Studies of hyper-recombination and mutator strains of *Escherichia coli* K12

THESIS

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This thesis has not been submitted for examination at any other University, and consists entirely of original work.

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ABSTRACT

This project concerns hyper-recombination and mutator mutants of Escherichia coli K12 which have been obtained by an examination of colonies on EMB-lactose agar of an F-merodiploid strain, H2 lac⁻/F lac⁻, with different mutations in the lacZ genes, for those which showed an increased number of lac⁺papillae.

Hyper-recombination mutants were a minority of the strains isolated by this method because the wild-type gene could only be formed by two cross-over events in a short segment of the chromosome; strains that were mutator or had duplications of a lacZ gene were frequent.

One of the hyper-recombination mutants, H2114, has been shown to carry a mutation, mapped on the Escherichia coli chromosome near thyA which in this strain and in other genetic backgrounds, increased the yield of P1-mediated transductants. The mutated gene in H2114 has been given the symbol, hypB, because it appeared to be different in phenotype and location from that described by Konrad and Lehman (1975). In crosses with certain Hfr strains the yields of recombinants were higher than those obtained with the parent strains. The yield of recombinants from crosses in which strain H2114 acted as donor were also higher than those obtained with the parental F merodiploids. Recombination within short regions of the chromosome was examined by measuring the yields of ara⁺ recombinants in P1-mediated crosses with various ara⁻ donors. The relative enhancement of the yield was greater, the smaller the interval in which one cross-over event was obligatory. Recombination between mutants of phage λ was also increased. The enhanced recombination is dependent upon the recBC genes because recombination in double mutants hypB recB was that expected of recB strains.

The hypB mutation appears to have some other effects; the mutation frequency is increased and the regulation of cell size and division is disturbed. Hyp$^-$ strains appeared smaller and divided more rapidly at all stages of growth. High yields of LAC$^+$ recombinants were obtained for the hyp$^-$ strain in stationary phase cultures; the yield fell nearly 100 fold during exponential growth.

Another mutant strain, H2110, has been identified as a mutator rather than hyper-recombination phenotype. H2110 gave the highest yield of azauracil resistant mutants and a relatively low yield of lac$^+$ cells compared to H2114. The strain H2110 has been shown to carry more than one mutation; one is the gene mutH, and other(s), still unidentified, prevented expression of the mutator phenotype when the strain carried a polA mutation. When the mutH gene was transduced into different genetic backgrounds, the double mutants, mutH polA were viable and polA did not have any effect on the mutator phenotype.

In the presence of F-primes and ColV, mutation frequency to nalidixic acid resistance was about 10 fold lower than the mutF$^-$ strains. It appears possible that an F gene product partially substitutes for the wild type mutH gene product. Plasmids unrelated to F, ColI and ColE, did not have this effect.

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SECTION I

I. INTRODUCTION

A. Introduction

At the molecular level we can define recombination as the production of new DNA molecules that carry genetic information derived from both parental molecules.

A feature common to all recombination events is that they involve physical rearrangement of the material of the parental DNA molecules, rather than copying of information present in parental DNA.

The observation of physical exchange of corresponding parts of chromosomes suggests that recombination might occur by a breakage and reunion model in which the two parental duplex molecules of DNA are broken at corresponding positions and then joined crosswise.

Recombination by copy-choice theories would imply a conservative mode of replication in which progeny chromosomes are synthesised de novo from the parental species; during the replication of paired chromosomes, with synthesis of the daughter genome on one template suddenly switching to the corresponding position on the other parental chromosome (Hayes, 1968; Lederberg, 1955).

As data has accumulated two important restrictions concerning molecular events in genetic processes - the semi-conservative replication of chromosomes of DNA, and the discovery that large segments of preformed DNA exchange with their neighbours in the various recombination processes have become clear. It has become very likely that copy-choice alone could not account for genetic recombination and that recombination models involving breakage and reunion are now more detailed and biochemically realistic.

The production of recombinants in eucaryotic cells appears to be correlated with the crossing over which occurs at the chiasmata formed between homologous chromosomes. If the probability of chiasma formation

between two loci on a chromosome depends upon their distance apart, genes located close to each other should tend to stay together: and as the distance between two loci increases so should the frequency of formation of recombinants.

The strong correlation between the behaviour of chiasmata and the formation of crossovers and the concept that they are the sites of genetic exchange is further strengthened by the phenomenon of interference. This is the term given to the difference in recombination events in close proximity to a point where one genetic exchange is scored from that expected by chance. Interference is said to be positive if the number of interchanges is less and negative if it is greater than the number expected on the basis of distance apart of the markers scored.

The majority of experimental work has been performed on procaryotic systems and the models that have been developed have been so as to explain various aspects of several experimental details. It seems that it is also possible to extend these models to higher systems, such as eucaryotes but the experimental evidence is not as great.

In the procaryotic systems, certain models have been proposed to explain recombination following the introduction of genetic material by conjugation, transformation and transduction but they also owe much to the work on gene conversion observed in the simple eucaryotic organisms, particularly Sordaria, Ustilago and yeasts.

B. Mechanisms of genetic transfer and recombination

The transfer of genetic information from one bacterium to another is mediated in several ways, including conjugation, transduction, transformation and episomal transfer. In this work conjugation and transduction were used as experimental procedures and these will now be explained in more detail.

1. Conjugation

Experiments by Hayes (1952) and by Cavalli and the Ledebergs (1952) demonstrated that genetic transfer by conjugation was a unidirectional transfer of genetic material from a donor cell (male) to a recipient cell (female). The donor property of F^+ cells of *E. coli* is determined by the presence in the cell of an infectious, virus-like agent called a sex factor or F-factor ("for fertility"). The F-factor is the essence of conjugation and it may be seen that, although host cell cooperation is essential, F is really concerned primarily with bringing about its own transmission rather than with chromosome transfer. The F-factor is a distinct, independent genetic entity, which possesses a high transfer frequency and whose presence is associated with a low probability of chromosomal transfer. F can interact with the chromosome and promote its transfer in two different ways : by a stable integration, producing an Hfr clone, and by an unstable type of attachment that does not give rise to an Hfr clone. The latter type of interaction which is greatly stimulated by UV irradiation (Evenchick, Stacey, Hayes, 1969) predominates in most F^+ cultures.

The attachment of the F to the bacterial chromosome does not represent a permanent breakage of the chromosome, but rather this event is visualized as a recombinational coalescence of two circular genomes into a single circular structure. Campbell proposed a model, in which the circular F chromosome pairs

with an homologous region on the circular bacterial chromosome. Since there are a number of different Hfr types, it can be assumed that F has sites similar to a number of homologous regions with the bacterial chromosome. Despite its homology F only rarely integrates into the chromosome. Yet F-merogenotes, which have acquired long contiguous stretches of host material, integrate into the chromosome much more frequently. The explanation of these regions of homology emerged from the observations of Starlinger and Saedler (see Saedler and Heiss, 1973) that some mutations in E. coli were due to the insertion of two classes of DNA, IS1 (about 800 nucleotide pairs) and IS2 (about 1400 nucleotide pairs), into the continuity of the chromosome, IS1 and IS2 are distinct DNA sequences and it has been found that about eight copies of IS1 and about five copies of IS2 are scattered throughout the chromosome of E. coli K12. Although no small plasmids exclusively containing the IS1 and IS2 sequences have been found in E. coli K12, their presence on F ensures their transmission. Similarly it can be said that recombination between unrelated plasmids could be mediated by these insertion sequences. After the pairing step, probably involving these insertion sequences, a reciprocal cross-over between F and the bacterial chromosome, by breakage and reunion, results in the insertion of F into the continuity of the host genome.

Transfer of the Hfr and the chromosome is mechanistically the same as the transfer of F factor, except that donor chromosomal material is transferred with the F factor which is integrated into the donor chromosome, and the chromosome maintains its closed circular form. Jacob and Adelberg (1959) described the isolation of conjugal fertility factors from Hfr donors which replicate autonomously, are transferred to F^- recipients at high frequency and contain a segment of chromosome that was adjacent to the Hfr-site. These elements are referred to as F-prime (F') factors (Scaife

and Gross, 1963) and have been of enormous value in establishing the dominant or recessive nature of different alleles of the same gene and have proved especially valuable in studying the regulatory interactions of genes because they permit the establishment of partially diploid cells. F'-factors probably arise by reciprocal cross-over between chromosomal sites on either side of an integrated sex factor or a reciprocal cross-over between an adjacent chromosomal site and a site in the integrated F, and represent presumably some form of illegitimate recombination. During conjugation (in Hfr transfer), one assumes that the "nicking" of the chromosome occurs within the F factor genome and DNA transfer proceeds. One end becomes the origin which begins to enter F^- cell in a linear fashion. It seems likely that the Ori end contains a small piece of F genetic material, but that the bulk of the F DNA remains at the terminus of the chromosome. Recombinants from $HfrXF^-$ crosses mostly remain F^-, and only if the recombinant cell receives the terminal part of the chromosome and the termination of the F is it converted into an Hfr cell. Therefore the origin of transfer seems to be a specific locus on the sex factor chromosome.

Conjugation requires some form of cell-to-cell contact and can be divided into several stages. These are mainly a) Specific pair formation, which is defined as the formation of donor : recipient cell union. The F'-pilus made up of the protein, pilin, is essential for this step. Specific pairs do not necessarily conjugate. b) Effective pair formation, which is defined as the establishment of a cellular connection between the donor and the recipient cells. c) Sex-factor mobilization and transfer. This is a specialized form of DNA replication which fits the rolling circle model of Gilbert and Dressler (1968). In this model it is suggested that one strand of a circular duplex remains closed during replication and serves as a template. The other strand is cut by an endonuclease at a specific site and is elongated by the covalent addition of new bases to the free 3'-hydroxyl end of the strand. As the bases

are added according to the dictates of the closed template, the 5'-phosphate free end of the strand is peeled out as a single stranded 'tail' which is thought to attach to a cellular site, probably the cell membrane. Thus the 'old' strand is peeled off, as a new strand is synthesised. This replication mode can be continuous and is called a rolling circle. d) The entry of single strand of sex factor DNA is accomplished by the synthesis of a complementary strand within the recipient cell. Once synthesis and replication have been completed, the recipient, in turn, becomes a genetic vehicle for sex factor transmission.

2. Generalized transduction

Generalized transduction can be defined as the transfer of genetic information from one bacterium to another by means of a phage vector. The transduction performed by phage P22 in S. typhimurium or phage P1 in E. coli are typical examples of the generalized type, in which almost any genetic marker of a donor strain can be transduced. Generalized transducing phage are rare for the Enterobacteriacea but common for Bacillus subtilis. The amount of the bacterial chromosome that can be carried by a single transducing fragment appears limited by the amount of DNA which can fit inside the protein head of the virus. A simple way of visualizing generalized transduction is that, during the autonomous replication of the virus, fragments of the host genetic material are packaged by "accident" into phage heads and become progeny particles. The frequency of this accident for a given gene is about 10^{-5} to 10^{-6} per plaque-forming unit; thus in a usual phage P22 lysate, about 0.1% of all phage particles will contain bacterial rather than viral DNA. On release from a lysed host cell these particles can be adsorbed to a sensitive host and the injected nucleic acid is now available for recombination. It is important to see that the transducing agent itself is not identical with the phage. A transducing fragment of this kind cannot lyse or lysogenize. The virus particle acts instead simply as a vector. It has been demonstrated that P1 transduces markers located at different positions on the chromosome with different frequencies (Masters, 1977). It thus seems that genes very close

to the origin of replication of the chromosome are more amenable to transduction than those located elsewhere on the chromosome.

Masters also demonstrated that introduction of an extra piece of DNA, in this case the F factor, into the chromosome, affected transduction frequencies on one side of the integrated F factor only. It does not seem to influence the transduction of those markers which are far enough away. One can speculate that transduced DNA containing the origin can replicate in the recipient cell and is thus afforded a greater chance of subsequent integration simply as a result of being present in greater concentration. Bachmann et al (1976) suggest that the gene frequency distribution may reflect three dimensional structure of the E. coli chromosome. Worcel and Burgi (1972) suggested if the chromosome is composed of non-freely rotating loops of DNA, some parts of the chromosome may be more accessible to transcription/ translation than others.

Generalised transduction provides a useful technique for fine structure mapping; two host markers can be transduced together only if they are close on the genetic map. For such markers, the frequency of joint transduction provides a measure of the map distance between them (Wu, 1970).

Not all transduced particles lead to permanent genetic alteration of the cell into which they are injected. Many abortive transductions can be observed if the gene products are sufficiently active to lead to micro-colonies. This suggests that in hyper-recombination mutants transduction might be much more efficient than in the wild-type because there might be fewer abortives and more stable transductants.

C. Breakage and reunion and pairing

Direct evidence for the breakage and reunion as the molecular basis of recombination was obtained by Meselson and Weigle (1961) in the case of recombinants between distinguishable types of bacteriophage λ . Two genetically marked parents, both labelled in the DNA with heavy density isotopes, were crossed in unlabelled cells and the progeny phage analysed by density-gradient equilibrium sedimentation in concentrated CsCl solution. Some recombinants were found which were virtually completely heavy, that is, they had arisen by the breakage and reassociation of parts of parental genomes. Therefore recombination can occur without extensive DNA synthesis. In fact, a small amount of DNA replication would have escaped detection. Although later studies by Stahl (1972) showed clearly that many of the recombination events in λ were accompanied by measurable DNA synthesis, the essential point, that recombination can occur by the breakage and reunion of unreplicated genomes, was secured. Further evidence that recombinant formation can proceed by a breakage-reunion mechanism by way of heteroduplex formation came from a study by Tomizawa and Anraku (1965) of the structure of recombinant DNA produced from crosses with phage T4. Phage with heavy density labelled DNA were crossed with phage containing radioactive DNA and at intervals after infection the intracellular DNA was extracted and analysed on CsCl density gradients. T4 DNA molecules could be observed which contained within the same structure both radioactive and density labels, i.e. were composed of segments from both parental molecules.

At early times this association of the parental segments was unstable, being destroyed by conditions which disrupt hydrogen bonds. At later times this association was stable to denaturation. Thus recombination appears to result from formation of hybrid molecules bearing a heteroduplex overlap segment in which initially the ends of the overlap are not joined (joint molecules) and which, at later times become covalently linked to form

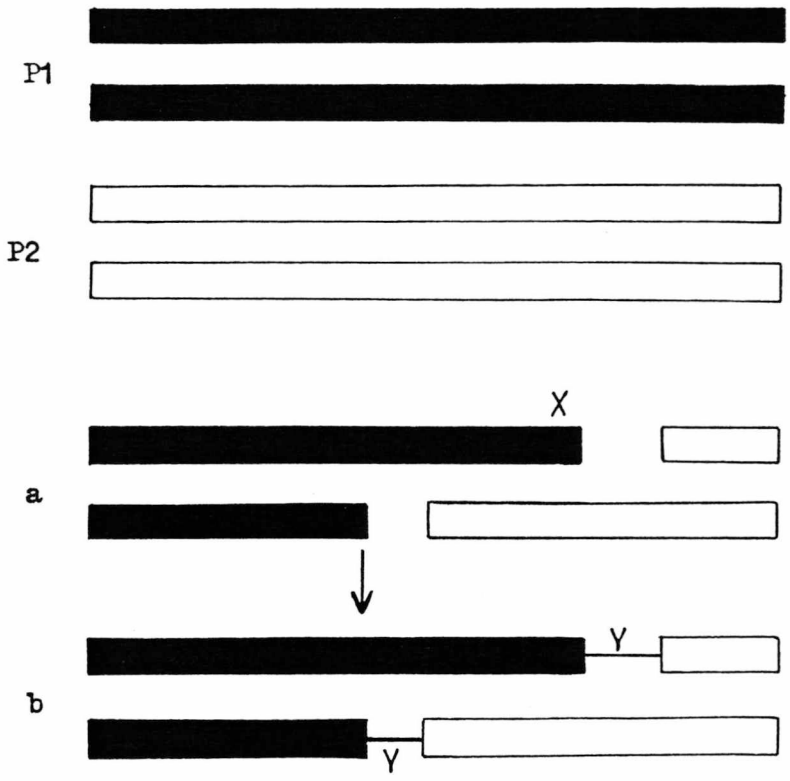


FIGURE 1

Structures of intermediates in recombinant formation. Parental genomes P1 and P2 give rise to "joint molecules" (a), held together by hydrogen-bonding in the hybrid region X. The action of DNA polymerase and DNA ligase at sites Y will convert joint molecules to recombinant or heteroduplex molecules. Duplication of b will cause segregation of the heterozygote.

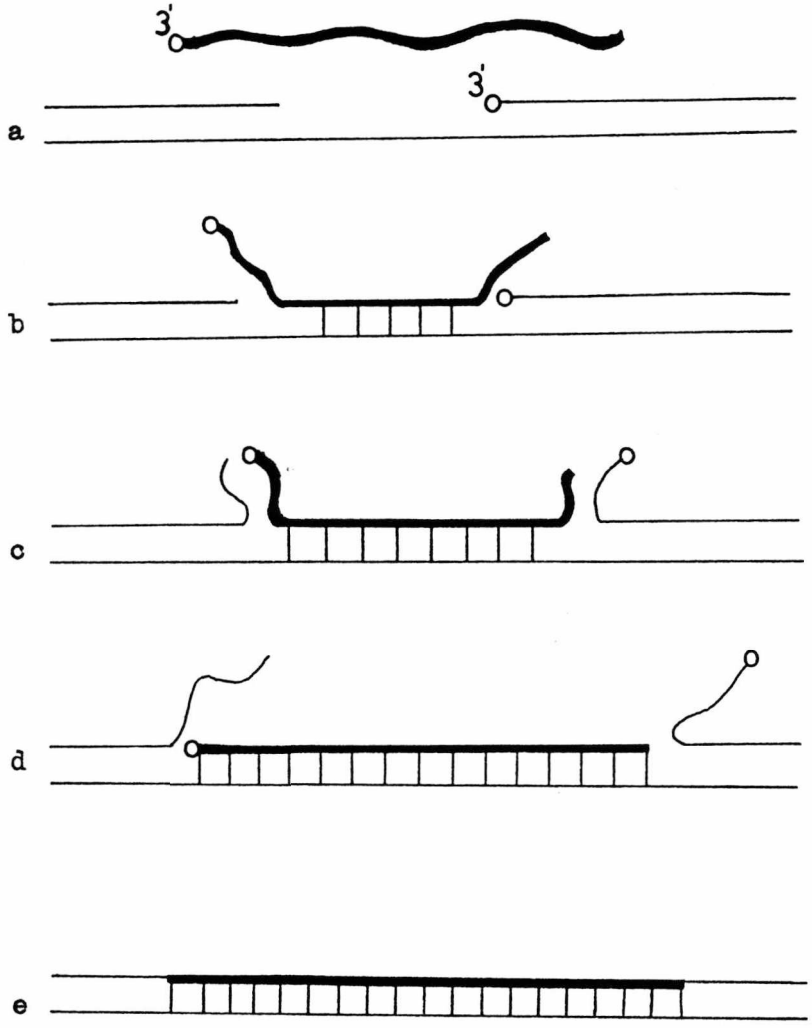


FIGURE 2 A recombination mechanism involving insertion of a single strand into a gap in duplex DNA.

recombinant molecules (Figure 1).

Similar events have been observed in E. coli zygotes after conjugation which the donor genetic material integrates into the resident genome by a process of breakage-reunion (Oppenheim and Riley, 1966, 1967). Fox and Allen (1964) suggest a model for pneumococcal transformation in which donor DNA enters the cell and is converted to single strands which are then integrated into the recipient genome (Figure 2).

Similar models have been suggested for transformation of B. subtilis (Davidoff-Abelson and Dubnau 1971) and H. influenzae (Notano and Goodgal 1960). Davidoff-Abelson and Dubnau (1973) reported that the single-stranded fragments contribute to the formation of hetero-duplex DNA in which one strand of the recipient DNA was replaced by a single-stranded segment of donor DNA. This mechanism has now been supported by the isolation of mutants blocked in various stages of recombination formation (Buitenwerf and Venema, 1977).

Current evidence points to a recombination mechanism in which DNA segments are recombined via a heteroduplex intermediate structure. Heteroduplex formation probably results from the nucleolytically induced breakage of parental single strands followed by their reassociation with heterologous complementary strands. Some DNA synthesis (repair synthesis) is therefore necessary to fill any resulting gaps and discontinuities (Figure 1).

In general, recombination in the various systems studied so far has exhibited some common features: the symmetry of exchange which is either reciprocal or non-reciprocal, its dependence on DNA homology, and whether recombination occurs at a particular site on the genome or at any position.

Pairing brings homologous regions of the two chromosomes into intimate contact for information exchange. However, it is not known if a minimum amount of homology is necessary to ensure effective pairing. In general recombination base homology is necessary and is stimulated by UV, whereas in site-specific recombination it is thought that particular nucleotide sequences are necessary for recombination and it is not stimulated by UV.

Recognition of attachment sites on bacterial DNA by a phage DNA which is to undertake integration appears to be mediated by a protein which recognises the appropriate sequences of DNA on each molecule. This leaves only recombination between bacterial DNAs themselves or between phage DNAs during replication to depend on pairing of homologous DNA duplex sequences as such; and we do not know how this may be achieved.

Pritchard (1955) first observed in A. nidulans that within very small intervals multiple exchanges occur with much greater than random frequency. He called this phenomenon localized negative interference and interpreted it in terms of frequent recombination within limited effective pairing regions (i.e. switch areas) which are randomly distributed among a population. Two forms of negative interference are seen in phage crosses. Chase and Doermann (1958) found that coefficients of coincidence increase considerably for multiple exchanges between closely linked r_{II} markers in T4. This is known as highly localized negative interference. Between distant markers, negative interference reflects the probability that more than one exchange event will occur, this is sometimes known as generalized negative interference. Pairing mechanisms that give rise to such interference as mentioned above are not clearly understood, however.

Sigal and Albert (1972) have pointed out that geometric restraints prohibit pairing between all four strands if intact. Interactions between DNA molecules may be based upon specific sequences of bases as well as homologous sequences of bases. The recognition of specific sequences as opposed to the recognition of homology, could conceivably play a role in generalised recombination by providing the basis for a preliminary and approximate alignment of homologous sequences (Radding, 1973). Further evidence for this is provided by Stahl who has shown that Chi mutants of λ have high rates of Rec-mediated crossing-over at particular sites.

Sobel (1973) has suggested that symetrical sequences of nucleotides could result in tertiary configurations of DNA which might be specific targets for endonucleolytic opening while still keeping the two arms of the DNA linked by hydrogen bonds. Such a sequence might be converted from its extended form to clover leaf by a recombination protein. By nicking each clover leaf, perhaps in a extended loop of single strand DNA, homologous clover leafs can come together by base pairing.

D. Recombination

Mainly three subclasses of recombination have been recognized.

a) General recombination in which exchange between homologous segments takes place anywhere along the length of the DNA molecule.

b) Site-specific recombination, in which exchange occurs at a specific site, as in the integration of phage λDNA into the E. coli chromosome, and the excision of the λDNA from it.

c) Illegitimate or nonhomologous recombination, in which foreign DNA is inserted at random, for example phage λDNA into E. coli DNA.

In view of the multiple kinds of recombination and the variety of genetic and physiological backgrounds in which recombination takes place, we must expect considerable variation in the biochemical mechanisms employed.

There may also be multiple pathways in a given cell; there are at least two in E. coli. Available evidence favours a breakage-reunion model of interacting DNA strands, yet among the operations of restoring intact chromosomes after breakage and reunion, there may be considerable gap-filling by replication on new templates. It is likely therefore that some of the enzymes thought to be involved in replication and repair serve in recombination. In addition, genetic studies implicate distinctive enzymes essential to recombination but dispensible for replication; all genes so far known to affect recombination also influence repair.

Integrase and gyrase are known to be all that are required for site-specific recombination of phage λ. Little is known about nonhomologous recombination. Regarding general recombination, several examples can be presented in which plausible suggestions have been made for a sequence of molecular events and will be discussed in page 17 - 20.

1. Site-specific recombination

Site specific recombination is the process responsible for the integration and excision of lambda. Circular λ molecules are integrated by union of a specific region of the phage DNA, called "att" at a precise location between the gal and bio genes on the E. coli chromosome. This site-specific recombination between phage and host DNA takes place in a unique way and requires λ gene products defined by the int locus. The int protein, called integrase, is distinct from the proteins specified by the λ red genes which are responsible for general recombination.

Excision of the phage DNA from the host chromosome is not simply a reversal of integration. It depends on a separate gene, xis, in addition to int. However, little more can be said about molecular details. One remarkable aspect of excision is the possibility of illegitimate excision which leads to the inclusion of part of the host chromosome along with viral DNA.

The distinction between integrative and excisive recombination and the varying frequencies with which site specific recombination occurs between phage carrying attachment sites derived from different sources, argue that the phage and bacterial attachment sites, and also, therefore, the prophage attachment sites, all represent different sequences. There are two possible models which distinguish the consequences of these types of integrative recombination. If there is no homology between the phage and bacterial sites, recombination must involve scission of the duplex between both attachment sites. This must be followed by crosswise union of the duplex ends. This model therefore requires site-specific recombination to proceed via an enzyme mechanism able to recognise duplex sequences of DNA, cut across the duplex and rejoin the opposing ends.

An alternative model is to suppose that each attachment site contains

a "core", a short region that is the same in all sites. Recombination takes place within this sequence. Introduction of staggered single strand breaks at each end of the core, followed by separation ~~to~~ yields single strand ends which can anneal crosswise to form hybrid DNA. This is comparable to the conventional model for recombination through the formation of hybrid DNA with the variation that the breaks generating the complementary single strands are made at specific sites.

2. Illegitimate recombination

The most clear cut distinction between general and illegitimate recombination is the lack of dependence of the latter upon the bacterial recombination genes and nucleotide sequence homology. In the main, illegitimate recombination has the effect of creating new DNA sequences around a point of fusion between different segments of a single genome or between nonmatching segments of different genomes. Thus a deletion or duplication can be viewed as an illegitimate recombination (or as a result of mistaken replication) in that the loss of a DNA segment is necessarily accompanied by fusion of DNA ends to form new DNA sequences.

Similarly, aberrant excision of F leading to F primes results in the fusion of segments of two nonmatching segments from different genomes. Illegitimate recombinations seems to occur at a low frequency and depend upon DNA scissions, exchanges and DNA joining, which are relatively nonspecific compared with the precise pairing between complementary base sequences seen in general recombination. It has recently been realised that the base sequence may play an important part in illegitimate recombination as shown by the cysC region of S. typhimurium which is more prone to deletion than other regions i.e. some regions are "hot spots" for illegitimate recombination (Weisberg, 1977).

3. Strand exchange

Transfer of a portion of a strand (single or double) from one DNA molecule to another involves breaking a set of hydrogen bonds and making a new set that is identical or nearly so.

Insertion of a single strand into a gap

This nonreciprocal form of recombination occurs during assimilation of DNA after it has been taken up from the medium (transformation) or transferred from a mating cell (conjugation) (Figure 2a). A model for the insertion process is outlined as follows. The entering single strand anneals to a homologous strand at a gap created (Figure 2b) in the recipient duplex by endonucleolytic incision followed by exonucleolytic excision. The hybrid region is then enlarged by displacement of the recipient strand (branch migration) in either direction (Figure 2c). Exonucleolytic removal (3'$\longrightarrow$5' or 5'$\longrightarrow$3') of the recipient strand may precede or follow assimilation of the donor strand (Figure 2d), and may also include part of the donor strand. Properly aligned termini, prepared by replicative gap filling, if necessary, are finally joined by ligase (Figure 2e).

This general scheme may also apply to recombination between the single stranded terminus at the end of a donor duplex and an internal gap of a recipient one. A single strand may anneal at a transiently melted region of a intact recipient duplex and successfully replace a stretch of the recipient strand, the latter being excised by repair endonucleases.

Proteins that bind tightly to single but not double stranded DNA have been described in bacteria and various systems and called "DNA melting", "DNA unwinding" or "DNA binding" proteins. The first protein to be isolated was the product of the gene 32 of phage T4 which converts a duplex DNA to single strands and is known to be essential for recombination as well as replication of T4 DNA (Alberts and Frey, 1970). The protein coded by gene 5

of M13 phage is essential for production of viral single strands from the duplex replicative form.

An untwisting protein protein called ω (swivelase) was purified from E. coli (Wang, 1971) on the basis of its ability to remove superhelical turns from highly twisted circular λ DNA. It apparently nicks one strand of supercoiled DNA, thus relieving the torsional stress by allowing some unwinding, and then reseals the nick.

Gellert et al (1976) described an enzyme activity, called DNA gyrase which is responsible for converting relaxed closed circular DNA into a negatively supercoiled DNA (in an ATP dependent reaction in E. coli). The reaction was stimulated by spermidine. The enzyme was also found to be active on relaxed-circular colicin E1, phage λ , and Simian virus 40 DNA. It was shown that DNA gyrase activity was inhibited by coumermycin and novobiocin (Gellert, O'Deha, Itoh, Tomizawa, 1976) and its activity in the presence of both drugs to be preserved when purified from a coumermycin-resistant mutant. Inhibition by novobiocin of ColE1 DNA replication in a cell free system was partially relieved by adding DNA gyrase from a resistant strain. DNA gyrase appears to play an essential function for the replication of double stranded circular DNA. But it is not clear whether a supercoiled DNA template is required for initiation of DNA synthesis, for chain elongation or for both.

Nalidixic acid and oxolinic acid inhibit DNA gyrase activity and induce formation of a relaxation complex analogue. Treatment of the complex with sodium dodecyl sulphate causes a double strand break in the DNA substrate. Complex formation, does not require ATP or relaxed circular DNA and is insensitive to novobiocin. DNA gyrase from a nalidixic acid resistant strain is 10^{-2} as sensitive to oxolinic and nalidixic acids than gyrase from sensitive strains with respect to inhibition of supertwisting and induction

of the pre-linearization complex. Addition of the nalA gene product; Pnal which has recently been purified from E. coli (Sugino et al, 1977) restores drug sensitivity and stimulates DNA gyrase activity. Gellert et al (1977) suggested that DNA gyrase does have a nicking-closing activity that is capable of relaxing supercoiled DNA, including positively supercoiled DNA. This activity might be due to the oxolinic acid sensitive component of the enzyme, because relaxation is inhibited by oxolinic acid. Nalidixic acid but not novobiocin, does have an effect on the recovery of recombinants in conjugal crosses even in nalidixic acid recipients (P. Oliver, private communication).

4. Mechanism of reciprocal recombination

Geometric constraints on pairing between two different duplexes require that a cut be made in at least one of the four strands. Schemes in which two cuts are made, one per duplex, and involving crossed strand exchange (Holliday, 1964; Whitehouse, 1963; Hotchkiss, 1971) (Figure 3,4,5) can account for recombinational events and also account for gene conversion. The cutting and pairing creates recombinant duplexes which have one chain from each parent duplex for the region around the crossover event. The duplex derived from two parents has been termed hybrid DNA. Two models have been proposed for the formation of hybrid DNA (Holliday, 1964; Whitehouse, 1963) which differ in the pattern of initial breakages which they stipulate. Holliday suggested that each duplex is probably broken in a strand of the same polarity. After breakage, the broken strands separate from their partners in the same direction along each chromosome; Figure 3 shows that crosswise annealing forms the hybrid DNA. If recombinants are to result, a second break must be introduced, this time in the hitherto unbroken strands. If breakage takes place again in the strands which have been broken previously, the original chromosomes can separate with each molecule possessing a region of hybrid DNA. Within the hybrid region the repair of mismatched bases may

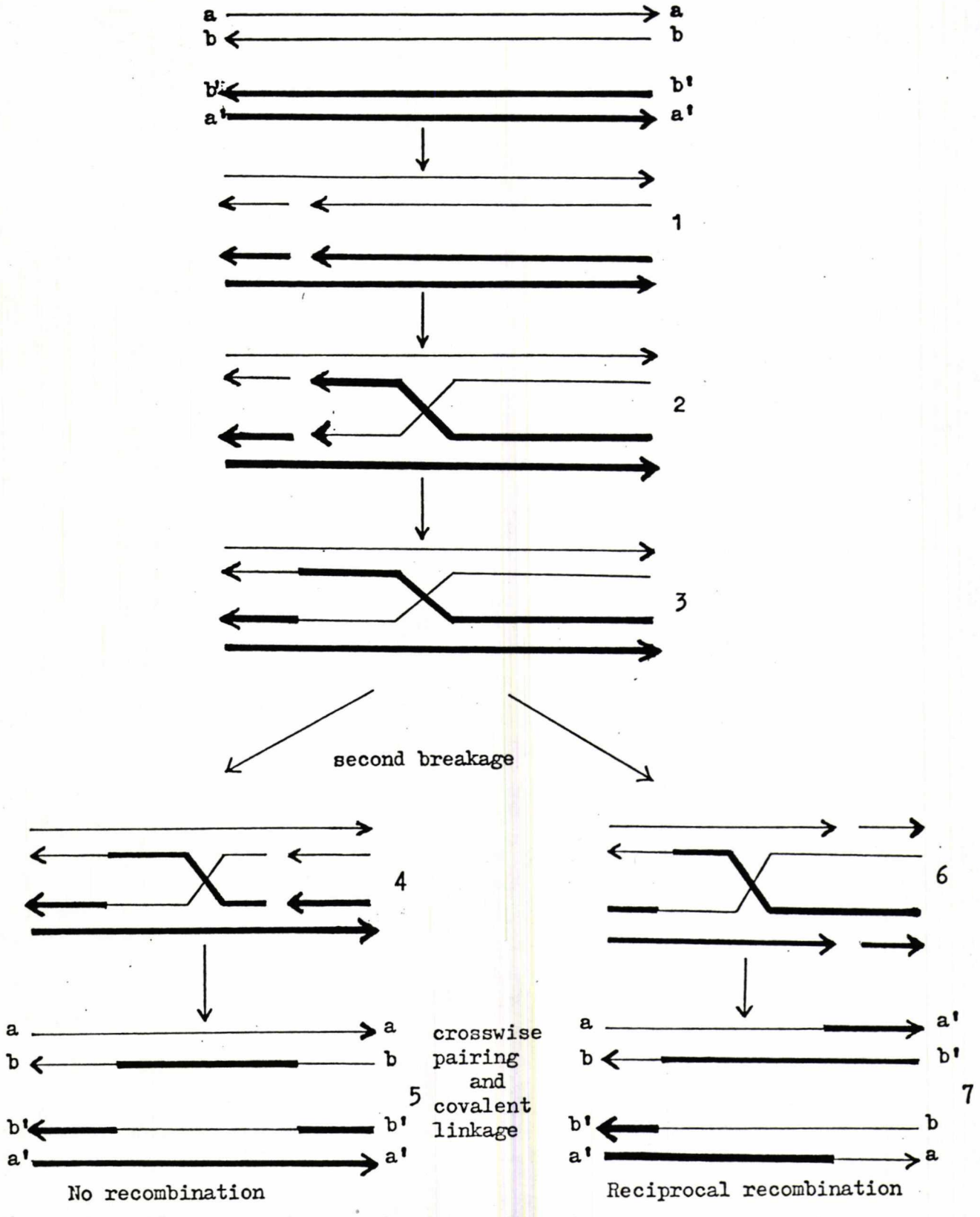

a
b
b'
a'
a
b
b'
a'
1
2
3
second breakage
4
5
6
7
crosswise pairing and covalent linkage
No recombination
Reciprocal recombination

FIGURE 3

FIGURE 3 Model for reciprocal recombination between duplex DNA molecules by formation of hybrid DNA.(Adapted from Holliday,1964).

1) First breakage in corresponding strands.

2) Broken strands pair with complement in other duplex.

3) Covalent links formed between strands.

4,5) Second breakage occurs in the same two strands implicated in the first exchange,as illustrated on the left,there is no recombination.

6,7) Second breakage takes place in the other two strands, as illustrated on the right,reciprocal recombinants are produced.

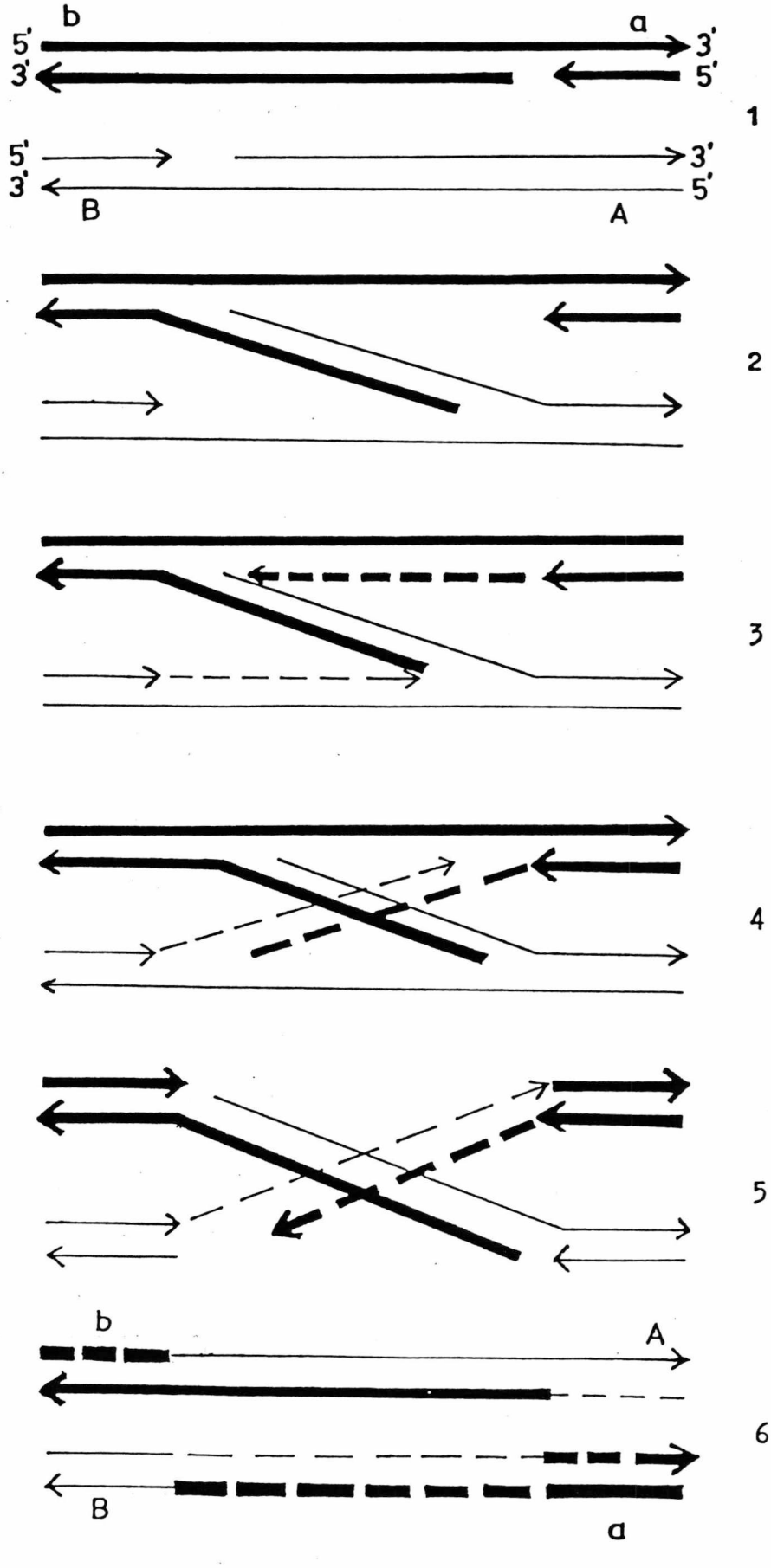


FIGURE 4

FIGURE 4 Hypothetical mechanism of recombination between DNA molecules. (Adapted from Whitehouse,1963).

