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GENERAL GENETIC RECOMBINATION IN BACTERIOPHAGE T4

(DNA exchange, DNA-protein-protein interactions)

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SUMMARY

We will discuss current models of genetic recombination emphasizing their general aspects and the specific points which have been demonstrated in phage T4. Recombination in this phage occurs preferentially (although not exclusively) near chromosomal ends. It depends on single-stranded regions (which are formed either by partial degradation or by partial synthesis). DNA replication stimulates recombination in T4 mainly because it generates single-stranded termini in partially replicated chromosomes. Such single-stranded termini invade homologous regions of duplex molecules, thereby generating recombinational forks. These forks can be resolved to yield insertion-type or crossover-type recombinants. Alternatively, they can be reconverted to replication forks. Thus, no clear-cut distinction between replicative or recombinational forks can be made. - Because the T4 chromosomes are circularly permuted, growing points reach ends and recombinational forks accumulate soon after the onset of DNA replication. The invading single-strands generate a network of DNA whose complexity increases with increasing numbers of infecting particles and with time after infection. The conversion of these forks to replication forks rapidly accelerates overall DNA synthesis in viral infected cells. The networks are finally resolved during maturation. - The interconversion of recombinational and replication forks is facilitated by the multiple roles of several proteins in replication and recombination. Activities of these proteins in both processes are modulated by specific interactions with the T4 gene-32 protein. This protein, which presumably coats all intracellular single-stranded DNA regions, provides an ordering principle for the coordinated action, in time and space, of the other proteins acting on DNA. Additional stabilization of these interactions occurs by the interaction of these proteins with membrane components.

INTRODUCTION

Exchange of genetic information among different organisms is one of the most fundamental aspects of evolution. It is also one of the basic tools of modern biology. Thus, understanding the mechanisms which generate such exchanges has theoetical as well as practical implications.

Ultimately we must understand these mechanisms in terms of interactions between DNA molecules, and we hope that all DNA-containing cells and viruses share some common aspects of these mechanisms. This hope is based on the observation that the formal analysis of genetic crosses in prokaryotes and in eukaryotes gives similar results which are best interpreted by similar models. Listening to Dr. PERKIN'S presentation (this volume) has certainly reinforced this rationale.

We want to discuss current knowledge and thoughts about general genetic recombination in prokaryotes, i.e., genetic exchanges which depend on extensive homology between participating DNA molecules. This is in contrast to "illegitimate recombination" (FRANKLIN 1971) or "site specific recombination" (SIGNER and WEIL 1968) which are independent of RecA function and use little or no sequence homology (for reviews see COHEN 1976, KLECKNER 1977, LANDY and ROSS 1977) to rearrange large blocks of genetic material. Even if there are certain links between these various modes of exchanges, it is premature to speculate about them. We shall first consider various models which summarize and explain numerous phenomena associated with genetic exchanges in various organisms. We shall then discuss specific aspects in phage T4.

GENERAL MODELS

General genetic recombination is usually extremely precise. It exchanges segments (and thus genetic alleles) between homologous DNA molecules, leaving the overall arrangement of genetic markers on the chromosomes unaltered. It involves breakage and rejoining of DNA, accompanied by some DNA synthesis (MESELSON and WEIGLE 1961, KELLENBERGER, ZICHICHI and WEIGLE 1961, STAHL et al. 1972). In most systems, exchanges occur at random positions in chromosomes and thus recombination frequencies can be used to estimate genetic distances. Certain exceptions distort the congruence of genetic and physical maps. Since these exceptions tend to confirm the rules for exchange, we shall consider them in the context of current recombination models.

There are several ways to follow the process of general recombination. In conventional genetic analyses, parental DNA is labeled with genetic markers (mutant *vs.* wild type alleles) and exchanges are detected using the distribution of corresponding phenotypes in the progeny of genetic crosses. In addition, the fate of DNA molecules undergoing genetic exchanges can be followed by biochemical means: parental DNA is differentially labeled with density- and radioisotopes; intermediate and final products are distinguished by density and sedimentation analyses and by electronmicroscopy. Each of these methods has obvious advantages and disadvantages: genetic analysis can detect extremely rare events ($\sim 10^{-6}$) but in most cases it requires gene expression and production of viable recombinants.

In addition, it cannot tell whether the markers were exchanged during breakage-rejoining (Fig. 1) or whether new marker arrangements were generated during subsequent partial repair of

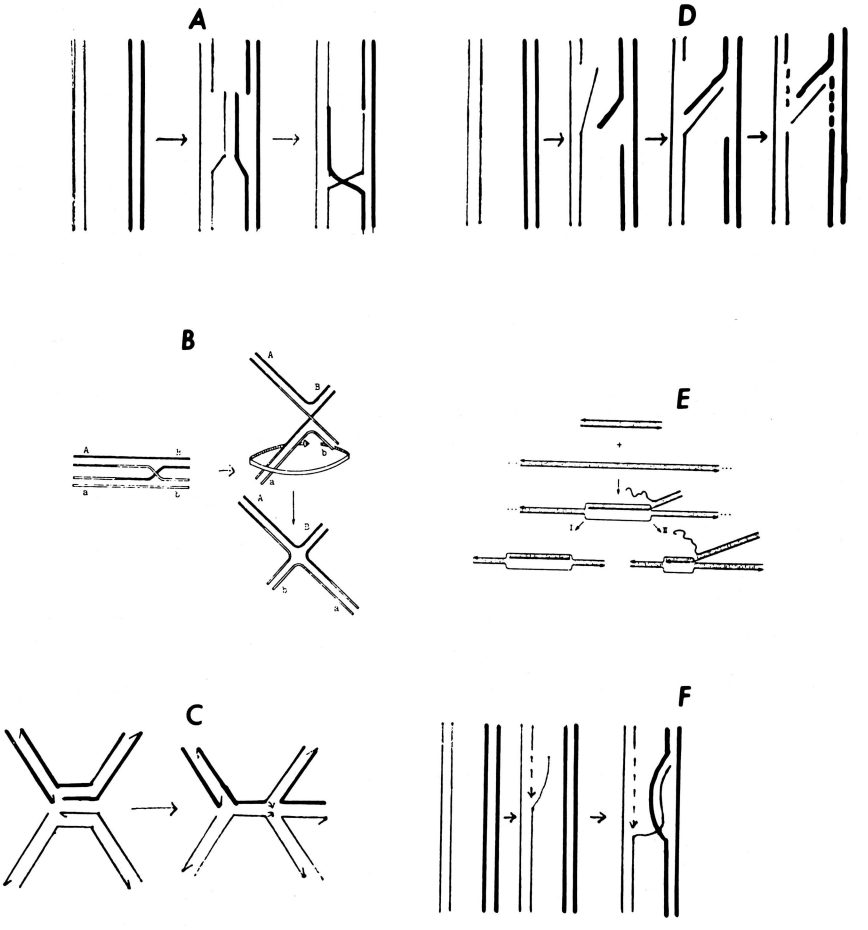

Figure 1. Models for initiation and propagation of heteroduplex regions in DNA. A: HOLLIDAY 1964 B: SOBELL 1972, POTTER and DRESSLER 1976 C: BROKER and LEHMAN 1971 D: WHITEHOUSE 1963 E: FOX 1966 F: MESELSON and RADDING 1975, HOTCHKISS 1974.

heteroduplex regions (Fig. 2B). Biochemical and biophysical methods can detect nonviable intermediates, but usually these methods are much less sensitive than crosses and they rarely permit precise localization of exchanges. Electronmicroscopy reveals recombinational intermediates but it does not distinguish parental molecules from final recombinants and thus does not reveal differences in crossover-type (Fig. 2,I) or insertion-type (Fig. 2,II) recombination. Obviously, the best understanding of the *in vivo* situation comes from combining both genetic and biochemical types of analyses in the same or similar experiments. This can best be done in microorganisms.

