vs imm^{21} results

TABLE 14 - Rescue of red function from coimmune halves by λredB and λbio72 helper phage.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA: imm^{λ} halves	susJ wholes	% rescue of halves
1.	152	$\lambda redB_{114}susR$	1	174	0.6
2.	152	λbio72susR	258	328	79.

Helpers and DNA were CI_{857}. Indicator was W3350.

$$\% \text{ rescue} = \frac{imm^{\lambda} \text{ halves}}{susJ \text{ wholes}} \times 100$$

was obtained in all other hosts tested. Although in all previous experiments, I have used whole λ*sus*J DNA as the control against which rescue of half molecules was compared, previous experiments also utilized heteroimmune helper phage. Since in these experiments, λ*sus*J wholes and CI_{857} helpers are coimmune, *sus*J recombination levels could be reduced. λimm^{21} halves were chosen as a standard of comparison since they are heteroimmune with both imm^{λ} and imm^{434} helper phage.

To ensure that the inefficient rescue of *red*B function was a general effect for the *red*B group and not specific to one *red*B mutation, several *red*B or *red*XB helper phage were tested. The following *red*B helpers, red_3, red_{270}, red_{113}, and red_{114}, exhibited low rescue in the *rec*A host when helper and half molecule were coimmune (0.03-0.2%, Table 16).

Although for all 4 *red*B helpers, rescue of coimmune halves is extremely low, rescue of heteroimmune imm^{21} halves is always high (i.e. equal to or greater than levels observed for coimmune λ*sus*J wholes, Table 19). Since in all helpers and DNA preparations, effect of *C*I repressor is minimal or absent due to mutation in the *C*I gene, one must explain the immunity effect by other means. The only other known immunity specific gene, located within the immunity region, is the *tof* gene. It is very possible that *tof* product repression of *red*B gene transcription is responsible for this inability to rescue *red*B function from coimmune halves. Sly et al.,

TABLE 15 - Rescue of imm^{21} halves as compared to imm$^{\lambda}$ halves.

	Host	Helper	sus^{+} infectious centers/10^{-6} A_{260} units of DNA				
			imm$^{\lambda}$ halves	imm^{21} halves	imm$^{\lambda}$susJ wholes	% Rescue of halves	
						imm$^{\lambda}$	imm^{21}
1.	152	$\lambda redB_{114} imm^{434} susR$	1654	1560	1608	103	97.
2.		$\lambda redB_{113} imm^{434} susR$	1648	1584	2952	56	53.
3.		$\lambda bio1 imm^{434} susR$	3	9	1736	0.6	0.5
4.		$\lambda susNN imm^{434}$	16	11	1236	1.3	0.9

Transformation assay was as described in Methods. Helpers were imm^{434}CI, DNA was λCI_{857} or $21C_{ts}$. Incubation temperature = 39.5°C. Indicator was W3350.

$$\% \text{ rescue} = \frac{\text{Halves}}{\text{susJ Wholes}} \times 100$$

TABLE 16 - Rescue of redB function under coimmune conditions.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA: imm^λ halves	imm^{21} halves	% rescue of halves
1.	152	$\lambda redB_{113} imm^\lambda susR$	22	1159	0.2
2.	"	$\lambda redB_{114} imm^\lambda susR$	1	922	0.1
3.	"	$\lambda redB_{270} imm^\lambda susR$	< 1	410	< .2
4.	"	$\lambda redXB_3 imm^\lambda susR$	3	1121	0.03
5.	"	$\lambda red^+ imm^\lambda susR$	940	2420	39.

Assay as described in Methods. All helpers and imm^λ and imm^{21} DNAs are CI_{857} or C_{ts}. Incubation temperature = 39.5°C. Indicator was W3350.

$$\% \text{ rescue} = \frac{imm^\lambda \text{ halves}}{imm^{21} \text{ halves}} \times 100$$

Variation in assay

	% rescue of halves
Line 1	0.2, 5.6
Line 2	0.1, 0.3
Line 3	< .2, < .7
Line 4	0.03, 0.2

(1971) have postulated that tof product is unable to act at the λV_2 mutation to turn off the l strand operon. If this is true, one might expect that λvir halves would be less affected by the immunity effect than are imm$^\lambda V_2^+$ halves (Table 17). Essentially no improved rescue of λvir halves, as compared to imm$^\lambda$ halves, is observed which is somewhat disconcerting. The transformation assay may not be directly comparable to the experiments done by Sly et al., (1971), since the half molecule is not replicating. Possibly the number of molecules of tof product, as related to number of DNA copies (only one in my experiment), is important if escape from tof inhibition by the V_2 mutation is to be observed.

(b) Rescue of redX function from halves.

Two types of redX helper phage were used, λredX$_{504}$, and deletion-addition phage λbio72 which is deleted into redX. Both these helpers are able to rescue redX function from coimmune halves (Table 18), although at levels lower than those for heteroimmune helpers. Thus, although redX function can be rescued from coimmune halves, it is only at 8.7% level for the point mutant helper, and 46% for the bio72 deletion helper, compared to a value of 73% for the heteroimmune helper. This is reproducibly better rescue than that observed for rescue of redB function (0.2% rescue of redB Table 16, line 1; compared to 8.7% rescue of redX, Table 18, line 1).

TABLE 17 - Rescue of redB function from λvir halves.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA: λvir halves	imm^{21} halves	% rescue of halves
1.	152	λ$redB_{113}imm^{\lambda}$ susR	36	1159	0.3
2.	"	λ$redB_{114}imm^{\lambda}$ susR	63	922	0.7
3.	"	λ$redB_{270}imm^{\lambda}$ susR	11	410	0.3
4.	"	λ$redXB_3imm^{\lambda}$ susR	26	1121	0.2
5.	"	λred^+imm^{λ} susR	1460	2420	60.

Helpers were CI_{857}. λDNA was λvir = C^+, imm^{21} = C_{ts}.

Indicator was W3350.

$$\% \text{ rescue} = \frac{\lambda vir \text{ halves}}{imm^{21} \text{ halves}} \times 100$$

TABLE 18 - Rescue of *redX* function from halves under coimmune conditions.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA: imm^λ halves	imm^{21} halves	% rescue of imm^λ halves
1.	152	$\lambda redX_{504}imm^\lambda susR$	131	1499	8.7
2.	"	$\lambda bio72imm^\lambda susR$	130	283	46
3.	"	$\lambda bio72imm^{434}susR$	1043	1435	73

Assay as described in Methods. Helpers were CI_{857} or CI, DNA was CI_{857} or C_{ts}. Incubation temperature = 39.5°C. Indicator was W3350.

$$\% \text{ rescue} = \frac{imm^\lambda \text{ halves}}{imm^{21} \text{ halves}} \times 100$$

Variability in assay

	% rescue of halves
Line 1	8.5, 8.7, 11
Line 2	32.6, 46, 57
Line 3	60, 73, 87

(c) Effect of coimmune inhibition on whole λDNA.