1) One parental molecule carries the markers AB,the other ab. Each parental molecule has a single strand cut at a different position,between markers A and B.
2) The parental duplexes have partially unwound,and a strand from one molecule has paired with a strand from the other molecule.
3) Resynthesis occurs along the single-stranded region of each parental molecule(dashed line).
4) The newly synthesised strands pair each other.
5) An endonuclease makes a single-strand cut in each parental duplex,and the cut 3' ends are sequentially digested by an exonuclease.
6) Resynthesis and rejoining produce two recombinant molecules,Ab and aB.

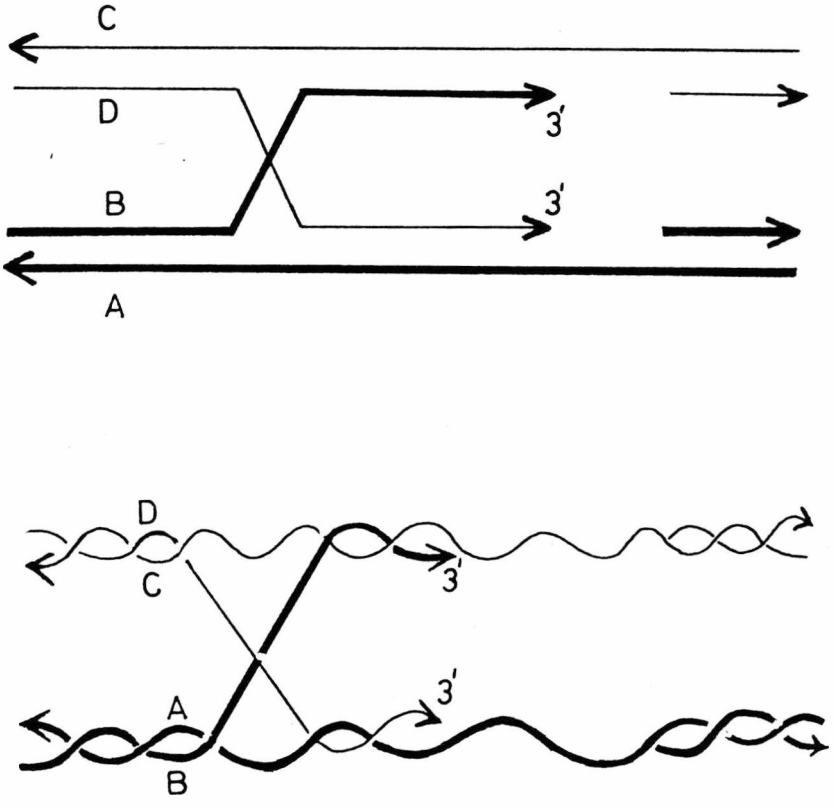


FIGURE 5 Crossed strand exchanges between helical duplexes required in recombinational events. The letters serve as symbols to identify the strands.

occur (see gene conversion) and this gene conversion may be important in recombination between closely linked markers in λ (Wildenberg and Meselson, 1975).

Sigal and Alberts (1972) constructed a molecular model of the Holliday crossed-strand exchange between two homologous DNA molecules, a likely intermediate in genetic recombination, and showed that it can be formed with all of the bases in the two double-helices remaining paired. There are two "outside" and two connecting or "bridging" strands in this structure. Since the positions of these two pairs of strands can be interchanged in the model by rotation, all four strands participating in the exchange must be regarded as equivalent. Meselson (1972) has shown that rotary diffusion arising from rotation of both duplices in the same sense about their helical axes, will drive the half chiasma far enough and quickly enough to generate quite long segments of hybrid DNA in a relatively short time. Cross and Lieb (1967) suggested, for the special case of insertion or excision of λ phage, into or out of the E. coli chromosome, that homologous regions come into close proximity and that there is an exchange of hydrogen bonding between complementary bases over a fairly short region. The result would be the formation of two complementary half chiasmata between which there has been exchange of partner strands. Since gyrase is involved in this process supertwisting may provide part of the energy needed to rotate the molecules.

Fogel and Hurst (1967) suggested that, following pairing of homologous chromatids, there may be a breakage of one or both noncomplementary strands at a site of heterozygosity. It is assumed that the base differences between the interacting chromatids create stress. Each broken strand now anneals with the complementary strand of the homologue giving rise to a half chiasma and to mispaired bases in each chromatid. Resolution proceeds by either of the paths suggested by Holliday after "terminalization" at the end of the gene locus, which is assumed to be a discontinuity.

Recombination Models

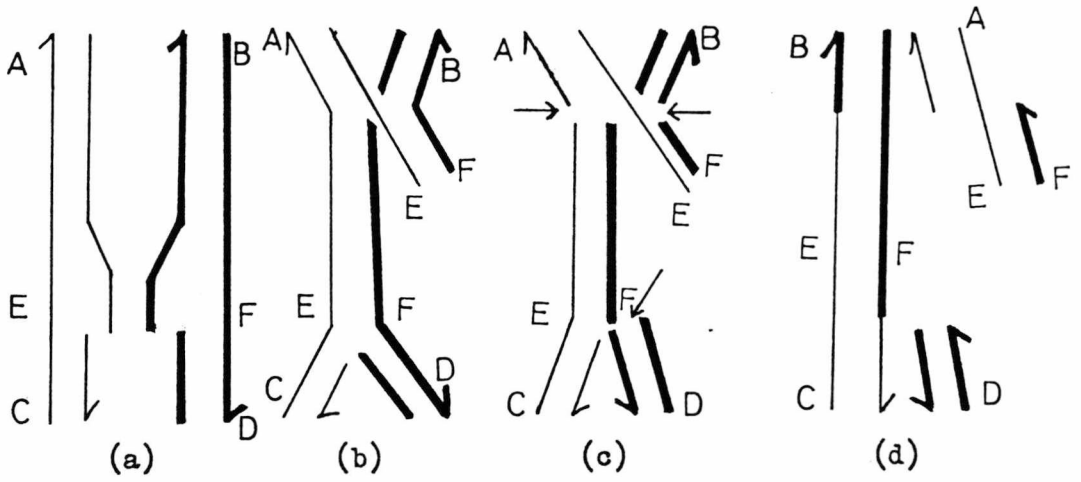


FIGURE 6 (Adapted from Broker and Lehman, 1971).

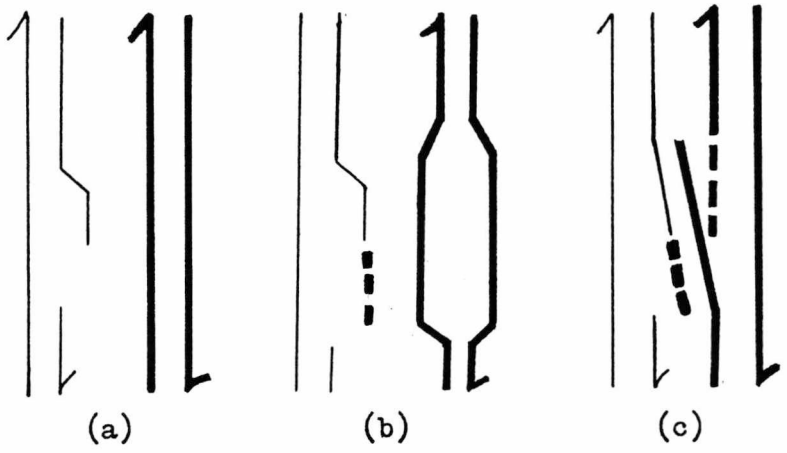


FIGURE 7 (Adapted from Paszewski, 1970).

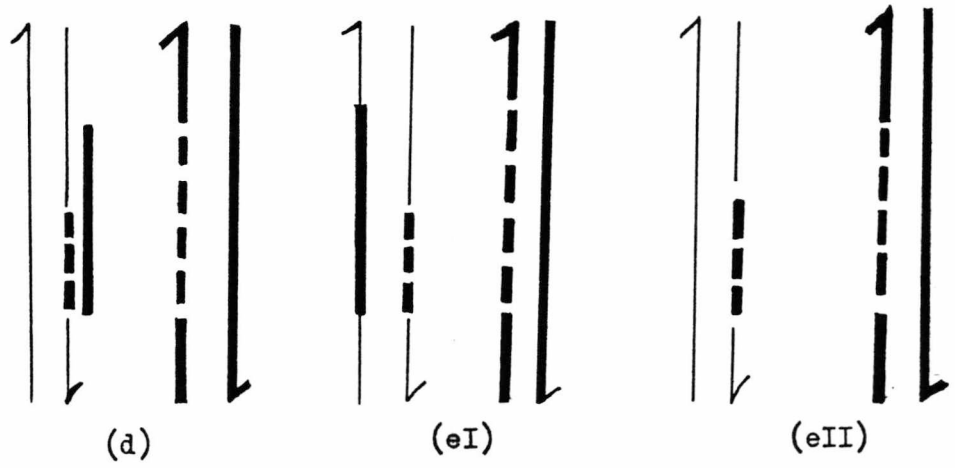


Figure 7 continued

Recombination Models

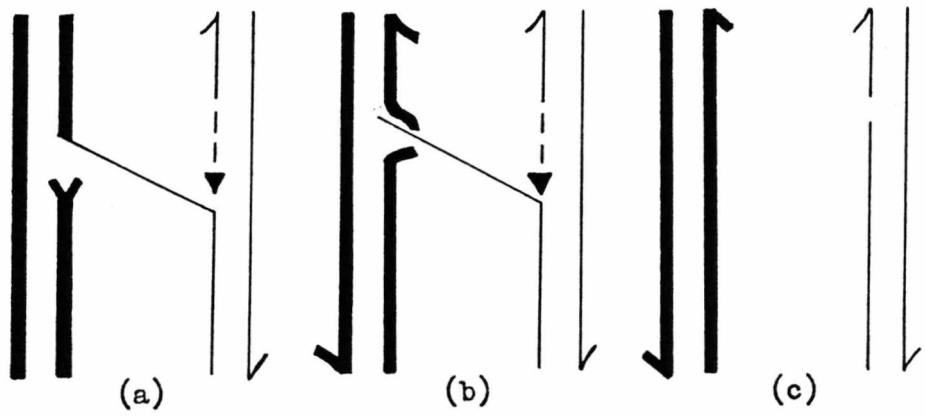


FIGURE 8 (Adapted from Hotchkiss, 1971).

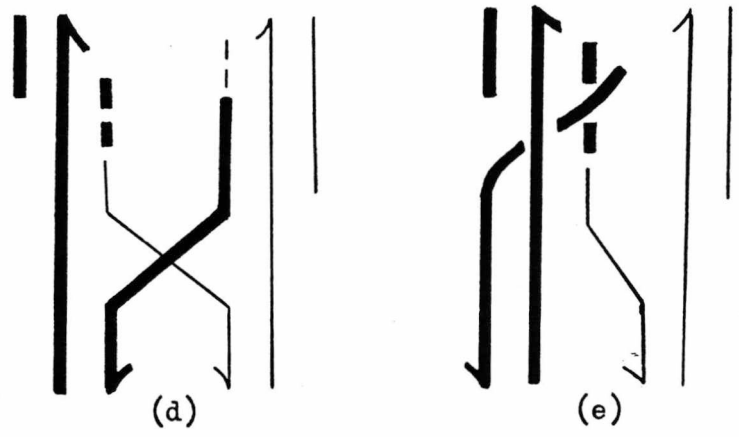


Figure 8 continued

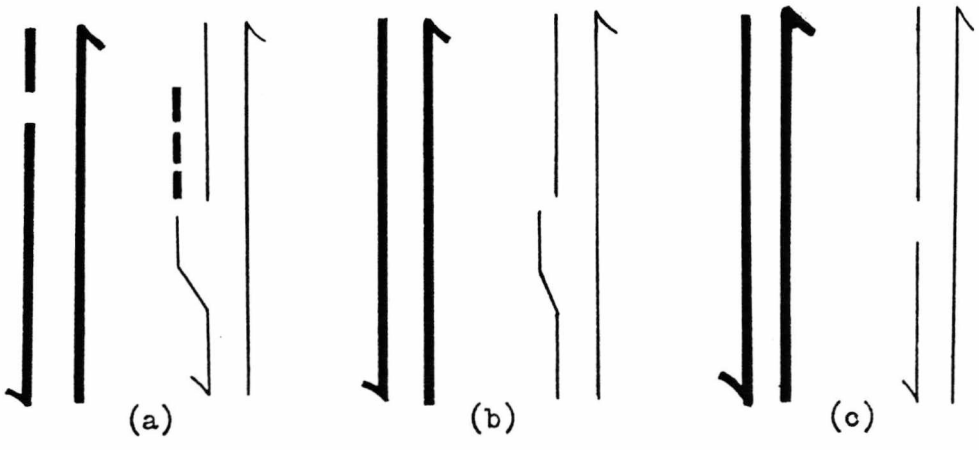


FIGURE 9 (Adapted from Meselson and Radding, 1975).

Whitehouse (1963) assumed that initial breakages occur in strands of opposite polarity. The model allows for DNA synthesis. Figure 4 illustrates that hypothetical mechanism of Whitehouse's recombination model.

On a Holliday mechanism, a half chiasma could be formed between the alphas (or betas) of the two homologues. On a Whitehouse mechanism exchange would occur between an α of one homologue and a β of the other. Broker and Lehman (1971) supposed that complementary broken strands on the same side of nicks pair, move outwards and expose gaps (Figure 6a). As the joint moves outward, the intact pair of strands move together and pair (Figure 6b). It is then possible to move branches along each other without loss of base pairing. The movement is continued until additional nicks or gaps are reached, at least three (Figure 6c) being needed to resolve the moving branch system (Figure 6d). Figure 6d illustrates the result of combination of nicks (in Figure 6c) which leads to a crossover spanning by hybrid region. Nicks in the other two strands at the upper end of Figure 6c would result in a non-crossover arrangement spanning the hybrid section.

Paszewski (1970) proposed that the invading strand (Figure 7a) was partly degraded, then paired with the complementary strand of the homologue and was extended by synthesis (Figure 7b). In so doing it captured a segment of the complementary strand to form a Whitehouse joint bridge (Figure 7c). The original invading strand then turned back to rejoin the original chromosome (Figure 7d), the captured segment of the other chromosome being discarded (Figure 7eII) or incorporated (Figure 7eI). Its place in the homologue was in the meantime reconstituted by synthesis. The proposals made by Paszewski cover only gene conversion without crossing over. The hypothesis could no doubt be extended to cover recombination of flanking regions. According to Holliday and Whitehouse (1970) this hypothesis does not account for inequalities in direction of conversion at a site, nor for map expansion.

Hotchkiss (1974) proposed that single strand displacement occurred by

3'-ward extension by polymerase (Figure 8a,b) which is likely to distort the recipient helix sufficiently to cause a further single strand break (Figure 8c). This newly liberated single strand would in turn "attack" the original helix (Figure 8d), producing a Holliday half chiasma (Figure 8e). This would be resolved by further breakage and reunion. These two hypotheses are similar, but differ in the polarity relationships of the strand with the primary and secondary nicks.

Meselson and Radding (1975) have proposed a single hypothesis which is an adaptation of the Holliday hypothesis. A single break in one strand of one homologue (Figure 9a) becomes the site of displacement by a DNA polymerase. The strand on one side of the break grows, displacing the strand on the other side. The displaced strand pairs with the complementary sequence in the homologue (Figure 9b) and induces a single strand break in the displaced (distorted) strand.

Two fragment strands are then ligated, leaving two other fragments one of which continues to grow by polymerase activity while the other is eroded by exonuclease action (Figure 9c). These two enzymic activities are conceived as different catalytic properties of the same enzyme complex which could be polI. These concerted actions result in further strand transfer. It produces a tract of heteroduplex DNA on only one of the two interacting molecules and so is designated as asymetrical strand transfer.

5. Gene conversion

In certain fungi, when recombination between two sites within a functional gene is studied reciprocal crossing over is rarely observed. Evidence in other systems is lacking: This is most clearly shown in Neurospora by Mitchell (1955). Two parental allelic genes, crossed in equal proportion are recovered in some tetrads in 3:1 rather than 2:2 ratios. These phenomenon of 3:1 (or 1:3) segregation where 2:2 is expected is known as gene conversion.

In principle, unequal recoveries of alleles could result from asymmetry in the mechanism of exchange or from revision of a heteroduplex joint molecule. This revision is usually conceived as the excision of part of one strand, stimulated by the presence of mismatched base pairs, and repair of the excised region by new DNA synthesis a theory called mismatch repair. The efficiency of mismatch repair varies for different mismatches. The position of a mutation in the region of conversion also influences the frequency of conversion.

Conversion within a locus is polarized decreasing in frequency from one end to the other, occuring with frequencies that differ widely from one locus to another. Models for hybrid DNA formation can explain polarized conversion frequencies by requiring that recombination is initiated only from fixed points rather than from random sites on the chromosome. Whitehouse (1966) suggested that the end of a gene might be a suitable place for this interaction to start; this would conform with the observation that the probability of conversion falls from one end of the gene to the other.

A model, already mentioned above in which palindromic sequences of DNA provide recognition sites for initiating recombination has been proposed by Sobell (1972). Recombination with gene conversion was discovered by crossing two different mutations located in the same gene in suitably marked strains and selecting the progeny for wild type; however, outside markers show all four possible arrangements instead of the one which would result from reciprocal recombination. By crossing mutants in two adjacent genes of Neurospora (Murray, 1970) found among wild type recombinants all four possible combinations of flanking outside markers. This suggests that gene conversion may be involved in recombination between closely linked genes as well as within a gene. Hurst, Fogel and Mortimer (1972) found that co-conversion can cover two, three or even four sites within a gene. Fogel and Mortimer (1969) have shown that the closer together two mutant

sites lie, the greater is the probability that both will be converted.

As the distance between two mutant sites in a gene or in adjacent genes increases, the probability of co-conversion decreases and the probability of independent conversion, with associated recombination between the sites, increases. The recombination frequency between very close mutants depends upon the particular base substitutions involved; and Norkin (1970) found that the introduction of a third mutation between two others in E. coli may change the recombination frequency between them. Gutz (1971) also found a site-specific effect in yeast, in which one allele showed a much higher frequency of gene conversion and recombination than neighbouring sites. The idea that conversion is achieved by correction of hybrid DNA sequences is supported by experiments in which Kitani and Olive (1970) added different DNA bases to supplement the medium on which Sordaria mutants were crossed. These treatments changed the frequencies and direction of gene conversion, presumably by distorting the nucleotide precursor pools on which the correction enzymes draw. This may be relevant to mechanisms of hyper-recombination.

The mismatch-repair theory of conversion is frequently compared to the repair of UV lesions by excision. But some experiments suggest that conversion and excision of UV lesions are only indirectly related. No enzyme has been directly implicated in conversion, but enzymes involved in the excision of UV lesions may provide a model for the excision of mismatched bases. The final step in the formation of recombinant molecule from a joint molecule is covalent closure by a polynucleotide ligase.

A consideration of strand exchange reveals that covalent closure may be accompanied without new DNA synthesis; the exchange process itself may leave interrupted strands with neither an excess nor a deficiency of nucleotides.

E. Recombination pathways of E. coli

Characterisation of mutants defective in genetic recombination can help clarify the molecular nature of recombination and its relationships to replication, repair and other processes of DNA metabolism (Clark, 1973; Symonds, 1974; and Eisenstark, 1977). Clark and Marquiles (1965) demonstrated that genetic analysis could be applied to the process of genetic recombination itself by isolating mutants of E. coli that were deficient in their ability to yield recombinants (10^{-4}% of wild type) after conjugation with an Hfr donor strain. Functionally, the first rec$^-$ mutants to be discovered fell into two classes : "cautious" and "reckless", depending on whether the mutants show less or more DNA breakdown, than the wild type after UV irradiation (Howard-Flanders and Theriot, 1966). The first rec$^-$ mutants were mapped at three loci. Two of these loci, recB and recC, map between thyA and argA and mutants are cautious. The third locus, recA maps between cysC and pheA and mutants of it are "reckless". Mutations in recA appear to block recombination in E. coli completely or nearly so and also has other pleiotropic effects. These include high sensitivity to UV (Clark and Margulies, 1965), X-irradiation (Howard-Flanders and Theriot, 1966), and insensitivity to the mutagenic action of UV (Witkin, 1969). Further, lambda prophage cannot be induced by UV if the host is recA.

Mutations in recA also block the action of tif, a temperature conditional mutant of λ prophage (Castellazzi, George and Buttin, 1972) at the non permissive temperature. The recA mutants also promote cell division and prevent filament formation in strains, like lon which could otherwise show this phenotype following irradiation with UV. In these strains it appears, therefore, that the presence of functional recA product leads to a block in cell division. Similar effects are caused by zab (Castellazzi and Buttin, 1972) and lexB mutations (Blanco, Levine and Devoret, 1975)

which map at the recA locus, except that these mutations permit some genetic recombination. This distinction between recombination proficiency in zab (or lexB) mutants and deficiency in recA mutants, however is not sharp, one zab mutation zab-4 causes extreme recombination deficiency at 30°C but not at 37°C. Transduction and complementation studies indicate that temperature sensitive mutants tif-1, rec A, zab and lexB are mutations of the same gene designated recA$^+$. This recA$^+$ gene product possesses two activities: one controlling genetic recombination through a recA$^+$ basic activity, the other controlling cell response to DNA damage through a zab$^+$lexB$^+$ activity. The pleiotropic effects of these mutations, and the genetic mapping data must be combined with the study of protein X which has recently been identified as recA$^+$ protein. Protein X is induced in E. coli by a variety of treatments which damage DNA or inhibit DNA synthesis (Gudas and Pardee, 1975; West and Emmerson, 1977; Little and Hanawalt, 1977).

These results implicate protein X in all recA$^+$ dependent processes. Thus radiation sensitivity, ability to induce prophage lambda, DNA degradation, mutability, co-ordination of cell division with DNA replication, recombination, control of respiration and ribosomal ambiguity may all depend on protein X. Roberts and Roberts (1975) also demonstrated that induction of a λ prophage by mitomycin C or UV light results in cleavage of the λ repressor molecules in the lysogen. This cleavage does not occur when induction is prevented by mutational alteration either of the phage repressor (λind$^-$) or of the host recA gene product. Proteolytic cleavage may be the primary mechanism of repressor inactivation in this induction pathway, or it may follow a different event which causes the initial inactivation. McEntee isolated plaque forming specialized transducing λ phages for recA region of E. coli chromosome. Those transducing λ phages were used to infect UV irradiated cells in order to label transducing phage

protein preferentially. By this method the protein product of the recA gene has been identified (McEntee, Hesse and Epstein, 1976). This protein is not synthesised after infection by a specialized transducing phage which carries the nearby srlC gene but does not carry the recA gene.

West and Emmerson (1977) suggested that protein X, induced by temperature shift-up in tif-1 was a mis-sense mutation in the gene coding for protein X. Since the tif-1 was a mutation of the $recA^+$ gene, so the protein X was the $recA^+$ gene product.

Protein X could not be detected in recA12, recA56, $recA99_{(am)}$ treated with mitomycin C; in the temperature sensitive recA mutant recA200 protein X was induced at 30° but not 42°C. Thus induction of protein X by mitomycin C treatment is strongly $recA^+$ dependent (West and Emmerson, 1977), as has also been found for induction by other agents which inhibit DNA synthesis such as Nalidixic acid (Gudas and Pardee, 1975), UV irradiation (Sedgwick, 1975; West and Emmerson, 1977), γ irradiation (West and Emmerson) and thymine starvation (Inouye, 1971). Gudas and Pardee (1975) said that protein X bound preferentially to single stranded DNA; it bound less tightly to double stranded DNA and they suggested that protein X could protect the single stranded DNA from the recBC coded exonuclease. An interesting analogy with the T4 gene 32 protein product has been suggested.

West and Emmerson (1977) have recently proposed a model to account for the relationship between protein X synthesis and the $recA^+$ and $lexA^+$ genes. In this model the $lexA^+$ gene produces a repressor which binds to the operator region of the $recA^+$ gene from which it can normally only be removed by the combined action of an inducer and protein X. Thus protein X in part controls its own synthesis. A mutation spr which maps at the lexA locus, is constitutive for expression of the inducible error-prone repair functions and induction of prophage λ. Two dimensional gel electrophoresis of proteins from a tif sfi lex spr recA strain revealed that it synthesises

large amounts of a mutated form of protein X, presumably constitutively because the spr mutation affects the ability of the lexA repressor to bind to the recA$^+$ operator region.

The product of the recB$^+$ and recC$^+$ genes is an ATP-dependent DNAase, exonuclease V. Cells which are either recB$^-$ or recC$^-$ lack this enzyme activity. The enzyme is remarkable in several ways. It is both an exo- and endonuclease. It degrades duplex molecules by cleaving off single-stranded fragments, several hundred nucleotides long, repeatedly from both the 3' and the 5' ends, leaving a duplex with a protuding single-stranded termini several thousand nucleotides long. The enzyme then switches to the protuding single strands and degrades them exonucleolytically to oligonucleotides about five residues long. Repetitions of this cycle on the shortened duplex degrades it completely. This scheme accounts for the processive nature of degradation of duplex DNA by the nuclease, and it is proposed that the requirement for ATP, and the hydrolysis of ATP are related to an unwinding of the duplex that must take place during the DNA digestion. Cultures of strains carrying recB or recC mutations are characterised by a high proportion of inviable cells (Barbour and Clark, 1970). Following conjugation and transduction of recB and recC mutants, recombinant colonies are observed at frequencies from 0.3% to 2% of that observed with wild type recipient. In certain crosses the high frequencies can be explained by the transfer of recB$^+$ or recC$^+$ alleles to zygotes. In other crosses the high frequencies can be explained by transfer of the rac$^+$ gene to zygotes in which it may be transiently derepressed. Recombination between F' plasmids and the chromosome of recB and recC mutant strains however, cannot be explained in this manner.

Chromosome mobilization by F'lac$^+$ in recB$^-$ and recC$^-$ single mutants, for example results in transfer of chromosome markers with frequencies

10% to 50% of those of a wild type strain. A different type of experiment also indicates that quite high levels of recombination can occur in recB and recC mutants. The amount of β-galactosidase produced by zygotes formed by crossing a lacZ⁻ Hfr (in which lacZ is transferred early to zygotes) with a series of F⁻ strains carrying a noncomplementing lacZ⁻ mutation has been measured (Low and Birge, 1974). (Intragenic recombination is presumed necessary for β-galactosidase production in this situation). The amounts of enzyme in recB⁻ and recC⁻ zygotes were nearly as much as in wild type zygotes. By contrast, recA⁻ zygotes produced no detectable enzyme. In these experiments, when the viability of the recombinant cell was required, the recombination frequencies were far lower than when the viability of the recombinant cell was not required. From this point of view exonuclease V appears to be more involved with cell viability than with recombination. RecB mutants give relatively efficient transduction if the phage is irradiated before infection (Stacey, private communication). Mutations in recB or recC may be suppressed by mutations at either of two further loci, sbcA and sbcB. Kushner et al (1971) showed that sbcA mutants possessed elevated levels of an ATP-independent exonuclease (Exo VIII) whose presumed structural gene is designated recE⁺ which may act as an analogue of the recBC product (exonuclase V) to restore the function of the recBC pathway. Mutants in sbcB lack the enzyme exonuclease I : thus the presence of exonuclease I prevents recombination in cells that lack the recBC nuclease.

Exonuclease I degrades single strands of DNA from 3' to 5', releasing mononucleotides, and causing the disappearance of free 3'-OH termini. Kushner et al (1972) proposed that this enzyme action must prevent recombination when exonuclease V is absent from the cell. A complicating feature in interpreting the properties of these mutants is the existence of xonA mutations which lack exoI and share with sbcB mutations the ability to suppress the deficiency in repair caused by the recB⁻ or recC⁻ mutation, but do not suppress the deficiency in recombination.

Presumably the sbcB and xonA mutations differ either quantitatively or qualitatively.

Nevertheless, there appear to be two ways by which, as it were, the function of exonuclease V in recombination can be replaced if it is defective. One is by the inactivation of exonuclease I in sbcB mutants, the other by the activation of exonuclease VIII in sbcA mutants. Clark interprets the interaction as a consequence of independent pathways, concluding that there is a recE pathway opened by the presence of of exonuclease VIII and a recF pathway opened by the absence of exonuclease I, as well as the usual recB recC pathway. However, the two supplementary paths of recombination are detectable only if exonuclease V is inactive.

It is likely that the recF pathway is important in recovery from the effects of UV irradiation in that recF strains are more sensitive than the wild type even though they possess the recB pathway intact.

F. Recombination pathways of phage λ

Phage λ specifies two pathways of genetic recombination: the Int pathway for site-specific recombination and Red pathway for general recombination. Phage λ can also recombine by means of the host cell general recombination (Rec) enzymes, since recombination between mutants of λ defective in the Red pathway (Red$^-$ mutants) still occurs in a *rec*$^+$ host (Unger and Clark, 1972). It is suggested that λ does not multiply in P2 lysogenic cells because it renders such cells phenotypically *recB*$^-$, and the P2 gene (*old*$^+$) product, expressed by the prophage inhibits growth of cells and superinfecting λ phage. Mutants of lambda capable of growth in P2 lysogenic strains (*spi*$^-$) multiply poorly in *recA*$^-$*recB*$^-$ strains. Apparently, in the absence of the *recA* product, the *recB* enzyme inhibits the multiplication of *spi*$^-$ strains of λ. Since *spi*$^+$ strains of λ are not inhibited in *recA*$^-$*recB*$^+$ strains, it is suggested that λ*spi*$^+$ possesses some gene whose product inhibits the *recBC* enzyme activity. The λ gene responsible for the decrease in exonuclease $\overline{V}$ activity appears to be the *gam* gene since *gam*$^-$ λ strains do not result in the decrease while *gam*$^+$ strains do (Unger and Clark, 1972).

Sakaki et al (1973) isolated the component of λ infected cells that is able to inhibit the *recBC* nuclease in vitro and showed it to be a protein which is effective in preventing all of the known degradative activities of the nuclease. Karu et al (1975) showed that the *gam* protein consists of two identical subunits. Unger, Echols and Clark (1972) have shown that mutations in the *gam* gene of λ increase the frequency of general recombination mediated by the *recBC* pathway.

The role of *gam* in contributing to the *spi*$^-$ genotype is clear; λ *gam*$^-$ fails to convert the P2 lysogen to the *recBC*$^-$ condition in which it is susceptible to the action of the *old*$^+$ gene.

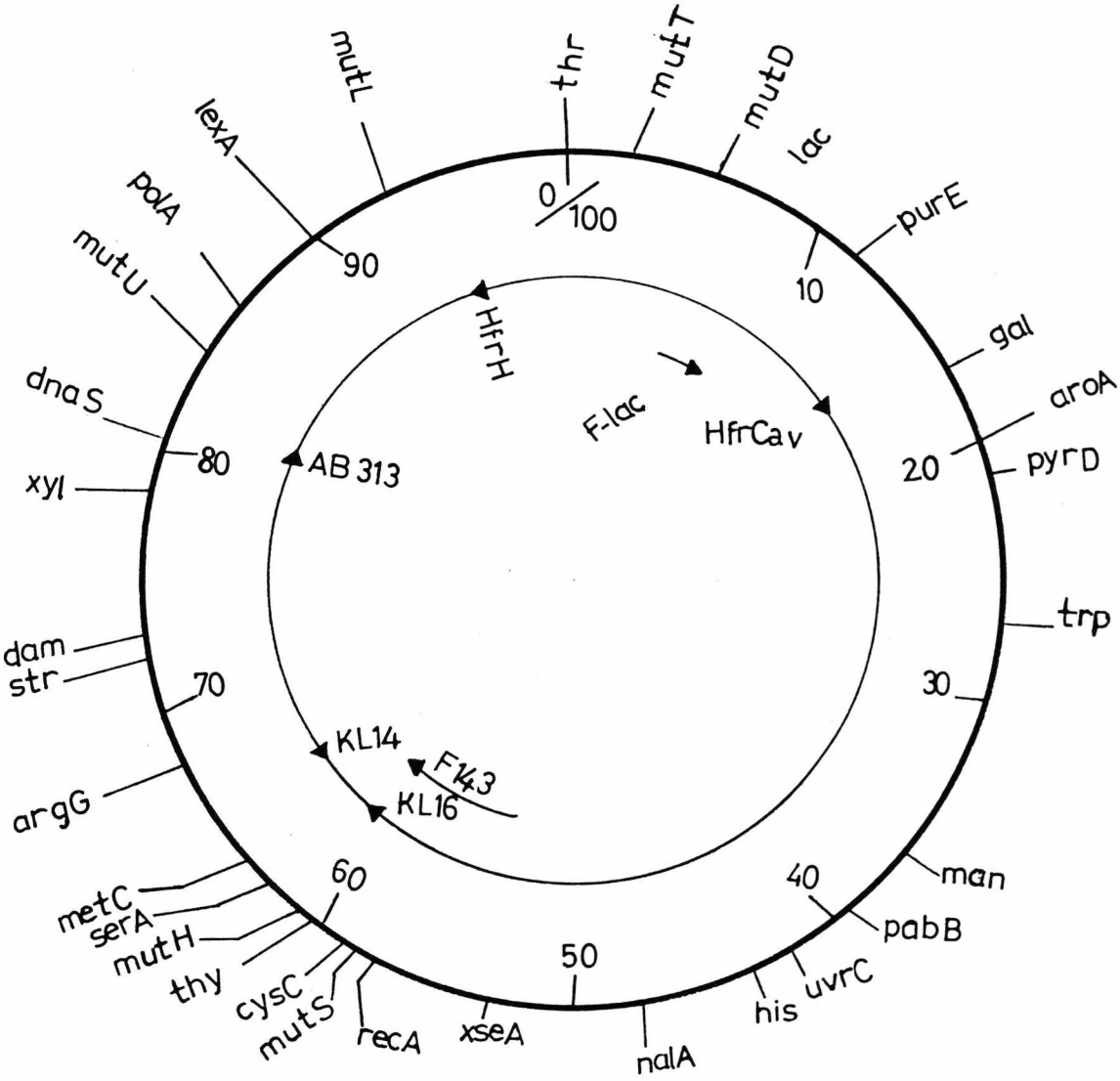

thr
mutT
mutD
lac
purE
gal
aroA
pyrD
trp
man
pabB
uvrC
his
nalA
xseA
recA
mutS
cysC
thy
mutH
serA
metC
argG
str
dam
xyl
dnaS
mutU
polA
lexA
mutL
0/100
10
20
30
40
50
60
70
80
90
HfrH
F-lac
HfrCav
AB 313
KL14
F143
KL16

FIGURE 10

FIGURE 10 Genetic linkage map of Escherichia coliK12 chromosome (Adapted from Bachmann, Low, and Taylor, 1976).

The outer circle shows the location of genetic markers on the circular linkage group, (based on a 100 minute time scale of conjugal transfer).

The inner circle shows the origin and direction of transfer of certain Hfr strains.

F-primes are shown by arrows.

G. Hyper recombination mutants of E. coli

Since 1964 various bacterial mutants defective in genetic recombination have been isolated and much used to clarify the mechanism(s) of genetic recombination. In recent years mutants of E. coli with an enhanced frequency of recombination (hyper-rec) have been reported (Konrad and Lehman, 1974, 1975; Konrad and Marinus, 1976; Konrad, 1977; Chase and Richardson, 1977; Stacey and Kalaycioglu, 1977).

1. Isolation and characterisation of hyper-rec mutants of E. coli

Konrad and Lehman (1974, 1975) were the first to report an E. coli mutant, polAex1, which showed an abnormally high frequency of recombination. E. coli polAex1 was found among temperature sensitive mutants. It was defective in the 5'⟶3' exonuclease activity of DNA polymerase I, but not in the polymerase activity. An E. coli-K12 strain of KS391 (HfrH lacM286 φ80dII lacBK1) was constructed by Konrad, introducing two lac⁻ mutations (deletions) into an HfrH strain (M286 was a deletion including lacY and lacZ, the deletion lacBK1 was carried on the prophage φ80dII lac and lacks part of the lacZ gene). The two deletions did not overlap and were expected to recombine with each other. A culture of KS391 was mutagenized with ethylmethane sulfonate and plated on lactose tetrazolium plates. After 3 days incubation at 30°C wild type colonies showed one or two white lac⁺ papillae while the hyper-rec phenotype yielded many more papillae on the surface of single colony.

The polAex1 mutation was mapped between 74-78 minutes on the E. coli chromosome map and presumed to lie between metE and rha. It is believed to be a single mutation. This polAex1 mutation behaved similarly to other polA mutants in its retarded sealing of "Okazaki fragments", its sensitivity to MMS and to UV irradiation and its inability to form plaques of λred⁻ phage.