All current models for general genetic recombination involve the formation of "hybrid" or "heteroduplex" DNA as intermediates in genetic recombination (HERSHEY 1958). Complementary single-stranded segments, derived from different parental molecules, are joined via base-pairing in heteroduplex regions of finite length (~100-5000 base pairs); when genetic markers are involved in the hybrid region, so-called partial heterozygotes are detected (HERSHEY and CHASE 1951). This requirement for extensive base pairing is responsible for the usually high accuracy of general recombination.

The models proposed by WHITEHOUSE (1963) and by HOLLIDAY (1964) first defined formation and resolution of hybrid DNA and its implications in genetic fine structure analysis. These models are the prototypes of all current models which differ in the details of how hybrid DNA is initiated, extended, and terminated and how these intermediates are resolved to yield final recombinant structures.

Two classes of models exist which attempt to explain how pairing of complementary strands is initiated between molecules of different parental origin. These models are summarized in Figure 1. In one class (Fig. 1,A-B) strands of the same polarity are exchanged at identical positions (perhaps by concerted action of recombination enzymes). These models all seem to imply that some pairing occurs prior to cutting and rejoining. Thus it is pleasing to see that pairing of homologous double strands to form four-stranded structures is indeed stereochemically feasible (WILSON 1979).

The other class of models (Fig. 1 C-F) postulates that a single-stranded segment of one parental duplex pairs with the complementary strand from another parental duplex either by pairing with an existing single- stranded region or by displacing the non-complementary strand. The result of the former is the so-called "H-structure" demonstrated by BROKER and LEHMAN (1971). Single-stranded regions can be generated: (i) by partial nucleolytic degradation of double-stranded, (ii) by partial unwinding of DNA (FOX 1966), or (iii) by displacement synthesis from nicks in DNA (WHITEHOUSE 1963, HOTCHKISS 1974, MESELSON and RADDING 1975). Single-stranded regions are also formed during DNA replication. This is one of the reasons why replication stimulates recombination (see below).

In support of the models shown in Fig. 1 (E-F), HOLLOMAN et al. (1975) have demonstrated uptake of single-stranded DNA by unnicked supercoiled DNA *in vitro* in the absence of proteins. Such triple-stranded structures generate recombinants

after transfection, although with rather low frequencies (HOLLOMAN and RADDING 1976). The presence of RecA protein, however, greatly facilitates single-strand uptake as well as other means of heteroduplex formation (McENTEE this volume).

The different models of heteroduplex formation are, of course, not mutally exclusive. Electronmicroscopy provides evidence for concerted exchange (POTTER and DRESSLER 1979), for so-called H-structures (BROKER and LEHMAN 1971, OGAWA et al. 1979) and for displaced single strands (BROKER and LEHMAN 1971, FORNILI and FOX 1976, MOSIG et al. 1979). A modified version of FOX' (1966) model (Fig. 1E) has provided a logical explanation of recombination in terminal regions of T4 DNA molecules (see below).

No matter how heteroduplex regions are initiated, they are extended by (single-strand or double-strand) branch migration. This process makes it extremely difficult to determine the point at which the first exchange was initiated. It is also difficult to determine (especially in electron micrographs) the extent of branch migration which has occurred *in vitro* (LEE et al. 1970) or *in vivo* (BROKER and LEHMAN 1971). The point at which two DNA strands of different parental origin cross over in an electronmicrograph is now usually called the "Holliday junction". This junction can be seen in "open" or in "closed" configuation (POTTER and DRESSLER 1976) see Fig 1B. These configurations are also called trans- and cis-isomers respectively. Like other biological processes, branch migration and the conversion of cis- and trans-isomers is probably facilitated by specific proteins.

Molecular model-building suggests that branch migration must stop at major nonhomologies in DNA, i.e. when one of the parental markers is a deletion, insertion or inversion. Thus, nonhomologies should interfere with recombination - and they do interfere, but only when replication is blocked (FOX et al. 1979). When replication is permitted the effect of nonhomologies on recombination frequencies is not as pronounced, presumably because longer heteroduplexes can be formed by direct pairing of single-stranded regions.

Depending on how these intermediate structures are resolved, different types of recombinants are formed. The intermediates can yield two single exchange (*crossover-type*) recombinants when the two new junctions occur in strands of opposite polarity (Figure 2 mode I, left panel). Alternatively, such intermediates generate one double-exchange (*insertion-type*) recombinant and one parental type, when the two new junctions occur in the same DNA strand (Figure 2 mode II, lower right panel). (Insertion-type recombinants should not be confused with IS elements!)

Note that only crossover-type recombination results in exchanges of markers outside of the heteroduplex region and thus is the main source of large interval recombinants. In contrast, insertion-type recombination is detectable mainly in short intervals. It generates correlated double exchanges within relatively short distances but rarely recombines markers that are far apart.

Heteroduplex regions may be partially repaired (step B, Figure 2). In many systems, most intragenic recombinants appear to be generated by heteroduplex repair (for reviews see STADLER 1973, HASTINGS 1975). Usually, heteroduplex repair alters the