Comparison of rescue of imm^{21} halves and imm$^{\lambda}$ susP or susJ whole DNA provides an assessment of the coimmune effect on rescue of redB and redX from whole molecules. While recombination of whole susP DNA will be totally dependent on expression of the resident red gene, λsusJ DNA can be recombined by int product as well as red product and thus provides a whole DNA control not necessarily dependent on red recombination. Whole susP DNA is affected by coimmune inhibition; redB function cannot be adequately rescued from whole susP DNA, and redX function is rescued from a deletion mutant, but only poorly from a point mutant defective (Table 19).

All the red$^-$ point mutants, redB and redX, exhibit low (0.7 - 10%) rescue of susP wholes, and high rescue (32 - 106%) of susJ wholes (Table 19, lines 1-5). In contrast, heteroimmune helpers and coimmune bio72 helper exhibit approximately equal rescue of susP and susJ wholes (36 - 103% Table 19, lines 6-8). Whole susP DNA reacts in similar fashion to halves under conditions of coimmune inhibition.

(d) Rescue of redX and redB function in rec$^+$ and recA host cells, and rescue by λfec helper phage in recB hosts.

In the rec$^+$ host, rescue of redX is more efficient than rescue of redB function under conditions of coimmune inhibition. As in the case of the recA host, no CI repressor should be active under the assay conditions employed. λredB helpers rescue redB function from halves at 3-20%, λredX helpers

TABLE 19 - Rescue of redB and redX from whole DNA under coimmune conditions.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA: susP wholes	susJ wholes	% rescue susP	% rescue susJ
1.	152	$\lambda redB_{113} imm^{\lambda} susR$	8	411	0.7	32.
2.	"	$\lambda redB_{114} imm^{\lambda} susR$	54	530	6.8	57.
3.	"	$\lambda redB_{270} imm^{\lambda} susR$	21	436	4.8	106
4.	"	$\lambda redXB_{3} imm^{\lambda} susR$	22	701	1.9	62.
5.	"	$\lambda redX_{504} imm^{\lambda} susR$	157	711	10.5	48
6.	"	$\lambda bio72 imm^{\lambda} susR$	128	158	45	55.
7.	"	$\lambda redB_{114} imm^{434} susR$	1130	1608	72.5	103
8.	"	$\lambda bio72 imm^{434} susR$	519	767	36.2	53.

Assay as described in Methods. Helpers and DNA are CI or C_{ts}. Indicator was W3350. Incubation temperature = 39.5°C.

$$\% \text{ rescue} = \frac{susP \text{ or } susJ}{imm^{21} \text{ halves}} \times 100$$

Value for imm^{21} halves was from Tables 17 & 19.

Variability in assay - % rescue of susP

Line 1 7.2, 0.7

Line 2 3.5, 6.8

Line 3 2.1, 4.8

Line 4 1.4, 1.9

Line 5 2.4, 9.1, 10.5

Cont'd.

Line 6 45

Line 7 44, 44. , 72.

Line 8 19.1, 36.

rescue _red_X function from halves at about 40-60% (Table 20). The host _rec_ system contributes only minor benefits to half molecule rescue. λ_imm_ ^{21} halves rescue was approximately equal to rescue of _sus_J wholes (See Appendix, Table 30).

In a _rec_A _rec_B host, all effects due to _rec_BC nuclease activity should be eliminated allowing observation of the extent of coimmune inhibition under conditions where half molecule rescue should be maximised. A differential effect of inhibition on _red_B as compared to _red_X still exists. _red_B mutants rescue _red_B function from halves at 2-11%, _red_X mutants rescue _red_X function from halves at 28-56% (Table 21, lines 1-4 vs lines 5 & 6). An apparent contradiction exists when one considers λ_sus_N helper which in this _su_ $^-$ host is _red_X $^-$ _red_B $^-$ and which rescues coimmune halves at 92% of the heteroimmune level (Table 21, line 7). Theoretically, this helper should yield behaviour similar to the _red_XB $_3$ (6%) or _bio_1 (.2%) helper phage (Table 21, line 4; Table 22, line 6). This will be discussed later in the discussion section of this chapter.

Rescue by coimmune _fec_ helper phage.

Since heteroimmune _fec_ helper phage do not rescue _red_ function, one would expect a similar defect for coimmune helpers. λ_fec_ helper phage do not effectively rescue any genotype of half molecule in a _rec_A or _rec_ $^+$ host (Appendix Tables 27 & 29); however one can observe the coimmune inhibition effect in a _rec_A _rec_B or _rec_B hosts (Table 22).

TABLE 20 - Rescue of redB and redX in rec^+ host.

	Host	Helper	sus^+ infectious centers/$10^{-6}A_{260}$ units of DNA		
			imm^{λ} halves	imm^{21} halves	% rescue imm^{λ}
1.	W3350 (rec+)	$\lambda redB_{113} imm^{\lambda} susR$	303	1397	21.6
2.	"	$\lambda redB_{114} imm^{\lambda} susR$	151	1111	13.6
3.	"	$\lambda redB_{270} imm^{\lambda} susR$	11	368	3.0
4.	"	$\lambda redXB_{3} imm^{\lambda} susR$	124	1115	11.1
5.	"	$\lambda redX_{504} imm^{\lambda} susR$	1013	2117	48.
6.	"	$\lambda bio72 imm^{\lambda} susR$	498	722	69.

Assay was as described in Methods. Helpers and DNA were CI_{857} or C_{ts} except λvir which was C^+. Incubation temperature was 39.5°C. Indicator was W3350.

$$\% \text{ rescue} = \frac{imm^{\lambda} \text{ halves}}{imm^{21} \text{ halves}} \times 100$$

Variability in assay - % rescue of imm^{λ} halves

Line 1 2.9, 5.1, 21.6

Line 2 13.7, 13.6

Line 3 3.0, 5.7

Line 4 4.0, 11.1

Line 5 24.4, 48.

