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Review

Mismatch repair in recombination of bacteriophage T4

Victor P. Shcherbakov

Institute of Problems of Chemical Physics RAS,
Chernogolovka 142432, Moscow Region, Russia

e-mail: svp@icp.ac.ru

Abstract

The review focuses on the mechanism of mismatch repair in bacteriophage T4. It was first observed in T4 as an extra recombination mechanism, which contributed to the general recombination only when particular rII mutations were used as genetic markers (high-recombination markers), whereas it was inactive toward other rII mutations (low-recombination markers). This marker-dependent recombination pathway was identified as a repair of mismatches in recombinational heteroduplexes. Comparison of the structure of markers enabled us to make several specific conclusions on the nature of the marker discrimination by the mismatch repair system operating during T4 crosses. First, heteroduplexes with one mismatched base pair (either of transition or of transversion type) as well as single-nucleotide mismatches of indel type are not efficiently repaired. Second, among the repairable mismatches, those with two or more contiguous mismatched nucleotides are the most effectively repaired, whereas insertion of one correct pair between two mismatched ones reduces the repairability. Third, heteroduplexes containing insertion mutations are repaired asymmetrically, the longer strand being preferentially removed. Fourth, the sequence environment is an important factor. Inspection of the sequences flanking mismatches shows that runs of A:T pairs directly neighboring the mismatches greatly promote repair. The mismatch is recognized by T4 endonuclease VII and nicked on the 3′ side. The nonpaired 3′ terminus is attacked by the proofreading 3′→5′ exonuclease of T4 DNA polymerase that removes the mismatched nucleotides along with several (~25) complementary nucleotides (the repair tract) and then switches to polymerization. The residual nick is ligated by DNA ligase (gp30). Most probably, the T4 system repairs replication and other mismatches as well; however, it might not discriminate old and new DNA strands and so does not seem to be aimed at repair of replication errors, in contrast to the most commonly studied examples of mismatch repair.

Keywords: bacteriophage T4; DNA polymerase; endonuclease VII; genetic recombination; mismatch repair.

Introduction

The existence of biological entities crucially depends on reliable reproduction of their genomes. Theoretically, it is clear that the fidelity of genome reproduction must be such that most progeny receives functional genetic information. This is provided by the high fidelity of DNA replication and by numerous DNA repair systems operating at various levels of DNA metabolism. The major contributors to the fidelity of DNA replication are the high selectivity of DNA polymerase against nucleotide misincorporation, the proofreading activity of replicative DNA polymerases, and the mismatch repair (MMR) [see Refs. (1–7) for reviews].

There are two major sources of mismatches: misincorporation of bases during DNA replication or repair (errors of replication) and recombinational heteroduplex intermediates. Modification of DNA bases is also known as an essential source of mismatches. For example, deamination of 5-methylcytosine to thymine leads to the formation of G/T mismatches, which are the major source of spontaneous transitions in *Escherichia coli* (8). The biological significance of the replication mismatches and those resulting from the base modification, on the one hand, and the recombinational mismatches, on the other hand, is quite different. The replication errors and base modifications are mutagenic, so their repair is biased against the miscoded strand, and it restores the parental sequence. Correction of the replication error requires that the newly synthesized strand be targeted for excision. In bacteria, the discrimination depends on the transient absence of adenine methylation at GATC sequences in the daughter DNA strand. The repair endonuclease cleaves the newly synthesized, temporarily unmethylated strand at hemimethylated GATC sites. Hence, postreplication repair, at least as a biased process, operates soon after the replication (before DNA methylation). MMR systems operating without definite connection to the DNA replication also exist (9).

Repair of the replication mismatches enhances the accuracy of DNA replication at least 1000-fold (10). In the case of the recombinational mismatches, both strands are parental; therefore, their repair, biased or unbiased, cannot enhance the overall fidelity of genome reproduction. The genetic consequence of this repair is gene conversion. Thus, its significance should be appreciated in the evolutionary context.

Historically, it was the repair of recombinational mismatches that was first discovered and characterized. The idea of enzymatic repair of mismatches was coined by Holliday (11) to account for the production of homozygous mutant clones during UV-induced mutagenesis. The notion of MMR was used to explain the marker effects and gene conversions

associated with meiotic recombination in fungi (12, 13). The evidence for MMR contribution to gene conversion was presented by Gutz (14) and Leblon and Rossignol (15), who studied separate and joint conversion and postmeiotic segregation of heteroalleles in *Schizosaccharomyces pombe* and *Ascobolus immerses*, respectively. Their data agreed well with the idea that the gene conversion is an indication of MMR, whereas postmeiotic segregation results from the absence of MMR.