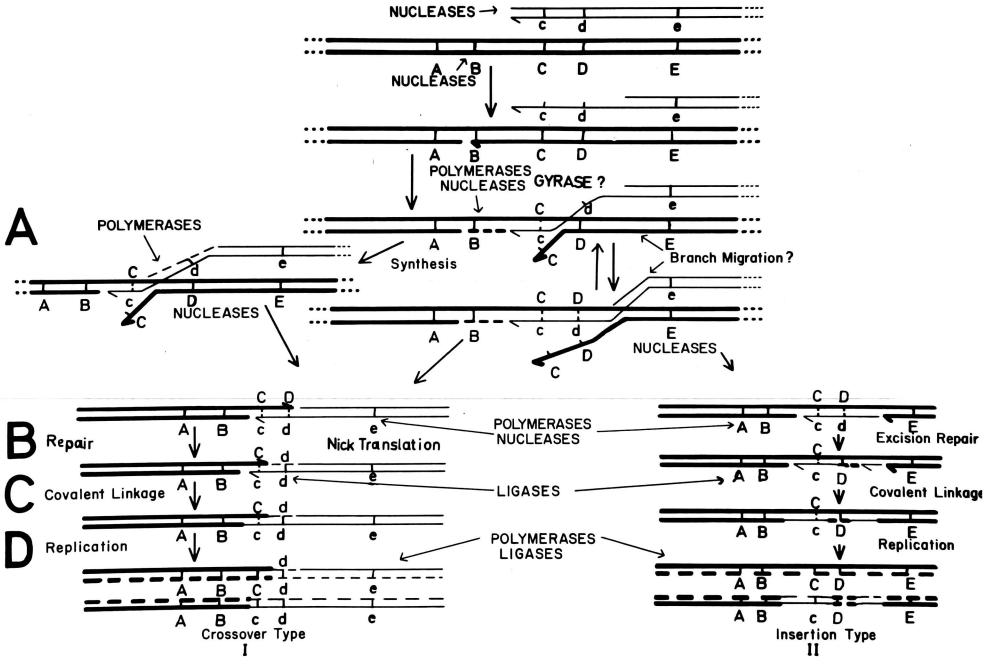

Figure 2. The resolution of recombinational intermediates (initiated as shown in Fig. 1E) into insertion-type or crossover-type recombinants are explained in the text. Parental DNA: ▬▬▬, ——— thick or thin solid lines, newly synthesized DNA: ▬ ▬ ▬, - - - broken lines. Different alleles of different parental origin are shown as capital or lower case letters respectively. Both DNA strands are marked only when heteroduplexes contain different alleles. 5' ends are marked with arrows.

ratio of alleles that are recovered in the progeny from the input ratio. Such changes of allele ratios were called *gene conversion*, long before heteroduplex repair was postulated. Note that heteroduplex repair may be accomplished in two ways: (i) it may be unspecifically initiated as nick translation from an unsealed junction of recombining DNA (as shown in the lower left panel of Figure 2) and "accidentally" correct a mismatched base pair; (ii) mismatched base pairs may be actively recognized and corrected by specific enzyme systems as shown in the lower right panel of Figure 2. [This kind of repair may be mismatch-specific and thus generate "marker effects" on

recombinant frequencies (EPHRUSSI-TAYLOR 1966, for reviews see STADLER 1973, HASTINGS 1975).] Specific mismatch repair within a heteroduplex region generates additional genetic exchanges within both insertion- and crossover-heteroduplexes; thus multiple clustered exchanges and "gene conversion" may or may not appear to be associated with exchange of markers outside of the heteroduplex region. On the other hand, unspecific heteroduplex repair by "nick-translation" does not generate additional genetic exchanges, but it changes allele ratios and reduces heteroduplex lengths. Thus, "gene conversion" is not necessarily associated with multiple genetic exchanges.

In step C, the exchanged DNA strands are covalently linked. Finally, DNA replication (step D) resolves any heteroduplexes (partially corrected or not) to final recombinants.

The temporal sequence of the recombination steps subsequent to heteroduplex formation is not precisely defined by experimental evidence. Presumably, *in vivo*, these steps do not occur independently: based on our recent results we propose that most, if not all, recombination steps are coordinated in time and space by binding of the responsible enzymes to a DNA binding protein, e.g. T4 gene-32 protein (see Figure 7 below). The specific result of a recombination experiment must, therefore, depend on the enzymatic make-up of the organism and on specific assay and growth conditions. When DNA replication is inhibited or slowed down, there is more chance for branch migration and for mismatch repair to occur than under replication proficient conditions. On the other hand, replication generates recombinogenic single-stranded regions which may form heteroduplexes with or without additional branch migration. It may also be important whether the participating molecules are under topological constraint (e.g. by being circular or by being membrane associated), so that topoisomerases can unwind the DNA.

RECOMBINATION IN BACTERIOPHAGE T4

We shall now focus on certain aspects of recombination processes which have been specifically demonstrated in phage T4: (i) the interrelationship of DNA replication and recombination, (ii) the effects of chromosomal ends on recombination, (iii) map distortions due to hot spots of recombination, (iv) pairing in *partially* homologous regions and its possible consequences on generating duplications and deletions, and (v) the roles and interactions of various gene products in genetic recombination. The distinct advantages for studying recombination in phage T4 are: (i) the frequency of recombination per unit length of DNA is unusually high. (ii) genetic mutations can be easily manipulated.

The total genetic information of T4 is stored in the sequence of 166,000 base pairs (KIM and DAVIDSON 1974). Each virus particle contains a single linear double-stranded DNA molecule representing a different permutation of the circular genetic map (Fig. 3). Approximately 3% of the terminal sequences are repeated at both ends as "terminal redundancy" (STREISINGER et al.1964, 1967, THOMAS and MacHATTIE 1967).

Conditional lethal mutations in more than 20 genes affecting DNA metabolism are known (WOOD and REVEL 1976). Many of

these affect DNA replication and recombination simultaneously. The effects of *different* mutations in the same gene, as well as the suppressing or synergistic effects of secondary mutations, can be readily investigated. ALBERTS and his co-workers have had spectacular success in purifying and characterizing replication proteins (LIU et al. 1979). Many of these proteins function also in recombination (BROKER and DOERMANN 1975). This is probably not fortuitous, but reflects the close relation ship of DNA replication and recombination in phage infected cells (MOSIG et al.1979).

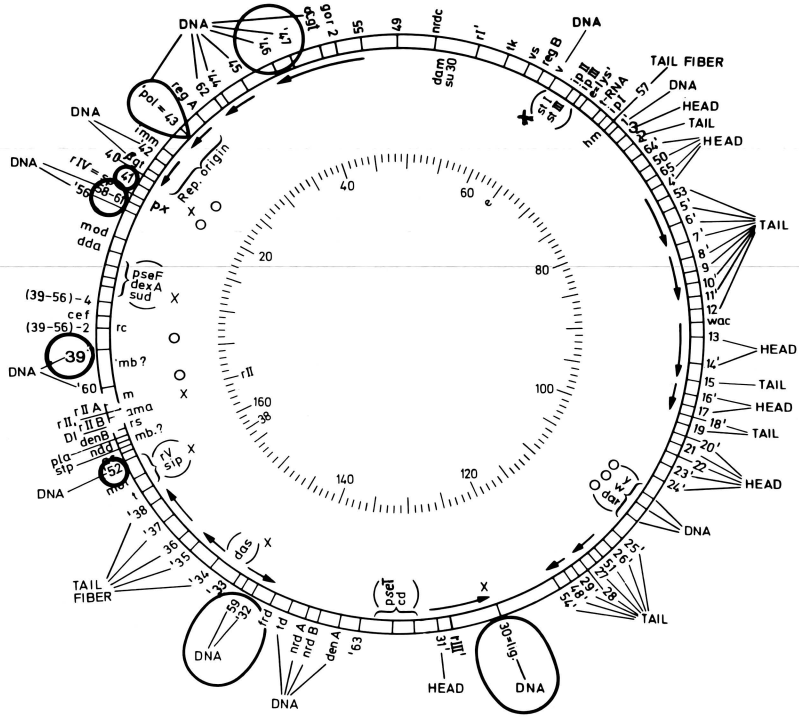

Figure 3. Linkage map of phage T4. Genes involved in recombination are marked with an 0. Genes which affect gene 32 expression are marked with an X. Genes whose products interact with the DNA binding protein are circled.