TABLE 21 - Rescue of redB and redX in a recA recB host.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA: imm^{λ} halves	imm^{21} halves	% rescue imm^{λ} halves
1.	E835 (recArecB)	$\lambda redB_{113}imm^{\lambda}susR$	190	1864	10.2
2.	"	$\lambda redB_{114}imm^{\lambda}susR$	205	1840	11.1
3.	"	$\lambda redB_{270}imm^{\lambda}susR$	42	2280	1.8
4.	"	$\lambda redXB_3imm^{\lambda}susR$	146	2326	6.4
5.	"	$\lambda redX_{504}imm^{\lambda}susR$	577	2047	28.2
6.	"	$\lambda bio72imm^{\lambda}susR$	702	1238	57.
7.	"	$\lambda imm^{\lambda}susNN$	1135	1231	92
8.	"	$\lambda red^+imm^{\lambda}susR$	636	1768	36

Assay was described in Methods. Helpers were CI_{857}. DNA was CI_{857} or C_{ts}, λvir was C^+. Assay incubation temperature = 39.5°C. Indicator was W3350.

$$\% \text{ rescue} = \frac{imm^{\lambda} \text{ halves}}{imm^{21} \text{ halves}} \times 100$$

Variability in assay. % imm^{λ} halves rescue

Line 1 8.4, 10.2

Line 2 7.3, 11.1

Line 3 1.8, 1.8

Line 4 6.4, 5.3

Line 5 21.0, 28.2

Line 6 65, 92, 57

Line 7 34, 36

TABLE 22 - Rescue of halves by coimmune λfec helper phage.

	Host	Helper	sus^+ infectious centers/10^{-6} A_{260} units of DNA		
			imm^{λ} halves	imm^{21} halves	% rescue imm^{λ} halves
1.	E835 (recArecB)	λbio11imm^{λ}susR	6	1528	0.04
2.	"	λbio1imm^{λ}susR	34	1881	0.2
3.	"	λbio1imm^{434}susR	1010	1210	83.
4.	DM22 (recB)	λbio1imm^{λ}susR	82	4464	0.2

Assay was as described in Methods. Helpers were CI_{857}, DNA was CI_{857} or C_{ts}. Incubation temperature = 39.5°C. Indicator was W3350.

$$\% \text{ rescue} = \frac{imm^{\lambda} \text{ halves}}{imm^{21} \text{ halves}} \times 100$$

Variability in assay: % rescue of imm^{λ} halves

Line 2   0.17, 0.18

Line 3   bio10imm^{434}susR = 99.

<u>imm</u>$^{\lambda}$ halves are rescued at only 0.04 - 0.2% as compared to <u>imm</u>^{21} halves. This is not due to a defect in ability of <u>fec</u> helpers to recombine with <u>imm</u>$^{\lambda}$ halves because <u>bio</u>l <u>imm</u>^{434} helper (Table 22, line 3) rescues <u>imm</u>$^{\lambda}$ halves at 83% compared to <u>imm</u>^{21} halves.

Since the most relevant measure of rescue of <u>red</u> function from coimmune halves is in the <u>rec</u>B hosts, where problems relating to degradation of halves are eliminated (see Chapter 3), I have analyzed my data as follows:

The helper phage can be divided into three groups according to their ability to rescue coimmune halves in the <u>rec</u>A <u>rec</u>B <u>su</u>$^-$ host.

(1) <u>bio</u>l & <u>bio</u>ll helpers → very low rescue (0.04-0.2%)

(2) <u>red</u>B & <u>red</u>XB helpers → intermediate rescue (2-11%)

(3) <u>red</u>X, <u>bio</u>72, <u>sus</u>N & <u>red</u>$^+$ helpers → high rescue (28-92%)

The helpers in group 1 cannot contribute any <u>red</u>X or <u>red</u>B product to the recombination process. The helpers of group 2 are uniformly unable to contribute <u>red</u>B product but vary in their ability to supply <u>red</u>X product. λ<u>red</u>B$_{113}$ produces normal levels of exonuclease activity, <u>red</u>B$_{114}$ and <u>red</u>B$_{270}$ produce low levels of exonuclease but do complement <u>red</u>X mutants, and <u>red</u>XB$_3$ cannot supply exonuclease at all. The unifying fact for these four helpers is their inability to supply <u>red</u>B activity (Signer et al., 1968, Signer, Pers. Comm. for <u>red</u>B$_{270}$). The helpers of group 3 are all able to contribute <u>red</u>B activity; λ<u>sus</u> <u>N</u> can contribute <u>red</u>B

and *red*X activity if *N* product from the half molecule "turns on" its *N* operon (see Discussion), and λ*red*$^{+}$ contributes both *red*X and *red*B proteins. The *red*X helpers, *bio*72 and *red*X$_{504}$, are unable to provide *red*X but do contribute *red*B protein. Consideration of all three groups suggests that the pivotal factor in rescue of coimmune halves is the presence or absence of *red*B protein from the helper phage genome.

(e) Effect of *imm*^{434} helpers on rescue of λ*vir* halves.

λ*imm*$^{\lambda}$ helpers generally rescue λ*vir* halves at equal to or better than the *imm*$^{\lambda}$ halves level (Table 16 & Table 17). In contrast to this, *imm*^{434} helpers rescue λ*vir* halves at a consistently lower value than the *imm*$^{\lambda}$ halves rescue (Table 23). This occurred in *rec*$^{+}$ hosts as well as *rec*A and *rec*AB hosts (Appendix, Table 30). The ratio of *imm*$^{\lambda}$: λ*vir* rescue ranged from 2.1 to 10.2 with an average value of 4.5 for 9 different helper-host combinations. This reduced rescue of λ*vir* halves was only observed if transcription of the half was demanded since *red*$^{+}$*imm*^{434} helpers rescued λ*vir* at an average ratio of 1.0. Similarly, *imm*$^{\lambda}$ *red*$^{+}$ and *red*$^{-}$ helpers rescued λ*vir* halves at an average ratio of 1.4 (Table 28, Table 32). This suggests that a λ gene located within the *imm*^{434} region acts to repress DNA carrying the V_2 mutation since λ*vir* half DNA expresses its *red* genes about 25% as well as does *imm*$^{\lambda}$ half DNA under identical helper-host conditions. This is very similar to the coimmune inhibition effect on *red*B function from half molecules.

TABLE 23 - Rescue of λ*vir* halves by λ*imm*^{434} helpers.