Konrad and Lehman (1975) also reported novel mutants of E. coli which accumulate very small DNA replicative intermediates. Strains carrying this new mutation, called dnaS1, dnaS2, and dnaS3, were isolated by screening hyper-rec mutants obtained at 30°C. All three mutations mapped very close to pyrE locus of E. coli chromosome at 72 minutes. (Mutations dnaS1 and dnaS3 were induced by ethylmethane sulfonate, and dnaS2 by nitrosoguanidine). These dnaS mutants were lethal at 44°C. It was presumed that this effect was due to secondary mutations since the transfer of dnaS mutation to a wild-type did not produce lethality at 44°C. Low (1972) has shown that the dnaS1 mutation was recessive, since introduction of F'111 covered the dnaS1 locus and restored the wild type phenotype.

During short pulses dnaS mutants were incorporated [^{3}H]thymidine into DNA fragments much smaller than the Okazaki fragments which were found in the wild type.

The levels of DNA ligase, DNA polymerase I and the 5'→3' exonuclease activity of DNA polI did not show any difference from the wild type level. These dnaS mutants were found to be resistant to methyl methane sulfonate, slightly sensitive to UV irradiation, and formed plaque with λ red$^-$ phage. Double mutant of dnaS1recA strain were viable.

The dam-3 mutant of E. coli was first isolated by Marinus and Morris (1973, 1974). It was reported that the mutant had a reduced amount of DNA adenine methylase. The residual adenine methylation observed in vivo was thought to be due to E. coli DNA modification methylase, a product of the hsp genes. The dam-3 mutant mapped at 65 minutes ~~of~~ on the E. coli chromosome between trpS and aroB. It was more sensitive to UV irradiation and to mitomycin C than ~~to~~ the wild type and it also shows higher mutability. This high mutability suggests that there might be increased DNA breakage and repair in dam-3 than wild type. The dam-3 mutant also contained excess single

stranded breaks that were amplified in dam-3 polA12 and dam-3 lig-7 double mutants. Combination of dam-3 with polA, recA, recB, recC was lethal. Recently Konrad and Marinus (1976) presented evidence that the dam-3 mutant also exhibits a hyper-rec phenotype. Segregation of F-prime homogenotes from F-prime heterogenotes has been scored as a measure of recombination. The lac$^-$ segregants from a F'lac$^+$/lac$^-$dam-3 and gal$^-$ segregants from a F'lac$^+$/gal$^-$dam-3 were shown to be F'lac$^-$/lac$^-$ and F'gal$^-$/gal$^-$ homogenotes. This homogenote formation was at least thirty fold higher in the dam-3 strain than in the isogenic wild type. These results suggested that the dam-3 mutation either increased recombination between plasmid and host chromosome or that it promoted aberrant segregation of plasmids. The dam-3 allele also caused reduction in the linkage of proximal unselected markers in exconjugants and increased the recombination frequency between a pair of closely linked markers. The dam-3 mutation did not affect the frequency of F-prime chromosome mobilization.

Konrad also plated wild type KS391 and its two hyper-rec mutants onto XGal glucose + IPTG plates (1). After three days incubation at 30°C lac$^+$ cells were counted. Among the 10^5 cells, wild type clones yielded one lac$^+$ colony whereas two of the mutants yielded 22 and 1200 lac$^+$ colonies. Konrad suggests that increased nicks or gaps in DNA might be the basis for the hyper-rec character. It is known that mutations of DNA polymerase I and DNA ligase increases the accumulation of nicks or gaps in the chromosome.

(1) X Gal glucose minimal plates (5-bromo-4-chloro-3-indolyl-B-D-galactoside) allow detection of various amounts of constitutive β-galactosidase inside E. coli colonies. If IPTG is included in the medium, then these plates become indicators for maximal levels of β-galactosidase rather than constitutive levels.

When Konrad introduced polA1 and lig-7 (ts) into KS391 background, both behaved as hyper-rec and yielded approximately 10 lac$^+$ papillae on the surface of a single colony.

Genetic control of recombination between two partially deleted lactose operons was examined by introducing several rec$^-$ genes into a hyper-rec derivative of KS391 (Zieg and Kushner, 1977). RecA13 and RecA142 produced dramatic decreases in recombination proficiency. Strains carrying recB, recC mutations showed decreased conjugational transductional proficiency, but only a five fold decrease in the number of lac$^+$ recombinants. Introduction of the sbc15 mutation into a recB21 strain regained the wild type levels of conjugation and transduction efficiency. The strain carrying recF143 was nearly identical to the wild-type in all aspects. The recL152 mutant exhibited increased conjugation proficiency and higher levels of lac$^+$ recombinant formation. This high level of lac$^+$ progeny suggested that possible differences in the enzymatic requirements occurs for the formation of viable progeny either after conjugation or after a crossover between nontandem duplications.

Kushner and Zieg have also examined the nature of the lac$^+$ papillae formation. Respreading analysis on minimal agar lactose plates confirmed that logarithmic increase in the number of lac$^+$ colonies coincided with the lac$^-$ cell population entering into early stationary phase. The presence of a small amount of glucose or L-broth in the plating medium led to an almost 100 fold increase in the total mass. When the cells were washed with M56/2 buffer prior to plating the number of lac$^+$ recombinants was significantly reduced but was still dependent of initial cell numbers.

Chase and Richardson (1977) have recently identified strains having reduced levels of exonuclease VII activity, xse$^-$. The high co-transduction frequencies of one group of strains with guaA make it reasonable to assume that the defects in these strains all occur within one gene, the

structural gene for exonuclease VII, which they designated xseA. All the xse$^-$ mutants isolated by Chase and Richardson were identified directly on the basis of having a reduced level of the 5'$\longrightarrow$3' hydrolytic activity of exonuclease VII.

Mutants with defects in the xseA gene show increased sensitivity to nalidixic acid and an abnormally high frequency of recombination (hyper-rec phenotype) as measured by the procedure of Konrad and Lehman as mentioned earlier. The hyper-Rec character of xseA$^-$ strains is approximately one half that of the polAex1 mutant defective in the 5'$\longrightarrow$3' hydrolytic activity of DNA polymerase I. The double mutant, polAex1xseA7, is twice as hyper-Rec as the polAex1 mutant alone. The xseA$^-$ strains are slightly more sensitive to ultraviolet irradiation than the parent strain. Bacteriophage T7, fd, and λred grow normally in xseA$^-$ strains.

The following experimental work was performed as the main part of the thesis, to study the mechanisms of recombination with particular reference to the ability of certain mutants to show a high level of recombination.

II. MATERIALS AND METHODS

MATERIALS AND METHODS

A. MATERIALS

1. Strain List

Table 1, 1(a), and 1(b) list the bacterial and bacteriophage strains used in this work.

The list shows the relevant genotype of each strain. In cases where the specific cistron or allele is unknown, no letter or number is given.

Genetic symbols used to describe the sex factor of bacteria are listed below.

F^- : the strain does not carry the sex factor, F

F^+ : the strain carries an autonomous sex factor

F' : the strain carries a sex factor which itself carries a genetically recognisible segment of the bacterial chromosome

Hfr : the strain carries a sex factor which is integrated within the chromosome.

A prophage is designated by using parentheses ((λ) indicates "carries λ as a prophage"). Drug sensitivity and resistance are designated by using letters r and s (Str^r and Str^s refer to streptomycin resistance and sensitivity respectively).

TABLE 1. Bacterial strains

Strain No.	Mating type	Characteristics	Source
X7184	F^-	*thi*$^-$ *sup*$^-$ *lacZ*$^-$ 10b,M15 *ara*$^-$ *mal*$^-$ *mtl*$^-$ λ^R $T6^S$ A_zU^S Str^R	a
AB1157	F^-	*thr*$^-$ *leu*$^-$ *his*$^-$ *proA*$^-$ *argE*$^-$ *thi*$^-$ *lac*$^-$ *gal*$^-$ *xyl*$^-$ *mtl*$^-$ *ara*$^-$ λ^- $T6^R$ Str^R	a
AB2070	F^-	*metE*$^-$ *ilv*$^-$ *trp*$^-$ *his*$^-$ *pro*$^-$ *leu*$^-$ *ara*$^-$ *thi*$^-$ Str^R	a
W945	F^-	*thr*$^-$ *leu*$^-$ *thi*$^-$ *gal*$^-$ *ara*$^-$ *xyl*$^-$ *mtl*$^-$ *lacY*$^-$ *supE* λ^R Str^R	a
C600(*ara*$^-$)	F^-	*thr*$^-$ *leu*$^-$ *thi*$^-$ *lacY*$^-$ *ara*$^-$ *tsx* $T3^R$ Str^R	C. Bushell
CR63(*ara*$^-$)		*ara*$^-$ λ^R	J. Hardy
D13.1	F^-	*ara*$^-$ *fol* Str^S λ^S	a
JG112	F^-	*thy*$^-$ *rha*$^-$ *lacY*$^-$ *polA1* Str^R	b

TABLE 1 (continued)

JG113	F^-	*thy*$^-$ *rha*$^-$ *lacY*$^-$ *pol*$^+$ Str^R	b
NY73	F^-	*leu*$^-$ *lac*$^+$ *dnaG*$^-$ *polA1* Str^R *metE thyA* Rif^R	c
NY73(1)	F^-	as NY73 except *lacZ*$^-$ *leu*$^+$	This work
S491	F^-	*supE*$^-$ *his*$^-$ *proA*$^-$ *gal*$^-_{(am)}$ *lac*$^-$ λ^- λ^S Str^S AzU^R	a
S491(1)	F^-	as S491 except *recB21*	c
AT2699 (CGSC4524)	F^-	*argG*$^-$ *metC*$^-$ *his*$^-$ *thyA*$^-$ *mal*$^-$ *gal*$^-$ *lac*$^-$ *supE*$^-$ λ^- λ^R Str^R	a
KL398	F^-	*metE*$^-$ *leu*$^-$ *proC*$^-$ *hisF*$^-$ *thyA*$^-$ *thi*$^-$ *lacZ36* *ara*$^-$ *mtl*$^-$ *xyl*$^-$ Str^R Spc^R	d
KL398mut$^-$	F^-	as KL398 except *thy*$^+$ *mutH21*	d
KL400	F^-	as KL398 except *met*$^+$	d

TABLE 1 (continued)

KL403	F^-	as KL400 except *polA1*	d
KL405	F^-	as KL400 except *polA12*(t.s.)	d
KL406	F^-	as KL400 except *polA107* (polymerase$^+$, 5 - 3 exo$^-$)	d
H2	F-prime	as X7184 except *pro*$^-$ F'*lacZ*$_{(am)U131}$	a
H2110	F-prime	as H2 except *mutH21*	a
H2114	F-prime	as H2 except *hyp*$^-$	a
KL398(*hyp*$^-$)	F-prime	as KL398 except *thyA*$^+$ F'*lac*$_Z^-$ (am)U131	This work
E'36	F-prime	Δ(lac-pro)$_{XIII}$ Str^S F'$_{(ts114)}$ *lac*$^+$	a
JG196	F-prime	*met*$^-$ *rha*$^-$ *recA*/F14 *met*$^+$ *rha*$^+$ *pol*$^+$	a
JG197	F-prime	*met*$^-$ *rha*$^-$ *recA*/F14 *met*$^+$ *rha*$^+$ *polA1*	a
KLF43	F-prime	*tyrA*$^-$ *pyrD*$^-$ *thi*$^-$ *his*$^-$ *trp*$^-$ *thyA*$^-$ *recA1* *mtl* *xyl* Str^R λ^R λ^- F143(*lysA*$^+$ *tyrA*$^+$)	a

TABLE 1 (continued)

KLF22	F-prime	*argG*$^-$ *metB*$^-$ *his*$^-$ *leu*$^-$ *thyA*$^-$ *recA1* *mtl*$^-$ *xyl*$^-$ F122(*argG*$^+$ *metC*$^+$) *lac*$^-$ Str^R λ^R λ^- *supE44*	a
DFF1	F-prime	*argG*$^-$ *metB*$^-$ *his*$^-$ *leu*$^-$ *thyA*$^-$ *recA1* *mtl*$^-$ *xyl*$^-$ F150(*his*$^+$ *zwf*$^+$) *lac*$^-$ Str^R λ^R λ^- *supE44*	a
KLF10	F-prime	*argG*$^-$ *metB*$^-$ *his*$^-$ *leu*$^-$ *thyA*$^-$ *recA1* *mtl*$^-$ *xyl*$^-$ F110(*polA*$^+$ *malB*$^+$) *lac*$^-$ Str^R λ^R λ^- *supE44*	d
KLF1	F-prime	*thr*$^-$ *leu*$^-$ *his*$^-$ *arg*$^-$ *pro*$^-$ *recA1* Str^R F101(*thr* *leu*)	a
Hfr Hayes	Hfr	(λ^+)	a
Hfr Cavalli	Hfr	*relA*$^-$ *met*$^-$	a
KL16	Hfr	(λ^+)	a
KL16-99	Hfr	as KL16 except *recA1*	d

TABLE 1 (continued)

AB313	Hfr	*thr*$^-$ *leu*$^-$ *thi*$^-$ *lac*$^-$ Str^R	a
AB312	Hfr	*thr*$^-$ *leu*$^-$ *thi*$^-$ *lac*$^-$ Str^R	a
KL14	Hfr	*thi*$^-$ *relA*$^-$ λ^-	c
Hfr P4X6	Hfr	*metB*$^-$ *relA*$^-$	a
AT2446 (CGSC4504)	Hfr	as HfrH except *metC*$^-$ *thi*$^-$ λ^-	a

TABLE 1 (a) Plasmids

Strain	Characteristic	Source
R1drd19	Su Str Tc Km Ap Pi$^+$	b
R-Utrecht	Cm	e
ColV-K30	colicinV	b
ColI_b-P9	colicinI	b
ColE1-K30	colicinE	b

TABLE 1 (b) Bacteriophage strains

Strain	Source
μ2	a
P1	a
λvsusP3	f
λvsus029	f
λvsus0206	f

a via K. A. Stacey

b via K. Hardy

c via P. Oliver

d via R. G. Lloyd

e via D. MacPhee

f via J. Hardy

2. Media

The various types of solid and liquid bacteriological media used in this work are listed here. Contents are given per litre of medium prepared in sterile distilled water.

Nutrient broth : 25g Oxoid broth No.2.

Nutrient agar : 25g Oxoid broth No.2, and 12.5g Davis New Zealand agar No.3.

Strength agar : ½ nutrient broth + ½ nutrient agar.

Tryptone broth : 10g of Oxoid tryptone, 8g NaCl, 1g glucose.

Tryptone agar : 10g of Oxoid tryptone, 8g NaCl, 1g glucose, 10g Davis New Zealand agar No.3.

Minimal salts : 7g K_2HPO_4, 3g KH_2PO_4, 0.1g $MgSO_4.7H_2O$, 1g $(NH_4)_2SO_4$, 0.5g sodium citrate $2H_2O$ (Davis and Mingioli, 1950).

Minimal agar : 20g of Davis New Zealand agar No.3, 20mg of L-amino acids, 5g of carbon source, 1mg of appropriate vitamins and 100ml of mineral salts (0.005 or 0.05 g of thymine if necessary).

Eosin Methylene Blue (EMB) agar : 8g Difco bacto casamino acids, 1g Difco bacto yeast extract, 5g NaCl, 2g K_2HPO_4, 0.4g Eosin yellow, 0.065g Methylene blue, 10g sugar, 15g agar.

The eosin, methylene blue, nutrient base and sugar solutions were prepared and sterilised separately and were mixed together just before pouring the plates.

Dorset egg medium : 30 beaten eggs, 250mls of nutrient broth Oxoid No.2, 0.5g thymine.

M9 liquid medium : 6g Na_2HPO_4, 3g KH_2PO_4, 0.5g NaCl, 1g NH_4Cl, 0.246g $MgSO_4.7H_2O$, 0.0147g $CaCl_2.2H_2O$, 0.01g gelatin, 4g sugar.

3. Buffer solutions

Phosphate buffer : 17.6g $Na_2HPO_4.12H_2O$, 3g KH_2PO_4, 5g NaCl, 10ml 0.1M $MgSO_4$, 10mls 0.01M $CaCl_2$, 10mls 1% gelatin made up to 1 litre.

P1 adsorption fluid : 90mls H_2O, 10mls tryptone broth, 1.1mls 1M $CaCl_2$ (Lloyd, 1972).

λ adsorption fluid : 0.01M magnesium sulphate.

4. Chemicals

N-methyl-N-nitro-N' nitrosoguanidine (NTG) (Aldrich) was dissolved in warm distilled water at varying concentrations.

Methyl methane sulphonate (MMS) (Aldrich Chemical Co. Ltd.) and acridine orange (BDH Chemicals Ltd.) were kept in the dark at 4°C. Appropriate amounts were mixed with molten agar when they were used in solid media.

Streptomycin (Glaxo) and spectinomycin (donated by Upjohn Ltd.) were dissolved in distilled water at 10mg/ml, 6 Azauracil (Sigma) at 3.5mg/ml and Trimethoprim lactate (Burroughs Wellcome) 1mg/ml.

Nalidixic acid (Calbiochem.) was dissolved in 100ml distilled water 2.5mg/ml by adding 2ml 2M NaOH.

Benzyl penicillin (Sigma) dissolved in distilled water at 10^4 units per ml.

Acriflavine HCl, Adenosine and Spermine tetrahydrochloride (Sigma) dissolved in distilled water.

B. METHODS

1. Bacterial Cultures

Stock cultures of bacterial strains were maintained in small screw capped bottles on Dorsett egg medium. To ensure their survival some of the strains have also been preserved by freeze-drying.

Colonies on nutrient agar plates were maintained at 4°C. Approximately every 3-4 months the stock plates were renewed. Before use each strain was purified by single colony isolation on minimal agar plates. Liquid cultures were inoculated from these plates and incubated overnight. On the day of the experiment the overnight culture was usually subcultured into a fresh liquid broth medium, and gently agitated either with an angled rotator or an MKV flat shaker at 37°C or 30°C.

2. Bacteriophage stocks

Bacteriophage stocks were maintained in buffer solutions at 4°C. New bacteriophage stocks were prepared by the plate-lysis method.

After incubation the top soft agar layers were scraped into a small 25ml bottle with a sterile glass spreading rod. An equal volume of buffer and 1-2 drops of chloroform were added and the mixture shaken vigorously for 20-30 seconds and allowed to stand for several minutes. After centrifugation to remove the cell debris, the supernatant was stored at 4°C.

3. Titrations

Viable bacterial cells were assayed by the spreading method on agar plates.

4. UV-Irradiation, MMS treatment, survival curves

UV radiation sensitivity curves were determined using a Hanovia (Model 16) UV lamp. All manipulations were made in a dark room. The lamp was set 50 cm distance from the plates. It was switched on for at least 40 minutes before use. Streak tests were often used in determining UV sensitivity. Mutant strains and the parent (wild type) strains were streaked alongside one another on nutrient agar plates. The plates were irradiated, wrapped with foil paper, and then incubated for 10-15 hours. Under these conditions significant differences in the growth of UV sensitive cells and the wild type were always obtained. A similar method was used for MMS sensitivity. A loopful of cells were streaked onto nutrient agar containing MMS and incubated for 10-15 hours. MMS sensitive cells did not form colonies or the number of colonies was reduced by 2 to 3 orders of magnitude.

The spot dilution method was also used. Log phase cultures in minimal media were centrifuged and resuspended in liquid minimal medium at $1\text{-}5\times10^{7}$ cells per ml. 0.01ml of appropriate serial dilutions were spotted on nutrient agar and minimal agar plates. As soon as the spots dried, the plates irradiated, wrapped in foil and incubated. The surviving fraction could be calculated from the number of colonies at the last dilution to give discrete colonies.

Also phage suspensions at 10^{9} particles were irradiated in glass petri dishes, and plated with appropriate indicator strains. After incubation it was clear whether or not a strain had the capacity for reactivation.

5. Conjugation, Phenocopies, Episome transfer

Nutrient broth cultures of a donor at 2×10^{8} cells/ml were mixed with a recipient at $2\text{-}4\times10^{8}$ cells/ml in the ratio of 1 donor cell to 10-20 recipient cells. Mating mixtures were incubated on an angled rotator or in a waterbath at 37°C. The incubation period usually depended upon the type of

donor and selective markers. 1ml of the mating mixture was withdrawn at appropriate times and agitated vigorously with a Gallenkamp flask shaker to interrupt mating.

Appropriate dilutions were plated on selective plates, and incubated 2-4 days at either 30°C or 37°C. Recombinants were picked and repurified on the same selective media before testing for the inheritance of unselected markers. Streptomycin, spectinomycin, nalidixic acid and azauracil were used, as appropriate, to counterselect the donor strain. To measure the number of revertants in both donor and recipient strains they were separately plated on each type of selective plate just before mating. The zero minute mating control was also used for each marker.

For the production of F^+ phenocopies male bacteria were aerated at room temperature for 24-36 hours by using an angled rotator, then mixed with the log phase donor in the ratio of 1 :1.

Episome transfer was effected with one of the following techniques :

a. A mixture of donor and recipient cells were incubated for 15-20 minutes at 37°C. After 15 minutes 150 γ/ml streptomycin was added and the culture incubated a further hour before plating on nutrient agar. The following morning a few colonies were picked at random and streaked onto nutrient agar. The streaks were spotted with a male specific phage and incubated for 10-15 hours. Cells sensitive to the phage showed a gap in the streak of bacterial growth.

b. Spot mating was also used for episome transfer. Selective minimal plates containing streptomycin or spectinomycin were divided into two sections. 0.05-0.1ml of log phase recipient was spread over the surface of one half of the agar plate and left for a few minutes to dry. Then 0.01ml of log phase donors was spotted onto both sections of plate and incubated.

Confluent growth was usually observed only in the spots in which donor and recipient were in contact after two days incubation. Single clones were isolated and tested with sex specific phages.

c. The third technique was used when the presence of the F prime in the recipient permitted direct selection. Mating mixtures were incubated for 40-50 minutes and plated on selective minimal plates. Recipient cells did not grow in the absence of a growth requirement while donor cells were killed by counterselection. Only those cells which had received the required marker carried by the episome formed colonies. A few of these were picked and tested with male specific phage.

6. Generalised P1 transduction

Suspensions of phage P1 were obtained by the confluent-lysis plate technique. Bacteria to be transduced were grown in 10ml nutrient broth until the titre was between $2\text{-}4\text{x}10^8$ at 37°C, centrifuged and resuspended in 1ml of P1 adsorption fluid. 0.1ml of a stock P1 phage suspension was mixed with 0.9ml bacterial cells and the mixture incubated for 25-30 minutes at 37°C in a water bath. The multiplicity of infection aimed at was usually about one. The mixture was then diluted and plated out on selective minimal agar plates. After 2-3 days incubation recombinants were counted, repurified and checked for co-transducible markers on minimal agar plates.

As a control, phage and bacterial cells were plated on the same type of selective plates before mixing.

7. Phage crosses

10ml log phase cultures of the host bacteria were concentrated 10 fold by centrifugation and resuspended in 0.01M $MgSO_4$ and incubated with aeration

for 30 minutes. Then approximately equal numbers of the two mutant phages were added. After 15 minutes the adsorption mixtures were centrifuged (infected centres and free phage assays were measured by plating the pellet and chloroformed supernatant with suitable indicators). The pellets were resuspended with warm tryptone broth, diluted 100 fold into 10mls of warm tryptone broth and incubated for 90 minutes. A few drops of chloroform were then added and 3-5 minutes later samples were plated on tryptone plates with suitable indicator strains and incubated overnight at 37°C.

8. Mutagenesis

1ml aliquots containing 20 g of nitrosoguanidine were added to 5ml log phase bacterial cultures in minimal medium and the culture was incubated for 20 minutes with aeration. The culture was then centrifuged or filtered and the cells washed and resuspended in buffer. The mutagenised cells were plated immediately onto EMB lactose agar and incubated at 37°C for four days at dilutions that gave about 300 colonies per plate. After the incubation those colonies which showed many papillae were picked and purified for further study.

9. Investigation of potential mutants with the mutator and hyper recombination phenotype

After the NTG mutagenesis of E coli K-12 strain with the genotype $\underline{lac}^{-}z_{10b}$ + M15 $F'\underline{lac}^{-}z_{u131}$, some survivors produced many papillae on EMB lactose agar.

Those potential mutants were restreaked, to see if the phenotype was stable, on EMB-lactose agar. Those that passed this test, were restreaked onto master plates and incubated to form master plates for replica plating.

Copies were made on a number of media to show

(a) the presence of new auxotrophic markers

(b) the mutation rate to azauracil resistance, and

(c) the rate of formation of cells able to grow on minimal media containing lactose instead of glucose.

This allowed a classification into mutator and potentially hyper recombination mutants. Hyper-rec and mutator phenotypes were determined by streaking to single colonies on EMB lactose agar plates. Hyper-rec and mutator colonies were those that showed many more lac^+ papillae on their surface whilst the wild type produced only one or two.

10. Episome curing

Episome curing was usually effected with acridine orange or the use of temperature sensitive F' factors.

(a) 0.1 ml of a 10^5 dilution of a fresh overnight culture inoculated into 5ml nutrient broth pH 7.6 containing 50μg/ml acridine orange. After overnight incubation at 37°C the culture was diluted and spread onto nutrient agar plates for single colony isolation. Colonies were picked and streaked on the nutrient agar plates and a drop of a suspension of a specific phage spotted onto each streak. After 10-15 hours incubation no gap was observed on the cured clones.

(b) The F' recipients were grown to produce F^- phenocopies as described earlier, before mixing with the donor culture $E'36\ F'\underline{lac}^+_{ts114}$. After 30-40 minutes the culture was agitated and appropriate dilutions plated on minimal-lactose agar and incubated for 48 hours at 30°C. Lactose recombinants were picked and purified on minimal lactose agar plates. 1000-2000 cells were inoculated into 5ml nutrient broth and incubated at

42°C overnight. The following morning the culture was diluted and plated on EMB lactose plates. After 3-4 days incubation at 42°C the pink clones, which can be presumed to be cured, were picked and tested with sex specific phages.

11. Penicillin treatment for the isolation of auxotrophic amino acid markers

An overnight culture was subcultured into nutrient broth by diluting $\frac{1}{10} - \frac{1}{20}$. The culture was aerated for 2-3 hours at 37°C and then filtered. The filter disc was transferred to a petri dish and washed with warm M9 medium. 0.1-0.3ml of washed cell suspension was transferred into a bubler tube containing 10ml of M9 glucose medium (the cell titres were usually about 5×10^7/ml). This tube was aerated 20 minutes at 37°C before adding 400 units penicillin per ml. Cells were incubated with aeration for 5-6 hours.

Aliquots of suitable 0.1ml dilutions were spread on nutrient agar plates and incubated overnight. Plates were then replica plated onto nutrient agar and minimal agar plates supplemented with the original requirements of the strain. After overnight incubation those colonies which appeared on the nutrient agar but not on the minimal agar were purified and their requirements identified.

Identification of amino acid auxotrophs was made using the amino acid pools made up according to protocol given in Clowes and Hayes (1968).

12. Selection of thymine requiring strains with trimethoprim

A fresh overnight bacterial culture was diluted into M9 glucose medium containing 50μg/ml thymine and 10μg/ml trimethoprim (Stacey and Simpson, 1965).

After an overnight incubation a few drops of the culture were subcultured into 5ml of the identical medium. They were allowed to grow

overnight to saturation. Suitable dilutions were spread on nutrient agar plates supplemented with 50μg/ml thymine and incubated overnight. Single colonies were picked and streaked onto two glucose minimal plates one supplemented with 50μg/ml thymine, and the other not. Thymine requiring clones did not grow on plates without thymine supplementations.

A plate method was also used for trimethoprim selection. 0.1ml aliquots of an overnight culture were spread onto glucose minimal plates containing 50μg/ml thymine and 10μg/ml trimethoprim. After two days incubation single colonies were picked and purified on the same type of plates. Then these colonies were tested for the thymine requirement on M9 glucose medium containing no thymine. Only non reverting mutants or those with very low reversion rates were retained for subsequent use.

13. Testing colicin production

Colicin factor transfer was effected using standard mating conditions. Then the presence of the Col factor in single clones of the recipient was tested in the following way.

5-50 single clones were spotted on a nutrient plate and restreaked onto a reference plate and the two plates incubated overnight. The colonies were sterilised by inverting the open plate over a watch glass containing 4-5 mls chloroform for 30 minutes. The plate was removed and left open to allow the chloroform to evaporate. Then 0.3mls of saturated culture of the indicator strain in 3ml of melted strength agar, were used to form an overlay.

After overnight incubation the colicinogenic colonies produced zones of inhibition in the confluent lawn of indicator bacteria. These were picked from the master plate, streaked and purified for further analysis.

14. Anaerobic growth

Bacterial cells were incubated anaerobically in a Baird and Tatlock

anaerobic jar.

Plates were placed upside-down inside the jar. A football bladder containing H_2 was connected to the jar and the gas transferred to the jar. The transfer was carried out (a few minutes) until all the O_2 had been removed by conversion to water in the presence of a catalyst. The degree of anaerobiosis was controlled with glass indicator capsules. The container was then incubated at the required temperatures for 6-7 days.

15. Mutation frequencies

Several testes were used to determine the mutatór character of recombinants and parent strains. Recombinants were usually grown with aeration in 2ml cultures in small tubes. Then suitable dilutions were spread on minimal glucose and selective plates. Mutation frequencies were calculated after discarding any plate in each series which had a very high number of resistant clones and revertants so as to avoid "jackpots".

a) Resistant mutants

The frequency of mutation to phage or drug resistance was determined by spreading 0.1ml samples on agar plates, containing a drug or previously spread with phage. Viable cell counts were also made. After an appropriate incubation period the numbers of parental and mutant cells were counted.

b) Reversion frequencies

0.1ml of each bacterial culture (approximately 10^9/ml) was plated on selective medium containing all the requirements for growth with the exception of one requirement. Samples were also plated for viable cell counts. The number of revertants was calculated after 2 days incubation at the required temperature.

16. Recombination rate

Overnight cultures of hyp$^-$ strains were grown in nutrient broth, casamino acids medium or M9 (minimal glucose medium) and diluted 100 fold into the same type of fresh medium and incubated with aeration at 37°C. Between 0-8 hours several samples were withdrawn and the recombination rate measured by plating serial dilutions on both minimal agar lactose and glucose plates which were then incubated for two days at 37°C.

III. RESULTS

A. Introduction

The mechanism(s) of recombination are as yet still obscure but since the isolation of mutants in the recombination pathway(s) by Clark and Margulies in 1965, a picture of how two DNA species recombine is slowly beginning to emerge. Although initially the analysis was purely genetic, recent work on the isolation of recombinant intermediates in bacteria blocked at various stages by the rec mutations have enabled models of recombination to be constructed.

An alternative approach is to look for strains which are more proficient at recombination than the wild-type. The first report of a genetic analysis of hyper-rec mutants was that of Konrad and Lehman (1975). This work similarly is concerned with the isolation and characterisation of novel hyper-rec mutants. They were detected by analysing the frequency of recombination in the lactose gene. The bacteria were seeded onto indicator plates which produced colonies with lactose positive papillae. Wild type colonies produced only one or two, whereas the hyper-rec strains produced many. It was these strains, producing more papillae than normal, that were selected for further study.

B. Isolation of the heterozygous F merodiploid lac$^-$z/F'lac$^-$z strains

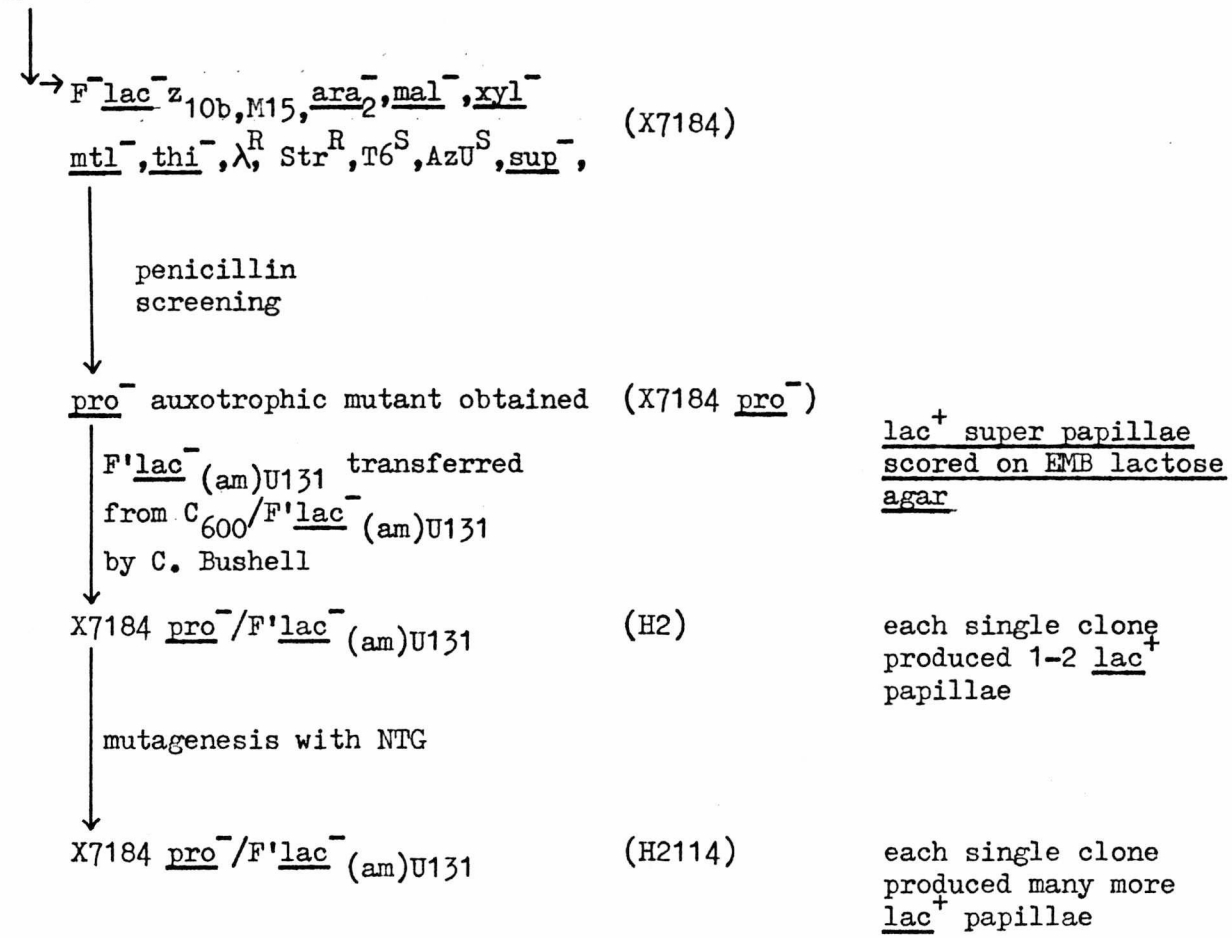


FIGURE 11

Geneaology of H2 and H2114

1. Isolation of superpapillate mutants on indicator plates after treatment with nitrosoguanidine

Most laboratory strains used in genetic analysis have been extensively mutagenized. Of the available strains in our bacterial stocks, F⁻X7184 did not appear at the time to have been subject to much mutagenesis. The F prime F'lac⁻ U131 was introduced so that the genotype of the diploid was lac⁻ z10bM15/F'lac⁻ z(am)U131 called H2 (Figure 11 and 11.a)

H2 was thought a suitable diploid for the work intended. Subsequent work showed this belief was wrong : several defects in H2 showed up in the course of this work. The number of papillae per colony was rarely more than 2 on EMB lactose agar after four days incubation. The frequency for lactose-positive cells was about $2\text{-}4\times10^{-8}$/ml after two days incubation on minimal agar lactose and glucose plates at 37°C. H2 was used for the isolation of the hyper-rec phenotype by looking for very papillate colonies on EMB lactose agar after mutagenesis with NTG (N-methyl-N-nitro-N'-nitrosoguanidine). The plates were incubated at 30°C for four days at dilutions that gave about 100-300 colonies per plate. The normal colonies at this stage showed 1-2 papillae. Those colonies with several papillae were picked and streaked to single colonies on the same indicator medium. Those plates that showed the character to be stable were kept as stock plates for further study.

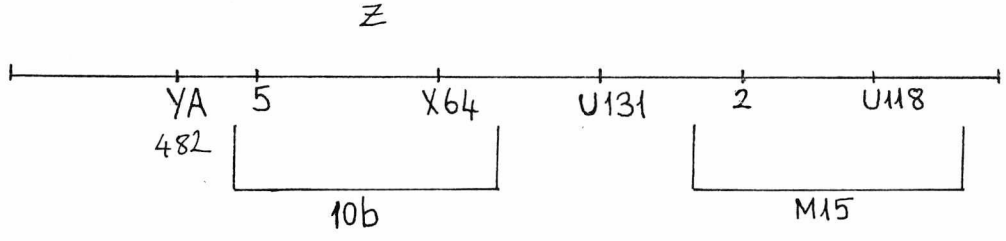


FIGURE 11.a

C. Isolation of hyper-rec mutant, H2114

Colonies with large numbers of papillae on EMB-lactose agar were picked into nutrient broth and grown overnight. Each culture was plated on minimal agar containing lactose in place of glucose. These cultures were also plated on minimal agar plates containing azauracil, to determine the forward mutation rate for one particular genetic marker and minimal agar glucose plates for a viable count. From the numbers of colonies that appeared after 48 hours the frequency of azauracil-resistant mutants and of lactose recombinants in these cultures were estimated. Many strains proved to have high mutation rates and normal or only slightly higher rates of recombination (The *mutH* strain isolated by this method will be discussed on page 109). Some proved to have rates for both properties increased perhaps two or three fold and these have not been further examined ; a few had substantially higher rates of recombination than the parent strain. In general, they too, had higher rates of mutation. Potential hyper-recombination strains were used as recipients in phage P1 transduction experiments. Some mutants with apparently high rates of recombination for the lactose genes gave normal yields of transductants with phage P1. One such strain was examined by Miss Atkinson in detail. She showed that the most likely explanation for its phenotype was an increased number of gene copies of the *lacZ* gene. Gene duplications are known to raise recombination by factors much larger than can be accounted for by the increase in number of copies.

One of the hyper-recombination strains, given the number H2114, has been examined in detail. H2 and H2114 were tested frequently with a male specific phage $\mu 2$. Both were sensitive and presumed still to be merodiploids for the lactose region.