After infection, the processes of DNA replication and recombination together generate [from the linear parental molecules] large, highly branched DNA complexes (HUBERMAN 1968). In T4, these structures are called "concatemers," even though it is now clear that they are branched. It is from these branched concatemers that the mature T4 DNA is packaged into phage heads and in the process is cut to linear unit length chromosomes (KEMPER and BROWN 1976). Thus the outcome of T4 recombination must to some extent depend on DNA replication and maturation.

INTERRELATIONSHIP OF DNA REPLICATION AND RECOMBINATION

The recombinational intermediates postulated in Fig. 1 should be visible in the electron micrograph. Since it seemed impossible to distinguish replicating from recombining DNA, BROKER and LEHMAN (1971) and BROKER (1973) chose to investigate recombining T4 DNA in mutants which were defective in T4 DNA polymerase (pol^-, gene 43) and thus were blocked in replication. Under these conditions, pairing between differentially labeled parental chromosomes occurs only late after infection. By this time a large proportion of the infecting molecules have been partially degraded and fragmented by the combined action of endo- and exonucleases (TOMIZAWA 1967); thus they have become recombinogenic. The conversion of these base-paired recombinational intermediates (so-called "joint molecules,") to covalently linked recombinants requires DNA polymerase(s); i.e. T4 DNA polymerase or E. coli DNA Polymerase I (ANRAKU et al. 1969, MOSIG 1974). Therefore, under pol^- conditions, the contribution of fragmentation and branch migration to recombination appears exaggerated.

Recombinational intermediates accumulate even more when the T4 pol^- mutations are combined with additional mutations in other genes, all of which primarily enhance formation of single-stranded nicks and gaps. Under these conditions, the majority of recombinational intermediates were "H-structures" (see Fig. 1C). Many of these showed double-strand branch migration. In a minority of forks, displaced *single-strands* protrude as whiskers from a duplex segment (BROKER and LEHMAN 1971, BROKER 1973).

In contrast, when replication is permitted, recombinational intermediates appear much earlier after infection (MOSIG et al. 1979). When we tried to analyse early stages of T4 DNA

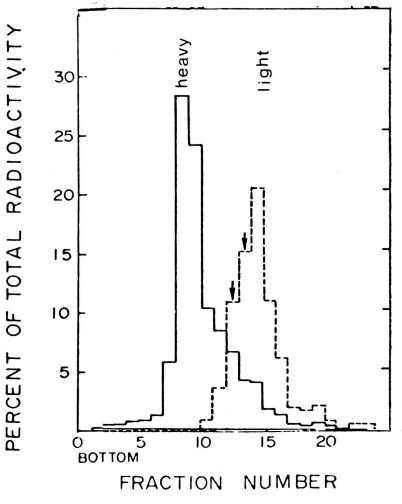

Figure 4. Separation of unsheared DNA in a neutral Cs_2SO_4 density gradient of unsheared DNA isolated 6.5 min after infection with wild type T4 particles. The parental particles were labeled with $^{32}P^{13}C^{15}N$ (heavy) (——) or with ^{3}H (light) (- - -). Bacterial DNA and progeny phage DNA were not labeled. The electronmicrographs shown in Fig. 5 came from the fractions marked with arrows.

replication by electronmicroscopy, we realized that after multiple infection with wild type phage, recombination starts less than one minute after the onset of parental DNA replication and certainly before all parental DNA had replicated once. To detect early recombination specifically, we infected unlabeled

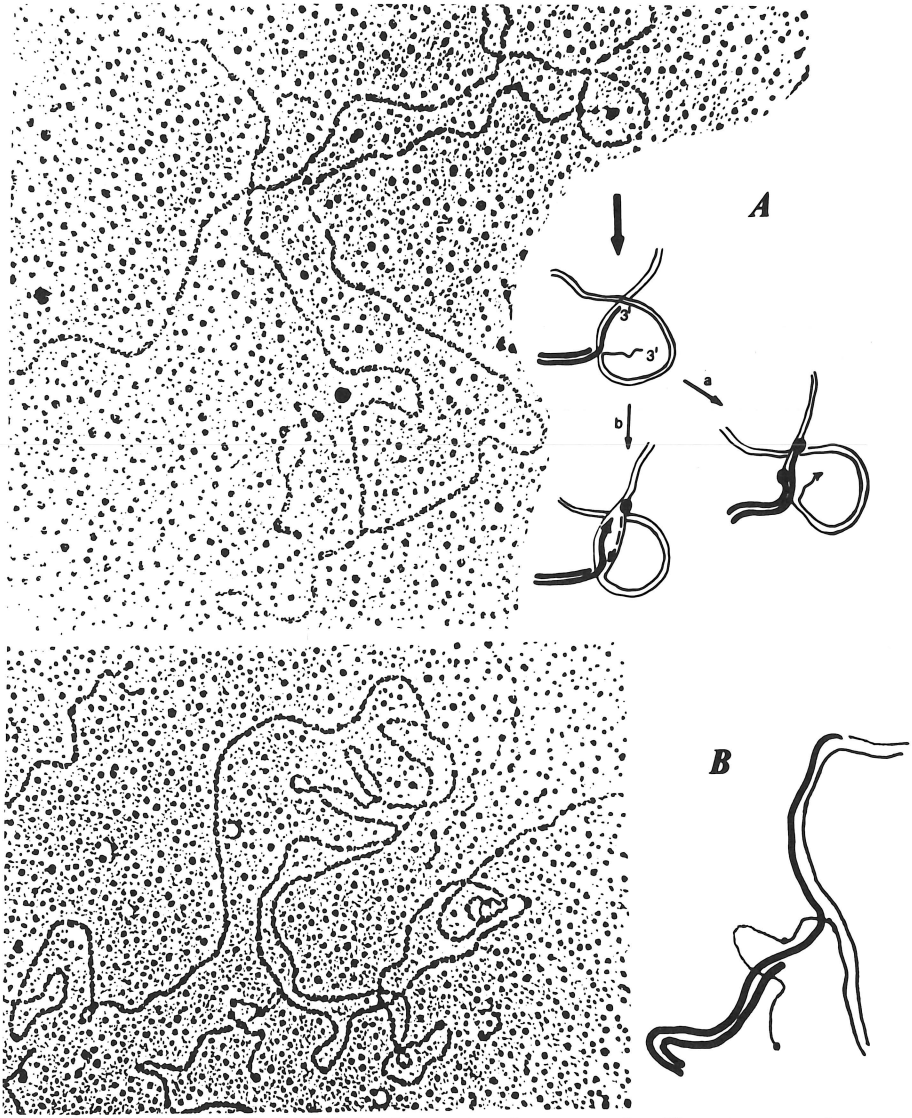

Figure 5. A replicated fork whose end (arrow) invades another DNA molecule. The insets illustrate the postulated conversion of this recombinational intermediate to a true recombinant (a) or to a replicated fork (b). B. A replication "eye" which is invaded by a single-stranded end of another molecule.