	Host	Helper	*sus*$^{+}$ infectious centers/10^{-6} A_{260} units of DNA			% rescue		Ratio
			imm$^{\lambda}$ halves	λ*vir* halves	*imm*^{21} halves	*imm*$^{\lambda}$	λ*vir*	*imm*λ : λ*vir*
1.	152 (*rec*A)	*red*B_{114}*imm*^{434}*sus*R	1728	319	2332	74.3	13.7	5.4
2.	"	*red*B_{113}*imm*^{434}*sus*R	1648	162	1584	104	10.2	10.2
3.	E835 (*rec*A*rec*B)	*red*B_{114}*imm*^{434}*sus*R	1312	470	1960	67.2	24.5	2.7
4.	"	*red*B_{113}*imm*^{434}*sus*R	1048	492	1712	61.0	28.7	2.1
5.	"	*bio*72*imm*^{434}*sus*R	1704	488	1408	124	31.7	3.9
6.	"	*bio*1*imm*^{434}*sus*R	1212	350	2136	57	16.4	3.5
7.	"	*bio*10*imm*^{434}*sus*R	1632	436	1648	99	26.5	3.7
8.	"	*bio*72*gam*$_{14}$*imm*^{434}*sus*R	1024	259	1488	69	17.4	3.9
9.	"	*red*$^{+}$*imm*^{434}*sus*R	2912	2488	2048	142	121	1.2
10.	"	*red*$^{+}$*imm*^{434}*sus*R	1976	2212	2440	81	90.7	0.9

Assay as described in Methods. All helpers are *imm*^{434}CI. Halves are CI_{857}, C_{ts}, λ*vir* is C^{+}.

$$\% \text{ rescue} = \frac{imm^{\lambda} \text{ halves}}{imm^{21} \text{ halves}} \times 100, \quad \frac{imm\lambda}{\lambda vir} = \text{ratio}$$

DISCUSSION

The most important fact to emerge from this set of experiments is the differential ability to rescue redB function, as compared to redX function, from a half molecule. The reduced rescue of redB function is observed only when both helper phage and DNA are coimmune, in the absence of CI repressor. This effect has been termed coimmune inhibition. The experimental evidence for a reduced ability to rescue redB function from coimmune DNA is as follows:-

1. imm^{λ} redB helpers rescue redB function from imm^{λ} halves at only 0.2% the rescue observed for imm^{21} halves in a recA host.
2. imm^{λ} redX helpers rescue redX function from imm^{λ} halves at 8.7-46%, the rescue observed for imm^{21} halves in a recA host.
3. Similar effects are observed in recB hosts, where degradation effects do to recBC nuclease have been eliminated.
4. This is not due to a defect in the imm^{λ} halves population since use of a heteroimmune imm^{434} redB helper results in high levels of rescue of redB function from imm^{λ} halves in all hosts.

The gene function responsible for the coimmune inhibition effect must be located within the region delineated by the imm^{434} substitution since the effect is not observed with this helper. Two known repression genes and their targets are located within this region, CI and tof. Since both helper and DNA preparations are mutant in the CI gene to minimize the effect of CI repressor,

tof inhibition of the N operon containing the redB and redX genes seems the most plausible explanation. tof has been shown to be immunity specific (Pero, 1970) and also to have a reduced inhibition activity on λV_2 mutants (Sly et al., 1971). In my system, the inhibition function shows immunity specificity, but unfortunately the V_2 mutation is also inhibited, possibly due to relative concentrations of DNA and tof product within the transformed cell. imm^{λ} helper phage do not "inhibit" imm^{21} halves and imm^{434} helpers do not "inhibit" imm^{λ} or imm^{21} halves. This indicates that λimm^{21} is not susceptible to inhibitory activity by tof^{434} or tof^{λ} products.

Two models can be devised to explain the reduced ability to rescue redB function from half molecules, as compared to the greater ability to rescue redX function from the same half molecules.

a. When a half molecule enters a coimmune redB helper infected cell, its transcription is slowed down or prevented entirely by tof product. It is thus in a $redB^-$ environment and is unable to produce its own redB product. One could postulate that redB function is a necessary co-factor to gam product protection of the half molecule from degradation. When the half molecule is rapidly transcribed, the presence of gam product alone is sufficient protection against exonuclease degradation. Under "tof-inhibited" conditions the half remains in a vulnerable state considerably longer and may require both redB and gam products for protection. An

adjunct to the theory that *red*B product is required for protection would be that *red*B product could exert a control effect over *red*X exonuclease activity (similar to the *E. coli* *rec*A/*rec*B relationship) and in its absence the λ exonuclease degrades half molecules.

b. A simpler hypothesis relates to the relative quantities of *red*X and *red*B products required for a successful recombination event. In the absence of *tof* inhibition, the half molecule can produce adequate amounts of either *red*X or *red*B proteins. In the presence of *tof* inhibition, with a coimmune helper phage, the DNA is able to provide much less *red*X and *red*B proteins. One could postulate that for successful recombination to occur only catalytic amounts of *red*X protein are needed, but much larger amounts of *red*B protein are required. When repressed by *tof* the half molecule produces enough *red*X to yield successful recombination but is unable to produce sufficient quantities of *red*B protein under the same circumstances.

The majority of my experimental evidence favors model (b) as an interpretation of the events in a coimmune transformed cell. The most important fact arguing against model (a) (*red*B protective activity) is the fact that even in a *rec*A*rec*B host, where the major exonuclease attacking halves is eliminated (Chapter 3), λ*red*B helpers still show reduced rescue of halves. This cannot be due to λ exonuclease activity since a similar reduction in rescue of halves is observed for λ*bio*1 helper phage which carry

a deletion of the *red* genes. It is quite probable that the drastically reduced rescue of halves in *rec*A hosts is due to *rec*BC exonuclease but this is observed for both *red*X and *red*B rescue with *red*B rescue being more strongly affected. A second factor pointing to reduced quantities of *red*B product being insufficient to catalyze recombination is the fact that whole *sus*P DNA is affected to approximately the same extent as are half molecules in the *rec*A host. λ*sus*P whole molecules are resistant to exonuclease attack in conditions where half molecules are degraded (with heteroimmune helpers); this argues that reduced rescue of *red*B function from *sus*P wholes is due to inability to recombine rather than degradation of the template. A third experiment pointing to model (b), is the λ*sus*N helper in the *rec*A *rec*B *su*$^-$ host; the half molecule enters a *tof*$^{\lambda+}$ *red*$^-$*N*$^-$ environment. Contrary to expectation, in these conditions, the coimmune half molecule is transcribed for *N* gene at efficiencies comparable to that for a heteroimmune *imm*^{21} half molecule. No coimmune inhibition effect is observed for *N* gene function; the most likely explanation for this is the fact that *N* gene protein is required in only catalytic amoünts which are synthesized even during coimmune inhibition conditions. A similar effect is seen for *int* function, which probably acts in catalytic amounts and is also unaffected by coimmune inhibition. The evidence for this is as follows. λ*bio*l helpers, which are *red*$^-$*int*$^-$, efficiently help λ*sus*J whole DNA (see Appendix, Table 28). Since *sus*P DNA is not helped efficiently, *red* product is not likely to be responsible for the rescue of λ*sus*J. It is logical to assume that *int* product from

the whole DNA template catalyzes the rescue and that int is not subject to the "tof-inhibition" effect. One could conclude that catalytic proteins N, int and redX do not suffer from tof repression because only small amounts of protein are required for activity. redB, an unknown activity involved in recombination, may be required in large amounts with the result that when the genome is repressed by tof the β protein levels are not sufficient to give recombination.