TABLE 1a,b,c,d,e

Rate of recombination for H2114 and H2

Str was used as a counterselective agent for the donor HfrCav, HfrH, HfrKL16

	Strain No.	Donor	Mating time (minutes)	Selected marker	Frequency of recombinants per donor	Efficiency of recombination frequency of H2114/H2
a.	H2	HfrCav	10	*lac*$^+$	2.4×10^{-3}	3.4
	H2114	"	"	"	8.2×10^{-3}	
b.	H2	HfrH	20	*lac*$^+$	2.0×10^{-3}	3.3
	H2114	"	"	"	6.7×10^{-3}	
c.	H2	HfrCav	25	*ara*$^+$	1.05×10^{-3}	16.3
	H2114	"	"	"	1.72×10^{-2}	
d.	H2	HfrH	10	*ara*$^+$	5.0×10^{-3}	6
	H2114	"	"	"	3.02×10^{-2}	
e.	H2 T^-	KL16	10	*thy*$^+$	2.5×10^{-4}	25
	H2114 T^-	"	"	"	6.25×10^{-3}	

TABLE 2

Transfer of R1drd19 DNA's efficiency to H2 and H2114

Strain No.	Donor	Selected Marker	Transfer of donor DNA fragment efficiency per ml.
H2	R1drd19	Cml	7.5×10^{-4}
H2114	"	"	8.5×10^{-4}

1. Rate of recombination for H2114 and H2

The yields of recombinants in standard crosses with HfrH and HfrCav were raised by factors of between 3.3 and 16.3 (Table 1a,b,c,d). KL16 gave 25 times as many *thy*$^+$ recombinants with H2114 than with H2 (Table 1e).

To ensure that the mating and DNA transfer was comparable in the two strains, the efficiency of transfer of the R factor, R1*drd*-19, to the two strains was measured (Table 2).

Log phase cultures of J5-3 R1*drd*-19 were mixed with the H2 and H2114 in a ratio of 1:1. After 25 minutes incubation at 37°C 0.1ml of serial dilutions of the mating mixtures was plated on minimal agar plates containing 5 μg ml^{-1} of chloramphenicol. After two days incubation colonies appeared on Cm plates which were presumed to carry the R1*drd*19 plasmid. H2 and H2114 were equally good recipients of R1*drd*19.

TABLE 3

Frequency of recombinants in crosses with H2 and H2114 as donors

Recipient	Selected Marker	Mating Time	Frequency of recombinants per donor	
			H2	H2114
AB1157	leu	20'	1.8×10^{-4}	8.3×10^{-4}
	argE	35'	7.7×10^{-5}	2×10^{-4}
KL398	leu	20'	8.2×10^{-4}	3.7×10^{-3}
	metE	35'	1.9×10^{-4}	8.7×10^{-4}

Footnote

Exponential phase cultures of donor H2114 and H2 were mixed with cultures of two recipients AB1157 and KL398.

0.1ml of appropriate dilutions of mating mixture were plated on minimal agar plates to select argE and leu recombinants of AB1157, metE and leu recombinants of KL398 and incubated two days at 37°C. Nalidixic acid (50 µg/ml) was used for counter selection.

2. Chromosome mobilisation

The yields of recombinants from crosses in which strain H2114 acted as donor were three to five fold higher than those obtained with the parental F merodiploids (Table 3).

TABLE 4

Transfer of *hyp*$^-$B to KL398 by H2114

Selected Marker	Colonies Scored	MetE$^+$	MetE$^+$*hyp*$^-$	Thy$^+$	Thy$^+$*hyp*$^-$	His$^+$	His$^+$*hyp*$^-$
metE	100	-	4	4	4	0	0
thyA	100	4	0	-	28	4	0
hisF	100	1	0	3	1	-	1

3. Mapping

Because these mutants were induced with methyl nitrosoguanidine, an agent notorious for causing multiple mutations it was necessary to study the mutation in another strain. The transfer of the mutation to another strain yields simultaneously the approximate location of the mutation on the E. coli chromosome map and a better defined genetic background. The F prime, F'lac_{U131} was used to mobilise and transfer the chromosome to KL398 and AB1157 T^-. The yield of hyp^- recombinants was low and the only marker to which it appeared to be linked was the thyA gene (Table 4). 100 threonine, arginine, thymine and histidine recombinants of AB1157 T^- were checked and only one thy^+ recombinant showed the hyper-recombination phenotype on EMB lactose. Since this strain grew slowly it was not investigated further. Therefore we attempted to put the hyp^- gene into a KL398 background which grows better and also has several auxotrophic markers. Other crosses made it clear that hyp^- mutation did not lie in the lac to xyl region.

H2114 was mixed with KL398 in the ratio of 1 to 10 and incubated at 37°C for 70 minutes. The mixture was plated on minimal media which selected for prototrophic recombinants for one of the markers of KL398 and counter-selective for the donor was 100 $\mu g\ ml^{-1}$ spectinomycin. 100 recombinants for each class were restreaked on the selective media and restreaked onto plates that were indicative of the unselected markers and on to EMB lactose. Table 4 shows that among the 100 thymine recombinants 28 of them inherited the hyp^- phenotype. One of the hyp^- thy^+ recombinants was chosen for further study of the hyp^- phenotype and called KL398hyp^-.

In a parallel experiment Thy^+ recombinants were obtained by counter selecting with 50 $\mu g\ ml^{-1}$ nalidixic acid in place of spectinomycin.

6 Thy^{+} spc^{s} recombinants were obtained and 5 had the Hyp^{-} phenotype.

This suggested that *hyp*$^{-}$ genotype was close to the *thyA* region (Table 4). This result at least gave us a clue about the possible location of *hyp*$^{-}$ gene on *E. coli* chromosome. If the *hyp*$^{-}$ gene was near thymine, we should have been able to introduce wild-type *hyp*$^{+}$ gene, by performing crosses and selecting for loci near to thymine. For that reason a *thy*$^{-}$ isolate of H2114 was obtained by trimethoprim selection. Ser^{-}, *try*$^{-}$ and *phe*$^{-}$ mutants were also obtained by penicillin selection. The serine mutant was thought to be *serA*$^{-}$ because HfrKL16 did not produce any serine recombinants in 20' mating whereas ~~the~~ Hfr313 yielded many.

(The *try*$^{-}$ and *phe*$^{-}$ double mutant strain was discarded because the strain produced mucoid colonies, and unusual colours on EMB lactose agar).

The thymine-requiring strain yielded fewer *lac*$^{+}$ papillae on EMB lactose agar than H2114*thy*$^{+}$. H2114*serA*$^{-}$ was plated onto minimal agar lactose and glucose plates. They did not produce any *lac*$^{+}$ recombinants on lactose plates although the viable count was as high as the original H2114. But the strain grew very slowly. After two days incubation at 37°C the size of colonies on glucose plates was about $\frac{1}{3}$ of the original H2114. When the EMB lactose agar medium was supplemented with 0.5% casamino acid streaks of H2114*serA*$^{-}$ yielded a few papillae after 4-5 days incubation at 37°C.

The wild type *thyA*$^{+}$ and *serA*$^{+}$ genes were introduced into H2114*serA*$^{-}$ and H2114*thyA*$^{-}$ either by conjugation or transduction. 100 recombinants of each were checked on EMB lactose agar and they all retained *hyp*$^{-}$ phenotype (Table 5,6,7). Both the *serA*$^{+}$ and *thyA*$^{+}$ recombinants produced the same level of *lac*$^{+}$ papillae on the surface of *lac*$^{-}$ colonies as H2114.

TABLE 5

Hfr x H2114 crosses

	Donor	Recipient	Selected phenotype	Mating time	Number of Recombinants Scored	Phenotype
a	Hfr AB312	H2114	Xyl^+	20'	100	Hyp^-
b	Hfr AB313	H2114 *thy*$^-$	Thy^+	25'	100	"
c	"	H2114 *serA*	Ser^+A^+	25'	50	"
d	"	H2114	Xyl^+	15'	100	"
e	Hfr KL16	H2114 *serA*	Ser^+	90'	50	"
f	"	H2114 *trp*$^-$	Trp^+	35'	100	"
g	"	H2114 *his*$^-$	His^+	25'	100	"
h	"	H2114 *thy*$^-$	Thy^+	10'	100	"
i	Hfr KL14	H2114	Xyl^+	10'	100	"
j	"	H2114	Xyl^+Sm^s	10'	20	"
k	Hfr P4X6	H2114	Xyl^+	40'	100	"

TABLE 5

Recombinants for several of the auxotrophic markers of H2114 recombinants were selected after conjugation with the Hfr 312, Hfr 313, Hfr KL16, Hfr KL14, Hfr P4X6. None of the selected recombinants had lost the Hyp^- phenotype.

$\underline{xyl}^+$ and $\underline{xyl}^+Str^s$ recombinants were obtained from the cross between Hfr's and original H2114. Other recombinants were obtained from crosses between Hfr's and H2114 auxotrophic derivatives.

TABLE 6

Hfr'sX KL398hyp$^-$ crosses

Donor	Recipient	Selected phenotype	Mating time	Number of recombinants scored	Phenotype
Hfr AB312	KL398 hyp$^-$	HisF$^+$	80'	100	Hyp$^-$
"	"	ThyA$^+$	90'	100	"
"	"	Xyl$^+$	20'	100	"
"	"	MetE$^+$	25'	100	"
Hfr AB313	KL398 hyp$^-$	HisF$^+$	45'	100	"
"	"	ThyA$^+$	25'	100	"
"	"	Xyl$^+$	15'	100	"
"	"	MetE$^+$	90'	100	"
Hfr KL16	KL398 hyp$^-$	HisF$^+$	25'	100	"
"	"	ThyA$^+$	10'	100	"
"	"	Xyl$^+$	90'	100	"
"	"	MetE$^+$	90'	100	"
Hfr KL14	KL398 hyp$^-$	HisF$^+$	90'	100	"
"	"	ThyA$^+$	90'	100	"
"	"	Xyl$^+$	20'	100	"
"	"	MetE$^+$	20'	100	"

TABLE 6

Several Hfr's crossed with KL398 *hyp*$^-$ and auxotrophic markers were selected. None of the selected recombinants had lost the Hyp$^-$ phenotype.

TABLE 7

Transduction of wild type *serA*$^+$ and *thyA*$^+$ gene into H2114 *serA*$^-$ and H2114 *thyA*$^-$.

Donor	Recipient	Selected marker	Phenotype
P1(HfrCav)	H2114 SerA$^-$	SerA$^+$	hyp$^-$
P1(H2)	"	"	hyp$^-$
P1(H2114)	"	"	hyp$^-$
P1(HfrCav)	H2114 T$^-$	ThyA$^+$	hyp$^-$
P1(H2)	"	"	hyp$^-$

Frequency of recombinants in transduction

Table 7 shows the result obtained when auxotrophic mutants of H2114 were used as recipients in transduction experiments with phage P1.

100 $ThyA^+$ and $SerA^+$ transductants were checked and all showed Hyp^- phenotype.

From the results so far one could conclude that there might be tremendous unlinking around the thy, ser region of hyp$^-$ chromosome, if the hyp$^-$ gene is very close to thymine.

Since the introduction of wild type serA$^+$ and thyA$^+$ genes did not abolish the hyper-rec phenotype, it was thought worthwhile to select recombinants for other markers near thyA. For this purpose a nal^R variant of the dnaG$^-$ polA lactose positive mutant, NY73, was isolated and mated with H2. 100 leucine recombinants were selected and plated onto minimal agar lactose and glucose plates. 4 of them did not grow on lactose plates. They were presumed to have inherited the lac$^-$ mutation from H2. They all produced 1-3 lactose papillae on EMB lactose agar and plated the male-specific phage μ2. One of these was designated NY73(1). 5ml of NY73(1) was incubated at room temperature for 24 hours to obtain a culture that contained a large fraction of phenocopies. This was then mixed with an exponential-phase culture of H2114 in the ratio of 1:1. The mating was interrupted after 70 minutes, and the mixture plated onto appropriately supplemented minimal agar plates but without thymine. After 2 days incubation at 37^{o}C, 50 Thy$^+$ recombinants were picked and repurified. 32 of them produced 1-3 papillae on EMB lactose agar and 18 of them many more than 1-3 super papillae. These 18 thymine independent recombinants were presumed to have inherited the hyper recombination gene. 6 of them were plated at 30^{o}C and 42^{o}C. All were still dnaG$^-$ and grew at 30^{o}C but not 42^{o}C and all were UV sensitive at 30^{o}C. NY73 hyp$^-$dnaG$^-$ was mated with Hfr KL14 and the met E$^+$ marker was selected. Among the 78 met E$^+$ marker recombinants 16 of them were dnaG$^+$ and grew at 42^{o}C. DNA G$^+$ revertants were also obtained spontaneously by incubating a plate of NY73 dnaG$^-$hyp$^-$ at 42^{o}C. In both cases the strain was still hyp$^-$ and the introduction of dnaG$^+$ did not have any affect on hyper-rec phenotype. We have also concluded

that a defect in polA did not affect the hyper-recombination phenotype.

Introduction of early markers from HfrKL16 and HfrKL14 into H2114, and KL398hyp$^-$ did not affect the hyp$^-$ phenotype (Table 5,6,7). This suggested the hyp$^-$ gene might lie between the two origins of HfrKL16, HfrKL14 and therefore metC mutants seemed to us to be the best marker in that region for which to select recombinants.

In crosses with the argG, metC, thyA recipient CGSC4524 none of the argG$^+$ recombinants were hyp$^-$ there were no metC$^+$ recombinants and none of the thy$^+$ recombinants were metC$^+$ (Table 8). MetC$^+$ recombinants were obtained with normal linkages to the other markers in crosses with HfrCav and HfrH (Table 8). These results suggested the hyp$^-$ phenotype might be the result of a deletion of some part of this segment of the chromosome in H2114 but control experiments with the unmutagenised parent strain H2 and another mutant, H2110, from the same parent gave very similar results (Table 8), although a very few metC$^+$ recombinants were obtained with H2.

These results suggested that the anomaly resided in the parent strain and was not connected with the mutant hyp$^-$ gene. Moreover the anomaly was transferred to the KL398hyp$^-$ clones that acquired the hyp$^-$ phenotype. This was demonstrated by mating KL398hyp$^-$/F'lac$^-$ with CGSC4524F$^-$ strain. No metC$^+$ recombinants from this cross were detected and none of the thy$^+$ and argG$^+$ recombinants were metC$^+$.

An attempt to transfer metC$^-$ mutation into hyp$^-$ strains also failed. CGSC4504,HfrHmetC$^-$ was mated to H2114thy$^-$ and KL398hyp$^-$thy$^-$.

Thy$^+$ and xyl$^+$ recombinants were selected from minimal agar plates supplemented with methionine. 150 thy$^+$ and xyl$^+$ recombinants were streaked onto minimal agar plates without methionine and all grew. It was presumed that none of the thy$^+$ and xyl$^+$ recombinants inherited metC$^-$ mutation. This may make sense of the HfrAB313 x H2114serA$^-$ cross which gave no hyp$^+$ recombinants which it should have done, if hyp$^-$ were near metC.

TABLE 8

The linkage of unselected markers in crosses with CGSC4524

Donor	Selected Marker	Colonies Scored	% coinheritence His	Thy	Met	Arg
H2114(1)	$HisF^+$	120	-	0	0	0
	$ThyA^+$	120	0	-	0	0
	$ArgG^+$	120	0	0	0	-
H2(2)	$HisF^+$	60	-	0	0	0
	$ThyA^+$	60	0	-	0	0
	$MetC^+$	60	0	0	-	7
	$ArgG^+$	60	0	0	0	-
H2110	$HisF^+$	30	-	0	0	0
	$ThyA^+$	30	0	-	0	16
	$MetC^+$	30	0	0	-	0
	$ArgG^+$	30	0	0	0	-
KL398hyp$^-$(3)	$ThyA^+$	50	-	-	0	28
	$ArgG^+$	50	-	12	0	-
HfrCav	$HisF^+$	50	-	64	16	40
	$ThyA^+$	50	4	-	12	25
	$MetC^+$	50	4	40	-	56
	$ArgG^+$	50	0	15	16	-
HfrH	$HisF^+$	50	-	34	100	50
	$ThyA^+$	50	20	-	100	60
	$MetC^+$	50	20	75	-	40
	$ArgG^+$	50	25	50	100	-

TABLE 8

Cross between hyp$^-$ strains and F$^-$ CGSC4524

Donor and recipient cultures were mixed in the ratio of 1:10 and incubated at 37°C for 30 minutes. The cultures were plated on minimal agar selective for each of the selected markers and counter selective for the donor. For the crosses with H2114, H2 and H2110 nalidixic acid was used, for HfrCav and HfrH streptomycin.

(1,3) No *metC*$^+$ recombinants were obtained

(2) H2 gave 10 fold more *arg*$^+$ and *his*$^+$ recombinants than H2114 and the number of *his*$^+$ recombinants was always 3-5 fold higher than *thy*$^+$ recombinants.

4. Mating with F143 and F122

Only among thymine independent recombinants of the recipient strains KL398, AB1157T$^-$ and NY73(1) were found any that had inherited the *hyp*$^-$ phenotype from H2114. Therefore two F' primes F143 and F122 were chosen to make partial diploids for the segments of the chromosome adjacent to the *thyA* gene of the *hyp*$^-$ chromosome. Attempts were made to cure KL398*hyp*$^-$ and H2114 of the F'*lac*$^-$ to provide suitable *hyp*$^-$ recipients by using the temperature sensitive F'*lac*$^+$ method. Exponential cultures of $C_{600}F'lac^+$ ts114 were mixed at a ratio of one donor to one recipient cell with overnight cultures of H2114 and KL398*hyp*$^-$ which had been grown with aeration at room temperature. The mating mixtures were incubated at 30°C for 30 minutes, mating pairs separated by violent agitation and suitable dilutions plated onto minimal agar lactose plates. After two days incubation at 30°C *lac*$^+$ colonies were repurified by streaking again on EMB lactose agar, inoculated into nutrient broth and incubated at 30°C. When cultures reached early log phase they were moved to a 42°C incubator. After overnight incubation appropriate dilutions were plated onto EMB lactose agar and incubated 3 days at 42°C. 50-70% of clones were either pink or had pink sectors. It was hoped that these pink clones had lost the temperature sensitive F'*lac*$^+$. Surprisingly all the clones tested were sensitive to phage μ2 and were therefore F$^+$. Presumably the *lac*$^+$ clones carried more than one copy of F and lost the temperature sensitive F'*lac*$^+$ at 42°C.

Although the curing method did not work it was still thought that direct selection of *thy*$^+$ recombinants after mating with donor strains carrying F122 or F143 might yield diploids which would allow a test for dominance of the *hyp*$^-$ allele.

TABLE 9

Cross between the F122 and F143 and hyp$^-$ strains

Donor	Recipient	Selected Marker	Colonies Scored	Number of hyp$^-$ clones	Number of mut$^+$ clones
F143	H2114 T$^-$	Thymine	150	148	2
F143	KL398hyp$^-$T$^-$	Thymine	150	148	2
F122	H2114 T$^-$	Thymine	150	150	0
F122	KL398hyp$^-$T$^-$	Thymine	150	150	0

TABLE 10 (Corresponding to Table 9)

Phenotypic behaviours of the non papillae sexductants

Strain(1)	Mating F' number	papillae on EMB	Mutation rate against to Nal.	Recombinants received by mating with KL398			
				Thy$^+$	his$^+$	leu$^+$	F' ?
H2114(1)	F143	none	1.5×10^{-7}	+	+	-	F143
H2114(2)	F143	none	1×10^{-7}	+	+	-	F143
H2114(3)	F143	many	1×10^{-6}	-	-	+	F'lac
H2114(4)	F122	many	1.1×10^{-6}	-	-	+	F'lac
H2114(5)	F122	many	1.3×10^{-6}	-	-	+	F'lac
				Mating with CSGC4524			
				ThyA$^+$	metC$^+$	argG$^+$	F' ?
KL398hyp$^-$(1)	F143	none	8.7×10^{-8}	+	-	+	?
KL398hyp$^-$(2)	F143	none	1.6×10^{-7}	+	-	+	?
KL398hyp$^-$(3)	F143	many	1.2×10^{-6}	-	-	-	F'lac
KL398hyp$^-$(4)	F122	many	1.3×10^{-6}	-	-	-	F'lac
H2114(1)	F143	none	1.5×10^{-7}	+	-	+	?
H2114(2)	F143	none	1×10^{-7}	+	-	+	?
H2114(3)	F143	many	1×10^{-6}	-	-	-	F'lac
H2114(4)	F122	many	1.1×10^{-6}	-	-	-	F'lac

Footnote

H2114(1),(2),(3),(4),(5) and KL398hyp$^-$(1),(2),(3),(4) were separate thy$^+$ clones selected from mating with F143 and F122. H2114(1),(2) and KL398hyp$^-$(1),(2) thy$^+$ clones (sexductants) did not show the mut$^-$ phenotype on EMB lactose agar.

Thymine requiring mutants of KL398*hyp*$^-$ and H2114 were isolated by trimethoprim selection. Log phase cultures of strain carrying F143 and F122 were mixed with an overnight culture of the *hyp*$^-$ *thy*$^-$ in a ratio of one to one. After 30 minutes mating at 37^{o}C thymine independent recombinants were sought using azauracil or spectinomycin as counter selective agents. All the *thy*$^+$ recombinants from the crosses between F122 and the *hyp*$^-$ strains had retained the hyper-recombination phenotype (Table 9). A few of them chosen at random, were tested for the mutator phenotype and all found to be mutators (Table 10). These *thy*$^+$ clones were also mated with a suitable female strain and all yielded *leu*$^+$ but not *thy*$^+$ recombinants after 30 minutes mating. We presumed that the F'*lac*$^-$ was still present in the *hyp*$^-$ strains and that the attempt to displace it with F122 had been unsuccessful. However, the results from the crosses between F143 and the *hyp*$^-$ strains were different. Two *thy*$^+$ clones from each cross had lost both the papillae appearance and mutator activity (Table 9). Of course it would not be expected to give many papillae when the F'*lac*$^-$ was lost and one might expect that the F143 would be dominant if it carried the wild type allele. This result was not consistent with the result of the previous crosses with Hfr*KL16*. Because the *hyp*$^+$ recombinants had not been obtained with either H2114 or KL398*hyp*$^-$ after selection for thymine independence either by conjugation and transduction although F143 was derived from *KL16* (Low, K.B., 1972). These two strains were therefore examined further.

Firstly their capacity as donors was examined. Crosses using these *thy*$^+$ sexductants (?) and CSGC4524 as recipient yielded *his*$^+$ and *thy*$^+$ recombinants (1) but not *met*C$^+$ recombinants.

But it was noted that some *arg*G$^+$ recombinants were obtained at early

Footnote :

(1) The yield of *thy*$^+$ recombinants was rather low.

times (Table 10). This latter result was rather surprising as *argG*$^+$ recombinants should have been detected only at the very end of chromosome transfer. Further the *argG*$^+$ recombinants had not inherited the *hisF*$^+$, *thyA*$^+$ and *metC*$^+$ loci. While none of the *thyA*$^+$ and *hisF*$^+$ recombinants were inherited the *argG*$^+$ or *metC*$^+$ genes. One possible explanation is that these *argG*$^+$ recombinants had been obtained from zygotes in which transfer of the episome has been reversed so that *argG*$^+$ was an early marker.

It has been shown that invertant males can occur and have been studied in strains that carry the *Ra1* episome. However, invertant males are usually only detected after UV irradiation (Joset, Fournier, 1966).

These results were inconclusive, although surprising, and the location of *hyp*$^-$B remained unresolved. However, if the F143 results were right, *hyp*$^-$ must be counter clockwise of *thyA*. It was unfortunate that it was not possible to pursue this point because it should be possible with these two F-primes to locate the *hyp*$^-$ gene unambiguously. In this point it was not possible to suggest whether the hyper-recombination and mutator phenotypes are the result of a single mutation or two closely-linked.

More recent work on KL398*hyp* has shown that *hyp*$^+$ is carried by F143 and that the mutator phenotype is recessive (G. Mackinnon - private communication).

5. Recombination of closely linked markers

A single marker is incorporated into the genome of the host by two recombination events to produce an insertional recombinant. Lysates of P1 grown in the arabinose-negative strains W945, D13.1, C_{600}*ara*$^-$, AB2070, AB1157, CR63*ara*$^-$ were used to transduce H2114 and H2 to Ara$^+$ (Table 11). These experiments were repeated when KL398*hyp*$^+$ and KL398*hyp*$^-$ (Table 12) became available. The yield of recombinants when the P1 had been grown on the *ara*$^+$ strain HfrCav was 3.4 fold higher in H2114 compared to H2 (Table 11).

When the generation of *ara*$^+$ recombinants required one cross-over event within the arabinose operon the difference between the two strains was more marked and the difference was greater the shorter the distance between the two mutant sites. The linkage of the arabinose and leucine genes, 75% in KL398*hyp*$^+$, is slightly low, however in KL398*hyp*$^-$ it was only 63% (Table 13). The linkage of *ara* and *leu* was measured in KL398*hyp*$^+$ and KL398*hyp*$^-$ by conjugation with HfrCav and HfrH and by P1 transduction. Leucine recombinants were selected, purified and streaked on minimal agar arabinose plates and incubated for two days. Arabinose positive recombinants were scored (Table 13). The same experiment was performed with Hfr*H* mating for ten minutes (Table 13).

TABLE 11

Yield of ara^+ recombinants following P1 transduction

Donor Strain	gene designation	Frequency of ara^+ transductant (1)		Efficiency of transduction frequency of $\frac{H2114}{H2}$
		H2	H2114	
Hfr Cav	*ara*$^+$	4×10^{-6}	1.35×10^{-5}	3.4
AB1157	*araC*$^-$	0	2.7×10^{-7}	-
W945	*araC*$^-$	0	1.1×10^{-7}	-
D13.1	*ara*$^-$	2×10^{-8}	5.2×10^{-7}	26
AB2070	*ara*$^-$	3.3×10^{-8}	5.7×10^{-7}	17.2
C_{600}*ara*$^-$	*ara*$^-$(2)	7.3×10^{-8}	1×10^{-7}	1.3
CR63*ara*$^-$	*ara*$^-$(2)	1.5×10^{-8}	1.9×10^{-7}	12.6

Footnote

(1) The frequency is expressed as recombinants per plaque forming unit.

(2) The gene(s) affected in these strains have not been identified.

TABLE 12

Efficiency of *ara*$^+$, *met*$^+$. *his*$^+$, *pro*$^+$ transduction by phage P1 grown in *ara*$^-$, *met*$^-$, *his*$^-$, *pro*$^-$ mutants.

Donor Strain	gene designation	Frequency of transductants		Efficiency of transduction frequency of $\frac{\text{KL398 hyp}^-}{\text{KL398 hyp}^+}$
		KL398 hyp$^+$	KL398 hyp$^-$	
AB2070	*ara*$^-$	0	2.4×10^{-6}	-
	met$^-$	1.3×10^{-7}	8.3×10^{-7}	6.3
	his$^-$	2×10^{-8}	2.35×10^{-7}	11.7
	pro$^-$	5.4×10^{-6}	5.2×10^{-6}	9×10^{-1}
CR63 *ara*	*ara*$^-$	0	1×10^{-6}	-
C_{600} *ara*	*ara*$^-$	1.5×10^{-8}	2.5×10^{-6}	166

TABLE 13

% linkage of arabinose gene to the leucine gene.

Donor Strain	Mating time (minutes)	colonies scored	selected marker	% linkage to ara^+	
				KL398 hyp^+	hyp^-
Hfr Cav	15	120	leucine	92	81
Hfr H	10	100	"	100	100
P1(Hfr Cav)	25	67	"	75	63

TABLE 14

Effect of hypB on mutation frequencies

Mutation	KL398 hyp^+	KL398 hyp^-
$leu^- \rightarrow leu^+$	1.1×10^{-8}	3×10^{-7}
$ara^- \rightarrow ara^+$	0	1.1×10^{-7}
$Nal^S \rightarrow Nal^R$	2.9×10^{-8}	1.2×10^{-6}

	H2	H2114	H2110 (1)
$ara^- \rightarrow ara^+$	0	2.2×10^{-8}	0
$Nal^S \rightarrow Nal^R$	3×10^{-8}	7×10^{-7}	1.5×10^{-5}

6. Mutation frequencies

The frequency of mutants resistant to nalidixic acid ~~were~~ was measured in cultures of H2114 and KL398 hyp$^-$ and compared with the corresponding frequencies in cultures of H2 and KL398 hyp$^+$ (Table 14). Leucine revertants of KL398 hyp$^-$ and KL398 hyp$^+$ were also measured (Table 14). Although no arabinose positive revertants of H2 and KL398 hyp$^+$ were obtained they did appear at a low frequency in H2114 and at a higher frequency in KL398 hyp$^-$ (Table 14).

Table 14

(1) H2110 was included here as an isogenic mutator strain, and will be the subject of the second section of the thesis.

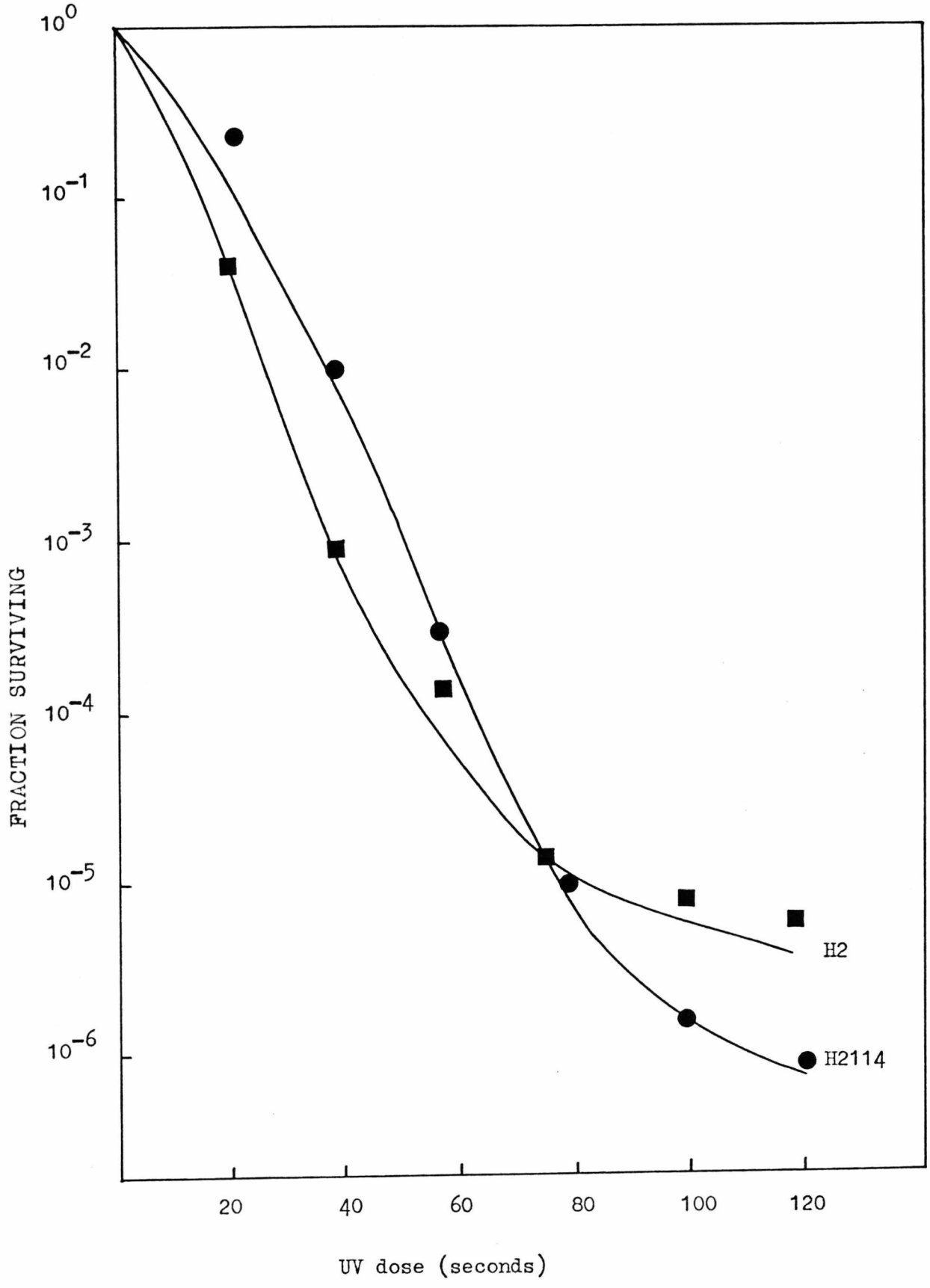

10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
FRACTION SURVIVING
20
40
60
80
100
120
UV dose (seconds)
H2
H2114

FIGURE 12

FIGURE12 UV survival curves

■ H2

● H2114

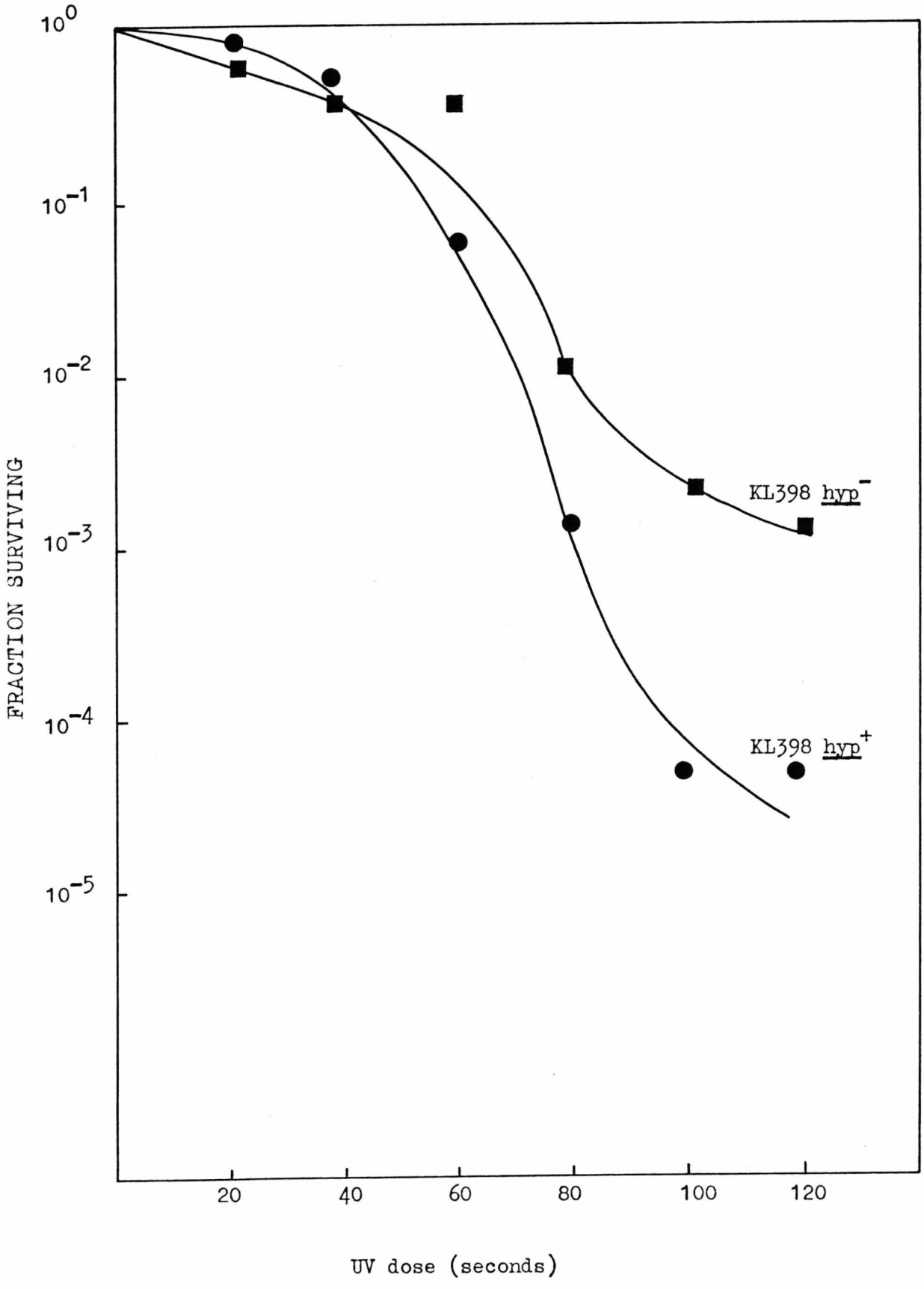

10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
FRACTION SURVIVING
KL398 hyp⁻
KL398 hyp⁺
20
40
60
80
100
120
UV dose (seconds)

FIGURE 13

FIGURE 13 UV survival curves

■ KL398 *hyp*$^{-}$

● KL398 *hyp*$^{+}$

7. UV survival curves

H2 is itself a UV sensitive strain in that its survival curve has no shoulder (Figure12) and although H2114 was not markedly different it was necessary to check the effect of the hyp$^-$ mutation once it had been transferred to a strain with a more nearly normal UV survival curve. However, the two strains KL398 hyp$^+$ and KL398 hyp$^-$ did not differ significantly in resistance to UV irradiation (Figure13). This result suggests that the product of the hyp gene is not involved in UV repair mechanisms.

8. Thymineless death

In thymineless death single strand gaps are left in the growing nucleotide chain, some of which are repaired probably in a similar manner to UV damage. Therefore one would expect that the responses to UV irradiation and thymine starvation would be similar. Thymineless death curves of thyA$^-$ derivatives of H2114 and H2 supported this hypothesis (Figure14,15). The hyper-rec gene had no effect on the process that causes thymineless death.

9. Sensitivity to methylmethane sulphonate

To determine the ~~survival~~ resistance to MMS, hyp$^+$ and hyp$^-$ strains were plated onto nutrient agar plates supplemented with MMS to a final concentration of 0.025M. MMS did not have any effect on either hyp$^+$ or hyp$^-$ strains.