bacteria with five heavy ^{13}C ^{15}N ^{32}P-labeled, and five light ^{3}H-labeled T4 particles. Under these conditions, the ^{32}P-label may move to light density by replication *or* by recombination with light DNA. Light ^{3}H DNA, however, can acquire heavier density only by recombination with heavy or hybrid density DNA. Our results show that DNA at the intermediate density (Fig. 4, arrows) is enriched in branched DNA molecules. Many of the forks display single-stranded whiskers (see Fig. 5), like some of the recombinational intermediates described by BROKER and LEHMAN (1971). A few of the DNA molecules exceed unit length. This suggests that recombination between circularly permuted molecules occurs early and does not require a pool of replicated unit length molecules (MOSIG et al. 1979, R. Dannenberg, Ph.D. thesis, Vanderbilt University 1979).

The forks with long single-stranded whiskers are not simply replication forks, but are generated by recombination between partially replicated molecules. This conclusion is based on two observations: the long whiskers are not seen when early recombination is prevented, i.e. when bacteria are infected with only a single phage particle (average 0.15 particles per bacterium) or when recombination deficient gene-32 mutants are used (MOSIG et al.1979, R. Dannenberg, Ph.D. thesis Vanderbilt University 1979).

While recombinational forks seen in replication proficient conditions resemble many of the forks in the unreplicated intermediates of BROKER and LEHMAN (1971), two differences are noteworthy: (i) at early times after infection we found no recombinational forks at the density position of unreplicated DNA, and (ii) at this time, few, if any, of the branched molecules could unambiguously be identified as "H-structures"; most of the forks displayed single-stranded "whiskers" (see Fig. 1C) like the minority class of BROKER and LEHMAN (1971). We conclude that replication greatly enhances the competence of DNA for recombination but that a limited extent of replication is sufficient to initiate heteroduplex formation by strand displacement.

How does replication enhance recombination? Electronmicrographs such as the two examples shown in Fig. 5 suggest an attractive explanation, i.e. that two factors contribute to recombination proficiency. (i) The end of one of the replication branches remains single-stranded, when the growing point reaches the end of the molecule because the lagging strand cannot be properly initiated (WATSON 1972, BROKER 1973). This single-stranded end is capable of invading another molecule at a homologous sequence as shown in Fig. 5A. (ii) Nascent DNA contains discontinuities (e.g. unjoined Okazaki pieces or nicks generated after misincorporation of uracil). Such discontinuities facilitate the invasion by homologous DNA. Fig. 5B shows the invasion of a single-stranded end into a recently replicated segment of another molecule, i.e. the end is invading one branch of the "replication eye".

Because of the circular permutation, the growing points initiated at an origin region (MARSH et al. 1971) will reach an end at different times after initiation. This explains why

recombinational intermediates can accumulate before most of the parental molecules have finished one round of replication(Fig. 4). Such recombinational forks can be reconverted into replication forks, as we will discuss below.

EFFECTS OF CHROMOSOMAL ENDS ON RECOMBINATION

Phage T4 produces some morphological variants due to errors in head assembly (MOSIG 1963, EISERLING et al. 1970). There are three classes of such small variants (MOSIG et al. 1972). They contain DNA molecules measuring 0.9, 0.77, or 0.67 of the unit length chromosomes. The incomplete chromosomes represent random segments of the genetic map. The small particles are nonviable in single infection, but they contribute *all* of their markers to progeny in simultaneous infection with complete chromosomes or with other incomplete chromosomes which provide the missing genetic segment (MOSIG 1963, DOERMANN and PARMA 1967, MOSIG et al.1971). Thus, the ends of the incomplete chromosomes can be identified genetically. The phage progeny of single bacteria which were infected with only two genetically marked phage chromosomes can be genetically analysed (single-burst analysis). This type of analysis has shown that the ends stimulate recombination (about 5-10 fold) in adjacent intervals. Fig. 6 summarizes the data from *one* representative single burst of a 19-factor cross between a complete multiply marked *am* chromosome and an incomplete chromosome carrying the am^+ alleles (MOSIG et al.1971). Fig. 6 also illustrates two additional general observations in the experiments of MOSIG et al. (1971). (i) The ends of the incomplete chromosomes can generate insertion-type or crossover-type recombinants (and thus show negative or positive interference respectively) with about equal

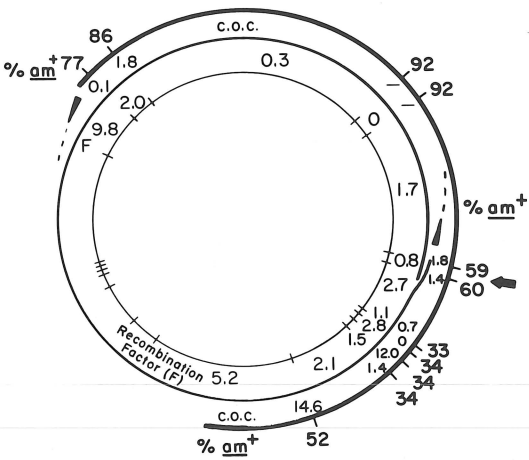

Figure 6. Summary of the recombination seen among the total phage progeny of a single bacterium which had been infected with a complete chromosome carrying 19 *am* mutations and an incomplete chromosome carrying the indicated $^+$ alleles. The inner circle shows the map position of the 19 markers. The outer circle shows the frequencies of am^+alleles (copied from the incomplete chromosome). The coefficients of coincidence (c.o.c.) and the factor (F) by which recombination is enhanced (or reduced) in a given interval (as compared to average recombination frequency in that interval) is also shown. The arrow marks the position where the end of a complete chromosome probably invaded the incomplete chromosome.

frequencies. This is precisely what is predicted when ends invade another molecule as shown in Fig. 5 and when those intermediates can be resolved to generate insertion-type *or* crossover-type recombinants as shown in Fig. 2E. (ii) Abrupt changes in allele frequencies contributed by the incomplete chromosome (Fig. 6, arrow) are evident in many of these bursts. This observation has suggested the possibility that the recombinational intermediates may initiate new growing points. We shall reconsider this point below.