There are very few reports in the literature relating to relative amounts of redX and redB protein required for successful recombination. λsexl mutants, which are defective in l strand mRNA production, have only 10% of normal N operon function (Franklin, 1971) but are $red^{-}gam^{+}$ phenotypically. A possible explanation of this behaviour would be that 10% of the normal β protein level is not sufficient for a red^{+} phenotype. Since sexl is also $CIII^{-}$ (Blattner & Dahlberg, 1972) this suggests that both redB and CIII are required in large amounts, while gam and redX are active at only 10% of their normal levels. A second suggestion that larger amounts of redB protein are produced than of redX protein comes from Radding & Shreffler (1966). In mitomycin induced extracts of λsus N lysogens, levels of α antigen (redX) were undetectable, however levels of β antigen (redB), while reduced, still gave a definite immunological reaction. This same publication also gives evidence that λ exonuclease and β protein are synthesized at a non-coordinate rate, or possibly time course, after induction of λtll mutants.

A minor point to emerge from these experiments is the inhibitory effect of _tof_^{434} on λ_vir_ halves. This suggests that _tof_$^{\lambda}$ and _tof_^{434} are very closely related proteins since a mutation in the V_2 target site causes reduced sensitivity to _tof_$^{\lambda}$ and increased sensitivity to _tof_^{434}. Since the inhibition effect was only observed if function was required from the half molecules, one can conclude that _tof_^{434} is actually reducing transcription of the λ_vir_ $\bar{l}$ strand operon, not simply preventing recombination.

In conclusion, from these experimental results, I suggest that for recombination to occur efficiently, considerably more _red_B protein is required than is the case for _red_X protein.

SECTION V.

GENERAL CONCLUSIONS

CONCLUSIONS

As detailed in the introduction to this thesis, the experiments just described were designed to explore the role played by DNA topology in λmRNA transcription. More specifically, I have analyzed the ability of a linear λDNA template to provide early gene functions *red* and *N*. Available evidence suggests that a circular template may be used for early mRNA transcription but does not indicate if this circular template is an essential prerequisite for mRNA synthesis. Several experimentally verifiable models can be devised to describe the role of template topology in λmRNA transcription.

Model 1 - A circular template is an essential requirement for transcription itself. For instance, a circular template might be required as a co-factor in binding of RNA polymerase to initiation sites.

Model 2 - A circular template plays no role in the actual process of transcription, however circular topology is essential for template survival in the presence of cell exonucleases. Indirectly, circular topology is required if transcription is to occur.

Model 3 - Circular topology plays no role in either transcription or template survival. The use of a circular template by λ is for convenience only since a circular molecule is probably required for λDNA replication.

My experiments, using whole λDNA as a source of a template which can circularize and half λDNA as the source of a linear template, suggest Model 2 is the most accurate representation of the role of topology in early mRNA synthesis. In the absence of the *recBC* exonuclease, linear half molecules are very efficient templates for both *N* and *red* genes. However in the uninhibited presence of this destructive exonuclease activity, half molecules cannot serve as template for *N* or *red* genes. This is interpreted to mean that although template topology plays no direct role in λmRNA synthesis, it plays a very essential indirect role ensuring survival of the template. This topological protection of the template is likely to be of maximum importance in transcription occurring immediately after infection since once gene *N* and the *N* operon are transcribed, a second λ survival mechanism comes into play. This is the *gam* gene whose product inhibits the *recBC* nuclease activity. The protective effect of *gam* gene product on linear molecules is very dramatically illustrated in my system. If a linear half molecule enters a host cell containing *gam* product (i.e. a λ*gam*$^+$ helper phage infected cell) it is very efficiently transcribed. In contrast, a half molecule entering a *gam*$^-$ host cell (i.e. λ*gam* helper infected cell) is degraded before transcription can occur. During a lytic infection cycle by free phage, injected λDNA enters a host cell which contains no *gam* product, corresponding to the λ*gam*$^-$ helper infected situation. Later in infection, after transcription of *N* and *gam*, the situation corresponds to the λ*gam*$^+$ helper

infected cell.

Since on infection, λDNA must initially enter a cell containing an active recBC nuclease, the most accurate experimental simulation of this event must involve entry of purified λDNA into a gam^- environment which contains active recBC nuclease. For this reason, the λgam^- helper infected competent cell was considered to be the most relevant environment in which to test ability of a linear DNA molecule to serve as template for early λ genes. As stated before, under these conditions, linear molecules have no template activity (i.e. half molecules in a λbiol helper infected cell).

Comparison of template activity of a linear molecule in a gam^- versus a gam^+ environment provides a very clear definition of the role template topology plays in λ early gene transcription.

1. If the linear template is protected from degradation by gam product or by absence of recBC nuclease, it is a very efficient template for λmRNA synthesis. RNA polymerase must bind, initiate and transcribe a linear template at an efficiency equal to that for a circular template. Template topology must play no role in the actual process of transcription itself.

2. In the absence of protective mechanisms, the linear molecule is highly susceptible to attack by recBC exonuclease. A circular molecule is unaffected under the same conditions. Therefore circular topology must play an essential indirect role in transcription by ensuring survival of the template

during the transcription process. Circular topology provides immunity against exonuclease degradation.

These experiments have also provided clues to the functioning of λ*gam* product and *rec*BC exonuclease *in vivo*. In agreement with *in vitro* studies, *rec*BC nuclease actively degrades linear molecules but has no activity on circular molecules. λ*gam* product inhibits this preferential degradation of linear halves. Again, in agreement with *in vitro* work, λ*gam* product is the only λ function required for *rec*BC inactivation. Since *rec*A product of *E. coli* appears to exert a control effect on *rec*BC activity, one might expect enhanced survival of the linear template in a *rec*$^+$ as compared to a *rec*A mutant host. This is not the case. The linear half molecule is poorly transcribed in the *rec*$^+$ host, in λ*gam*$^-$ conditions; the functional *rec*A product of the *rec*$^+$ host does not limit the degradation of halves by *rec*BC nuclease. On the contrary, it appears that more *gam* product is needed for protection in a *rec*$^+$ host than is required in a *rec*A host. Perhaps *gam* and *rec*A products compete for the same allosteric site on the *rec*BC nuclease molecule, but alter its activity in different ways. *rec*A product inhibits excessive nuclease activity on bacterial DNA: λ*gam* product inhibits nuclease degradation of λDNA.