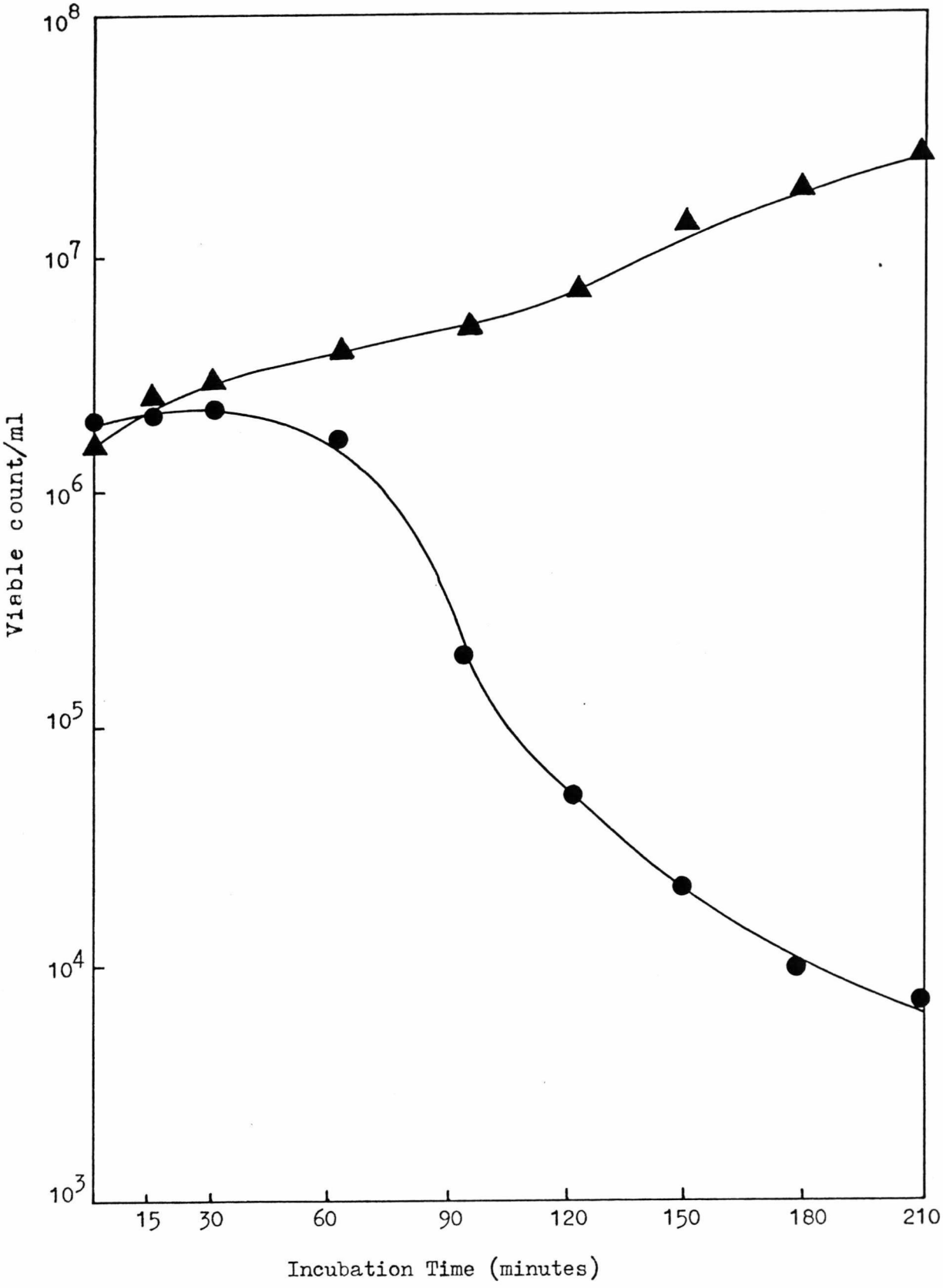

10^8
10^7
10^6
10^5
10^4
10^3
Viable count/ml
15
30
60
90
120
150
180
210
Incubation Time (minutes)

FIGURE 14

FIGURE 14

● Survival of a thyA derivative of H2114 after thymine deprivation in M9 + glucose minimal medium

▲ Growth curve of a thyA derivative of H2114 in M9 + glucose + thymine minimal medium

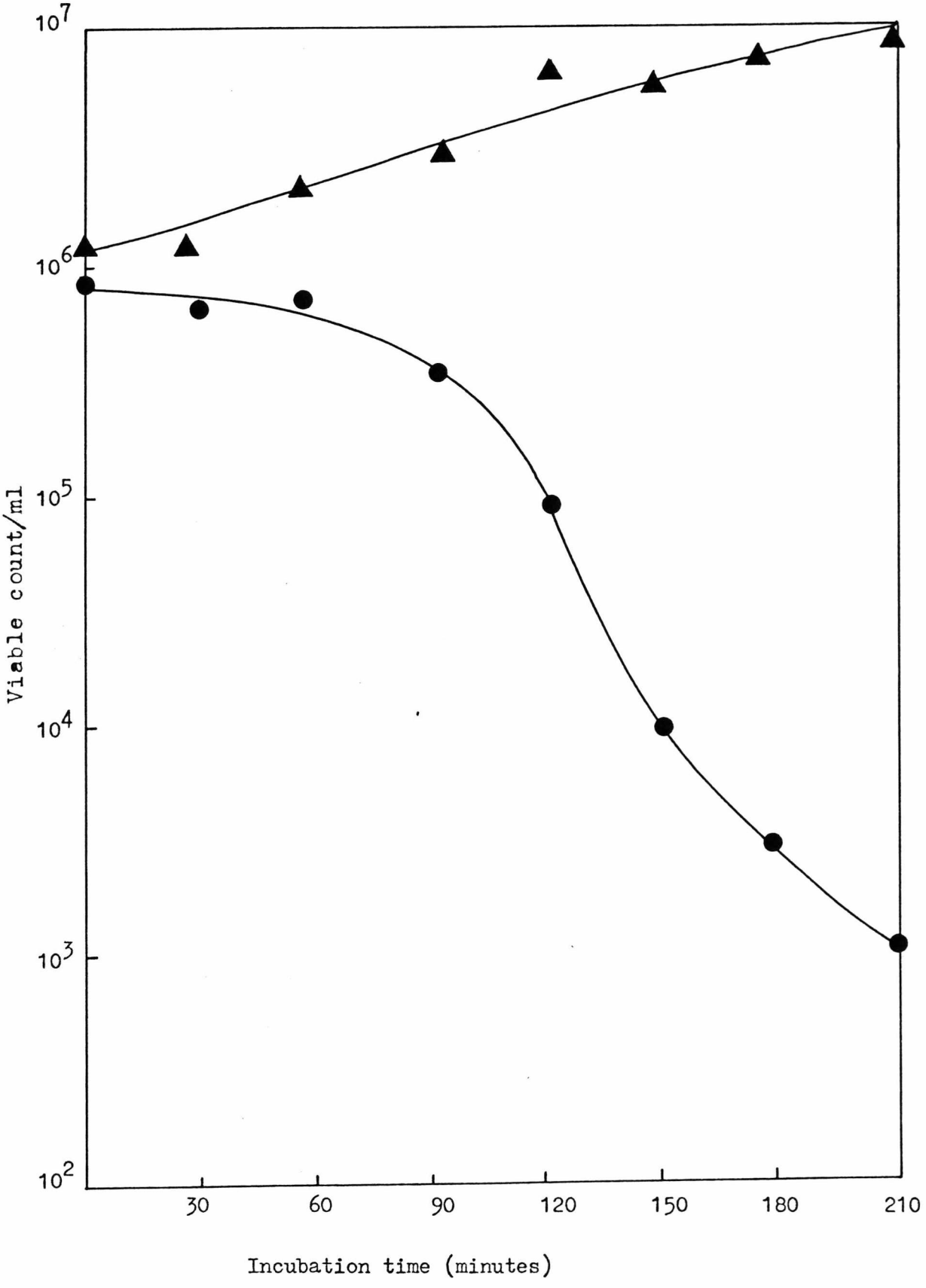

10^7
10^6
10^5
10^4
10^3
10^2
Viable count/ml
30
60
90
120
150
180
210
Incubation time (minutes)

FIGURE 15

FIGURE 15

● Survival of thyA derivative of H2 after thymine deprivation in M9 + glucose minimal medium

▲ Growth curve of thyA derivative of H2 in M9 + glucose + thymine minimal medium

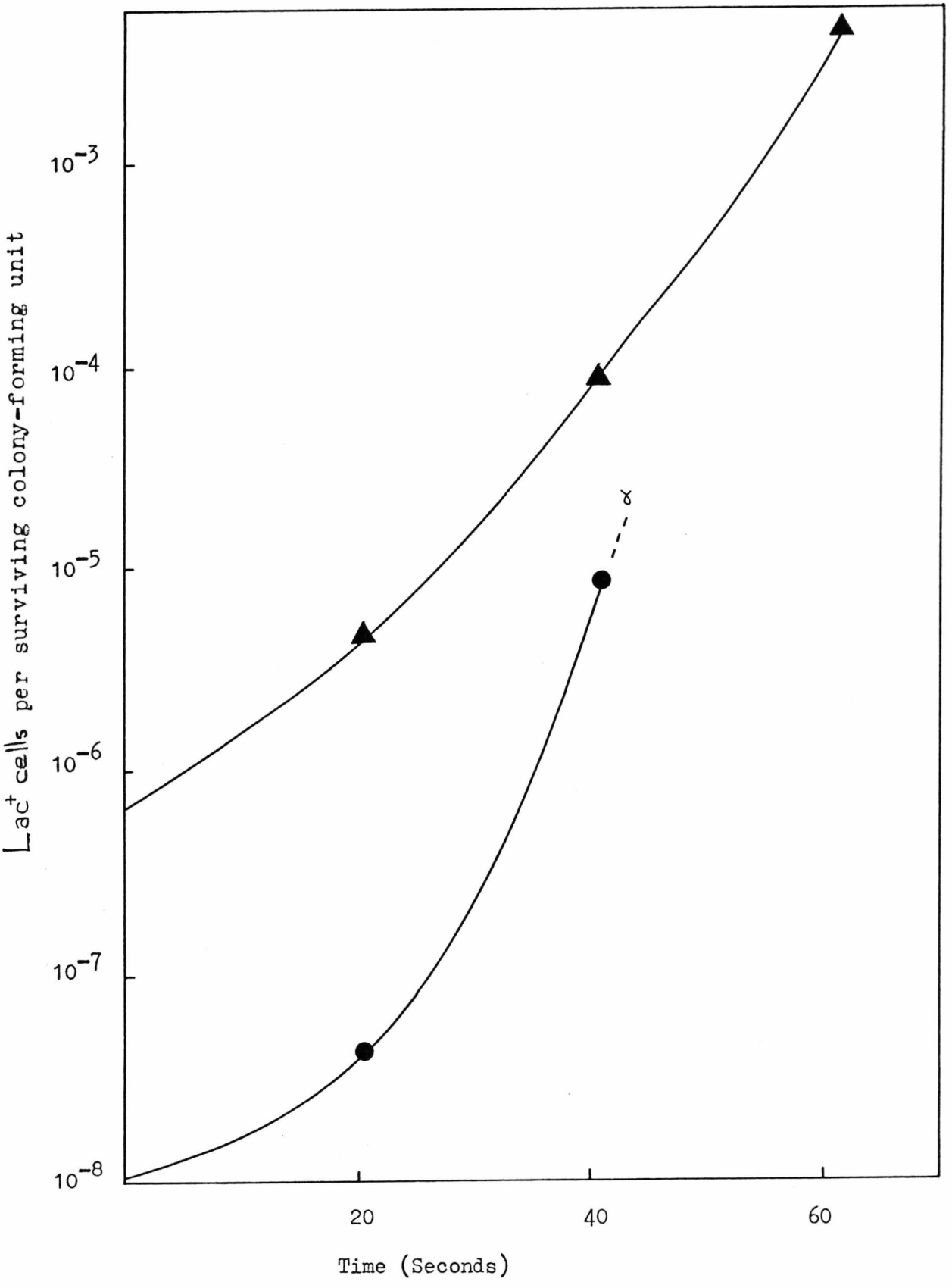

Lac+ cells per surviving colony-forming unit
10-3
10-4
10-5
10-6
10-7
10-8
20
40
60
Time (Seconds)

FIGURE 16

FIGURE 16

Stimulation of production of recombinant *lac*$^+$ cells after UV irradiation

● H2

▲ H2114

10. UV stimulation of Lac$^+$ cells

UV and thymineless death increase recombination probably by causing single strand gaps which act as pointsfor recombination. Therefore the number of recombinants in the survivors will be high. Thymine starvation did not much affect recombination in H2114, however, UV stimulated recombination in both H2114 and H2 (Figure 16).

TABLE 15

Effect of a recB21 mutation on the yield of recombinants in a hyp strain

Donor	Marker Selected	RECIPIENTS (Frequency of recombinants)			
		KL398 hyp$^-$	KL398 hyp$^-$ recB21	H2114	H2114 recB21
HfrH	Leu	5.6×10^{-3}	6.5×10^{-6}		
	His	1×10^{-5}	3.75×10^{-8}		
P1 grown in	Leu	7×10^{-5}	1×10^{-9}		
HfrCav	Ara			7.75×10^{-6}	1.25×10^{-9}
HfrH	Ara			1.5×10^{-5}	2.5×10^{-7}
HfrKL14	Xyl			3×10^{-5}	1.25×10^{-6}

11. Phenotype of hyp⁻ recB⁻ double mutant

The following experiments were performed in order to determine ~~in~~ which pathway of recombination, the hyp⁻ mutation affected.

P1 grown on S491recB21 was used to transduce KL398 hyp⁻ thy⁻ and H2114 thy⁻ for thymine independence. Few recombinants were obtained because P1 grew poorly in the recB21 strain but among them were those that had coinherited the recB mutation. They were used as recipients in conjugation with HfrH and HfrKL14 and in transduction by phage P1. In both types of cross the yield of recombinants was reduced by factors of up to 10^3 in the recB strain compared to recB⁺ isogenic parent. No effect of the hyp⁻ mutation could be reliably discerned (Table 15).

From this result we presumed that when the recBC pathway was blocked ~~and~~ the difference between the hyp⁻ and hyp⁺ was abolished. Therefore the hyp⁻ effect presumably involves the recBC pathway.

TABLE 16

Cross between λvsusP3 and λvsus029

Bacterial strain	Strains in cross	m.o.i.	Burst size	% sus$^+$ recombinants
KL398 hyp$^+$	SusP3 x Sus029	7.5	175	0.34
KL398 hyp$^-$	SusP3 x Sus029	3.7	151	0.61

TABLE 17

Cross between λvsusP3 and λvsus0206

Bacterial strain	Strains in cross	m.o.i.	Burst size	% sus$^+$ recombinants
KL398 hyp$^+$	SusP3 x Sus0206	12.5	133	0.10
KL398 hyp$^-$	SusP3 x Sus0206	3.41	87	0.19

12. Recombination of phage λ

It is believed that vegetative recombination in λ may involve either the Rec system of the host, or the Red system of the phage (Echol, Gingery, 1968; Signer, Weil, 1968). And again it is believed that λ provides for a specific inhibition of the RecBC recombination pathway through an inhibition of the activity of the RecBC nuclease by the product of the gam gene (γprotein), (Unger and Clark, 1972). It was therefore of interest to see if the hyp⁻ mutation affected λ recombination. The yields of sus⁺ recombinants from mixed infections with λvsus0 and λvsusP in KL398 hyp⁺ and KL398 hyp⁻ were measured to see if the effect of the hyp mutation persisted when the recombination systems are modified by the introduction of the red⁺ and γ genes of phage λ. The yield of sus⁺ recombinants in the hyp⁻ strain was twice that in the hyp⁺ parent, although the total phage yield was reduced to 60-80% of that in the parent strain (Tables 16 and 17).

In these experiments C_{600} was used as the permissive indicator strain for the assay of the λsus progeny and S491 was the restrictive indicator on which only λsus⁺ recombinants formed plaques. For these crosses KL398 hyp⁻ and hyp⁺ were used because these strains were λ^S whereas H2114 was λ^R. H2114 gave very peculiar mucoid colonies on minimal agar maltose plates so no attempt was made to select mal⁺ revertants or mal⁺ recombinants of H2114. We were not able, therefore, to look for mal⁺λ^S colonies of H2114.

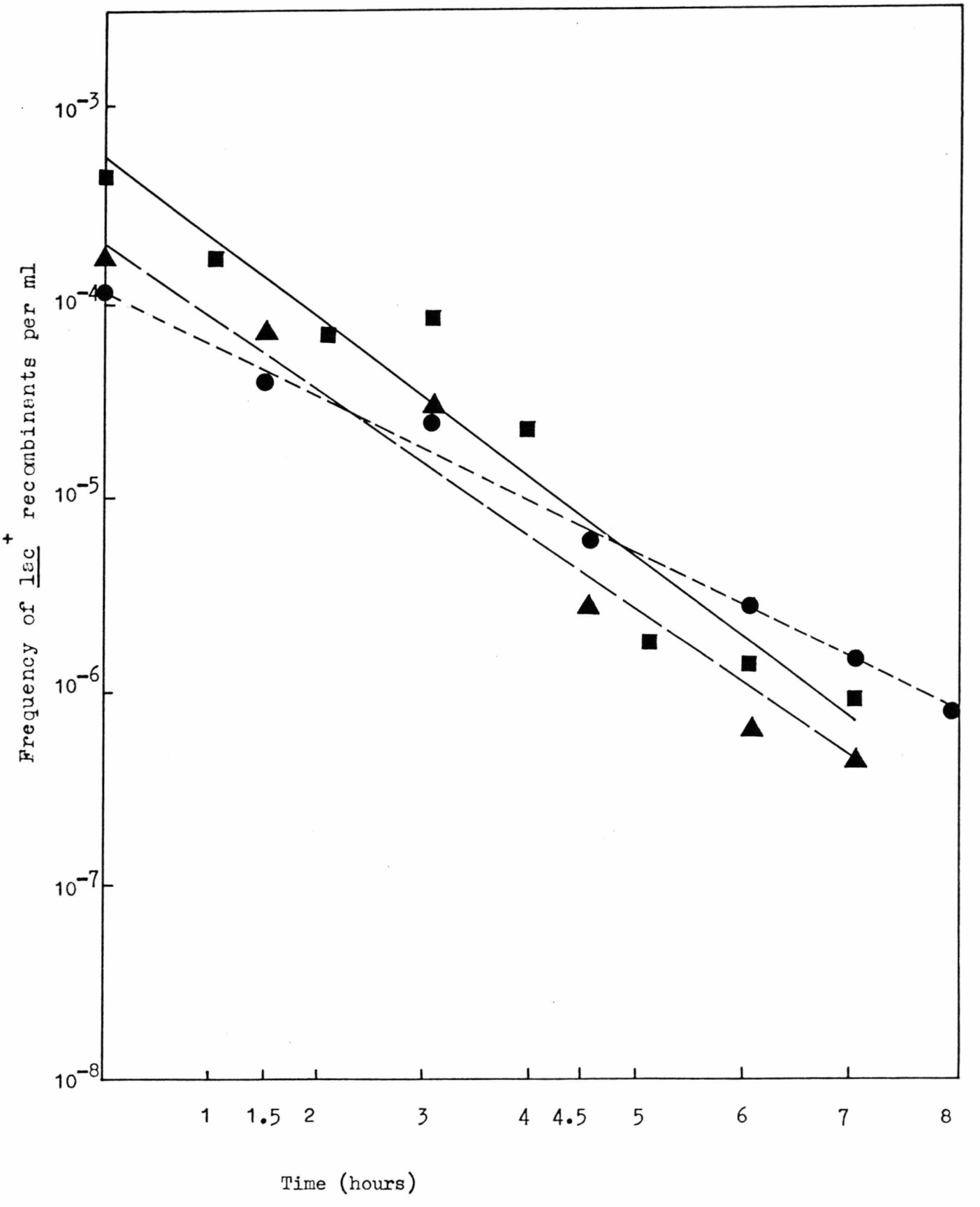

Frequency of lac+ recombinants per ml
10-3
10-4
10-5
10-6
10-7
10-8
1
1.5
2
3
4
4.5
5
6
7
8
Time (hours)

FIGURE 17

FIGURE 17

Variation of the frequency of *lac*$^+$ recombinants during bacterial growth of H2114 in different types of medium.

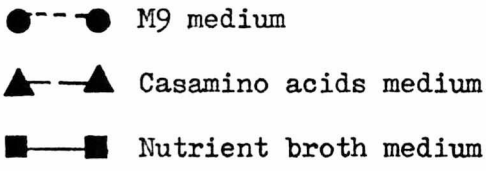


TABLE 18

Effect of medium on the expression of the hyp^- phenotype

Strain	Type of medium	Recombination frequency for lactose marker
H2114	(M9)	8.33×10^{-6}
	(Casamino acids)	4×10^{-6}
	(Nutrient broth)	5×10^{-6}
	(Nutrient broth anaerobically)	3×10^{-6}

Footnote :

Assays were performed by plating the overnight stationary culture onto minimal agar lactose and minimal agar glucose plates.

13. Effect of growth rate on the hyp$^-$ phenotype

Reference has already been made to hyp$^-$ strains that were slow growing and showed a reduced number of lac$^+$ papillae on EMB agar. However, attempts to vary the growth rate in liquid medium by comparing the yield of lac$^+$ cells from H2114 in nutrient broth, casamino acid medium and in minimal glucose medium gave no indication that the rate of recombination was affected by differences in growth rate during exponential growth. Indeed this experiment showed that high yields of recombinants were obtained for the hyp$^-$ strain only in stationary phase cultures; the yield fell during exponential growth (Figure17). The recombination frequency at zero time was 100 fold higher than the result obtained for cultures entering the stationary phase. This was first thought to be an effect of clumping on prolonged incubation which might have increased the apparent recombination rate. However, this was subsequently shown not to be so. Stationary phase cultures were agitated and checked under the light microscope and found not to contain many clumps. It was very similar to the log phase culture seen under the microscope. Figure17 shows the variation of the recombinant frequency during bacterial growth.

The type of medium did not affect the frequency of recombinants (Table 18). H2114 was also grown anaerobically as described in the materials and methods. 5ml of nutrient broth culture of H2114 was incubated for 48 hours by using a Baird and Tatlock anaerobic jar. After incubation the culture was removed from the jar and 0.1ml of H2114 was plated onto minimal agar lactose and glucose plates. After 2 days incubation the numbers of lactose positive cultures were counted.

The recombination frequencies for lactose did not show a significant difference from that obtained for cultures grown aerobically (Table 18).

H2114 was streaked onto EMB lactose agar and incubated anaerobically

for one week at 37°C. After incubation each streak showed several black dots on the surface of *lac*$^-$ clones. These black and greenish dots were streaked onto minimal agar lactose plates and all found to be lactose positive.

14. EM photographs

It was noted that the growth characteristics of the wild type *hyp*$^+$ and the hyper-rec differed, the latter having an apparently faster growth rate. It was noticed initially by a greater turbidity of a liquid culture when compared to the *hyp*$^+$. It was also noted that the cells seemed to be smaller, judging from the size of the centrifuged pellet, although the actual total viable count was greater than that of the wild type. These results were confirmed from light microscopy of KL398 *hyp*$^+$ and KL398 *hyp*$^-$. The hyper-rec strain appeared to have smaller cells.

Electron micrographs of cells from both cultures suggest that in the *hyp*$^-$ cells there seemed to be a higher concentration of DNA per cell as when compared to the *hyp*$^+$ cells but no structural difference could be seen (Figure 18,19,20,21).

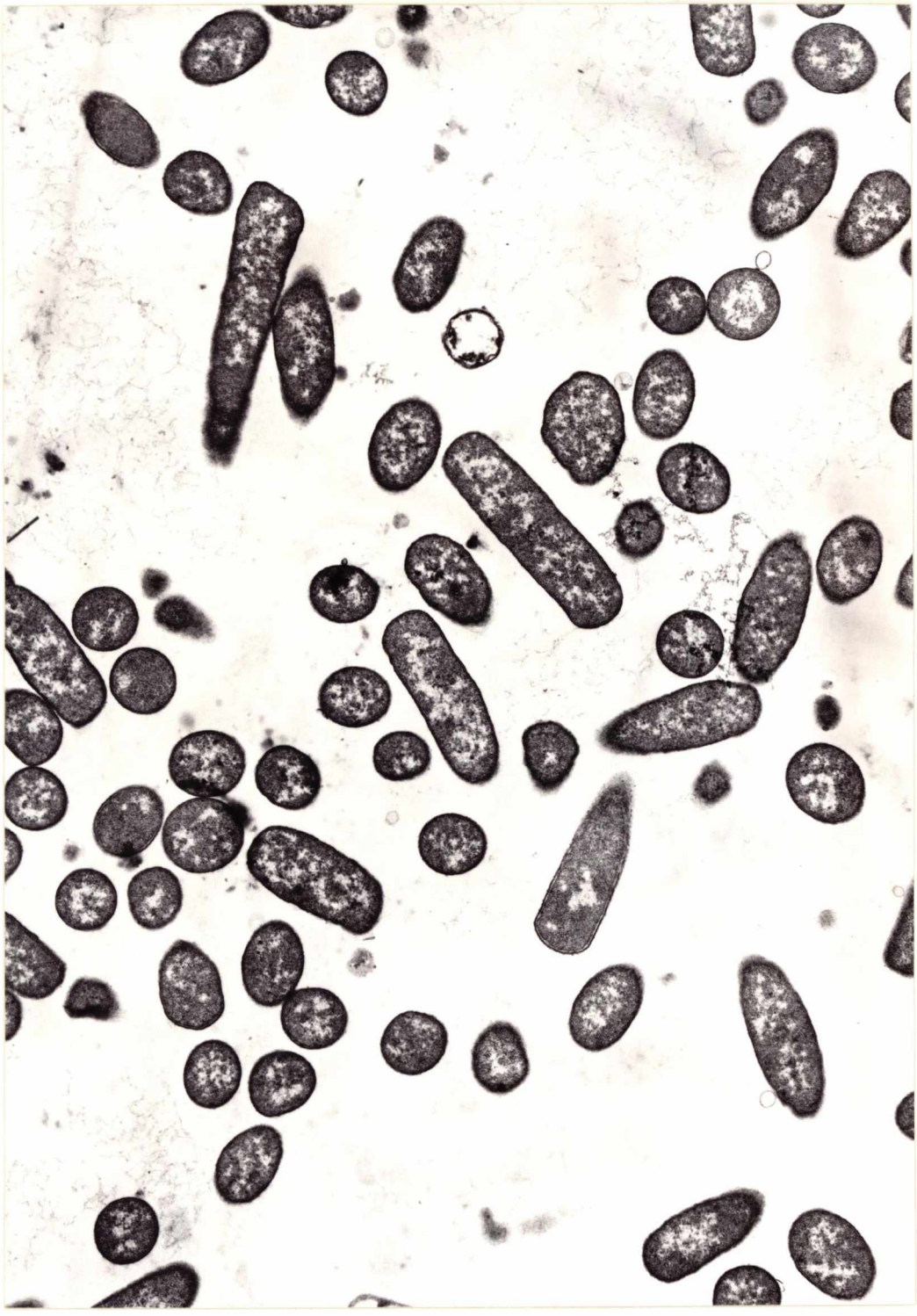


FIGURE 18 Electron micrograph of thin section of KL398 *hyp*$^{+}$

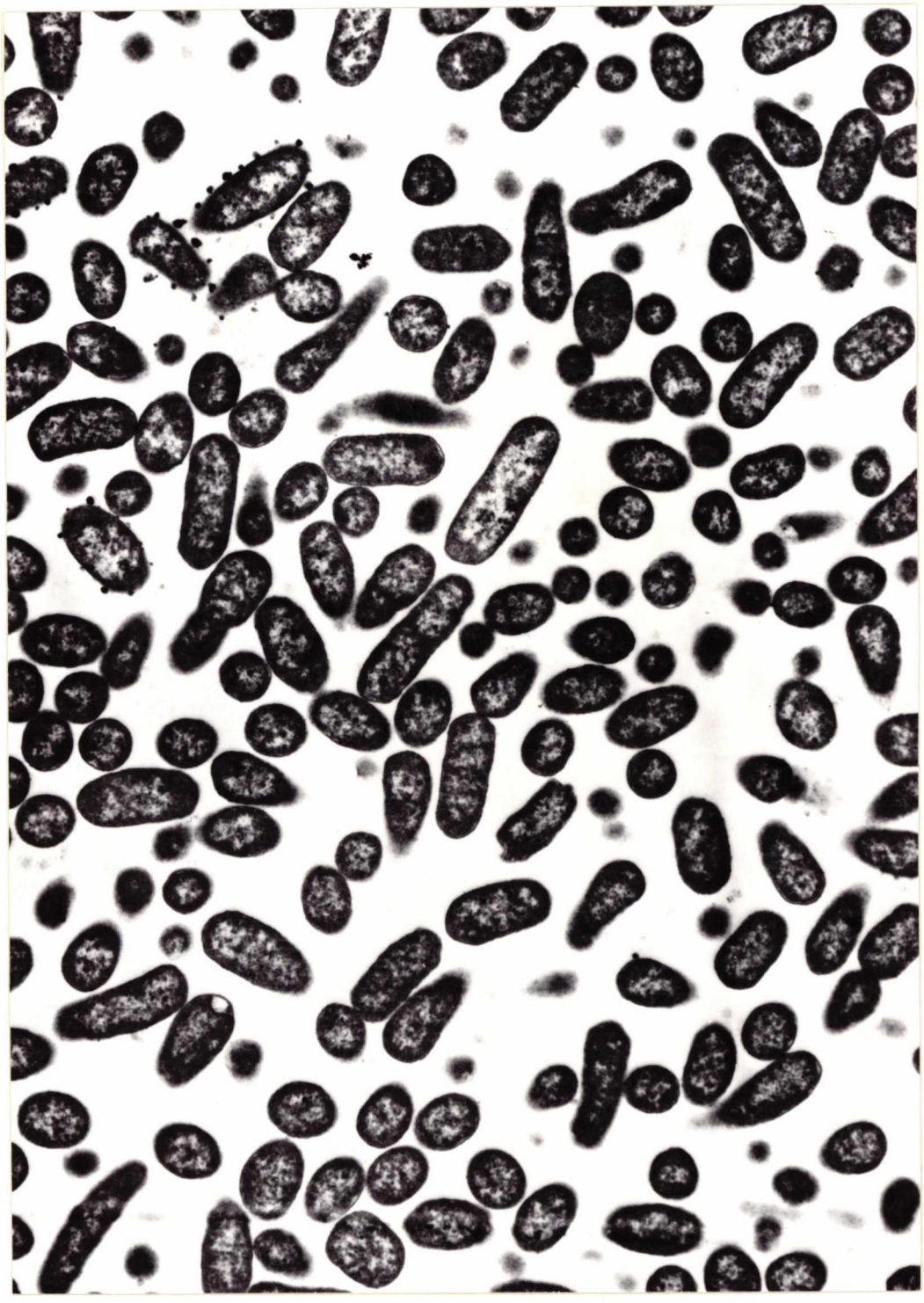


FIGURE 19 Electron micrograph of thin section of KL398 *hyp*$^-$

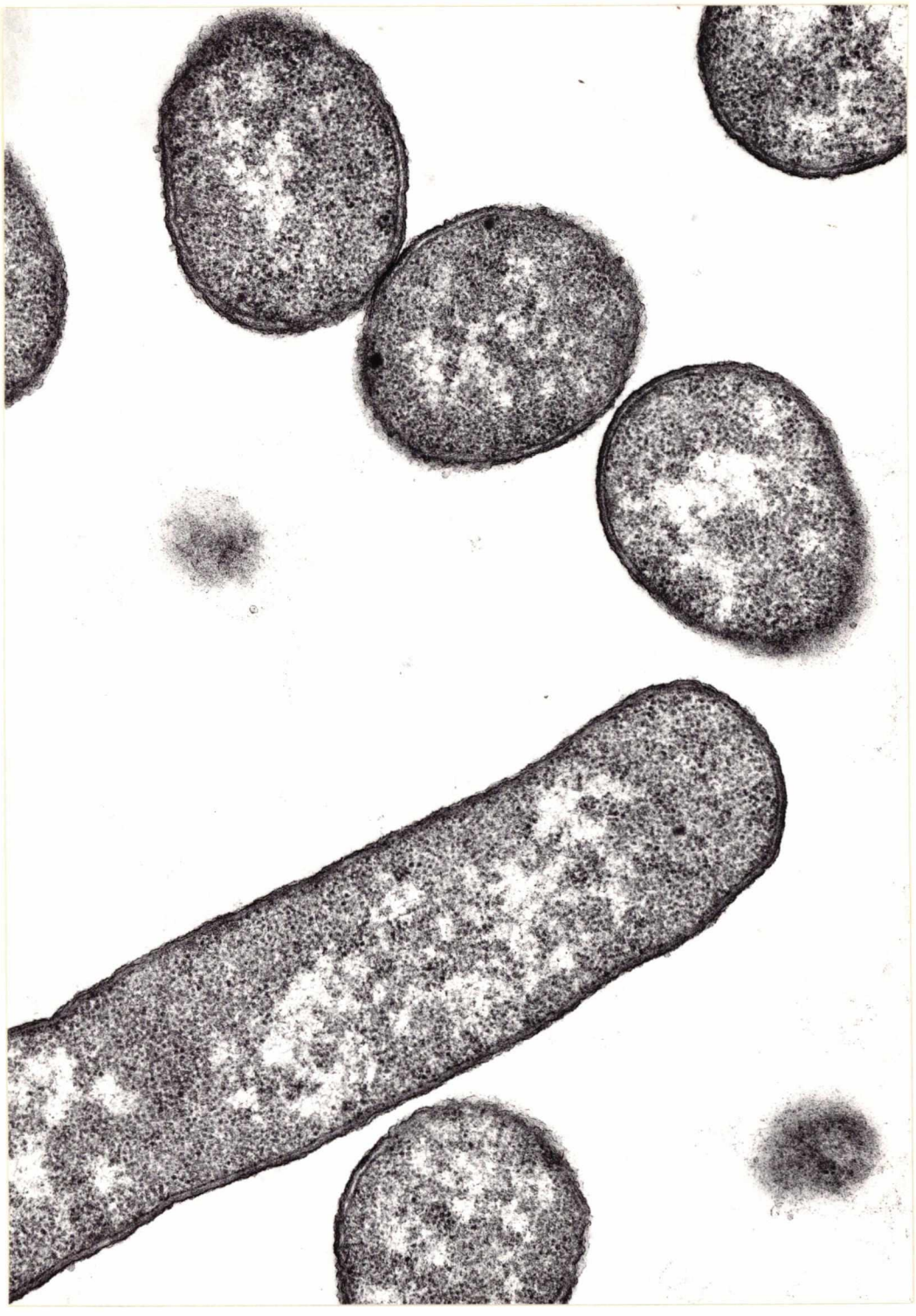


FIGURE 20 Electron micrograph of thin section of KL398 hyp$^+$

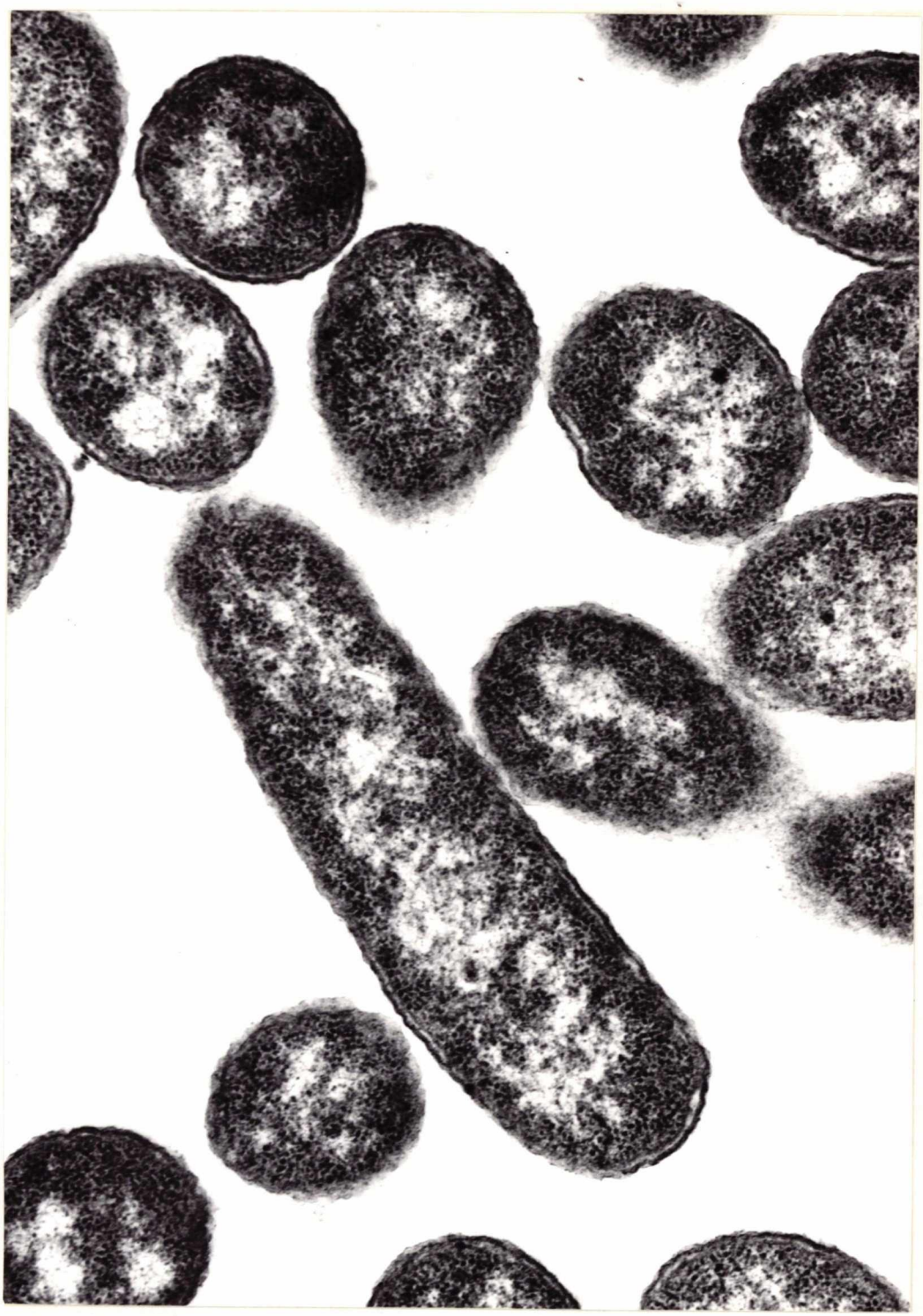


FIGURE 21 Electron micrograph of thin section of KL398 hyp$^-$

SECTION II

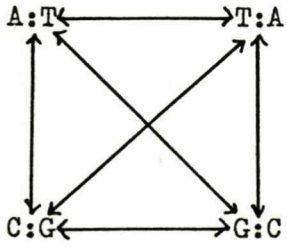
I. INTRODUCTION

1. INTRODUCTION

Strains of *E. coli* are known in which the spontaneous mutation frequency is from one to three orders of magnitude higher than in the wild type. These are called mutator strains, and the increase in mutation rate occurs in the absence of mutagens such as ionizing radiation, ultraviolet light and base analogs. Many of these genes have been mapped on the chromosome and their products, or lack of products, cause a variety of mutations ranging from single base-pair changes through small additions and deletions to quite large deletions and insertions. Mutators have been shown to increase the mutation rate in most, if not all, genes on the chromosome. How new mutants arise is poorly understood, but there are growing experimental data to suggest that many, if not all, spontaneous mutations arise as errors in all three metabolic processes affecting DNA, replication, recombination and repair.