HOT SPOTS OF RECOMBINATION

When distances between genes are measured by methods other than recombination frequencies, hot spots and silent regions of recombination can be detected. Two methods to measure distances in the T4 genome have been used: (i) heteroduplexes can be prepared *in vitro* between T2 and T4 DNA (KIM and DAVIDSON 1974). Distances between loops at regions of nonhomology can be aligned with distances between genetic markers. (ii) Frequencies with which ends of incomplete chromosomes occur in any genetic interval can be measured (MOSIG 1966, MOSIG et al. 1968, CHILDS 1971, MOSIG et al. 1971). Comparison of the resulting map with the recombination map revealed that the region encompassing genes 34 and 35 is recombinogenic. BECKENDORF and WILSON (1972) proposed that there is a preferred initiation site for recombination between genes 34 and 35 and that (due to branch migration) recombination appears enhanced over a finite distance from that site in both directions. The unusually high recombination frequencies in this region depend, at least in part, on T4-specific glucosylation of the DNA (LEVY and GOLDBERG, Genetics in press). It appears that both initiation of heteroduplex formation and heteroduplex repair contribute to the high recombination activity in this region.

PAIRING OF PARTIALLY HOMOLOGOUS REGIONS

As in other organisms, some heteroduplexes between deletion and non-deletion mutants seem to be formed in T4, although few of them are found in mature particles (DRAKE 1966). BENZ and BERGER (1973) have proposed that the single-stranded loops in such heteroduplexes are preferentially excised, so that the wild-type alleles are lost. As discussed before, such heteroduplexes cannot be generated by simple branch migration, but they could be formed by direct pairing of partially complementary single-stranded segments. It has been suggested that similar pairings between *partially* complementary sequences at *different* positions of the genome are responsible for formation of duplications and deletions (SYMONDS et al. 1972, HILL and COMBRIATO 1973, ANDERSON and ROTH 1979, see Fig. 7). Most of the duplications investigated in T4 involve the rII genes. They appear with relatively low frequencies (about 10^{-7}) and they are usually unstable: they generate, by recombination, single-copy derivatives as well as higher copy numbers. When the total length of the reiterated region exceeds the length of the terminal redundancy, compensating deletions (in non-essential or duplicated regions) are selected. Interestingly, if such compensating deletions eliminate an essential segment of the original duplication, the remaining duplicated segments appear stable (WEIL and TERZAGHI 1970, SYMONDS et al. 1972,

PARMA et al. 1972, van de VATE and SYMONDS 1974, van de VATE et al. 1974, HOMYK and WEIL 1974, ROTHMAN et al. 1975).

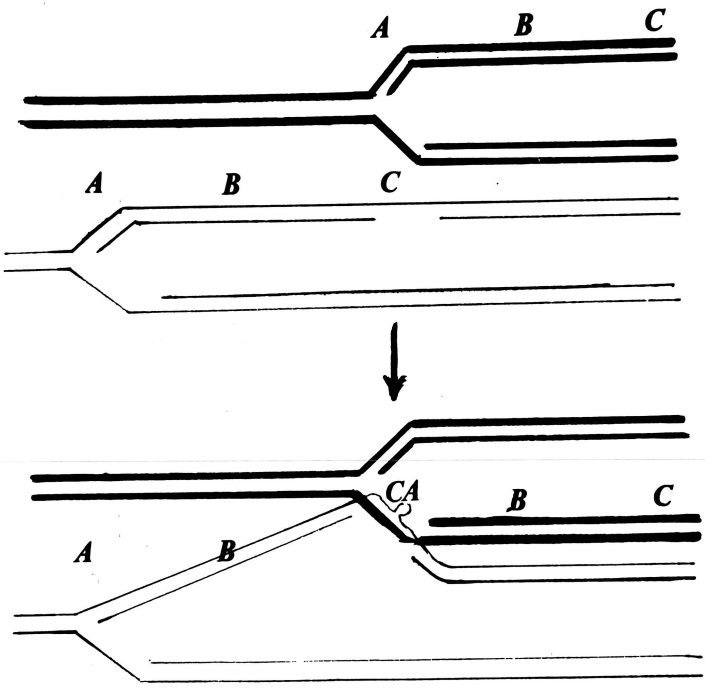

Figure 7. A model to explain the formation of duplications (and deletions). Partially complementary sequences from different genetic locations pair within sister chromatids. Subsequent exchange results in chromosomal aberrations.

We have recently found (unpublished observations) duplications involving a large segment of gene 32 (single-stranded DNA binding protein). The duplications have similar properties as the *rII* duplications characterized earlier but they appear with much higher frequencies (approaching 0.1%) in the progeny of crosses between two gene-32 mutants. It is possible that the gene-32 mutations generate mispairings with unusually high frequencies. On the other hand it is also possible that partial sequence homology exists within or in the vicinity of gene- 32 and that such partial homology facilitates formation of specific duplications.

INTERACTIONS OF GENE PRODUCTS IN RECOMBINATION

While precursors and products of recombination are rather well characterized, the precise roles of various enzymes are less well defined. DNA polymerase (gene 43), ligase (gene 30), recombination nucleases (genes 46, 47, and probably

49), single-stranded DNA binding protein = helix-destabilizing protein (gene 32), topoisomerase (genes 39, 52, 60) (MUFTI and BERNSTEIN 1974, STETLER et al. 1979, LIU et al. 1979, McCARTHY 1979) must participate because mutations in the corresponding genes affect recombination. Additional recombination genes whose functions are not yet known (for reviews see BROKER and DOERMANN 1975, MILLER 1975, CUNNINGHAM and BERGER 1977) are marked in Figure 3.

It is now generally accepted that proteins involved in DNA replication function as complexes (for reviews see KORNBERG 1974, ALBERTS et al. 1975). Based on our recent experiments we propose that many, if not all, of these proteins also interact with each other when they catalyze the different recombination steps outlined in figures 1 and 2. However, interactions with each other and with DNA are probably different in

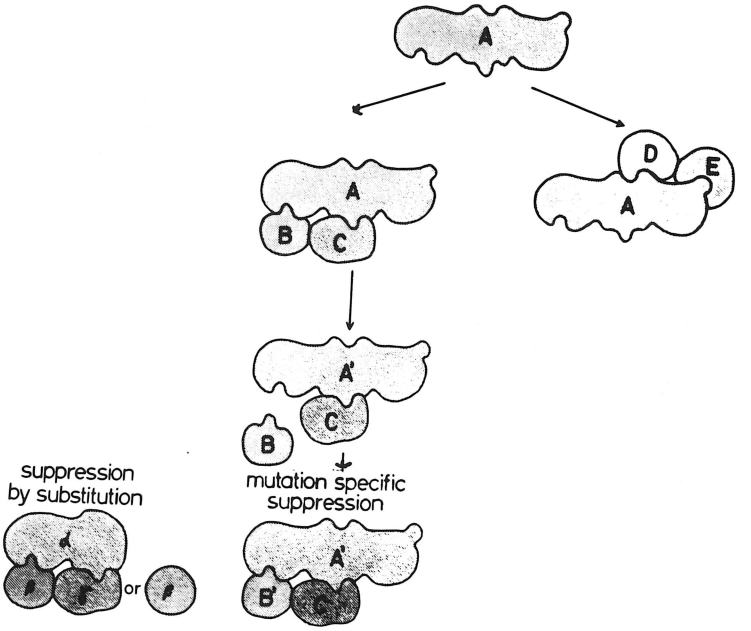

Figure 8. A genetic approach to analyze interactions of gene products by suppressor studies. If protein A participates in two different processes by interacting with different cellular components, we expect that certain mutations differentially inactivate interactions with B, thus inactivating B function, without necessarily affecting other functions. In this situation, we expect that certain site-specific mutations in gene B partially restore interaction of B' with the altered A' protein. Other mutations in gene B may have the opposite or no effect. Alternatively a protein β or a protein complex containing β may substitute for the defective interaction of A with B.