Studies on helper phage activity for coimmune as opposed to heteroimmune λDNA molecules suggests that *tof* product represses transcription of λDNA, preventing rescue of λ*red*B product function. Since *red*X function is more efficiently rescued, as is *N* gene function, it appears that high levels of *red*B product are

needed for efficient recombination; redX and N products are required at considerably lower levels. These experiments also provide an explanation for the probably universal observation that in the transformation assay, heteroimmune helper phage yield the highest efficiency of infection for λDNA. Under coimmune conditions, tof product inhibits infecting DNA.

In summary, the following conclusions can be drawn concerning this work.

1. The level of redB protein required for recombination is considerably higher than the levels of redX product required for that same recombination event.

2. In vivo, λgam product inhibits recBC exonuclease activity on linear λDNA. gam is the only λ function required for this inhibition.

3. The role of template topology in early λmRNA synthesis is an indirect one. A circular template is essential for transcription because it provides protection for the λ genome from exonuclease attack. Circularity ensures survival of the template in a hostile cell environment until gam protection is established.

SECTION VI.

APPENDICES.

The material in these two appendices is included solely for the purpose of future data retrival. Part 1 reccrds experimental data accumulated in an attempt to explain the large range of half molecule rescue by a *gam*⁻ helper phage. Although indicative of a role for host physiology in this variation, no firm conclusions can be drawn from this work. Part 2 is a comprehensive list of the ability of helper phage tested in any given host, to help the DNA genotypes used as transfecting DNA. This is included as an easy reference to experimental data, especially data not shown in tables of the results section.

APPENDIX (Part 1)

Variability in rescue of halves by λ*bio*72 *sus gam* helper in a *rec*A host.

STANDARD CONDITIONS

Variability observed in rescue of half molecules by λbio72 sus gam helper in a recAsu⁻ host.

TABLE 24

Host	Helper		sus+ infectious centers/ $10^{-6}A_{260}$ units of DNA			% rescue
			λ+ halves	susJ wholes	bio72susP wholes	
152	λbio72sus gam_{14}susR	1.	45	620	6	7.2[illegible]
		2.	130	559	1	23.0
		3.	192	1548	19	12.4
		4.	170	1252	-	13.6
		5.	16	804	9	2.0

All five assays were performed on different days, with different cultures of 152, but otherwise the experimental techniques were identical to those described in Methods, Section 7, for all five sets of data. Helper was imm^{434}CI $susR_{54}R_{60}$.

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The function of the gam product has been postulated to be inactivation of the recB exonuclease activity which is present in uncontrolled excess in a recA mutant host. During the procedures leading to competence the host cell is in a starved condition and as such undergoes two 37°C incubation periods. It was possible that the variations of sus gam helper activity were due to a variable amount of recBC exonuclease within the competent cell.

Although every effort was made to keep conditions of the transformation assay constant from experiment to experiment, minor variations occur which could contribute to a variable level of exonuclease if this activity were to decay during the competence procedure, or if leak of λgam were intensified. The timing of three sections of the transformation procedure were varied to determine their effects on rescue of halves by sus gam helper phage.

(a) Variability in rescue of halves due to growth phase of the host bacteria during initial multiplication in H1 and maltose was determined by transformation of cells grown to:

1. $A_{600} = 0.8$ Early log phase
2. $A_{600} = 1.7$ Late log phase
3. $A_{600} = 2.1$ Late log phase

Ageing of the cells might be expected to significantly after both enzyme activities and energy levels within the cell which could be reflected in the efficiency of transformation with λDNA.

Comparison of % rescue of halves in Table 25 suggests that growth phase of the host cell does slightly influence ability of a gam^- helper to allow transcription of halves. Early log phase host cells permit only ½ as much rescue of halves by gam^- helper as do late phase host cells. This indicates that variable rescue of halves can be generated by variations in the growth phase at which host cells were chilled prior to entering the transformation procedure.

TABLE 25 - Effect of growth phase on rescue of halves by gam$^-$ helper.

Host 152	Helper	sus^+ infectious centers/ $10^{-6} A_{260}$ units of DNA: λ^+ halves	susJ wholes	% rescue of halves (c)
1. A_{600}=0.85 (a)	λbio72gam$_{14}$	87	1675 (d)	5.2
2. A_{600}=1.0	λbio72gam$_{14}$	121	1792	6.6
3. A_{600}=1.7	λbio72gam$_{14}$	170	1252	14.2
4. A_{600}=2.1	λbio72gam$_{14}$	39	326	11.8

(a) Host cells were grown to A_{600} = 0.85, and concentrated 4 fold in I medium instead of the usual 2X. Otherwise the procedures followed were as described in Materials and Methods.

(b) Helper was imm^{434}CI susR$_{54}$R$_{60}$.

(c) % rescue = $\frac{\lambda^+ \text{ halves}}{\lambda+ \text{ wholes}}$ x 100

(d) This value was λ^+ wholes rather than susJ wholes. Indicator was W3350. DNA samples were CI$_{857}$.

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It is possible that the factors responsible for degradation of halves in the absence of normal gam product levels are either more active or present in larger amounts in early log as compared to late log phase cells.

(b) Host cells in I medium undergo two 10 minutes - 37°C incubation periods. These were varied to 5 minutes and 15 minutes

at 37°C. Competent cells were then allowed to remain the standard 120 minutes at 0°C before being infected with DNA.

Period 1 - Host cells in I medium.

Period 2 - Helper infected host cells in I medium.

It was assumed that period 1 would affect only exonuclease decay, if any, and period 2 would involve both exonuclease decay and *gam* product inhibition of this activity, if *gam* product is able to be produced in such a starved cell.

Several facts are apparent in Table 26.

1. Rescue of halves by the *gam*$^-$ helper is variable (0.7 - 10.6%) by a factor of 10.

2. Half DNA does not follow the pattern set by whole DNA in response to variations in periods 1 and 2. Whole DNA exhibits a 3-8 fold range for the *gam*$^-$ helper (the effect of physiological state of the host cells on rescue and recombination of whole DNA with *gam*$^+$ helper is discussed in Methods, Section 7a). The variation in level of whole DNA rescue varies in response to length of period 2, the highest values occurring at the 5 minutes time point (Table 26, lines 1 & 3). Similar variations were observed for whole *sus*P DNA.

3. The above two considerations suggest that variability in rescue of halves by the *gam*$^-$ helper may be partially due to host physiological effects on

TABLE 26 - Variations in 37° incubation periods. Effect of gam$^-$ helper rescue of half DNA molecules.