The mutator strain was isolated by the hyper-rec method (see page 60) and believed to be a novel one and thus ~~it~~ its mapping was attempted ~~to be mapped~~, however, in the course of this work it became apparent that the mutation was *mutH*. Two new features were noted - the *polA* problem and the F effect.

Double Stranded DNA



Single Stranded DNA

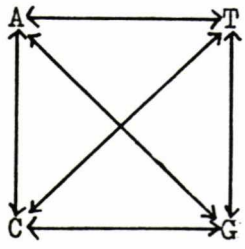
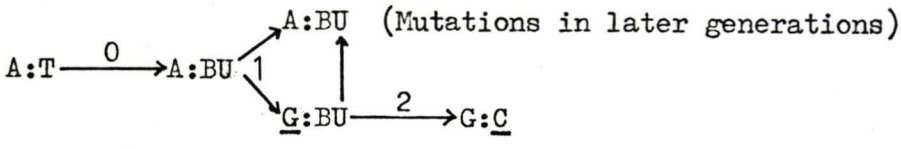


FIGURE 22 Base pair substitutions

Diagonals = Transitions

Horizontals and Verticals = Transversions



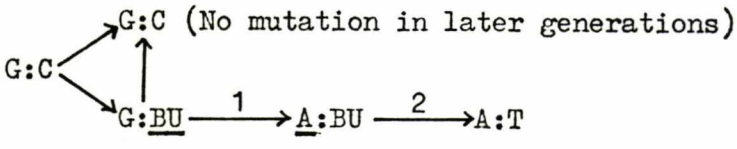


FIGURE 23 5-bromouracil mutagenesis. The first opportunity for a base to direct the transcription of mutant messenger RNA is indicated by underlined symbols. The generation at which this can occur depends upon the purine-pyrimidine orientation of the affected base pair.

(Figures 22 and 23 adapted from Drake, 1970)

A. Mutations

Mutations are changes in the sequence of nucleotides in the DNA, which usually cause changes in the way DNA controls the production of other molecules, such as enzymes. Mutations happen spontaneously in nature but the experimenter can increase the mutation rate by using certain chemicals which interact with DNA.

There are two major classes of mutational mechanism, direct and indirect. In the former spontaneous or induced mispairing results in specific-base substitution. Indirect mechansims generate mutations one or more steps removed from the initial event. The heritable alteration or loss of some gene function is attributed to a forward mutation, which may occur at any one of a large number of sites within a gene or a gene cluster. Most of the highly mutable nucleotide pairs, "hot spots," probably do not arise by normal duplication, and are connected with mispairing during crossing-over. When a mutant individual is grown into a large population, secondary mutations which restore whatever activity was lost at the time of the original mutation may often be observed. Such events are called reversions or back mutations. If reversion occurs at the original site and restores the original wild type DNA configuration, it is a true reversion. If reversion occurs either at some secondary site, or at the original site but not so as to restore the original wild type DNA configuration, it is called a false or pseudo reversion. These mutations most likely reflect failures in the replication process which leads to mistaken pairing. If a purine is replaced by another purine, and/or a pyrimidine by another pyrimidine, it is called a transition. If a purine is replaced by a pyrimidine and/or a pyrimidine by a purine, it is called a transversion (Freese, 1959; Drake, 1970) (Figure 22).

Other spontaneous mutations involve the loss (deletions) or gain (insertions) of nucleotides. Sometimes hundreds ~~to~~ or thousands of nucleotides are involved in deletions, and in rare cases whole genes are lost. Reverse (back) mutation to the normal gene arrangement is clearly impossible for large deletions and insertions, and occurs only at low rates for simple one nucleotide deletions (insertions). Jordan, Saedler and Starlinger (1968) have shown that IS sequences (see page 4) cause loss mutations but can revert to wild type by excision. Insertion or deletion events are frequently connected with mistakes in crossing-over. Error levels per incorporated nucleotide range from 10^{-6} to 10^{-9}. At first it was thought that these error frequencies were a result of the inherent accuracy of A:T and G:C base pair formation. The discovery of the proofreading capability of DNA polymerases, a process by which an incorrectly selected nucleotide or amino-acyl tRNA can be removed and replaced by the correct molecule, removed this dilemma.

Base analogues cause mutations as a result of their incorporation into the DNA molecule itself and it is believed that they only induce transitions. Their structural similarity to the normal DNA bases, allow their incorporation into DNA without destroying its capacity for replication. Their different structures, however, often cause less accurate base pair formation than normal, leading to mistakes during the replication process. Litman and Pardee (1956, 1960) observed that a number of thymine analogues 5-chlorouracil and 5-iodouracil, as well as 5-bromouracil were mutagenic (Figure 23). Freese (1959) has shown that 2-aminopurine can pair with thymine and cytosine. Hydroxylamine attacks both cytosine and uracil (in RNA) and, to a much lesser extent, thymine (Freese et al, 1961).

When a base is mutated in the DNA of the genome, a codon in RNA is changed and this may result in the substitution of a different amino acid

from wild type in the corresponding chain. If this substitution is acceptable to the protein there is no phenotype effect, but if it is unacceptable a missense mutation can be detected. The activity of the protein produced by the mutant gene depends upon the physico-chemical relationship between the original amino acid and its replacement. When the base substitution creates a nonsense codon, however, synthesis of the polypeptide chain is terminated prematurely at the mutant site. Mutations of either kind can be restored to the original phenotype either by reversion at the original mutant site or by mutation at a different locus. The latter situation is termed supᵖression.Suppressor mutations fall into two main categories : those due to nucleotide changes within the same gene as the original mutation but at a different site on this gene (intragenic suppression, and those occuring in another gene (intergenic suppression). Those genes which cause supᵖression of mutations in other genes are called supᵖressor genes.

Frameshift mutations are created in DNA by certain mutagens which cause the insertion or deletion of an extra base pair in DNA; this changes the reading frame of the message so that triplets are translated in the wrong sets beyond the mutation. Frameshift mutations are efficiently and specifically induced in bacteriophage T4 and in a few cellular systems by a variety of acridines (Drake, 1970; Roth, 1974). They are more efficiently induced in bacteria when the acridines are attached to coupling (alkylating) agents, the ICR compounds. Most mutations caused by ICR 191 are not induced to revert by base analogues, but are induced to revert by ICR 191.

Certain mutations (intergenic suppressor mutations) appeared to increase the frequency of mistakes in reading the genetic code. As a result of this increase in the mistake level, a mutant gene may occasionally produce a normal product. Suppressor genes exist for both missense (amino acid

replacement) and nonsense (chain-terminating) codons as well as for frameshift mutations caused by single nucleotide insertions. tRNA's with altered anticodons are the molecular basis of many suppressor gene actions.

B. DNA repair mechanisms

Damage to DNA is in the form of strand scissions, or modification or loss of bases sustained from chemical or physical agents in the environment. The chief damage to DNA by ultraviolet light, the best understood of DNA lesions, is dimerization of the pyrimidines. Adjacent thymine residues, for example are joined by a cyclobutane ring and can no longer form hydrogen bonds with adenines in the opposing strand. The helix in that region is consequently distorted. Unless the thymine dimer is removed, replication and transcription are prevented or disturbed.

DNA lesions are repaired by one at least of the four different mechanisms in Escherichia coli.

1. Photoreactivation

The pyrimidine dimers are removed in situ by visible light in an enzymatically catalysed cleavage of the cyclobutane ring. Photoreactivation can repair any dimer that can be repaired by excision in the dark. Photoreactivating enzyme (PRE) binds pyrimidine dimers in the dark but requires photoreactivating light (PRL), (Wulff and Rupert, 1962).

2. Excision repair

In the dark pyrimidine dimers are not separated chemically but may be removed from the DNA by a multienzymatic mechanism, excision repair (Boyce and Howard-Flanders,1964). The distortion of the double helix caused by a pyrimidine dimer (or by some other kinds of DNA lesions) is recognised by a specific endonuclease II, the product of the uvrA and uvrB genes of E. coli (Braun and Grossman, 1974).

The enzyme introduces a single strand nick or incission, at or near the lesions, usually to the 5' side of it. An exonucleolytic excision releases the oligonucleotide bearing the pyrimidine dimer plus some

bases on either side of it, and the "excision gap" is patched by repair replication (Pettijohn and Hannawalt, 1964) (Figure 24), the intact region of the strand opposite the gap serving as template for accurate replacement of the missing nucleotides. In wild type E. coli, the excision and resynthesis steps are probably affected simultaneously by DNA polymerase I, although efficient excision requires additional gene products, including those of uvrC (Howard-Flanders and Boyce, 1966), uvrE (Siegel, 1973; Smirnov, Sinzinis and Saenko, 1973) and mfd genes (George and Witkin, 1974). The final step is sealing of the sugar-phosphate linkage by polynucleotide ligase. Alternate pathways of excision repair, utilising DNA polymerase III (Young and Smith, 1973) or DNA polymerase II (Masker, Hanawalt and Shizuya, 1973) have been demonstrated in mutants lacking active DNA polymerase I. The "short patch" pathway of excision repair at least as it occurs in recA$^-$ and lexA$^-$ strains must be accurate since it does not contribute to a detectable level of UV induced mutations. The low frequency of mutations which have been ascribed to errors in the repair of excision gaps (Bridges and Mattershead, 1971) may be associated with "long patch" excision repair, which like UV mutagenesis, requires the recA$^+$lexA$^+$ genotype.

3. Postreplication repair

Some UV sensitive mutants that are capable of excision repair are also deficient in recombination.

A recombination-like postreplication (Rupp and Howard-Flanders, 1968) repair process has been described that involves the formation of rather large gaps (10^3 or more bases) in progeny strands opposite the induced lesions in the template strand (Stallcup, Sharrock and Raleinowitz, 1974), followed by the filling of these gaps by a strand transfer process. E. coli is capable of performing postreplication repair via a number

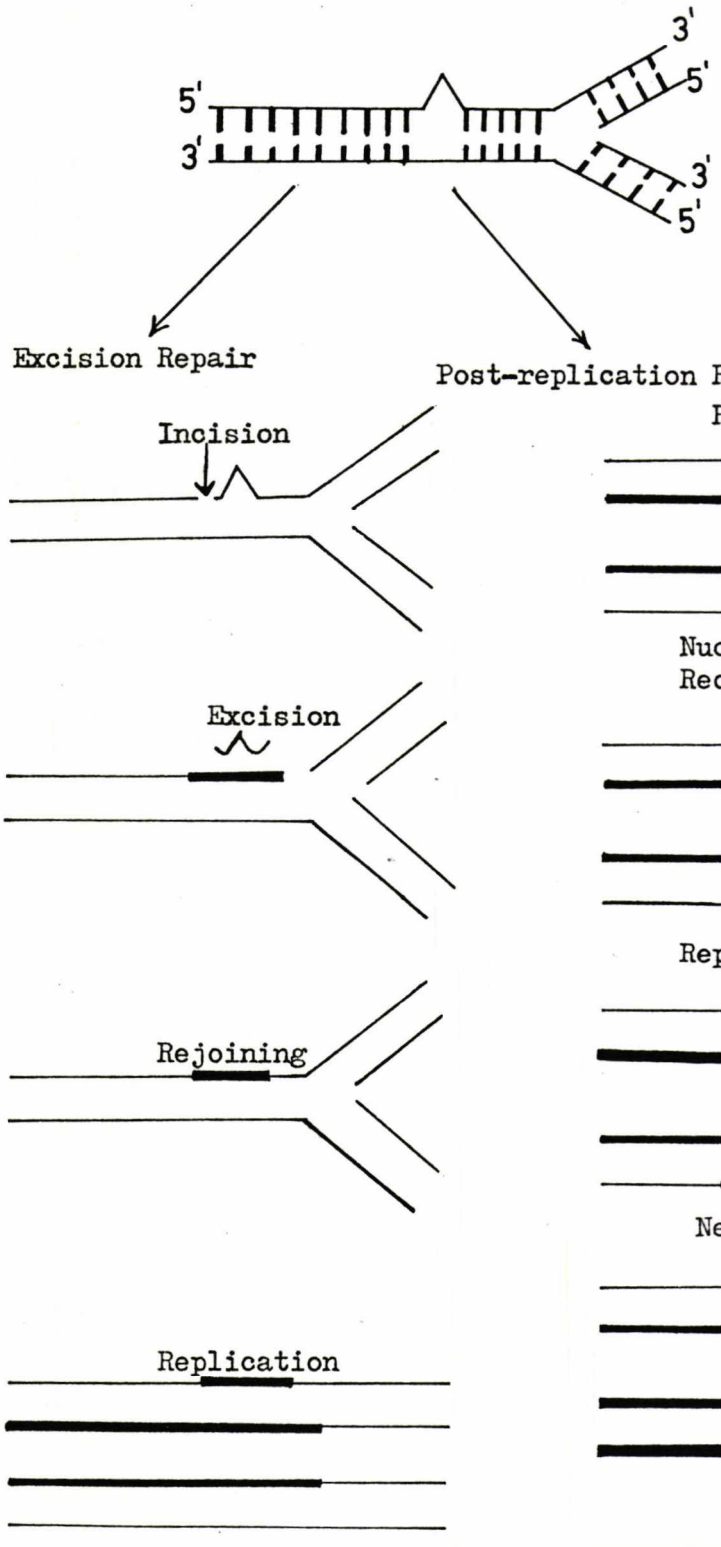


FIGURE 24

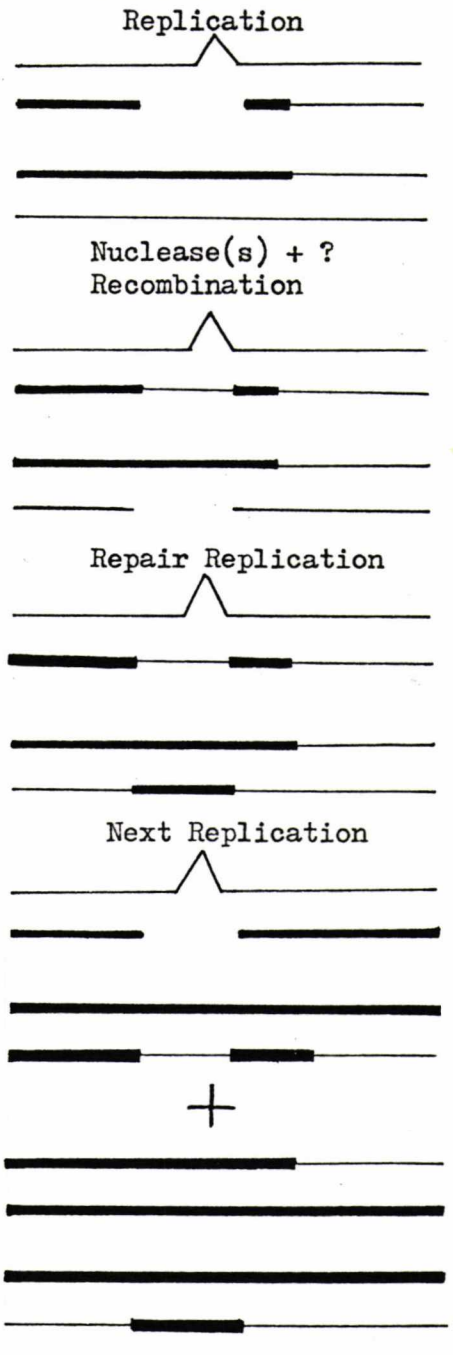


FIGURE 25

FIGURE 24. 25 (Adapted from Hanawalt, 1975).

The ultraviolet irradiation induced cyclobutane dimer is represented by the inverted "V" in one of the DNA strands in the replication segment of DNA. All replication subsequent to the introduction of the dimer is indicated by the heavy lines. Not illustrated is the discontinuous nature of the normal semiconservative replication of double-stranded DNA.

of distinct pathways, all of which require the recA$^+$ genotype (Figure 25). The requirement for the recA$^+$ gene product, does not necessarily imply that all pathways of postreplication repair are recombinational, and some pathways of postreplication repair will be considered with the error-prone repair systems (~~Also~~ see this page).

4. Error-prone repair

The three repair processes that have already been described appear to be accurate and all detectable mutations induced by agents such as UV appear to arise through the operation of a fourth (minor) recA$^+$lexA$^+$ dependent pathway of E. coli (Witkin, 1969). DNA repair requiring both the recA$^+$ and lexA$^+$ gene products are probably error-prone and responsible for UV mutability (Bridges and Mottershead, 1971). Postreplication repair, at least as it occurs in uvr$^-$ strains is far more error-prone than excision repair. The existence of two distinct pathways of postreplication repair, one error-proof and lexA$^+$ independent, the other error-prone and lexA$^+$ dependent, was postulated to account for the properties of certain derivatives of UV-sensitive UV-nonmutable lexA$^-$ strains.

Two hypotheses concerning the nature of error-proof and error-prone pathways of postreplication repair have been considered. Witkin proposed that recombinational repair is accurate, and that the lexA$^+$-dependent mechanism of daughter-strand gap-filling is an error-prone DNA polymerase activity capable of inserting nucleotides opposite pyrimidine dimers or other noncoding UV photoproducts without template instruction. Gaps opposite pyrimidine dimers could be filled by repair replication with a high probability of error. A second hypothesis (Witkin, 1969) proposed that UV mutagenesis may be due to errors in a lexA$^+$ dependent error-prone pathway of recombinational repair.

It is believed that error-prone DNA repair activity is not constitutive,

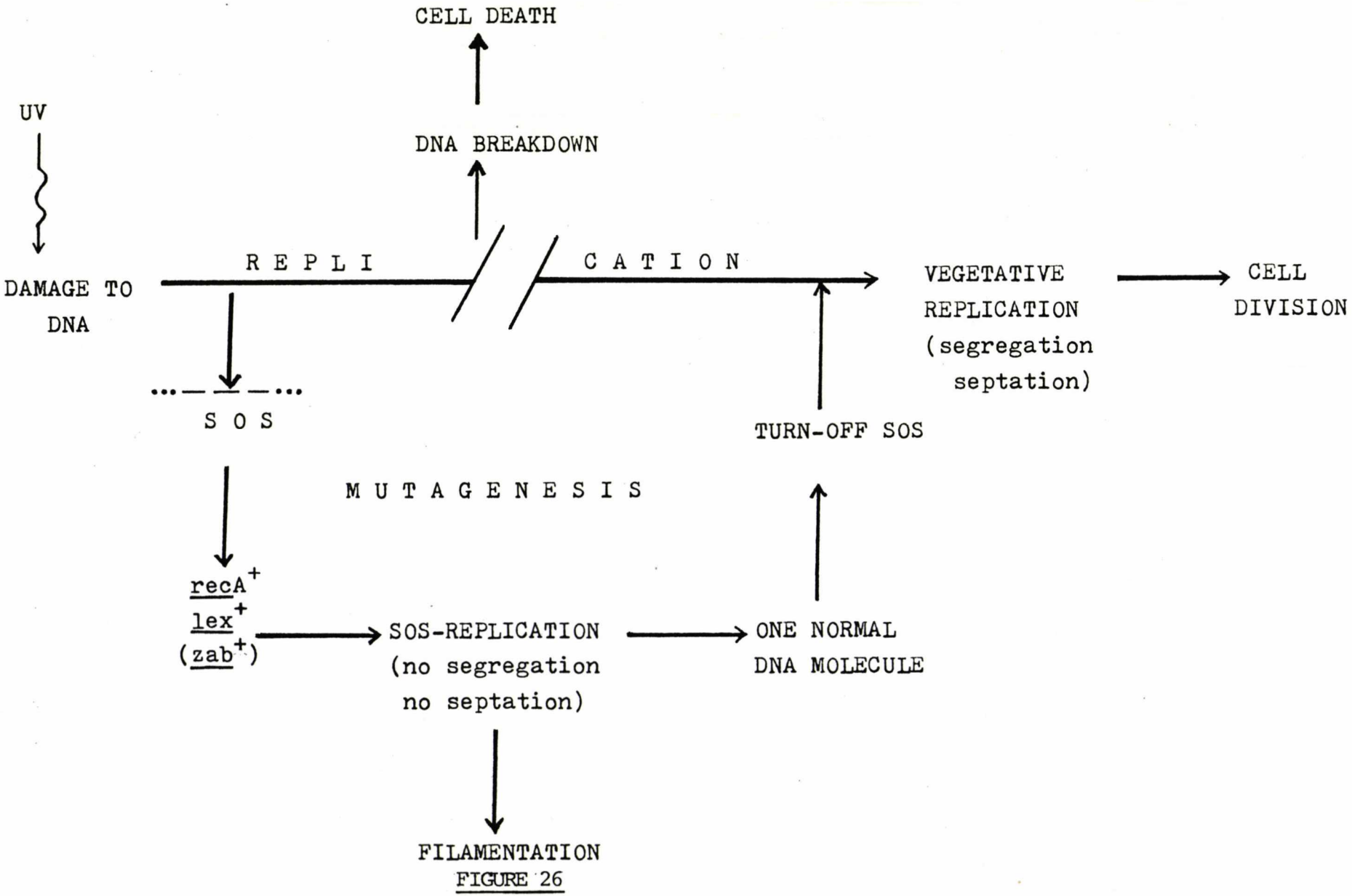

CELL DEATH
UV
DNA BREAKDOWN
DAMAGE TO DNA
REPLI
CATION
VEGETATIVE REPLICATION (segregation septation)
CELL DIVISION
S O S
TURN-OFF SOS
MUTAGENESIS
recA+
lex+
(zab+)
SOS-REPLICATION (no segregation no septation)
ONE NORMAL DNA MOLECULE
FILAMENTATION

FIGURE 26

but is induced by DNA damage. Because of its "response" to DNA damaging treatment this hypothetical repair is called "SOS" repair (Radman, 1975) (Figure 26). Some characteristics of SOS repair are (1) it is induced or activated following damage to DNA, (2) it requires de novo protein synthesis, (3) it requires several genetic functions of which the best studied are *recA*$^+$ and *lex*$^+$ of *E. coli* and (4) the physiological and genetic requirements for the expression of SOS repair are suspiciously similar to those necessary for the prophage induction. This is probably because induction of the recA protein, protein X, is required for both processes. During the action of SOS repair, mutation frequency is increased. The simplest assumption is that the SOS repair mechanism is error-prone; on the other hand mutagenesis may be just a secondary consequence of the physiological conditions under which SOS repair operates. In order for SOS repair to function it should require some specific genetic elements, the inducing signal, and de novo protein synthesis.

C. Mutator genes in E. coli

The mutT1 allele of the mutT gene was first recognised and studied by Treffers, Spinelli and Belser (1954) and mapped by Skaar (1956) and by Cox and Yanofsky in the thr-leu region of the chromosome. Bacon and Treffers (1961) noticed that all mutT1 induced revertants of an ornithine-requiring strain were second site revertants. The explanation for their observation is that the mutT1 allele caused a unique base-pair change, an A:T $\longrightarrow$ C:G transversion. (Yanofsky and co-workers observed only this mutational error analysing several sites in trpA mutants, in a mutT1 strain). The reverse mutation C:G $\longrightarrow$ A:T appears to occur at the wild type level or lower. It was concluded that the allele of the mutT gene does not increase the transition frequency, nor does it reverse A:T $\longrightarrow$ C:G transversions. Siegel and Kamel (1974) have shown that mutT1 had no effect on the reversion of lacZ frameshift mutations. Several alleles of mutT1 (mut-33, mut-44, mut-61, mut-79) were isolated and all increased the $Sm^S \longrightarrow Sm^R$, and $Trp^- \longrightarrow Trp^+$ mutation rate to the same extent as the original isolate, mutT1. The mutT1 allele reverts markers on F'trp and F'lac exogenotes as well as amber mutations of phage λ and T7. Cox suggested that the mutT1 gene is involved directly in DNA replication. Three kinds of evidence support this conclusion. First, unreplicated phage λ particles rescued from an immune mutT1 lysogen have not mutated, whereas once- and twice-replicated λ genomes have. Secondly, non-replicating λb2 susP3 phage DNA in a non-supressing mutT1 host is not subject to mutation. Thirdly, dnaE293, a mutation believed to be in the polymerase III structural gene, interacts with mutT1.

In mutT1dnaE293 double mutants DNA replication is considerably less temperature sensitive, and broth grown cells are considerably longer. The mutation rate is, however, that characteristic of mutT1dnaE$^+$ strains.

Cox has also shown that mutT1 action can be suppressed by an unlinked suppressor, sum-44. But it is not known whether sum-44 is a missense suppressor or is specific for the suppression of mutT. It is also not known whether mutT has a protein product. The mutT1 activity is not detectably influenced by mutations in recA, polA or the ligase. Clarke and Shankel (1977) proved that caffeine, quinacrine and spermine, but not guanosine acted as antimutagens in mutT induced Ara^+ reversion. Siegel et al (1976) demonstrated that some Mu-1 lysogens had mutator phenotypes. One (designated mut-49) resembled mutT1 in the frequency and types of mutations induced. The failure of mut-49 to increase the frequency of spc mutations and the induction of the same two classes of trpA78 revertants as mutT1 indicate that mut-49 has the same specificity as mutT1. This may show that mutT must be loss of mutT function.

The mutator gene, mutD5, whose phenotype is conditional, has been identified in E. coli by Degnen and Cox (1974). MutD5 was mapped by P1 transduction at approximately 5 minutes, and counter-clockwise of proA. When mutD5 cultures are grown in rich medium (L-broth), the mutation rate is several thousand fold higher than in a coisogenic wild-type. In a minimal medium (VB medium), the mutation rate is much lower, although still fifty to one hundred fold higher than wild type control. (str^r frequencies of mutD5 is about $5x10^{-6}$ to 10^{-5} in broth medium whereas 10^{-7} in minimal medium).

Thymidine has been identified as at least one of the mutational effectors present in rich medium, and it is active as an inducer of the high mutating state at concentrations as low as 10^{-7}M. A phosphorylated derivative of thymidine is the true effector since strains blocked in thymidine kinase no longer respond with an increased mutation rate upon the addition of thymidine to minimal salts medium. Free bases, nucleosides,

and deoxynucleosides have been tested as mutational effectors. Many of these compounds can be excluded as the possible effector. Deoxyuridine was found active over a wide range of concentration, and was inactive in a thymidine kinase-mutD5 double mutant, suggesting that deoxyuridine acts as a thymidilate precursor. Added thymidine does not, however, simply alter the relative concentrations of thymidine mono-, di- and triphosphate relative to wild type. This result can be interpreted to mean that the mutD phenotype results from the failure of a component of DNA replication to interact correctly with a phosphorylated derivative of thymidine. A protein active during replication, rather than a step in a recombination or repair, seems the most consistent interpretation of the data, because the mutD5 allele is inactive in the absence of DNA synthesis, and active in a recA background. Cox isolated supressors of mutD (sumD) by selecting for 5-aminoacridine-resistant derivatives of mutD5. He also isolated a second supressor of mutD5 which maps in the nalA gene. (It is now known to be the gyrase gene which implies something to do with DNA coiling - see page 16). All possible (base pair mutations) are increased in a mutD5 strain grown in rich medium. A:T$\leftrightarrow$G:C transitions as well as A:T$\leftrightarrow$C:G, A:T$\leftrightarrow$T:A and G:C$\leftrightarrow$C:G transversions are stimulated.

Transitions occur more frequently than homologous and heterologous transversions. MutD5 also increases the reversion frequencies of trpA frameshift mutations by causing base pair additions and, possibly, base-pair deletions. The mutD phenotype - high mutation rate, acridine sensitivity, interaction with nalidixic acid resistant mutants - suggest that the mutD product normally act at the replication fork, where it functions to prevent the replication of incorrect base pairs.

An azaserine-resistant derivative of E. coli B was found to carry a mutator gene, and designated mutS3 (Siegel and Bryson, 1967).

Reversion studies with *his*$^-$, *thr*$^-$, *leu*$^-$, *lys*$^-$ auxotrophs, on minimal agar plates supplemented with 2-aminopurine suggested that mutS3 could affect two types of transition A:T $\leftrightarrow$ G:C (Siegel and Bryson, 1967). The *mutS3* was mapped more precisely by Cox, Degnen and Scheppe, between *cysC* and *recA*. They demonstrated that the *mutS1* allele strongly increased the frequency of full revertants of *trpA23*, *trpA46*, *trpA58*, *trpA446* (1).

The frequency of full revertants of *trpA78* is unchanged and *trpA223* is slightly raised relative to the wild-type level. Full reversion frequency of *trpA446* (G:C $\longrightarrow$ A:T) is increased, as well as that of *trpA23*, *trpA46* and *trpA446* (A:T $\longrightarrow$ G:C), proving that this mutator causes reversible transitions (A:T $\leftrightarrow$ G:C). Not all A:T pairs are equally susceptible to *mutS* action, however, since the A:T pair at the *trpA223* site reverts at a frequency similar to *mut*$^+$. This might be a consequence of neighbouring bases, because other A:T pairs are reverted by *mutS* in the same gene : and an A:T pair in the *lac* operon is reverted at two widely separated points on the chromosome and in two orientations relative to the *trp* sense strand. Siegel and Kamel reported that *mutS3* increased the frequencies of *lacZ* frameshift mutations. It has also been reported that *mutS* alleles can be induced by the frameshift mutagen ICR-191, thus suggesting the *mutS*, is a nonessential function. The double mutant *mutS3recA* was found to be viable.

Hoess and Herman (1975) devised a selection procedure that selects for mutants showing enhanced frequencies of spontaneous reversion of frameshift mutations. One of them mapped between *lysA* and *thyA* ~~and~~

Footnote :

(1) The codon and amino acid interchanges between *trpA* mutants and all their known full revertants has been demonstrated by Yanofsky and coworkers (1966).

thyA and designated mutR (now mutH because mutR is probably identical with the mutH mutant isolated by Hill in E. coli B). Hoess and Fan (1975) found that mutR is carried on a specialized transducing phage (λpc1857thyA) generated by the excision of λc1857 integrated at a secondary attachment site between lysA and thyA. MutR increases both base substitution mutations and frameshift mutations.

Base substitution activity has been measured by using mutation to streptomycin resistance. The reversion frequency of different frameshift mutations varies considerably.

The reversion pattern of a series of trpA mutations was analysed. It appeared that mutR34 increases the frequency of transition mutations over transversion mutations. The transition A:T $\longrightarrow$ G:C appears to occur more frequently than the transition G:C $\longrightarrow$ A:T when compared with the reversion pattern in trpA58 and trpA446. MutR34 activity appears the same, when grown in minimal or rich medium. When the deoxyribonucleoside pools were examined no difference could be found between mutR34 and mutR$^+$. No evidence was found to suggest that mutR is involved in repair of damage produced by UV irradiation or mitomycin C, and no deficiency could be found in recombination. When mutR34 recipient strain with several lacZ mutations, were crossed with an Hfr carrying a lacZ nonsense mutation, intragenic recombination was increased two to three fold above the frequency obtained with wild type.

This increase appeared independent of the distance between the two markers within the gene in which the cross-over took place. Hoess and Fan (1975) suggested that mutR is a nonessential gene. Because 1) Deletions of the mutator can be found among survivors of heat induction of λc1857 when the phage is integrated between lysA and thyA. 2) Mutations in mutR can be induced with the frameshift mutagen ICR-191. 3) An amber mutant in

mutR has been found. MutR is viable with polB, polApolB, ligts7, and uvrA mutations and still mutates in these genetic backgrounds.

Nestmann (1977) has checked the antimutagenic effects of spermine and guanosine in continuous cultures. He has shown that, mutation to $T5^r$ in a mutH strain is lowered from 180 to 67 and 120 $T5^r$ mutants/10^8 bacteria/ generation by spermine and guanosine, respectively.

Liberfarb and Bryson (1970) isolated several mutator genes and five of them were cotransducible with purA at frequencies 80% to 100%. Mutator activity was characterised by rates of mutation to streptomycin resistance and T-phage resistance, both of which were significantly higher than the parental (mut^+) rates. The homology of the genetic maps of S. typhimurium and E. coli suggests that Liberfarb and Bryson identified in E. coli the same mutator locus that Kirchner and Rudden had studied in S. typhimurium LT7.

Siegel and Ivers (1975) detected mut-25 mutation (now mutL25) after scoring for high numbers of Ade^+ revertants from mutagenised cultures. Transduction analysis indicated that the gene order was ampA mut-25 purA. The mut-25 retained its mutator activity when coupled with purA auxotrophic mutations, indicated that mut-25 is not a mutation in the purA gene although closely linked to it. The reversion studies with the mutations trpA46, and trpA446 showed that mut-25 induced the transitions A:T$\longleftrightarrow$G:C. The mut-25 also increased the frequencies of reversion of lacZ frameshift mutations. Siegel and Ivers also established that mut-25 does not interact with uvrA, recA and exrA, that it is normally UV resistant and active in trans with F'trp.

The mutU4, UV-sensitive mutator strain was isolated by Siegel (1973). After mutagenesis, survivors of a streptomycin sensitive E. coli K12 strain were plated on nutrient agar plates and incubated overnight. These plates were then replica-plated onto nutrient agar plates containing streptomycin (200 μ g/ml). These streptomycin plates were incubated for

three days. Mutator colonies yielded some growth. Several mutator strains were isolated, and one of them was found to be UV sensitive. Siegel suggested that UV sensitivity and mutator activity were due to one mutation and mapped near ilv and metE.

Smirnov and Skavronskaya (1971) located a UV sensitive mutator gene (uvrE502) between metE and ilv. Liberfarb and Bryson also identified two, mut-9 and mut-10, UV sensitive mutators in the same region. It is not clear yet whether mut-9, mut-10, uvrE-502 and mutU4 are in the same cistron.

MutU4 and uvrE502 are similar in many respects. Both increase the spontaneous mutation rates for a variety of point mutations and frameshifts. The frequency of reversion of trpA46 on an F-prime was increased by mutU4. The mutU4 mutation induced transitional base changes, but did not affect recombination, and host cell reactivation of UV irradiated phage T1 and was resistant to MMS. MutU4 did not increase mutation frequencies in virulent phages or lytically grown phage λ. The mutU4rec$^-$ double mutants were extremely UV sensitive. This suggested that mutU4 involved in excision repair rather than postreplication repair. The mutU4 uvrA6 double mutant was only slightly more UV sensitive than a single uvrA6 mutant.

These combinations of mutU4 with recA, B, or C mutations, or with the uvrA6 mutation were phenotypically mutator. Neither a functional repair or postreplication repair system was needed for the expression of the mutator gene. Siegel has demonstrated that mutU4 was inviable with polA strains. Double mutants of mutU4 and polA4, which determines a cold sensitive polymerase, were unable to grow at 24°C, the nonpermissive temperature. Since the nature of defective step(s) in a mutU4 strain has not yet been determined, it makes it difficult to explain the mutator nature of mutU4 or its inviability with polA. The cell may require the mut$^+$ or polA$^+$ product to repair a DNA lesion that occurs during normal

cell growth. Since the mutU4 strains repaired single-strand breaks relatively normally, unlike polA or rec⁻ strains, the lesion may be different from that involved in recApolA lethality. Possibly, the repair of this lesion exclusively by DNA polymerase I in a pol⁺mutU4 strain results in the mutator phenotype. Simirnov and Skavronskaya (1973) proved that the missense supressor, su58, increased the UV resistance of the uvrE502, when introduced into uvr502 chromosome. Since missense supression acts at the level of translation of mRNA into protein it can be inferred that the product of the uvrE502 gene is a protein. Mattern and Houtman (1974) demonstrated that the double mutants uvrE502resA1, and uvrE502polA1 are inviable, but uvrE502polA107 viable. (PolA107 mutant lacks the 5'⟶3' exonuclease activity, but shows normal polymerase and 3'⟶5' exonuclease activity). These results suggested that only the polymerase and/or 3'⟶5' exonuclease activity of DNA polymerase I is essential for growth in a uvrE strain.

Apparently, the uvrE gene product and these enzymatic activities have similar functions in the cell during normal replication and can be substitute each other in some way. Clark and Rothman (1977) recently suggested that uvrE is in fact the same gene as recL. Although recL mutants are not mutators while uvrE mutants are.

The ast mutator (Zamenhof, 1966; 1967) maps at about 5 minutes on the E. coli chromosome and may be an allele of mutD. The ast mutator produces several percent auxotrophs in a single colony and also affects virulent bacteriophages reproducing in an host cell. The ast did not increase the rate of mutation to streptomycin resistance in its original host, the Harvard strain, but did so when transferred to K12. A possible explanation can be made by assuming that ast induces a specific type of mutational change which, because of difference in base pair sequence in the str region of the Harvard and K12 strains, might result in a resistant phenotype in K12 only.

D. DNA polymerases of E. coli

Three DNA polymerases, polI, II and III, have been found in extracts of bacterial cells. DNA polymerase I has roles in both DNA repair and replication. The function of DNA polymerase II is not yet clear, while DNA polymerase III is necessary for replication of the bacterial chromosome and carries out most of the DNA synthesis of this process. Structural genes for DNA polymerases I-II-III are dseignated polA, polB, polC (dnaE).