recombination or replication. This implies that these interactions may be transient and weak. In such situations it will be often difficult to detect biochemical interactions and to correlate them with respective *in vivo* functions. Thus, we have used a genetic approach, outlined in Figure 8, to help understand the biologically important functions of such protein interactions.

We have focused our analysis on interactions of gene-32 protein of phage T4 (gp 32) because gene 32 plays a key role in DNA replication and recombination (EPSTEIN et al. 1963, TOMIZAWA 1967, KOZINSKI and FELGENHAUER 1967). It is also the prototype of single-stranded DNA binding proteins (ALBERTS and FREY 1970).

We are studying: (i) mutations in gene 32 that inactivate some but not all functions of the gene product, (ii) compensating suppressor mutations in other genes, and (iii) mutations that enhance the defect in the first mutation (negative suppressors). From the results, we can determine which other gene products might be involved in these complexes. Such positive or negative suppression should be mutation-specific if the corresponding gene products interact directly and this criterion can be used to distinguish "conformational suppression" from more indirect means of suppression. Steric and allosteric interactions, however, cannot be distinguished, for example, although protein X does not directly interact with protein Y, disturbing protein X's interaction with any member of the complex may destroy protein Y's ability to function properly in the complex.

We have combined this genetic approach with biochemical analysis of the DNA in unsuppressed or suppressed mutants. The appropriate labeling with density and radioisotopes followed by density and sedimentation analyses as well as electron microscopy of the DNA permits us to recognize which step of DNA replication and recombination is affected. All of our results (MOSIG and BRESCHKIN 1975, MOSIG and BOCK 1976, BRESCHKIN and MOSIG 1977a,b, MOSIG et al, 1979, and unpublished results) indicate that different regions of 32-protein during recombination interact with DNA, with recombination enzymes, and with membrane components. Our experiments have also shown that many (single or multiple) mutations which interfere with recombination are to the same extent defective in initiation of secondary DNA replication (although they exert little effect on the first round, i.e., primary DNA replication). After correlating defects and map positions of the mutations (see Fig. 9), we have proposed that portions of the N-terminal domain of 32-protein are involved in binding to DNA, to membrane proteins, and to proteins that initiate DNA replication and recombination. Portions of the C-terminal domain modulate the activities of recombination nucleases and thus protect DNA from excessive degradation (MOSIG et al. 1979). The interaction of certain gene-32 mutations with mutations in genes for membrane proteins suggests that some, if not all steps in recombination occur at the membrane. Membrane attachment could provide topological constraints and thus permit unwinding of linear and nicked DNA by DNA gyrase and/or by other

swivelases. We suggest that by virtue of its binding to other enzymes and membrane components, gene-32 protein facilitates the coordinated action of recombination enzymes on DNA in the steps outlined in Figures 1 and 2.

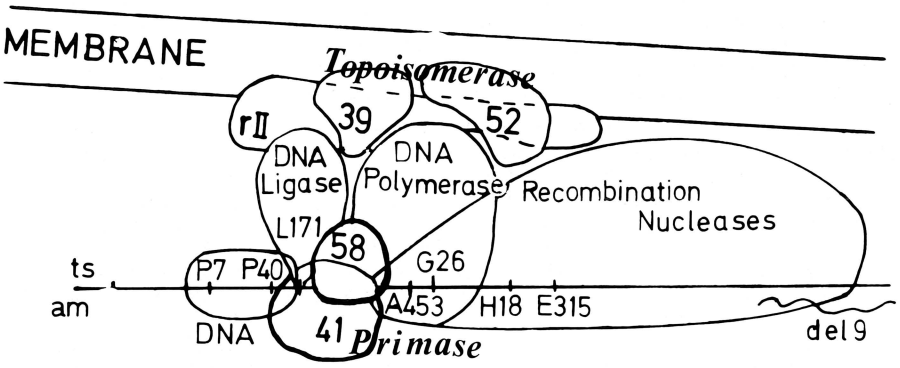

Figure 9. Representation of interactions of gene-32 protein with other proteins. The bottom line shows the position of the gene-32 mutants which we have used. *ts* mutations are shown above the line, *am* mutations below the line. Genes 39 and 52 code for subunits of the T4 topoisomerase (STETLER et al. 1979, LIU et al. 1979). Genes 58 and 41 are thought to code for a T4 specific primase (LIU et al. 1979).

These interactions among gene-32 protein, DNA and other replication and recombination proteins provide the potential to convert recombinational intermediates into final products and also to facilitate the transition from recombination to initiation of replicative forks. The interactions with gene-32 protein also help to coordinate the activities of the different recombination proteins even though their expression is under different genetic control.

CONCLUSIONS

Many previous studies have shown that recombination in phage T4 occurs preferentially (although not exclusively) near chromosomal ends. Many aspects of the exchanges in terminal regions are compatible with the model proposed by FOX (1966). We have modified this model with respect to T4 to accomodate the following observations (some of which are ours and were described above): (i) Initiation of recombination is greatly facilitated and thus occurs much earlier when DNA replication is permitted, than under replication deficient

conditions (used by TOMIZAWA 1967 and by BROKER and LEHMAN 1971). (ii) While the first initiation of replication in the infecting chromosomes does not require recombination functions (MARSH et al. 1971, BRESCHKIN and MOSIG 1977a), initiation of secondary DNA replication forks is largely dependent on recombination functions. This implies that the mechanism for initiating subsequent rounds of DNA replication can be different from that used to initiate the first round of replication. (iii) Specific regions of gene-32 protein interact with DNA and with several proteins which function both in DNA replication and in recombination. Some of these proteins are also associated with membrane components.

Based on these and other results, we suggest the following model: The first round of T4 DNA replication generates replication forks which result in single-stranded regions at the chromosomal termini. These single-stranded segments invade homologous regions of other chromosomes to form recombinational forks (Fig, 5A,B). Recombinational forks can be converted to secondary replication forks; e.g., this happens when a free 3'OH group of an invading DNA segment is used as a primer to initiate further DNA replication. (Since the single-stranded end was formed by a replication complex, this process may be considered as the switch of a replication fork from one template to another.) The different steps in recombination and the conversion of recombinational intermediates into replication forks are facilitated by specific interactions of enzymes acting on DNA, with gene-32 protein (Fig. 9), which covers the single-stranded regions of DNA. The composition of this complex changes as the substrate is altered. We view the interactions with the membrane components in the context discussed by TANFORD (1978): the membrane provides an "ordering principle".