In an attempt to obtain a more direct proof of MMR, many researchers used transfection or transformation of cells with heteroduplex DNAs prepared *in vitro* and assessed the progeny for homozygous clones or definite recombinants that could appear only if the mismatches were repaired before the first DNA replication. Such experiments were made with *in vitro* prepared heteroduplex DNAs of phages λ (16–19), SPP1 (20), ϕX174 (21), and f1 (22); bacteria *Bacillus subtilis* (23) and *Streptococcus pneumoniae* (24); and animal viruses (25). All the researchers concluded that MMR systems operate in bacterial and animal cells. The data were interpreted in terms of excision repair-like mechanisms of MMR. Despite the general similarity of MMR to the excision repair of UV lesions, it was clear that these processes differ in enzymology (17, 26, 27). The results of the transfection and transformation experiments were regarded as direct evidence for MMR.

Marker-specific variations in the transformation efficiency were observed in *S. pneumoniae* (28–30) and *Haemophilus influenzae* (31), in phage crosses (32) and in *E. coli* recombination (33–35). They were interpreted in terms of MMR. This interpretation was supported by the isolation of mutator strains (hex) of *S. pneumoniae* (30, 26) and *H. influenzae* (31) and mut of *E. coli* (17, 36). The mutator strains were deficient in marker-discrimination ability (36). The *E. coli* MMR pathway (genes *MutL*, *MutS*, *MutH*, *and UvrD*) has been extensively studied and is well characterized (6). Homologues of *E. coli MutS* and *MutL* were also found in eukaryotic organisms including yeast and human cells (3, 4). A broad summary of the genetics and enzymology of DNA repair systems in prokaryotic and eukaryotic organisms is presented in a recent review by Morita et al. (37).

Marker-dependent recombination in bacteriophage T4

This review focuses on the mechanism of MMR in bacteriophage T4, which is special in many respects. An MMR event in recombinational heteroduplexes results in double genetic exchange. (We define exchange or genetic exchange as a point in the DNA strand where the sequences of two parents encounter.) This understanding was drawn to explain the phenomenon of high negative interference (HNI) in bacteriophage λ (38–40). The HNI (detected as a great excess of multiple exchanges over their frequency predicted from a random coincidence of single exchanges) was first observed in T4 crosses (41). It was interpreted in purely statistical terms (hence, its improper designation). It is known that the apparent excess of multiple exchanges does not result from repeated recombinational events, as if one recombinational event increased the probability of the other recombinational event in the nearest vicinity. The multiple exchanges is the intrinsic property of recombination via Holliday junction (HJ) (12, 42, 43), a resolution of which gives hybrid regions with recombinant and parental flanks termed splices and patches (44). [A hybrid region is a recombination intermediate in which two DNA strands originated from different parents (11).] A patch is equivalent to a double exchange. If a genetic marker falls within the hybrid region, it produces a mismatch (heteroduplex) that can be repaired, giving an additional double exchange. If different mismatches are repaired with different efficiency, the recombination frequencies are expected to show marker specificity. Proceeding from this consideration, we carried out a large series of two- and three-factor crosses between closely linked T4 *rII* mutants. Ten of the 30 markers used in these crosses were found to be susceptible to an extra recombination mechanism to which the other markers seemed to be not susceptible (45–48).

Recombinational analysis of rII mutants of phage T4

Bacteriophage T4, along with other T-even phages, represents one of the most suitable model organisms for molecular genetic studies. The genetics of phages (even the molecular genetics in general) started with the work by Hershey and Rotman (49) on T2. Phage T4 was used in numerous genetic, biochemical, and physicochemical investigations. The wild-type T4 multiplies on *E. coli* strains B and K12, including the λ-lysogenic strains. On the bacterial lawn, it produces plaques with a small transparent center surrounded by a turbid halo. T4 strains with mutations in the adjacent *rIIA* and *rIIB* genes are distinguished by two properties important for the genetic analysis: they produce large transparent plaques on *E. coli* B (49), and they do not multiply on the λ-lysogenic *E. coli* strains (50). This enables one to observe *rII* mutants easily and selectively detect the wild-type revertants and recombinants in crosses between different *rII* mutants. This endowed the recombination analysis with a very high resolution, enabling one to discriminate mutations at adjacent nucleotides. Another favorable circumstance is the availability of *E. coli* strains with suppressors of nonsense mutations. The suppressor transfer RNAs recognize the nonsense codons UAA (ochre), UAG (amber), and UGA (opal) as sense codons.