All three DNA polymerases are single polypeptides, and carry out DNA synthesis in the 5'→3' direction. A phosphodiester bridge is formed between the 3' hydroxyl group at the growing end of a DNA chain, called primer DNA, and the 5' phosphate group of the incoming deoxynucleotide. Each deoxynucleotide added to the chain is selected by base pair matching with a template strand. No known DNA polymerase is able to initiate chains. Correct base pairing is required at the primer terminus, as well as in the matching of the incoming nucleotide to the template. If a base at the primer terminus is unpaired because of mismatching or "fraying", it is removed by the 3'→5' exonuclease activity, which hydrolyses 5' mono nucleotides from single-stranded DNA from the 3' terminus. In addition DNA polymerase I has a 5'→3' directed exonucleolytic activity which specifically attacks double stranded chains. The 5'→3' nuclease cleaves a diester bond only at a base-paired region. Furthermore, the 5'→3' nuclease can excise oligonucleotides up to ten residues long from the 5' end, whereas 3'→5' nuclease removes only a single nucleotide at a time.

A nick, ~~is~~ defined as a break introduced in a DNA strand, by an endonuclease, is required for the single strand scissions. Any base sequence in the template will be copied with no particular reference or specificity. The mutation, designated polA1 (DeLucia and Cairns, 1969)

is a nonsense mutation and suppressed by the amber suppressors and recessive in partial diploids to the wild type *polA*$^+$ gene. This *polA* mutant grows at the same rate as its *polA*$^+$ parent but is defective in its capacity to repair the DNA damage caused by UV irradiation and MMS. The temperature sensitive mutants of the DNA polymerase I, such as *polA12* has provided the best evidence that *polA* locus is the structural gene for DNA polymerase I. Strains with *polA12* mutation have the normal level and temperature response of the 5'$\longrightarrow$3' exonuclease activity (Monk and Kinross, 1972).

The probability of mismatch has been estimated at about one in 10^4 pairings, a frequency far greater than observed for spontaneous mutations but a possible source of mispairing is a defect in the polymerase itself. Speyer (1965) and Drake and their coworkers showed that temperature sensitive mutants in the structural gene (43) of phage T4 DNA polymerase at permissive temperatures have either an increased (Speyer, Karam and Lenny, 1966) or decreased (Drake and Allen, 1968) frequency of mutation. Genetic tests showed that the mutator polymerases promote both transitions and transversions, and that the extent of the change in mutation frequency depends upon which mutation in which particular gene is tested and upon which polymerase mutant is used.

Mutator DNA polymerase incorporates noncomplementary nucleotides at a greater frequency than the wild type enzyme. Muzycka et al (1974) demonstrated that antimutators incorporate less 2-aminopurine into DNA than does the wild type, and mutators incorporate more than the wild type. Mutations altering the normal ratio of polymerase activity to 3'$\longrightarrow$5' exonuclease activity might be expected to be mutators when the ratio is increased, and antimutators when the ratio is decreased (Muzycka et al, 1972).

Geiger and Speyer (1977) recently reported an antimutator phenotype, Mud, in *E. coli*. A pleiotropic mutation in the *purB* gene is responsible for the observed antimutator effect. This antimutator effect is very large for some genetic loci, but is absent at other sites. This mutation in *purB* is temperature sensitive for both adenine auxotrophy and the antimutator action and can be reversed by the addition of adenine or adenosine to the growth medium when the strain is grown in permissive conditions (30°C in minimal medium). The antimutator effect is recessive in strains merodiploid for the *purB* region. It also acts in trans.

A single mutation, (*tdi*), has been recently reported by Stacey and Oliver (1977), characterised by its antimutator effects. The strain which is *tdi*$^-$ could neither be transduced by bacteriophage P1 nor give rise to transducing particles : it could only rarely be transformed by R factor DNA and exhibited a very low mutation rate.

II. RESULTS

A. Summary

A mutant strain, H2110, has been isolated as a mutator strain. It has been shown to carry two mutations; one in the gene, *mutH*, and a second, still unidentified, which prevented expression of the mutator phenotype when a *polA* mutation was introduced by conjugation. The two mutations were separated by transfer of the *mutH* allele to a different genetic background. Strain H2110 was isolated carrying an F-prime; "curing" enhanced the mutation frequency and restoring the F prime reduced it. It appears possible that an F factor gene product partially substitutes for the wild type *mutH* gene product. Two other plasmids, $ColI_b$ and ColV were also tested. $ColI_b$ had no effect on mutation rates while ColV had an even greater effect. It reduced the frequency of Nal^R mutants to 3% of that found in H2110 F^- cultures.

TABLE 19

Effect of H2110 and H2 on mutation frequencies

	H2	H2110
$Ara^- \longrightarrow Ara^+$	$<3.3 \times 10^{-7}$	$<2.4 \times 10^{-7}$
Amp^R (10μg/ml)	4.2×10^{-7}	7.6×10^{-6}
Tm^R (10μg/ml)	$<3 \times 10^{-7}$	7×10^{-7}
(1) Tm^R (10μg/ml)	4×10^{-6}	3.2×10^{-4}
Nal^R	2.3×10^{-8}	1.5×10^{-5}

Footnote

(1) Minimal agar plates also supplemented with thymine (50μg/ml).

B. Isolation of H2110

During the isolation procedure for hyper-rec strain (see page 59 , 60), it was noted that several of the colonies producing many papillae proved to be mutators. One of these H2110 was picked (because it was the strongest mutator) into broth, incubated overnight and the cultures plated onto minimal agar containing 100 μg/ml azauracil on minimal lactose agar and on minimal glucose agar to measure the frequencies of azauracil resistant mutants and lactose positive cells. H2110 gave the highest yield of azauracil resistant mutants and, by comparison, a relatively low yield of *lac*$^+$ cells. It seemed likely that this was a mutator strain rather than one with a hyper-recombination phenotype.

1. Mutation frequencies

Table 19 shows that the frequency of mutants in cultures of H2110 was highest for loss mutations but the yield of missense mutations (reversions) was also high. Penicillin selection with a culture of H2110 yielded a variety of auxotrophic mutants while the same procedure applied to a culture of H2 gave only rare *his*$^-$ mutants (Table 20). The frequency of Nal^R mutants was not greatly affected by UV irradiation or by periods of thymine starvation. In fact, 10 seconds UV doses decreased the frequency of Nal^R mutants 10 fold whereas the viable count only dropped about two fold.

In order to map the mutator locus of H2110, it was necessary to cure the F'*lac*$^-$ in order to allow introduction of F14 and some other F-prime. It was noted incidentally, that the F$^-$ clones were better mutators than those carrying H2110 F'*lac*$^-$. The mutation frequencies to resistance to 50μg/ml nalidixic acid, in cultures of H2110 F$^-$ was 5-6 fold higher than that measured for the original strain while the frequency of clones of H2110 F*lac*$^-$

showing no mutator phenotype was nearly 1%, in H2110 F^- it was less than 0.1%. The type of growth medium (minimal or nutrient broth) did not change the mutation frequencies when both the liquid medium and minimal agar plates were supplemented with 0.04mM thymidine, mutation frequencies were still nearly the same.

TABLE 20

5 hour penicillin (400 units/ml) treatment of H2 and H2110 (1)

Time (hours)	Viable count	Type and number of mutants obtained
	H2	
0	7×10^7	
2	8×10^6	
5	8×10^4	3 His^-
	H2110	
0	6.3×10^7	
2	1.4×10^7	
5	3.5×10^5	1 Thr^-, 1 Cys^-, 1 Ilv^-, 8 His^-, 4 Trp^-, 4 Met^-

TABLE 20

(1) Twenty five nutrient agar plates, each containing 80-100 clones, were replicated onto minimal agar and nutrient agar plates.

TABLE 21

a) Mapping data for H2110, linkage analysis of selected and unselected markers

From the cross between H2110 x AB1157/nal (50μg/ml)

Selected Markers	Colonies Scored	% linkage leu	arg	thr	his	mut⁻
Thr	200	55	2	-	0	0
Leu	200	-	2	60	2	0
Arg	200	68	-	78	3	0
His	50	0	4	0	-	0

b) Linkage analysis of selected and unselected markers

from the cross between H2 x AB1157/nal

Selected Markers	Colonies Scored	% linkage Leu	arg	thr	his
Thr	200	57	0	-	0
Leu	200	-	0	80	0
Arg	200	64	-	77	0
His	50	8	6	8	-

TABLE 22

a) Mapping data for H2110; linkage analysis of selected and unselected markers from the cross between H2110 x AB2070/nal

Selected Markers	Colonies Scored	% linkage				
		ilv	trp	his	met	mut⁻
Met	100	98	0	0	-	0
Ilv	100	-	0	0	60	0
His	50	0	0	-	0	0

b) Linkage analysis of selected and unselected markers from the cross between H2 x AB2070/nal

Selected Markers	Colonies Scored	% linkage			
		ilv	trp	his	met
Met	100	100	0	0	-
Ilv	100	-	0	0	42
His	50	0	0	-	0

TABLE 23

Effect of polA1 on the mutator strain H2110

	Mutation frequency (50 μg/ml Nal)
JG197/F14 *polA1*	$<2\times10^{-7}$
H2110 F^{-} *met*$^{-}$	6.2×10^{-5}
H2110 F^{+} *met*$^{+}$ *pol*$^{+}$	8.3×10^{-6}
H2110 F^{+} *met*$^{+}$ *pol*$^{-}$	1.1×10^{-7}

2. Mapping

Since ~~the~~ H2110 was mutagenised with nitrosoguanidine, an agent notorious for causing multiple mutations, it was necessary to study the mutation in another strain, and at the same time to see whether H2110 carried a new mutator gene. Therefore several crosses were performed between H2110 and some E. coli K12 recipients.

In crosses between H2110 and AB1157 F^- recombinants selected for Thr^+, Leu^+, Arg^+, His^+, were not mutators (Table 21). And none of the selected recombinants from crosses between H2110 x AB2070 ~~were~~ inherited the mutator gene (Table 22). All these recombinants were scored for the mutation frequencies of other auxotrophic markers and Tm^R, Val^R and AzU^R (Tables 21 and 22 also show the linkage percentage for unselected markers). An attempt was made to introduce F14 into a metE derivative of H2110 F^- strain using JG196/F14 and JG197/F14 polA1 as donors.

Mutator strains with altered polymerases are known in phage T4 (Speyer, Karam and Lenny, 1966; Bessman, Muzyczka, Goodman and Schnaer, 1969). Some of the met^+ recombinants had partially lost mutator activity from the cross between JG197/F14 polA1 x H2110 F^-met^-. None of the met^+ recombinants lost the mutator activity (Table 23) from a cross performed between JG196/F14 pol^+ x H2110F^-met^-. (All of the mut^+met^+polA1 clones were also plated with μ2). All of these mut^+ clones proved to be pol^-, after UV irradiation (Figure 27). This led us to believe that the polA gene was involved but all attempts to transduce the mutator gene by P1 transduction from H2110 to both pol^+ and pol^- recipient strains, selecting either for $metE^+$ or rha^+, failed.

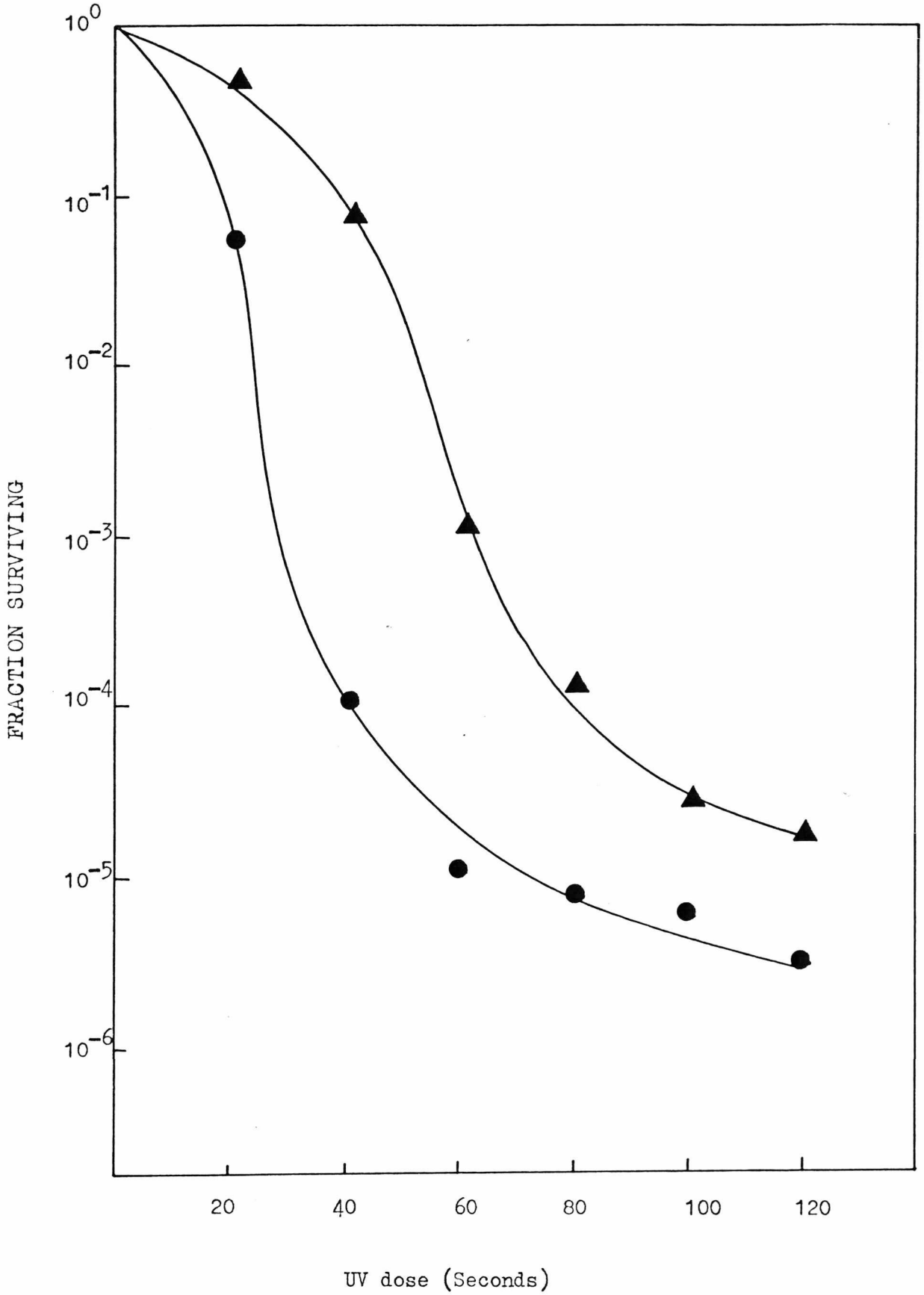

10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
FRACTION SURVIVING
20
40
60
80
100
120
UV dose (Seconds)

FIGURE 27

FIGURE 27 UV survival curves

▲ H2110

● H2110 polA1

TABLE 24

Transduction of mutH21 into different recipients

P1(H2110)	Colonies Scored (Thy^+)	% linkage Mut^- phenotype	Mutation frequency (50μg/ml nal)
x KL398 *pol*$^+$	100	64	3.19×10^{-5}
x KL403 *polA1*	100	66	3.7×10^{-5}
x KL405 *polA12*	100	58	1×10^{-5}
x KL406 *polA107* ($polymerase^+$ $5' \rightarrow 3'$ exo^-)	100	62	8.2×10^{-5}

TABLE 25

a) Cross between H2110 and KL400/spc (150 μg/ml)

Selected Markers	Colonies Scored	% linkage his	thy	uraP⁻	leu	mut⁻	
(1) Leu	100	2	60	22	-	42	(2)
Thy	100	0	-	30	25	75	
His	100	-	40	25	10	30	(2)

b) Cross between H2110 and KL398/spc (150 μg/ml)

Selected Markers	Colonies Scored	% linkage leu	his	thy	uraP⁻	met	mut⁻	
Met	100	32	0	88	8	-	4	(2)
Thy	100	20	0	-	2	12	76	
His	100	16	-	88	8	4	4	(2)

Footnote

(1) These results suggest that there was considerable mating on the plate despite the level of spectinomycin used to counter select the donor

(2) All the mut⁻ recombinants were also thy⁺

TABLE 26

Frequencies of mutants in *mut*$^+$ and *mut*$^-$ cultures of KL398 *mutH21* and KL398 *mut*$^+$

Mutant phenotype	KL398 *mut*$^+$	KL398 *mutH21*
met$^-$ $\longrightarrow$ Met$^+$	$< 2.6 \times 10^{-8}$	1.2×10^{-7}
pro$^-$ $\longrightarrow$ Pro$^+$	3.4×10^{-7}	2.4×10^{-7}
leu$^-$ $\longrightarrow$ Leu$^+$	$< 2.6 \times 10^{-8}$	3.8×10^{-6}
lac$^-$ $\longrightarrow$ Lac$^+$	$< 2.6 \times 10^{-8}$	3.7×10^{-6}
nal^S $\longrightarrow$ Nal^R	2.6×10^{-8}	4.2×10^{-5}

100 *met*$^+$ (AB2070); and rhamnose positive (JG112 and JG113) were checked against for mutation to nalidixic acid resistance, and none had inherited the mutator gene activity.

Further conjugation experiments suggested that the mutant gene was linked to *thyA*. At this point we learnt of the work of Hoess and Herman (1975) and tried, therefore, to transduce *thy*$^-$ strains using phage P1 grown on H2110 and obtained 60% cotransduction of high mutability (Table 24). The tight linkage of the mutator activity and *thyA* was confirmed by crosses between H2110 as donor and KL400 and KL398 as recipients (Table 25a,b) and between KL16 as donor and H2110 *thyA*$^-$, as recipient. In this last cross 83% of *thy*$^+$ recombinants were *mut*$^+$. Those results so thoroughly resemble those obtained by Hoess and Herman (1975) that we presume that the mutator phenotype is the result of a mutation in the *mutH* gene and we have designated it *mutH21*. As expected diploids of F143 which carried the wild type alleles of *mutH*$^+$ and *thyA*$^+$; H2110 *thyA*$^-$ *mutH21*/F143 had the same mutation frequencies as the original strain, H2. Table 26 shows the mutation frequencies of some markers in KL398 *mut*$^-$ and the isogenic parent *mut*$^+$ strain.

3. Involvement of polA

Once the mutant allele had been segregated into a different genetic background it was possible to test again the mutability of mutH21 polA double mutants and this time they proved just as mutable as pol$^+$ strains, in agreement with the observations of Hoess and Fan (1975). The mutH21 allele was introduced into strains carrying polA1, polA107, and polA12; in each case the double mutant was viable and a mutator (Table 24). The H2110 polA double mutant behaved quite differently from the KL398 mutH polA double mutant and it was thought worthwhile to try and explain this effect, And further experiments with H2110 showed that the loss of mutability of H2110 polA was not an accident that arose as a result of recombination between F14 and the chromosome of H2110.

The maintenance of the plasmid ColE1 is dependent upon the polymerase function of polymerase I (Kingsbury and Helinsky, 1970a,b) and selecting Col$^+$ strains from cultures of polA1 parent strain is a means of isolating polA$^+$ revertants.(Conversly mutation to polA$^-$ causes loss of ColE). H2 accepted ColE1 readily; the frequency of Col$^+$ clones from mating with a ColE1 donor was 90-100%; however only 50-55% of the H2110 and H2110 F$^-$ clones isolated under the same conditions were stably ColE1$^+$. Since ColE1 itself is not self transmissible HfrH ColE1 was used for the matings. ColE1 was equally transferred from the H2 ColE1 and H2110 ColE1 strains, to the AB2070 and AB1157. Starting with H2110 polA1 a search was made by the overlay method for colonies which produced colicin.

From 6-7 plates only one single colony produced a clear zone on the surface of nutrient agar plates which had been covered with a colicin sensitive indicator bacteria. This single colony was picked, purified and found to be UV and MMS resistant. Therefore it was presumed that H2110 pol$^+$ was a spontaneous revertant of H2110 polA1. This spontaneous revertant

also regained the mutator phenotype. It again produced many Lac^+ papillae on the surface of Lac^- cells on EMB lactose agar plates. The frequency of $\underline{nal}^R$ mutants was 1.2×10^{-5}, whereas the frequency for H2110 polA1 was 8×10^{-8}.

Similarly the transfer of $\underline{polA}^+$ by Hfr Cav, selecting for Xyl^+, to H2110 polA1 restored the mutator phenotype. 10 Xyl^+ recombinants were tested with UV and 3 of them were UV resistant. They were presumed to be $\underline{pol}^+$. The frequency of $\underline{nal}^R$ mutants in cultures of the recombinant H2110 $\underline{xyl}^+\underline{polA}^+$ was $2\text{-}3 \times 10^{-5}$ and for the recombinant H2110 $\underline{xyl}^+$polA1, 1.4×10^{-7}. Curing the F14 from H2110 polA1 strain by substituting $F'_{ts}\underline{lac}^+$, and then curing at 42°C did not change the strain's phenotype. It was still $\underline{mut}^+\underline{met}^+$ but F^-. However, the mutation rate increased 5-6 fold. This was presumably due to antimutator effect of F' and it suggested that even though the polA mutation reduced the mutation rate to nearly that of the wild type some of the mutations observed were still due to mutator gene.

TABLE 27

Nal^R mutation frequencies in plasmid bearing strains.

Strain	Plasmid	Mutation frequency (50 μg/ml)
H2	none	$2x10^{-8}$
	$ColI_b$	$2.3x10^{-8}$
	RUtrecht	$9.1x10^{-8}$
H2110	none	$5.6x10^{-5}$
	$ColI_b$	$6.6x10^{-5}$
	F110	$5.8x10^{-6}$
	F150	$3.4x10^{-6}$
	ColV	$1.3x10^{-6}$
	RUtrecht	$8.6x10^{-5}$
KL398 *mut*H21	none	$1x10^{-5}$
	KLF1	$3.4x10^{-6}$
	F150	$1.3x10^{-6}$
	F'*lac*$^-$	$3.3x10^{-6}$
	F14	$3x10^{-6}$
	F143	$2.2x10^{-8}$

4. The effect of plasmids on the mutator phenotype

The increased rates of mutation which followed loss of F factor in H2110 was not seen with the parent strain H2 so it was likely that the effect was specific for the mutH phenotype. This effect may have been due to a further mutation on the F-prime. Thus a different non mutagenised F' was reintroduced to test this hypothesis. Further it must be emphasised that the F143 had its effect because it brought back into the strain a functional $mutH^+$ product. All the F-primes introduced into H2110 F^- had quite similar effects in reducing the mutation frequency (Table 27). It seems improbable that the antimutator effect is associated with a chromosomal gene and likely that it is the result of a function encoded by the F factor itself. ColV derivatives of H2110 showed an even more striking reduction in the mutation frequency (Table 27). It is known that ColV is very like F and that ColI is quite dissimilar (Meynell and Lawn, 1967). $ColI_b$ derivatives of H2110 F^- showed the same frequencies of Nal^R mutants as the F^- strain itself. The plasmid R Utrecht itself normally mutagenic for the strains that carry it (MacPhee, 1973) was, nevertheless, an antimutator in H2110 F^-.

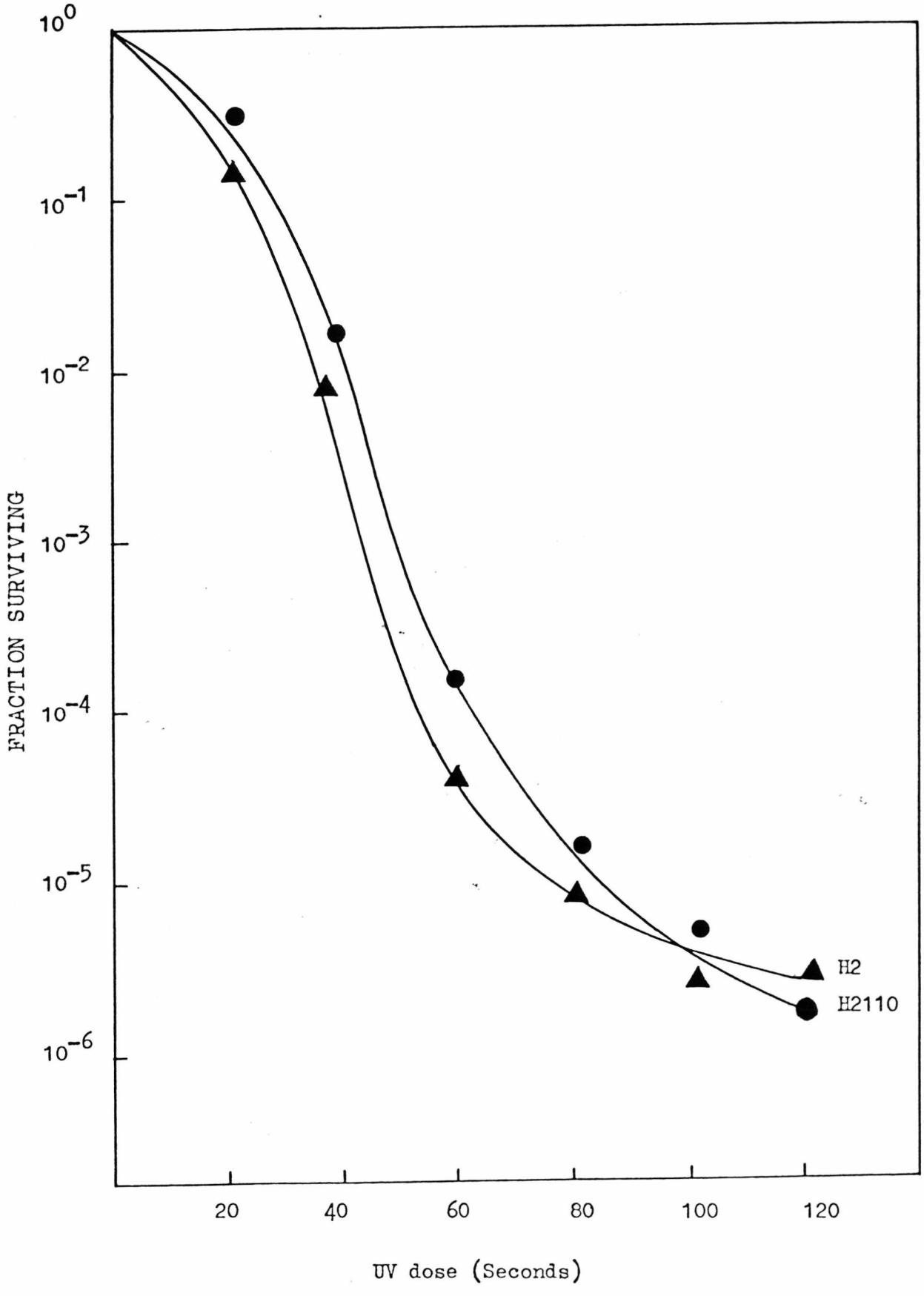

10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
FRACTION SURVIVING
20
40
60
80
100
120
UV dose (Seconds)
H2
H2110

FIGURE 28

FIGURE 28 UV survival curves

▲ H2

● H2110

5. Other phenotypic properties of mutH21

H2110 F'lac$^-$ did not differ from its parent strain in its sensitivity to UV irradiation (Figure 28).

Four recA1mutH21 double mutants have been isolated, two in H2110 and two in KL398 mutH21. KL16-99 recA1 were mated with KL398 mutH21 for 20 minutes at 37°C. (Streptomycin was used as the counter selective agent against the donor). 30 his$^+$ recombinants were tested with UV. 5 of them were UV^S and presumed to have inherited the recA gene. Among the 5 recAmutH21 strains, 3 ~~of them~~ lost mutator phenotype. (This was presumably due to introducing the wild type mut$^+$ gene, since ~~the~~ mutH is very close to the KL16 origin). The other two were still mut$^-$ in the presence of recA. All four double mutants showed higher spontaneous mutation rates than strains carrying recA alone. The rates resembled those obtained in recA$^+$ strains.

The mutation frequencies for KL398recA1mutH were 9×10^{-6} (Nal^R) and 6.6×10^{-6} (Lac$^+$) and for KL398mutH the corresponding values were 4.2×10^{-5} and 3.7×10^{-6}. When the recA1 gene was introduced into H2110cys$^-$, similar results were obtained. Two recA derivatives of H2110 were checked and the mutation frequency didn't change much compared to H2110 cys$^-$. (This cys$^-$ mutant, obtained by penicillin screening, was presumed to be in the cys operon at minute 59, because it yielded a normal level of cys$^+$ recombinants in 10 minute matings with KL16).

H2110 grew more slowly than H2 and behaved differently with respect to phage P1. It was more easily lysogenised by phage P1cml, and lysates generated from lawns of H2110 contained fewer transducing particles than phage grown on H2 (Table 28).

The marker showing the least difference was that nearest the origin, arg. P1cml was mixed with H2110 (multiplicity of infection, 2.2) and H2 (multiplicity of infection, 1.9). After 30 minutes the mixture was plated

TABLE 28

Transduction frequencies (1) for phage P1

Marker Scored (2)	Donor (3)	
	H2	H2110
Thr	1.2×10^{-5}	2.4×10^{-7}
Leu	3.9×10^{-5}	2.5×10^{-6}
Arg	7.2×10^{-6}	1×10^{-7}
His	1.8×10^{-6}	3×10^{-8}

(1) Frequencies expressed as transductants per plaque forming unit

(2) The recipient was AB1157

(3) The phage was grown 2 cycles of plate lysis on the donor strain

TABLE 29

Frequencies of mutants in $\underline{mut}^-$ and $\underline{mut}^+$ cultures grown in the presence of antimutagens

	KL398 mut^+		KL398 mut^-	
Type of medium	$leu^- \rightarrow leu^+$	Nal (50µg/ml)	$leu^- \rightarrow leu^+$	Nal
Minimal medium	$<8\times10^{-8}$	8×10^{-8}	1.3×10^{-7}	7.3×10^{-6}
Minimal med. + Spermine 4HCl	5.4×10^{-9}	8.9×10^{-7}	1.9×10^{-7}	4.7×10^{-6}
Minimal med. + adenosine	$<1.2\times10^{-9}$	3.8×10^{-9}	1.3×10^{-7}	6.2×10^{-6}
Minimal med. + acriflavine	$<4\times10^{-8}$	4×10^{-7}	3.8×10^{-7}	7.8×10^{-6}

	H2		H2110	
	Nal	$lac^- \rightarrow lac^+$	Nal	$lac^- \rightarrow lac^+$
Minimal medium	2.4×10^{-8}	$<2.4\times10^{-8}$	1.6×10^{-5}	5×10^{-5}
Minimal med. + spermine 4HCl	1.7×10^{-8}	$< 1.7\times10^{-8}$	1.5×10^{-5}	5.4×10^{-5}
Minimal med. + acriflavine	1.4×10^{-8}	$< 1.4\times10^{-8}$	2.4×10^{-5}	1.4×10^{-4}

onto minimal agar and chloramphenicol (25 μg/ml) plates. After two days incubation at 37°C H2110 lysogens were obtained with a frequency of 3.3×10^{-4} lysogens/viable cell whilst the corresponding frequency for H2 was 5.3×10^{-5} lysogens/viable cell.

The experiments on antimutagens were performed to determine if the mutator effect of mutH21 was dependent upon DNA replication, and therefore similar to Nestmans(1977) results (see page 103). However, the results proved to be different to those of Nestman although. It must be stated that experimental procedures used were different. Nestman used continuous culture whereas my results were obtained with batch culture. The mutH21 and mut^+ strains were grown on minimal medium in the presence of antimutagenic chemicals; Acriflavine HCl (5 μg/ml), Adenosine (200 μg/ml), Spermine tetrahydrochloride (250 μg/ml). It has been shown that these antimutagens did not have any serious effect on the mutator phenotype (Table 29).

III. GENERAL DISCUSSION

General Discussion

Clark and Margulies believed it should be possible to look at genetic recombination as if it were a normal biochemical pathway, and so they looked for mutants that were deficient in forming recombinants after conjugation. They isolated recA mutants in 1965 and since then Clark and other workers have isolated a variety of mutants deficient in recombination. Several other genes have been identified, recB and recC, recF, recL etc. (Clark 1973, Symonds 1974, Eisenstark 1977). An alternative way of looking at recombination has been to find bacteria in which recombination occurred more readily than normal. A hyper-rec phenotype might result from a variety of mutational alterations. These might include an increased frequency of single strand breaks ("nicks") or gaps in the DNA, which could provide sites for the initiation of recombination, a higher than normal efficiency of pairing or synapsis due to an increased concentration of DNA binding protein an increased number of sites that are hot spots for recombination (Chi-sites) or an altered concentration of a specific endonuclease required in a post-synaptic event. Konrad and Lehman (1974, 1975) with the isolation of polAex1 and dnaS mutants have identified two reasons for hyper-recombination.

We have also isolated a hyper-recombination mutant of E. coli K12 by examining colonies on EMB lactose agar (page 60) of an F merodiploid strain H2 lac$^-$/F lac$^-$ after mutagenesis by NTG for ones that showed more than the normal number of papillae. Hyper recombination mutants and specifically, mutants in which recombination occurs with unusually short regions between crossovers were sought with an F merodiploid in which a functional lacZ gene could only be formed by crossovers in two short regions on either side of an amber mutation, U_{131} in the lacZ gene of the F prime. This mutation is located approximately mid-way between two small deletions

in the operator-proximal region of the Z-gene on the chromosome. Possibly because of this arrangement, mutator strains and ones bearing duplications of lacZ were found more frequently than hyp$^-$ mutants among the clones picked for the frequency with which Lac$^+$ papillae appeared during growth on EMB lactose agar. We have examined in detail only one hyp$^-$ strain, H2114, but other mutants were obtained which appeared to have rather similar properties. Since the hyp$^-$ mutants were obtained after mutagenesis by NTG, which induces closely linked double and even multiple mutations, it was necessary to study the mutation in another strain. Therefore the hypB mutation, was transferred to KL398 by selecting thy$^+$ recombinants by conjugation (Table 4). The mutator and growth properties of H2114 were transferred at the same time. The hypB mutation appeared to affect recombination without any significant alteration in the plating efficiency on solid media and resistance to UV irradiation and to thymine starvation, all properties which are affected by mutations in other rec genes (see Clark 1973) including polAex1. H2114 and H2 had no shoulder on UV survival curves but KL398hyp$^-$ and KL398hyp$^+$ were normal. The effect of the mutation was evident on both the absolute levels of recombination, particularly in transduction and on the frequency with which particular crossover events occurred. The efficiency of P1 transduction, at least for certain markers, was greater than that produced by UV irradiation of the P1 lysate which Arber (1960) found increased the number of stable transductants some 3 to 5 fold. A substantial fraction of P1 transductants are abortive so that there is a large pool of potential transductants which can be made stable by any improvement in recombination efficiency.

In the case of recombination within the arabinose operon the activity of the hyp$^-$ mutation became more obvious as the length of the region between the two ara$^-$ mutations tested was reduced (Table 11).

Intracistronic (*araC*) recombination was particularly affected. This behaviour was consistent with the method of isolation which required multiple cross overs in a short region to produce Lac^+ cells, and with the finding that a lower linkage was obtained in Hyp^- strains between the *ara* and *leu* markers in KL398.

The enhanced level of recombination probably involves the *recBC* pathway (Clark, 1973) because blocking this pathway by the introduction of a *recB21* mutation abolished the difference between hyp^+ and hyp^- strains (Table 15). This result was consistent with the result obtained for phage λ crosses. There was a small effect on the recombination frequency, a doubling for closely linked mutations in the O and P genes. Although the *recBC* nuclease is inhibited by the *gam* gene product, the *red* gene products have similar functions to those of Exonuclease V. The fractional yield of sus^+ mutants from crosses of closely linked markers in KL398 hyp^- was twice that in KL398 hyp^+ although the burst size was always lower in the Hyp^- strain (Table 16, 17). Since H2114 was λ^R, we were not able to do this experiment with the original mutant.

The hyp^- mutation in H2114 is different from that described by Konrad and Lehman, because it differs in its properties and because the gene involved appears to be located around *thyA* rather than *pyrF*. For this reason we wish to use the gene symbol $hypB^-$ for the mutated gene in H2114 until the biochemical lesion can be identified. To delineate the position of hyp^- more accurately, conjugation experiments with KL16 and KL14 were performed. The former carries markers in an anticlockwise direction, and the latter in a clockwise direction. After selecting for early markers transferred by the two Hfr strains, KL14 and KL16, Hyp^+ was not found as an unselected marker and it was presumed that the locus was between the two origins of transfer (Table 5). During the mapping experiments we

were confused by the observation that hyp$^-$ strains had a chromosomal anomaly (presumably a deletion or transposition) in the region near metC. But we have concluded that this anomaly was already present in the parent strain H2 (Table 8). Replacing F'lac in hyp$^-$ strains by F143 suggested that at least the Mut$^-$ character of the hyp$^-$ strains was located somewhere between the lysA and tyrA, because H2114/F143 and KL398hyp$^-$/F143 strains had lost the mutator activity (Table 9). The Mut$^+$ allele carried by F143 diploids was dominant so mutator must be counter clockwise of thymine. But these results did seriously raise the question whether the Mut$^-$ character of hyp$^-$ strains was the result of a mutation to produce ung a separate mutator gene, or whether it was a pleiotropic effect of the hyp$^-$ gene. I was unable for lack of time to make any serious attempt to see if these two phenotypic characters are separable. However it seems not at all unlikely that hypB is pleiotropic and that the changed regulation of cell size and cell division are in some way connected with a disturbance in DNA metabolism. All through the growth cycle, only cells smaller than those found in Hyp$^+$ cultures could be seen in Hyp$^-$ cultures so cell division must occur before the usual cell size is reached in the Hyp$^-$ cell. It is possible that this change in the cell mass at which division occurs is due to premature initiation of chromosome replication. EM photographs of KL398 hyp$^-$ and hyp$^+$ (Figure 18, 19, 20, 21) cultures the impression that hyp$^-$ strain contains more DNA than hyp$^+$ strain.

The effects of the mutation in hyp$^-$ could be interpreted as arising from the introduction of DNA breaks which act as initiators of recombination. Other agents which lead, either directly or via repair or replication to single strand interuptions in the chromosome are recombinogenic. This would lead to both the enhancement in the yield of transductants and possibly to the apparent map expansion seen with closely linked ara$^-$ markers as

well as the reduced linkage values.