[In replicated T4 DNA some additional recombination must be initiated by a different mechanism, i.e., by direct pairing of single-stranded regions and not by strand displacement because: (i) heteroduplexes involving partial homologies are found, (ii) duplications and deletions are formed by recombination between partially homologous regions and (iii) most late recombinational exchanges in T4 appear to be of the insertion-type (Fig. 2, II).]

Our model explains all of our results. It incorporates the findings of others on functions and interactions of gene-32 protein (ALBERTS and FREY 1970, HUBERMANN et al. 1971, NOSSAL 1974, HUANG and LEHMAN 1972, ALBERTS et al 1975, LIU et al. 1979, KRISCH et al. 1974, GOLD et al. 1976, KRISCH and van HOWE 1976) and on the role of other recombination functions in DNA replication (for review see BROKER and DOERMANN 1975). It also uses the suggestion of BOON and ZINDER (1971) and STAHL et al. (1974) that recombination functions initiate DNA replication. The model explains the phenotype of mutants in the DNA-delay genes which code for a T4 topoisomerase (STETLER et al. 1979, LIU et al, 1979). These mutants are (i) recombinogenic (MUFTI and BERNSTEIN 1974, HAMLETT and BERGER 1975, CUNNINGHAM and BERGER 1977), (ii) defective in generating replication forks (McCARTHY et

al. 1976), but (iii) can replicate their *parental* DNA under the most restrictive conditions for growth (MOSIG et al. 1979). These findings are readily explained if the T4 topoisomerase is involved in the choice between recombination and initiation of secondary replication forks as shown in Fig. 5A (*a* or *b*). In addition, this choice may depend on "competition"of ligase, polymerase and nucleases.

This choice between recombination and replication must also depend on the structure of the recombinational forks. The single strands displaced by the invading strands can form a displacement loop or an open single-stranded whisker. Although our electronmicrographs (e.g., Fig. 5) showed only whiskers, it is likely that some of these whiskers were generated from loops by our lysis procedure. We used SDS together with proteinase K to deproteinize the DNA. These conditions are known to generate breaks in DNA at positions where topoisomerases are bound. The implication of our model that ends of parental DNA are used as primers and therefore are covalently linked to newly synthesized DNA has recently been confirmed (MOSIG et al. 1980).

It has been postulated that T4 DNA replication occurs in two different modes: an early mode which generates many unit length daughter molecules and a late mode which depends on the formation of large concatemers (BROKER and DOERMANN 1975). Our model implies that there is no defined switch from an early to a late mode. Instead, initiation of replication forks from recombinational intermediates provides an additional mode of initiating growing points. This occurs as soon as the first primary replication forks (initiated at origin sequences) have reached a chromosomal end. We believe that this mechanism is responsible for the rapid accumulation of growing points in T4 infected cells (WERNER 1968). Our model also explains why exchanges in many terminal intervals of parental T4 chromosomes are *not* clustered (i.e , show coefficients of coincidence below 1; MOSIG et al. 1971, see Fig. 6): the use of the chromosomal ends as primers for DNA replication eliminates their high recombination potential in subsequent matings.

The invasion of single-stranded termini into homologous regions of circularly permuted chromosomes generates a network of branched and looped DNA whose complexity increases with increasing multiplicities of infection and with time. Because of the arguments presented above, we believe that many of the loops seen in replicating T4 DNA by DELIUS et al. (1971) were generated by this mechanism.

The interconversion of recombination and replication forks implies that after the first round of replication has occurred *pure* recombinational or replication complexes (or forks) might not exist. The modulation of enzyme activities by interactions in complexes whose composition changes as the substrate is altered, allows a smooth transition during choices between alternative pathways of DNA metabolism.

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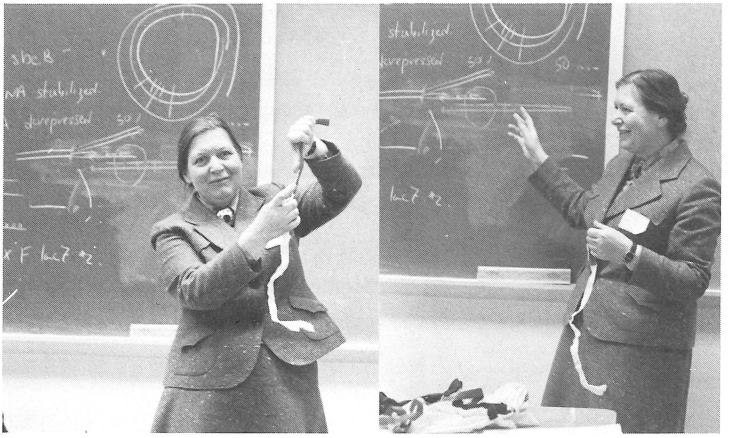
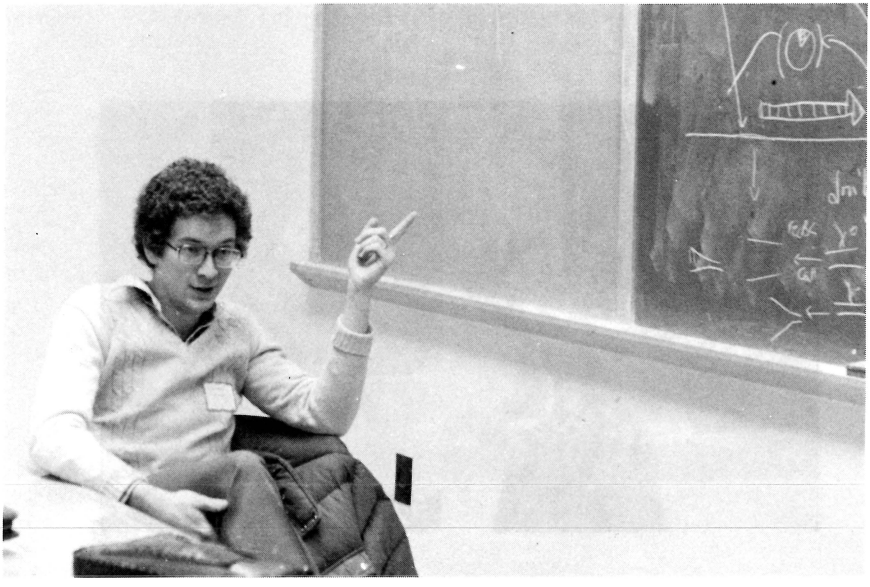


Dr. Mosig explains the mechanism of recombination.

Dr. Judy C. Wall (right) listens to Dr. Mosig.



Dr. Kevin McEntee makes a point.