	Host	Helper	Period 1 min. at 37°C	Period 2 min. at 37°C	sus$^+$ infectious centers/10^{-6} A_{260} units of DNA: λ^+ halves	λ^+ wholes	bio72susP wholes	susP wholes	% rescue of halves (1)
1.	152	bio72gam$_{14}$	5	5	11	1600	22	1032	0.7
2.	"	"	5	15	70	656	3	148	10.6
3.	"	"	15	5	27	1760	27	1000	1.5
4.	"	"	15	15	23	237	1	55	9.7

(1) $\% \text{ rescue} = \frac{\lambda^+ \text{halves}}{\lambda^+ \text{wholes}} \times 100$

Helper was imm^{434}CI susR$_{54}$R$_{60}$. All DNA samples were CI$_{857}$. Indicator was W3350. Except for variations in periods 1 and 2, the experimental conditions were as described in Materials and Methods.

whole DNA, which serves as a standard of comparison for halves, as much as to variation in the actual number of half molecule recombinants. The % rescue depends as much on variation in levels of λ^+ whole DNA transformation as it does on transformation by λ^+ halves.

(c) Helper infected cells have been reported to gain competence by incubation at 0°C in TCM. The standard period used in this thesis was 120 minutes. Since variation of the 37° incubation periods of the competence procedure did give rise to variable rescue of halves by both *gam*$^+$ and *gam*$^-$ helpers, experiments were performed to assess rescue of halves after variation of the 0°C incubation in TCM during which helper infected cells develop competence.

======

TABLE 27

	Host	Helper	Minutes at 0°C in TCM	*sus*$^+$ infectious centers/ $10^{-6}A_{260}$ units of DNA: λ^+ halves	λ^+ wholes	%rescue of halves
1.	152	λ*bio*72*sus* *gam*$_{14}$	1	5	498	1.0
2.	"	"	30	6	521	1.1
3.	"	"	60	7	532	1.3
4.	"	"	120	16	804	2.0
5.	"	"	180	8	671	1.2

Helper was *imm*^{434}CI *sus*$R_{54}R_{60}$. DNA was CI_{857}. Indicator was W3350. Residual recombination was measured with *bio*72*sus*P_{228} whole DNA, at the 120 minutes time point, with a value of 8 (line 4).

This experiment shows that, at least for this host and helper, full levels of competence are acquired almost instantly. However, variations in time of incubation at 0° in TCM produce no significant variations in rescue of half molecules by λgam⁻ helper.

In conclusion, growth phase of the cells during growth in minimal medium, and duration of the 37°C incubation periods in buffer, affect rescue of halves by a bio72gam⁻ helper, yielding a range of 0.7 to 14% rescue of halves. In contrast, incubation of competent cells in buffer at 0°C for 0-3 hours has no effect on half molecule rescue. This suggests that either the nuclease activity responsible for half molecule degradation exhibits decay in activity as cells reach late log phase and also during starvation periods at 37°C, or leak translation of the λsus gam mutation is greater under those same conditions.

Two factors appear to be involved in these experiments. One, the nuclease activity on half molecules which can be observed only in the absence of gam product; and two, the effect of cell physiology on transformation by whole λDNA which can be observed with both λbio72gam⁺ and λbio72gam⁻ helpers. Both of these factors may contribute to the variable comparative rescue of half molecules.

APPENDIX (Part 2)

Complete data on all helpers in all hosts tested.

TABLE 28 - CI_{857}imm$^{\lambda}$ helpers in recAsu^{-} host.

Host	Helper	sus^{+} infectious centers/10^{-6} A_{260} units of DNA								
		λ^{+} Halves	λvir Halves	imm^{21} Halves	λ^{+}	susP	susJ	red_{114}susP	bio72susP	λdg
152	red_{3}susR	3	26	1121	725	22	701	3	-	797
	red_{270}susR	< 1	11	410	504	21	436	2	-	620
	red_{113}susR	22	36	1159	411	8	411	< 1	-	323
	red_{114}susR	1	63	922	580	54	530	11	-	662
	red_{504}susR	131	260	1499	-	157	711	-	< 1	368
	bio72susR	130	60	283	281	128	158	26	1	-
	bio11susR	< 1	-	-	433	9	109	-	< 1	-
	bio1susR	< 1	< 1	12	450	29	298	-	16	188
	$susN_{7}N_{53}$	8	15	18	780	241	800	73	107	916
	$susR_{54}R_{60}$	940	1460	2420	938	528	1344	537	692	1212

TABLE 29 - imm^{434}CI helpers in recAsu^{-} host.

Host	Helper	sus^{+} infectious centers/10^{-6} A_{260} units of DNA								
		Halves			Wholes					
		λ^{+}	λvir	imm^{21}	λ^{+}	susP	susJ	red$_{114}$susP	bio72susP	λdg
152	red$_{113}$susR	1648	162	1582	1944	1112	2952	1	-	2160
	red$_{114}$susR	1728	319	2332	1932	1048	2664	12	804	2064
	bio72susR	615	171	1023	1232	519	1471	312	13	1087
	bio72gam$_{14}$susR	166	40	141	884	356	1248	166	-	1348
	bio1susR	10	3	9	-	-	1736	-	72	980
	bio10susR	10	-	-	247	110	335	-	3	1100
	bio72gam$_{3}$susNN	19	-	22	928	274	1080	91	42	1465
	susN$_{7}$N$_{53}$	16	7	11	880	440	1236	408	332	1948
	gam$_{210}$susR	305	172	350	-	1140	3128	-	1048	-
	gam$_{5}$susR	603	541	396	-	1164	2880	-	620	-

TABLE 30 - CI_{857}imm$^{\lambda}$ helpers in rec^{+}su^{-} host.

Host	Helper	sus^{+} infectious centers/10^{-6} A_{260} uhits of DNA								
		Halves			Wholes					
		λ^{+}	vir	imm^{21}	λ^{+}	susP	susJ	red$_{114}$susP	bio72susP	λdg
W3350	red$_{3}$susR	125	231	1115	590	127	1115	30	-	824
	red$_{270}$susR	11	29	368	484	92	696	10	-	380
	red$_{113}$susR	303	315	1397	1137	369	1357	209	-	1277
	red$_{114}$susR	151	168	1111	519	243	867	87	722	471
	red$_{504}$susR	1013	865	2117	1029	1009	2277	-	557	1234
	bio72susR	1066	334	922	1250	746	1774	-	98	-
	bio11susR	<1	-	100	744	452	968	308	648	-
	bio1susR	18	<1	86	994	451	1450	303	411	292
	susN$_{7}$N$_{53}$	61	3	81	874	774	1278	-	674	798
	susR$_{54}$R$_{60}$	1185	3170	2305	2020	880	2705	585	720	1760

TABLE 31 - imm^{434}CI helpers in rec^{+}su^{-}host.