The proximal part of gene *rIIB* (~200 bp) is not essential for multiplication of *rII* mutants in λ-lysogenic cells (51). Only nonsense and frameshift mutants in this segment have the rII phenotype; double frameshifts with opposite signs have the wild-type phenotype. This enables one to observe the reciprocal (double mutant) recombinants easily. Most of our MMR study was performed with the mutants located in this nonessential segment of *rIIB* gene (Figure 1).

Despite the great popularity of T4 in the early molecular genetic studies, the mechanism of MMR in this organism had been studied only by two groups. *In vivo* studies

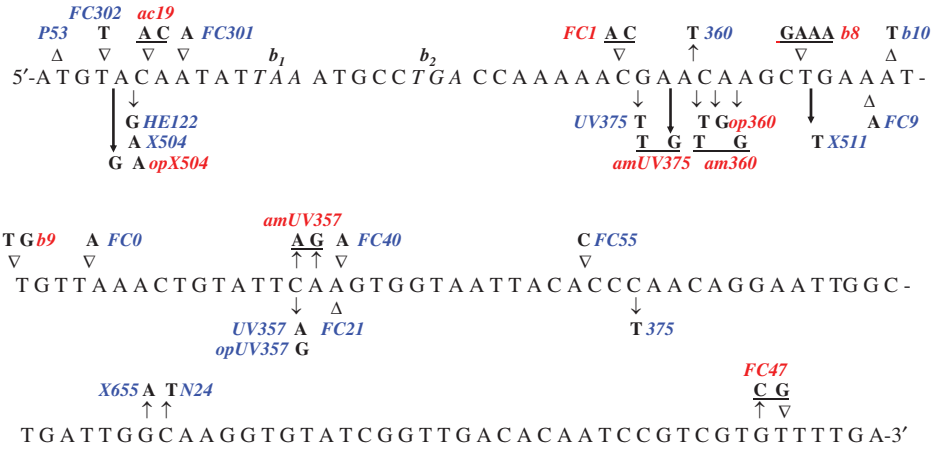

Figure 1 Genetic map of the initial part of *rIIB* gene of T4 phage; modified from Ref. (46).
The names of the mutations are given in italic type. Mutants with single-base change and those with more than one base change are shown in blue and in red, respectively. ↑ or ↓ means base substitution; ∇ and Δ designate insertion or deletion, respectively, of one or more base pairs. New (mutant) nucleotides are in bold; b_1 and b_2 designate termination codons (italic) out of phase.

were carried out by my group at the Institute of Problems of Chemical Physics of the Russian Academy of Sciences in Chernogolovka; *in vitro* studies were carried out in the laboratory of Borries Kemper at the Institute of Genetics, University of Cologne. I would like to specially acknowledge here the contribution by Oleg Toompuu, an Estonian scientist who worked in my group as a visiting researcher. Having a rather good mathematical background, Oleg helped us to develop a strictly formalized recombinational analysis (52), which was especially important at the time we started our investigation (the middle of the 70s) when the physical distances and DNA sequences were not available. We successfully used the genetic distances in the analysis of the recombinational data and measured marker effects as a deviation from additivity. Our first results were published in Russian (53–59).

One useful concept was a notion of 'basic recombination'. Basic recombination is the recombination resulting from DNA strand exchanges without any contribution from MMR or marker interference. In case of recombination via HJs at distances between the genetic markers smaller than the length of the patch, the basic recombinant frequency should be a linear function of the genetic distance between the markers. A deviation from the linearity is an indication of the marker effect. Another useful concept was the concept of 'indicator distances'. We defined the indicator distance as an interval of small length compared to the mean length of the hybrid region, but exceeding the length of the DNA segment involved in a single repair event. The mismatches separated by the indicator distance are spaced closely enough to fall preferentially into the same hybrid region, but are sufficiently far apart to rule out their joint repair. Within indicator distances, the MMR contribution to recombination reaches its maximum: when the distance between two markers is shorter than the repair region, their joint repair (not resulting in recombinant formation) becomes possible; when the distance increases to a length comparable to that of a hybrid region, the MMR contribution also diminishes because of the reduced probability that both markers fall simultaneously into the same hybrid region. It is clear that the concept of indicator distances could work only if the repair tract is much shorter than the length of the hybrid region. This lucky situation happens to be real in T4. The mean length of the hybrid regions in T4 estimated by various methods (52, 59, 60) is 330–420 bp, whereas the length of the repair region is ~25 bp (46).