The first report on hyper recombination strains of *E. coli* came from Konrad and Lehman (1974). The temperature sensitive *polA*ex1 mutation was defective in the 5'⟶3' exonuclease activity of DNA polymerase I (page33). Konrad and Lehman (1975) also reported that *dnaS* mutants of *E. coli* accumulate very small DNA replicative intermediates. It has been demonstrated that the *dam*-3 mutant of *E. coli* shows higher mutability and higher recombination rates than normal level (Konrad and Marinus 1976). In this mutant DNA is undermethylated and probably the vacant sites are targets for nucleolytic attack. Another hyper-rec mutant *xse*$^-$, was isolated by Chase and Richardson (1977). All the *xse*$^-$ mutants have reduced level of 5'→ 3' hydrolytic activity of exonuclease VII (page37).

However the data do not exclude the possibility that recombination is itself a mutagenic process in this strain. The idea that recombination is mutagenic has been proposed (Magni 1963), to account for the higher spontaneous mutation rates observed in meiosis than in mitosis in yeast. Magni has provided clear evidence that the increased mutation frequencies in meiosis are associated with genetic exchange. He considered the mechanism of "exchange-associated" mutation to be unequal crossing over, and his prediction that mutations of the frameshift type should be generated exclusively by this process now seems to have been borne out. If UV induced mutations were recombination errors, the mutagenic effect of recombination in *E. coli* however, could not be due to inequality of exchanges, since the majority of UV-induced mutations, are single base substitutions. However the mutagenic specificity of misrepair mutagenesis is very wide, so that base pair substitutions, frameshifts and even deletions are induced.

Most of the UV damage produced in the DNA of surviving bacteria is repaired by relatively "error-proof" mechanisms (e.g., photoreactivation, "short-patch" excision repair, the major pathways of recombinational postreplication repair), which do not contribute substantially to UV mutagenesis. Some kinds of DNA damage (probably single strand gaps not repairable by any constitutive accurate mechanism) are targets for the activity of inducible "error-prone" repair activity ("SOS repair), which is entirely responsible for UV mutagenesis in E. coli and in λ bacteriophage. Another phenomenon suggesting the mutagenicity of recombination in bacteria is "selfing" (Demerec 1962) in which some auxotrophic strains can be "transduced" to prototrophy by phages grown on the same strain. Demerec first interpreted this as an example of unequal exchange but later (1963) ruled out this interpretation by showing that "selfing" occurs even if the donor carries a deletion covering a large part of the marker gene. He concluded that the mutability of some genes is increased by the proximity of a paired transducing fragment, but a more likely explanation now is that the reversions are induced by a mutagenic step in this process of recombination itself.

Clark suggested that selfing might be due to recombination involving a chance homology with some other short region of the chromosome. This is however no better founded than Demerec's explanation. The possibility that a recombinational event may itself generate an induced mutation is relevant also to the phenomenon of negative interference (Pritchard 1960). Negative interference is the apparent clustering of recombinational events in very small regions, corresponding approximately to about 1200 nucleotide pairs (Amati and Meselson 1965), or the approximate length of a single cistron. Little is known about what controls the distance between the cross over points, but it is possible that a difference in concentrations of the

enzymes concerned with recombination e.g. gyrase or ligase may be involved. It seems possible that recombination-induced reversions, scored as recombination events could distort estimates of negative interference. This, of course, would apply only if the locus involved is initially of a type susceptible to recombination-induced reversion, if the strain involved is capable of producing such mutations, and if the probability of induced mutation per recombinational event is very high.

Acridines such as proflavin and the ICR series of compounds induce frameshifts (Crick et al. 1961), probably by intercalating between adjacent base pairs in the DNA double helix and enhacing errors in DNA repair or in recombination (due to unequal crossing over, (Lerman 1961). Lerman's suggestion found support from Sesnowitz-Horn and Adelberg (1969). They demonstrated that proflavin is mutagenic for E. coli if cells are exposed during the course of a conjugational cross. The results were puzzling, however since recombination did not need to occur within the region being mutagenized. Furthermore, many (23%) of the mutants isolated were apparently base substitution types, not frameshift mutations.
It is to be hoped that in future work on this strain it will be possible to establish the nature of the DNA lesions, (if any) because this may give a clue to the biochemical nature of the hyp$^-$ gene product. However the arrangement of the mutations in the lacZ genes in H2 are such that in selecting for increased recombination the selection might be biased towards mutants which show more negative interference than wild type strains. It has been also observed that the recombination frequency was dependent upon growth phase. The frequency (Lac$^+$ cells) was highest in overnight cultures and declined (about 100 fold) during exponential phase.

The other problem this work was concerned with was the mutH mutant.

The genetic control of average mutation rates appears to be demonstrated by two broad phenomena: (1) large differences in average mutation rates per base pair in different organisms, and (2) mutations in specific organisms, which drastically alter average mutation rates. It has been observed that methane-utilising bacteria have very low mutation rates (Williams and Bainbridge 1972). Mutator (and antimutator) mutations, have been observed in numerous organisms, although in bacteria antimutators are rare.

Mutator mutations were very poorly understood until 1965, when Speyer reported that a mutation in gene 43 of phage T4 resulted in a temperature sensitive T4 DNA polymerase which produced a huge increase in the average mutation rate. The frequencies of mispairing due to unusual tautomers of the normal bases are probably far higher than observed mutation rates. It seems reasonable, therefore, that the enzymes involved in DNA synthesis, and DNA polymerase in particular, should act to reject or edit out incorrect base pairings. Speyer's observation that a defective polymerase was involved in the mutational process gave powerful support to this idea. Shortly thereafter, however, a much less predictable mutant form of T4 DNA polymerase discovered (Drake and Allen 1968). This mutant, also originally detected because of its temperature sensitivity, exhibited antimutator properties. In addition to confirming the crucial role of DNA polymerase in maintaining fidelity, this observation suggested that under natural selection, phage T4 has "adopted" a mutation rate greater than that which could be achieved by the most accurate enzyme. Furthermore not only spontaneous but also at least some kinds of chemically induced mutations are strongly influenced in their rates by mutant T4 DNA polymerases (Drake and Greening 1970). Other genes besides that coding for the T4 DNA polymerase are also involved in maintaining the fidelity of T4 DNA replication and repair (Drake 1973).

Numerous temperature sensitive mutants of the T4 DNA polymerase have been characterized with respect to their effects upon mutation rates; they are classified as mutators, antimutators, or neutrals and in some cases, as to the specific mutational pathways affected (Drake et al, 1969). What is really needed, however, is an understanding of the enzymological basis for these specificities. The first major advance in this direction came from Muzyczka, Poland and Bessman (1972). It had been known , previously that the T4 DNA polymerase and several other prokaryotic DNA polymerases contain, in addition to their polymerizing activity, a 3'-5' exonuclease activity which can directly reverse the polymerization step. This nuclease activity acts preferentially on incorrectly inserted bases and has been assigned a probable copy-editing or proofreading function. Bessman's group demonstrated that the fidelity of DNA replication results at least in part from a balance between the polymerase and the nuclease activities.

Mutator mutations have also been analysed in bacteria, the powerful E. coli mutator mutation mutT promotes a specific base pair substitution pathway (Yanofsky, Cox, Horn 1966). This pathway happens to be a transversion (A:T$\longrightarrow$C:G), and this is one of the very few cases in which a transversional mechanism is specifically enhanced; unfortunately, the mechanism of action of mutT and in particular, the normal function of the mutated gene remain unknown even now. In E. coli some other mutator genes have been identified by several workers (such as mutD, mutS, mutR, mutL, mutU Figure 10). The gene-product of none of the many genes so far reported has, as yet, been identified. Genetic studies of these E. coli genes were briefly discussed on page 98.

Although the F merodiploid method was set up for the isolation of

hyper recombination mutants the majority of clones with large numbers of papillae isolated from H2 were mutators. One strain obtained by this method H2110, has been studied in some detail. We had begun to associate the mutator genotype with a gene near thyA when the paper of Hoess and Fan (1975) high-lighted what appeared at that time to be a new gene, which they called mutR. We then showed H2110 to be like their mutants. It has been re-named mutH because Bachman, Low and Taylor (1976) re-designated it because Ruth Hill had already reported a mutator gene in E. coli B near thyA.

H2110 grew rather slowly compared with wild type H2. Viable cell counts were always 4-5 fold higher in H2 than H2110 when both cultures reached the maximum level early in stationary phase. The mutator gene was co-transduced by phage P1 with the thyA gene with frequencies close to 60%. The phenotype it produced, especially when the allele had been transferred to another genetic background, resembled closely that described by Hoess and Fan (1975); as in their mutants the mutator activity was greatest for missense mutations. The mutH, and also the hyp$^-$ strain, H2114, appeared to be aberrant in terms of UV survival and thymineless death. However they are not different from their parent strain H2 which suggests that neither act on repair pathways. The frequency of mutants was not increased by UV and thymine starvation, indeed the frequency of Nal^R mutants was lower among the survivors of low doses of UV irradiation than it was in the unirradiated cultures. (This result is interesting although the explanation is still obscure). Presumably the mutagenesis induced by these treatments is so much less than that produced by the absence of the mutH product that it cannot be reliably detected. At first sight the mutability of H2110 appeared to reside in polA gene in that mutH21 polA strains did not mutate much more than normal.

In view of the mutagenic polymerases known in T4 this observation seemed to suggest an explanation for the mutator properties of this strain. However subsequent analysis showed that this was not so; a quite independent mutation prevented the expression of high mutability in H2110 polA1 strains. In other genetic backgrounds polA1 mutH21 double mutants were as highly mutable as Hoess and Fan (1975) had found (Table 24). This second mutation in H2110 has not been located but it does not appear to be linked to any of the markers we have tested on the segment between the origin of Hfr Cav and the thyA gene. It is not closely linked to mutH The absence of the mutH gene product causes an effect which does not normally require polymerase I for the fixation of new mutations. However, because the effect of this second mutation is to make the polymerase activity of polymerase I essential for the survival of mutH induced mutants it seems likely that some form of DNA damage is involved. However we have not detected any effect of this second mutation on any other property of H2110. However a small effect on viability would not have been detected.

But an alternative hypothesis can be advanced. If there were two equally efficient pathways which could lead to the fixation of the mutation induced by loss of a functional mutH gene product then if one involves polA the other must be blocked in polA mutants by the second mutation.

The influence of the F prime on the mutator phenotype was unexpected and it implied that an F-directed activity can partially substitute for the gene product specified by mutH. The same or a similar activity is associated with the rather similar plasmid ColV but not with $ColI_b$. ColV is very closely related to F by hybridization studies, but ColI is not. The F function may be associated with replication or maintenance, and Dr. A. J. Clark is examining the effect on mutation rates of a number restriction endonuclease shortened F factors in the hope that this will locate the gene

responsible on the genetic map of the plasmid. R-Utrecht, itself mutagenic for the strains that harbour it, also largely reverses the effects of mutH21 (Table 27).

The antimutagens, acriflavine, adenosine and spermine did not seriously affect the mutation frequency in cultures grown in their presence (Table 29).

Although at the end of this work, I was unable to establish anything of the biochemical nature of the causes of the effects reported in this thesis because of lack of time, there are several avenues of work which have been revealed that would be worthwhile to pursue.

REFERENCES

REFERENCES

Alberts, B. M., and Frey, L. (1970). T4 phage gene 32: a structural protein in the replication and recombination of DNA. Nature 227, 1313

Amati, P. and Meselson, M. (1965). Localized negative interference in bacteriophage λ. Genetics 51, 369

Arber, W. (1960). Transduction of chromosomal genes and episomes in Escherichia coli Virology 11, 273.

Bachmann, B. J., Low, K. B., and Taylor, A. L. (1976). Recalibrated linkage map of Escherichia coli. Bacteriol. Rev. 40, 116.

Bacon, D. F., and Treffers, H. P. (1961). Spontaneous and mutator-induced reversions of an Escherichia coli auxotroph. J. Bacteriol. 81, 786.

Barbour, S. D., and Clark, A. J. (1970). Biochemical and genetic studies of recombination proficiency in Escherichia coli. Enzymatic activity associated with recB$^+$ and recC$^+$ genes. Proc. Natl. Acad. Sci. U.S. 65, 955.

Birge, E. A., and Low, K. D. (1974). Detection of transcribable recombination products following conjugation in rec$^+$, recB$^-$ and recC$^-$ strains of Escherichia coli K12. J. Molec.Biol. 83, 447.

Blanco, M., Levine, A., and Devoret, R. (1975). LexB: a new gene governing radiation sensitivity and sensitivity and lysogenic induction in Escherichia coli K12. (In Molecular Mechanisms for the Repair of DNA, p.379. Plenum Press, New York)

Boyce, R. P., and Howard-Flanders, P. (1964). Release of ultraviolet light-induced thymine dimers from DNA in Escherichia coli K12. Proc. Natl. Acad. Sci. U.S. 51, 293.

Braun, A., and Grossman, L. (1974). An endonuclease from Escherichia coli that acts preferentially on UV-irradiated DNA and is absent from the uvrA and uvrB mutants. Proc. Natl. Acad. Sci. U.S. 71 1838.

Bridges, B. A., and Mottershead, R. (1971). $RecA^+$ dependent mutagenesis occurring before DNA replication in UV and γ irradiated Escherichia coli. Mutat. Res. 13, 1.

Broker, T. R., and Lehman, I. R. (1971). Branched DNA molecules: intermediates in T4 recombination. J. Molec. Biol. 60, 131.

Buitenwerf, J., and Venema, G. (1977). Transformation in Bacillus subtilis. Fate of transforming DNA in transformation deficient mutants. Molec. Gen. Genetics. 151, 203.

Castellazzi, M., George, J., and Buttin, G. (1972). a) prophage induction and cell division in Escherichia coli: Further characterization of the thermo sensitive mutation tif-1 whose expression mimics the effect UV irradiation. Molec. Gen. Genetics. 119, 139.
b) Linked (recA,zab) and unlinked (lex) suppressors of tif-1 mediated induction and filamentation. 1972. Molec. Gen. Genetics. 119, 153.

Chase, M., and Doermann, A. H. (1958). High negative interference over short segments of the genetics structure of phage T4. Genetics. 43, 332.

Clark, A. J., and Margulies, A. (1965). Isolation and characterization of recombination deficient mutants of Escherichia coli. Proc. Natl. Acad. Sci. U.S. 53, 451.

Clark, A. J. (1973). Recombination deficient mutants of Escherichia coli and other bacteria. Ann. Rev. Genetics. 7, 67.

Clarke, C. H., and Shankel, D. M. (1977). Antimutagens against mutT. Molec. Gen. Genetics. 154, 335.

Crick, F. H. C., Orgel, A., Barnett, L., and Brenner, S. (1961). The theory of mutagenesis J. Molec. Biol. 3, 121.

Cross, R. A., and Lieb, M. (1967). Heat inducible λ prophage. Induction of prophages with mutations in genes O, P and R. Genetics 57, 549.

Cox, E. C., Degnen, E. G., and Scheppe, M. L. (1972). Mutator gene studies in Escherichia coli: The mutS gene. Genetics. 72, 551.

Cox, E. C. (1973). Mutator gene studies in Escherichia coli. The mutT gene. Genetics Suppl. 73, 67.

Cox, E. C. (1976). Bacterial mutator genes and the control of spontaneous mutation. Ann. Rev. Genetics. 10, 135.

Davidoff-Abelson, R., and Dubnau, D. (1971). Fate of transforming DNA after uptake by competent Bacillus subtilis: failure of donor DNA to replicate in a recombination-deficient recipient. Proc. Natl. Acad. Sci. U.S. 68, 1070.

Davidoff-Abelson, R., and Dubnau, D. (1973). Kinetic analysis of the products of donor deoxyribonucleate in transformed cells of Bacillus subtilis. J. Bacteriol. 116, 154.

Demerec, M. (1962). "Selfers" attributed to unequal crossovers in Salmonella. Proc. Natl. Acad. Sci. 48, 1696.

Demerec, M. (1963). Selfer mutants of Salmonella typhimurium. Genetics. 48, 1519.

Degnen, G. E., and Cox, E. C. (1974). A conditional mutator gene in Escherichia coli: isolation, mapping and effector studies. J. Bacteriol. 117, 477.

De Lucia, P., and Cairns, J. (1969). Isolation of an Escherichia coli strain with a mutation affecting DNA polymerase. Nature 224, 1164.

Drake, J. W., and Allen, E. F. (1968). Antimutagenic DNA polymerases of Bacteriophage T4. Cold Spring Harbor Symp. Quant. Biol. 33, 339.

Drake, J. W., Allen, E. F., Forsberg, S. A., Preparata, M., and Greening, E. O. (1969). Genetic control of mutation rates in bacteriophage T4. Nature 221, 1128.

Drake, J. W. and Greening, E. O. (1970). Suppression of chemical mutagenesis in bacteriophage T4 by genetically modified DNA polymerases. Proc. Natl. Acad. Sci. U.S. 66, 823.

Drake, J. W. (1970). The molecular basis of mutation. Holden-Day. San Francisco.

Drake, J. W. (1973). The genetic control of spontaneous and induced mutations rates in bacteriophage T4. Genetics Suppl. 73, 45.

Echols, H. and Gingery, R. (1968). Mutants of phage λ defective in vegetative recombination. J. Molec. Biol. 34, 239.

Eisenstark, A. (1977). Genetic recombination in bacteria. Ann. Rev. Genetics. 11, 369.

Evenchick, Z., Stacey, K. A., and Hayes, W. (1969). UV induction of chromosome transfer by autonomous sex factor in Escherichia coli. J. Gen. Microbiol. 56, 1.

Fogel, S., and Hurst, D. D. (1967). Meiotic gene conversion in yeast tetrads and the theory of recombination. Genetics. 57, 455.

Fogel, S., and Mortimer, R. K. (1969). Informational transfer in meiotic gene conversion. Proc. Natl. Acad. Sci. U.S. 62, 96.

Fowler, R. G., Degnen, G. E., Cox, E. C. (1974). Mutational specificity of a conditional Escherichia coli mutator, mutD5. Molec. Gen. Genetics. 133, 179

Fox, M. S., and Allen, M. (1964). On the mechanism of deoxyribonucleate integration in pneumococcus transformation. Proc. Natl. Acad. Sci. U.S. 52, 412.

Freese, E. (1959). The specific mutagenic effect of base analogues on phage T4. J. Molec. Biol. 1, 87.

Freese, E., Freese-Bautz, E., and Bautz, E. (1961). Hydroxylamine as a mutagenic and inactivating agent. J. Molec. Biol. 3, 133.

Geiger, J. R., and Speyer, J. F. (1977). A conditional antimutator in Escherichia coli. Molec. Gen. Genetics. 153, 87.

Gellert, M., Mizuuchi, K., O'Dea, M. H., and Nash, H. A. (1976). DNA gyrase: An enzyme that introduces superhelical turns into DNA (1976). Proc. Natl. Acad. Sci. U.S. 73, 3872.

Gellert, M., O'Deha, M. H., Itoh, T.,and Tomizawa, J. I. (1976). Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc. Natl. Acad. Sci. U.S. 73, 4474.

Gellert, M., Mizuuchi, K., O'Dea, M. H., Itoh, T., and Tomizawa, J. I. (1977). Nalidixic acid resistance: A second genetic character involved in DNA gyrase activity. Proc. Natl. Acad. Sci. U.S. 74, 4772.

George, D. L., and Witkin, E. M. (1974). Slow excision repair in an mfd mutant of Escherichia coli B/r. Molec. Gen. Genetics. 133, 283.

Gilbert, W., and Dressler, D. (1968). DNA replication: the rolling circle model. Cold Spring Harbor Symp. Quant. Biol. 33, 347.

Gudas, L. J., Pardee, A. B. (1975). A model for the regulation of Escherichia coli DNA repair functions. Proc. Natl. Acad. Sci. 72, 2330.

Gutz, H. (1971). Gene conversion: remarks on the quantitative implications of hybrid DNA models. Genet. Res. 17, 45.

Harward, J. H., Williams, E., and Bainbridge, B. W. (1972). Mutation of the methane-oxidising bacterium Methylococcus capsulatos. J. appl. Bacteriol. 35, 99.

Hayes, W. (1952). Recombination in Bact. coli: unidirectional transfer of genetic material. Nature 169, 118.

Hayes, W. (1968). The genetics of bacteria and their viruses (Blackwell Scientific Publications: Oxford).

Hill, R. F. (1972). Synergistic effect of an Escherichia coli mutator gene on mutagenesis by UV radiation and by alkylating agents. Mutat. Res. 14, 23.

Holliday, R. (1964). A mechanism for gene conversion in fungi. Genet. Res. 5, 282.

Hotchkiss, R. D. (1971). Towards a general theory of genetic recombination in DNA. Adv. in Genetics. 16, 325.

Hotchkiss, R. D. (1974). Models of genetic recombination. Ann. Rev. Microbiol. 28, 445.

Hoess, R. H. and Herman, R. K. (1975). Isolation and characterization of mutator strains of Escherichia coli K12. J. Bacteriol. 122, 474.

Hoess, R. H. and Fan, D. P. (1975). Further characterization of a non-essential mutator gene in Escherichia coli K12. J. Bacteriol. 124, 650.

Howard-Flanders, P. and Boyce, R. P. (1966). DNA repair and genetic recombination: studies on mutants of Escherichia coli defective in these processes. Radiat. Res. 6: (Suppl.), 156.

Howard-Flanders, P. and Theriot, L. (1966). Mutants of Escherichia coli defective in DNA repair and in genetic recombination. Genetics. 53, 1137.

Hurst, P. D., Fogel, F. and Mortimer, R. K. (1972). Conversion associated recombination in yeast. Proc. Natl. Acad. Sci. U.S. 69, 101.

Inouye, M. (1971). Pleiotropic effect of the recA gene of Escherichia coli: uncoupling of cell division from DNA replication. J. Bacteriol. 106, 539.

Jordan, E., Saedler, H. and Starlinger, P. (1968). 0^{o} and strong polar mutations in the gal operon are insertions. Molec. Gen. Genetics. 102, 353.

Joset, F. E. and Fournier, R. (1966). Induction of invertant males in Escherichia coli K12. Genet. Res. 7, 353.

Karu, A. E., Sakaki, Y., Goldmark, P. J. and Linn, S. (1975). The gamma protein specified by phage λ. Structure and inhibitory activity for the recBC enzyme of Escherichia coli. J. Biol. Chem. 250, 7377.

Kingsbury, D. T. and Helinski, D. R. (1970). "DNA polymerase as a requirement for the maintenance of the bacterialplasmid colicinogenic factor E1". Biochem. Biophys. Res. Commun. 7, 237.

Kirchner, C. E. J. and Rudden, M. J. (1966). Location of a mutator gene in Salmonella typhimurium by cotransduction. J. Bacteriol. 92, 1453.

Kitani, Y, and Olive, L. S. (1970). Alteration of gene conversion patterns in Sordaria fimicola by supplementation with DNA bases. Proc. Natl. Acad. Sci. U.S. 66, 1290.

Konrad, E. B. and Lehman, I. R. (1974). A conditional lethal mutant of Escherichia coli K12 defective in the 5'⟶3' exonuclease associated with DNA polymerase I. Proc. Natl. Acad. Sci. U.S. 71, 2048.

Konrad, E. B. and Lehman, I. R. (1975). Novel mutants of Escherichia coli that accumulate very small DNA replicative intermediates. Proc. Natl. Acad. Sci. U.S. 72, 2150.

Konrad, E. B. (1977). Method for the isolation of Escherichia coli mutants with enhanced recombination between chromosomal duplications. J. Bacteriol. 130, 167.

Kushner, S. R., Nagaishi, H. and Clark, A. J. (1971). Indirect suppresion of recB or recC mutations by exonuclease deficiency. Proc. Natl. Acad. Sci. U.S. 68, 1366.

Kushner, S. R., Nagaishi, H., Templin, A., and Clark, A. J. (1972). Genetic recombination in Escherichia coli: the role of exonuclease. Proc. Natl. Acad. Sci. U.S. 69, 824.

Kushner, S. R., and Zieg, J. (1977). Analysis of genetic recombination between two partially deleted lactose operons of Escherichia coli K12. J. Bacteriol. 131, 123.

Lederberg, J., Cavalli, L. L. and Lederberg, E. M. (1952). Sex compatability in Escherichia coli. Genetics. 37, 720.

Lederberg, J. (1955). Recombination mechanisms in Bacteria. J. Cell. Comp. Physiol. 45, 75.

Lerman, L. S. (1963). The structure of the DNA-acridine complex. 1963. Proc. Natl. Acad. Sci. 49, 94.

Liberfarb, R. M. and Bryson, V. (1970). Isolation, characterization and genetic analysis of mutator genes in Escherichia coli B and K12. J. Bacteriol. 104, 363.

Litman, R. M. and Pardee, A. B. (1956) The induction of mutants of bacteriophage T2 by 5-bromouracil. Biochim. Biophys. Acta. 42, 131.

Litman, R. M. and Pardee, A. B. (196o). The induction of mutants of bacterio-phage T2 by 5-bromouracil, 4 kinetics of bromouracil-induced mutagenesis, Biochem.Biophys.Acta. 42, 131.

Little, J. W. and Hanawalt, P. C. (1977). Induction of protein X in Escherichia coli. Molec. Gen. Genetics. 150, 237.

Lloyd, R. G. (1971). Ph.D. thesis.

Low, K. B. (1972). Escherichia coli K12 F prime factors, old and new. Bacteriol. Rev. 36, 587.

MacPhee, D. G. (1973). Effect of rec mutations on the ultraviolet-protecting and mutation-enhancing properties of the plasmid R-Utrecht in Salmonella typhimurium. Mutat. Res. 19, 357.

Magni, G. E. (1963). The origin of spontaneous mutations during meisosis. Proc. Natl. Acad. Sci. 50, 975.

Marinus, M. G. and Morris, N. R. (1973). Isolation of DNA methylase mutants from Escherichia coli K12 J. Bacteriol. 114, 1143.

Marinus, M. G. and Konrad, E. B. (1976). Hyper-recombination in dam mutants of Escherichia coli K12. Molec. Gen. Genetics. 149, 273.

Masker, W., Hanawalt, P. and Shizuya, H. (1973). Role of DNA polymerase II in repair replication in Escherichia coli. New Biol. 244, 242.

Masters, M. (1977). The frequency of P1 transduction of the genes of Escherichia coli as a function of chromosomal position: Preferential transduction of the origin of replication. Molec. Gen. Genetics. 155, 197.

Mattern, I. E. and Houtman, P. C. (1974). Properties of uvrE mutants of Escherichia coli K12. Construction and properties of pol and rec derivatives. Mutat. Res. 25, 281.

Meselson, M. and Weigle, J. J. (1961). Chromosome breakage accompanying genetic recombination in bacteriophage. Proc. Natl. Acad. Sci. U.S. 47, 857.

Meselson, M. (1972). Formation of hybrid DNA by rotary diffusion during genetic recombination. J. Molec. Biol. 71, 795.

Meselson, M. and Radding, C. R. (1975). A general model for genetic recombination. Proc. Natl. Acad. Sci. U.S. 72, 358.

Meynell, G. G. and Lawn, A. M. (1967). Sex pili and common pili in the conjugational transfer of colicin factor Ib by Salmonella typhimurium. Genet. Res. 9, 359.

Mitchell, M. B. (1955). Aberrant recombination of pyridoxine mutants of Neurospora. Proc. Natl. Acad. Sci. U.S. 41, 215.

Monk, M. and Kinross, J. (1972). Conditional lethality of recA and recB derivatives of a strain of Escherichia coli K12 with a temperature sensitive DNA polymerase I. J. Bacteriol. 109, 971.

Murray, N. E. (1970). Recombination events that span sites within neighbouring gene loci of Neurospora. Genet. Res. Camb. 15, 109.

Muzyczka, N., Poland, R. L. and Bessman, M. J. (1972). Studies on the biochemical basis of spontaneous mutation. J. Biol. Chem. 247, 7116.

Muzyczka, N. and Bessman, M. C., Goodman, M. and Schnaar, R. L. (1974). Studies on the biochemical basis of spontaneous mutation. J. Molec. Biol. 88, 409.

McEntee, K., Hesse, J. E. and Epstein, W. (1976). Identification and radiochemical purification of the recA protein of Escherichia coli K12. Proc. Natl. Acad. Sci. U.S. 73, 3979.

Nestman, E. R. (1977). Antimutagenic effects on spermine and guanosine in continuous cultures of Escherichia coli mutator strain mutH$^-$. Molec. Gen. Genetics. 152, 109.

Norkin, L. C. (1970). Marker-specific effects in genetic recombination. J. Molec. Biol. 51, 633.

Notani, N., and Goodgal, S. H. (1966). On the nature of recombinants formed during transformations in H. influenzae. J. Gen. Physiol. 49, suppl. 197.

Oppenheim, A. B. and Riley, M. (1966). Molecular recombination following conjugation in Escherichia coli. J. Molec. Biol. 20, 331.

Oppenheim, A. B. and Riley, M. (1967). Covalent union of parental DNA following conjugation in Escherichia coli. J. Molec. Biol. 28, 503.

Paszewski, A. (1970). Gene conversion: observations on the DNA hybrid models. Genet. Res. 15, 55.

Pettijohn, D. and Hanawalt, P. (1964). Evidence for repair replication of ultraviolet damaged DNA in bacteria. J. Molec. Biol. 9, 395.

Pritchard, R. H. (1955). The linear arrangement of a series of alleles of Aspergillus nidulans. Heredity 9, 353.

Pritchard, R. H. (1960). Localised negative interference and its bearing on models of gene recombination. Genet. Res. Camb. 1, 1.

Radding, C. M. (1973). Molecular mechanisms in recombination. Ann. Rev. Genetics. 7, 87.

Radman, M. (1975). SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis. (In molecular mechanisms for the repair of DNA, Plenum Press, New York).

Richardson, C.C. and Chase, J. W. (1977). Escherichia coli mutants deficient in exonuclease VII. J. Bacteriol. 129, 934.

Roberts, J. W. and Roberts, C. W. (1975). Proteolytic cleavage of bacteriophage Lambda repressor in induction. Proc. Natl. Acad. Sci. U.S. 72, 147.

Roth, J. R. (1974). Frameshift mutations. Ann. Rev. of Genetics. 8, 319.

Rothman, R. H. and Clark, A. J. (1977). Defective excision and postreplication repair of UV-damaged DNA in a recL mutant strain of Escherichia coli K12. Molec. Gen. Genetics. 155, 267.

Rupp, W. D. and Howard-Flanders, P. (1968). Discontinuities in the DNA synthesized in an excision defective strains of Escherichia coli following ultraviolet irradiation. J. Molec. Biol. 31, 291.

Saedler, H. and Heiss, B. (1973). Multiple copies of the insertion DNA sequences IS1 and IS2 in the chromosome of Escherichia coli K12. Molec. Gen. Genetics 122, 267.

Sakaki, Y., Karu, A. E., Linn, S. and Echols, H. (1973). Purification and properties of the γ protein specified by phage λ: an inhibitor of the host recBC recombination enzyme. Proc. Natl. Acad. Sci. U.S. 70, 2215.

Scaife, J. and Gross, J. (1963). The mechanism of chromosome mobilization by F prime factor in Escherichia coli K12. Genet. Res. Camb. 4, 328.

Sedgwick, S. G. (1975). Inducible error-prone repair in Escherichia coli. Proc. Natl. Acad. Sci. 2753.

Sesnowitz-Horn, S. and Adelberg, E. A. (1968). Proflavin treatment of Escherichia coli: generation of frameshift mutations. Cold Spring Harbor Symp. Quant. Biol. 33, 393.

Siegel, E. C. and Bryson, V. (1967). Mutator gene of Escherichia coli B. J. Bacteriol. 94, 1.

Siegel, E. C. (1973). Ultraviolet-sensitive mutator strains of Escherichia coli K12. J. Bacteriol. 113, 145.

Siegel, E. C. (1973). Ultraviolet-sensitive mutator mutU4 of Escherichia coli inviable with polA. J. Bacteriol. 113, 161.

Siegel, E. C. and Kamel, F. (1974). Reversion of frameshift mutations by mutator genes in Escherichia coli. J. Bacteriol. 117, 994.

Siegel, E. C. and Ivers, J. J. (1975). Mut-25, a mutation to mutator linked to purA in Escherichia coli. J. Bacteriol. 121, 524.

Siegel, E. C., Conrad, S. E. and Dussik, K. T. (1976). Bacteriophage Mu-1-induced mutation to mutT in Escherichia coli. J. Bacteriol. 125, 1018.

Sigal, N. and Alberts, B. (1972). Genetic recombination : the nature of a crossed strand exchange between two homologous DNA molecules. J. Molec. Biol. 71, 789.

Signer, E. R. and Weil, J. (1968). Recombination in phage λ. I. Mutants deficient in general recombination. J. Molec. Biol. 34, 261.

Sinzinis, B. I., Smirnov, G. B. and Saenko, A. S. (1973). Repair deficiency in Escherichia coli UV-sensitive mutator strain uvr502. Biochem. Biophys. Res. Commun. 53, 309.

Skaar, P. D. (1956). A binary mutability system in Escherichia coli. Proc. Natl. Acad. Sci. U.S. 42, 245.

Smirnov, G. B. and Skavronskaya, A. G. (1971). Location for uvr502 mutation on the chromosome of Escherichia coli K12. Molec. Gen. Genetics. 113, 217. Molec. Gen. Genetics. 113, 217.

Smirnov, G. B. and Skavronskaya, A. G. (1971). Suppression of UV sensitivity in Escherichia coli uvr502 by the su58 missense suppressor. J. Bacteriol. 115, 486.

Sobel, H. M. (1972). Molecular mechanism for genetic recombination. Proc. Natl. Acad. Sci. U.S. 69, 2483.

Sobel, H. M. (1973). Symmetry in protein-nucleic acid interaction and its genetic implications. Adv. in Genetics 17, 411.

Speyer, J. F. (1965). Mutagenic DNA polymerase. Biochem. Biophys. Res. Commun. 21, 6.

Speyer, J. F., Karam, J. D. and Lenny, A. B. (1966). On the role of DNA polymerase in base selection. Cold Spring Harbor Symp. Quant. Biol. 31, 693.

Stacey, K. A., and Simson, E. (1965). Improved method for the isolation of thymine-requiring mutants of Escherichia coli. J. Bact. 90, 554.

Stacey, K. A. and Lloyd, R. G. (1976). Isolation of Rec$^-$ mutants from a F-prime merodiploid strain of Escherichia coli K12. Molec. Gen. Genetics. 143, 223.

Stacey, K. A., and Oliver, P. (1977). A novel pleiotropic mutation in Escherichia coli K12 which affects transduction, transformation and rates of mutation. J. Gen. Microbiol. 98, 569.

Stacey, K. A. and Kalaycioglu, A. (1977). A novel hyper-recombination mutant of Escherichia coli K12. Proc. Soc. Gen. Microbiol. (1977). 136.

Stahl, F. W., McMilin, K. D., Stahl, M. and Nozu, Y. (1972). An enhancing role for DNA synthesis in formation of phage λ recombinants. Proc. Natl. Acad. Sci. U.S. 69, 3598.

Stallcup, M. R., Sharrock, W. J., and Rabinowitz, J. S. Ribosome and messenger specificity in protein synthesis by bacteria. Biochem. Biophys. Res. Commun. 58, 92.

Sugino, A., Peebles, C. L., Kreuzer, K. N. and Cozzarelli N. R. (1977). Mechanism of action of nalidixic acid: purification of Escherichia coli nalA gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. U.S. 74, 4767.

Symonds, N. (1974). Recombination. MTP international Review of Science, Biochemistry of Nucleic acids. Volume 6, 165.

Tomizawa, J. I. and Anraku, N. (1965). Molecular mechanism of genetic recombination of phage. Absence of polynucleotide interruption in DNA of T4 and lambda phage particles with special reference to heterozygosis. J. Molec. Biol. 11, 509.

Treffers, H. P., Spinelli, V. and Belser, N. O. (1954). A factor (or mutator gene) influencing mutation rates in Escherichia coli. Proc. Natl. Acad. Sci. U.S. 40, 1064.

Unger, R. C., Echols, H. and Clark, A. J. (1972). Interaction of the recombination pathways of phage λ and host Escherichia coli: effects on λ recombination. J. Molec. Biol. 70, 531.

Unger, R. C., and Clark, A. J. (1972). Interaction of the recombination pathways of phage λ and its host Escherichia coli K12. effects on exonuclease V activity. J. Molec. Biol. 70, 539.

Wang, J. C. (1971). Interaction between DNA and an *Escherichia coli* protein. J. Molec. Biol. 55, 523.

Weisberg, R. A. (1977). Illegitimate recombination in bacteria and bacteriophage. Ann. Rev. of Genetics. 11, 451.

West, S. C. and Emmerson, P. T. (1977). Induction of protein synthesis in *Escherichia coli* following UV - or γ irradiation, mitomycin C treatment or *tif* expression. Molec. Gen. Genetics. 151, 57.

West, S. C. and Emmerson, P. T. (1977). Identification of protein X of *Escherichia coli* as the *recA*$^+$/*tif*$^+$ gene product. Molec. Gen. Genetics. 155, 77.

Whitehouse, H. L. K. (1963). A theory of crossing over by means of hybrid DNA. Nature 199, 1034.

Whitehouse, H. L. K. (1966). An operator model of crossing-over. Nature 211, 708.

Wildenberg, J. and Meselson, M. S. (1975). Mismatch repair in heteroduplex DNA. Proc. Natl. Acad. Sci. U.S. 72, 2202.

Witkin, E. M. (1969). The mutability toward UV light of recombination deficient strains of *Escherichia coli*. Mutat. Res. 8, 9.

Worcel, A. and Burgi, E. (1972). On the structure of the folded chromosome of *Escherichia coli*. J. Molec. Biol. 71, 127.

Wu, R. (1970). Nucleotide sequence analysis of DNA. I partial sequence of the cohesive ends of bacteriophage λ or 186 DNA. J. Molec. Biol. 51, 501.

Wulff, D. L. and Rupert, C. S. (1962). Disappearance of thymine photodimer in ultraviolet irradiated DNA upon treatment with a photoreactivating enzyme from baker's yeast. Biochem. Biophys. Res. Commun. 7, 237.

Yanofsky, C. and Cox, E. C. (1969). Mutator gene studies in Escherichia coli. J. Bacteriol. 100, 390.

Youngs, D. A. and Smith, K. C. (1973). Involvement of DNA polymerase III in excission repair after UV irradiation. New. Biol. 244, 240.

Zamenhof, P. J. (1966). A genetic locus responsible for generalized high mutability in Escherichia coli. Proc. Natl. Acad. Sci. U.S. 56, 845.