Host	Helper	sus^{+} infectious centers/10^{-6} A_{260} units of DNA: Halves			Wholes					
		λ^{+}	vir	imm^{21}	λ^{+}	susP	susJ	red$_{114}$susP	bio72susP	λdg
W3350	red$_{113}$susR	342	77	250	996	488	1324	16	748	1032
	red$_{114}$susR	876	300	872	1184	484	1360	33	440	1204
	bio72susR	1066	334	922	1250	746	1774	-	98	-
	bio72gam$_{14}$susR	71	7	56	1840	550	1575	-	154	1604
	bio1susR	43	13	55	950	381	1980	128	140	1310
	bio72gam$_{3}$susNN	112	23	147	1530	468	1915	-	65	1960
	susN$_{7}$N$_{53}$	100	47	85	1423	756	1833	-	444	1838
	gam$_{210}$susR	120	39	94	-	1524	2640	-	-	-
	gam$_{5}$susR	518	234	376	-	940	2976	-	-	-

TABLE 32 - imm$^{\lambda}$ helpers in recA recB su^{-} host.

Host	Helper	sus^{+} infectious centers/10^{-6} A_{260} units of DNA						
		Halves						
		λ^{+}	vir	imm^{21}	λ^{+}	susJ	red$_{114}$susP	bio72susP
E835	red$_{3}$susR	146	218	2332	-	870	< 1	-
	red$_{270}$susR	42	149	2280	-	384	< 1	-
	red$_{113}$susR	90	126	1864	-	446	4	-
	red$_{114}$susR	205	212	1840	-	648	< 1	-
	red$_{504}$susR	577	571	2047	-	773	-	< 1
	bio72susR	702	316	1240	-	790	-	< 1
	bio11susR	6	24	1528	165	45	-	7
	bio1susR	34	136	1881	773	287	< 1	9
	susN$_{7}$N$_{53}$	1135	679	2128	-	1135	115	239
	susR$_{54}$R$_{60}$	636	1172	1768	-	904	-	220

TABLE 33 - imm^{434}CI helpers in recA recB su^{-} host.

Host	Helper	sus^{+} infectious centers/10^{-6} A_{260} units of DNA					
		Halves			Wholes		
		λ^{+}	vir	imm^{21}	susJ	red$_{114}$susP	bio72susP
E835	red$_{113}$susR	1048	492	1712	2224	1	-
	red$_{114}$susR	1312	470	1960	2280	3	-
	bio72susR	1691	475	1395	1763	-	2
	bio72gam$_{14}$susR	1024	259	1488	1816	-	< 1
	bio1susR	1212	348	2134	2212	76	-
	bio10susR	1630	434	1646	1816	-	22
	bio72gam$_{3}$susNN	1640	282	1584	2192	-	10
	susN$_{7}$N$_{53}$	1893	661	2349	2077	271	-
	gam$_{210}$susR	2912	2488	2048	3176	-	-
	gam$_{5}$susR	1976	2212	2440	2704	-	-

TABLE 34 - Transformation in the *recBsu*$^-$ host

Host	Helper	*sus*$^+$ infectious centers/$10^{-6}A_{260}$ units of DNA								
		Halves			Wholes					
		λ^+	*vir*	*imm*^{21}	λ^+	*susP*	*susJ*	*red*$_{114}$*susP*	*bio72susP*	λ*dg*
DM22	*red*$_3$*imm*$^\lambda$*susR*	80	-	2566	314	20	376	4	34	-
	red$_{114}$*imm*$^\lambda$*susR*	1342	-	3560	1030	510	1702	28	550	-
	bio72imm$^\lambda$*susR*	1530	-	2780	1071	942	1942	220	30	-
	bio1imm$^\lambda$*susR*	82	-	4470	336	62	498	4	8	151
	susN$_7$*N*$_{53}$*imm*$^\lambda$	1713	1004	-	1353	677	1245	259	421	797
	susR$_{54}$*R*$_{60}$*imm*$^\lambda$	1796	2784	3850	1150	828	1170	476	1036	1672
	red$_{113}$*imm*^{434}*susR*	2156	874	-	1242	780	2060	27	-	-
	red$_{114}$*imm*^{434}*susR*	2326	-	3420	2360	760	2050	14	1104	-
	bio1imm^{434}*susR*	4340	-	4836	4140	2520	4156	190	212	-
	bio10imm^{434}*susR*	1786	-	-	1578	1094	1246	-	<1	2002

TABLE 35 - Transformation in $rec^{+}su^{+}$ host.

Host	Helper	sus^{+} infectious centers/$10^{-6}A_{260}$ units of DNA							
		Halves			Wholes				
		λ^{+}	vir	imm^{21}	λ^{+}	susP	susJ	red_{114}susP	λdg
R-A9605	$red_{3}imm^{\lambda}susR$	1	-	-	748	148	161	121	-
1200	$red_{270}imm^{\lambda}susR$	387	-	-	452	160	190	68	-
R-A9605	$susN_{7}N_{53}imm^{\lambda}$	988	-	-	816	-	764	-	-
1200	$susN_{7}N_{53}imm^{\lambda}$	692	519	-	1120	-	740	-	235
R-A9605	$susR_{54}R_{60}imm^{\lambda}$	1149	-	-	1017	-	937	251	359
R-A9605	$bio72imm^{434}susR$	396	-	-	1010	342	738	-	-
"	$bio72gam_{14}imm^{434}susR$	1070	-	-	-	662	938	-	-
"	$bio10imm^{434}susR$	56	-	-	111	144	-	22	-
"	$bio72gam_{3}susNNimm^{434}$	482	72	436	820	-	1552	-	944
"	$susN_{7}N_{53}imm^{434}$	1888	692	2432	-	776	2264	-	1824
"	$gam_{210}imm^{434}susR$	1104	-	1105	-	-	2345	-	-
"	$gam_{5}imm^{434}susR$	376	-	346	-	-	1582	-	-

TABLE 36 - Transformation in 152 (imm^{434}) host.

Host	Helper	sus^{+} infectious centers/ $10^{-6}A_{260}$ units of DNA		
		λ^{+} Halves	susJ Wholes	λ^{+} Wholes
152(imm^{434})	$CI_{857}susN_{7}N_{53}$	7	-	276
"	$bio72imm^{434}susR_{5}$	23	441	437
"	$red_{113}imm^{434}CI\ susR_{54}R_{60}$	274	802	648
"	$imm^{434}CI\ susN_{7}N_{53}$	77	429	523

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