The results of two-factor crosses between the mutants shown in Figure 1 are presented in Figure 2. The recombinant frequencies obtained in crosses between the strains with single-base substitution or single-nucleotide indel type mutations

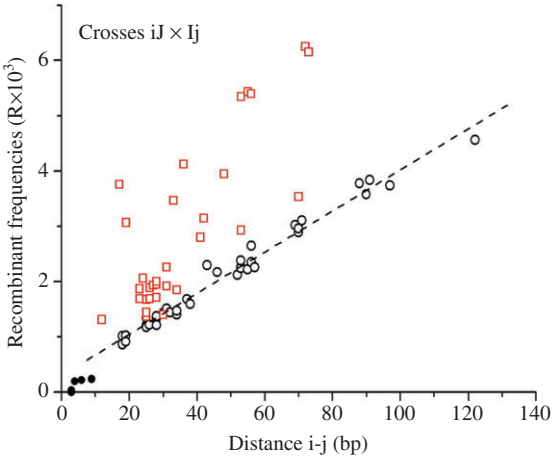

Figure 2 Distance-frequency relationship and marker effects in two-factor iJ×Ij crosses (46).
The markers i and j are mutations in the proximal part of *rIIB* gene. *R* designates the frequency of rII^+ recombinants. Open circles mark crosses between single-nucleotide mutants of base substitution or indel type; squares mark crosses in which either one or both markers were mutations with more than one changed nucleotides of base substitution or indel type (Figure 1). The regression line was drawn through the points marked by open circles (basic recombination) by the least squares method. Filled circles correspond to the crosses at ultrashort distances. They deviate significantly from the regression line because of marker interference (61).

(shown by empty circles) demonstrate a very good linear relationship between the wild-type recombinant frequencies R and the physical distance D, giving regression line

$$R=(3.02\pm0.037D)\times10^{-4} \quad (1)$$

The observed linearity means that within the *rIIB* gene segment under study (122 bp), recombination events are distributed uniformly, and the single-nucleotide mismatches formed by the markers used in these crosses are rarely corrected (if at all) to the wild-type allele. These markers were named low-recombination (LR) type. Four filled circles correspond to the crosses at ultrashort distances. These frequencies deviate significantly from the regression line because of marker interference (61). Note that the empirical straight line does not run through the origin of coordinates, cutting off a segment of the ordinate equal to 3.0×10^{-4}. Although its absolute value is small, it differs significantly from zero. We discussed this phenomenon in Ref. (48) and presented arguments in favor of the existence of a subpopulation of very short (below 20 bp) patches.

The heterogeneous group of crosses, in which markers i or j or both (shown by squares on the graph) have more than one nucleotide changed, demonstrated various deviations from linearity. All these crosses gave higher frequencies of rII^+ recombinants than is predicted by the basic line. We called them high-recombination (HR) markers and considered them to be a subject of MMR.

Ways to measure mismatch repairability

Within indicator distances, the contribution of MMR to recombination reaches its maximum. When the distance between two markers is shorter than the repair region, their joint repair (not resulting in recombinant formation) becomes possible. When the distance increases to a length comparable to that of a hybrid region, the contribution of MMR diminishes because of the reduced probability that both markers fall simultaneously into the same hybrid region.

Taking advantage of the empiric plot for distance-related recombination (Figure 2), and provided the physical position of a given mutation is known, one can estimate the contribution of MMR to the recombinant frequency by the equation

$$\kappa_j=R_{\text{JI}}-R_{\text{basic}} \quad (2)$$

where κ_j is the repairability of marker j (input of MMR to the measured recombinant frequency), R_{JI} is the measured frequency in the cross jI×iJ, and R_{basic} is the recombinant frequency corresponding to the distance i–j [according to Eq. (1)]. The repairability defined this way is the difference between the measured recombinant frequency and its value expected in the absence of MMR. The distance j–i should be an indicator distance; i.e., it should be short when compared to the mean length of the patch but should exceed the length of the repair tract. At indicator distances, MMR makes its maximum contribution to the recombinant frequency. This is illustrated in Figure 3 for the HR marker FC1. In a series

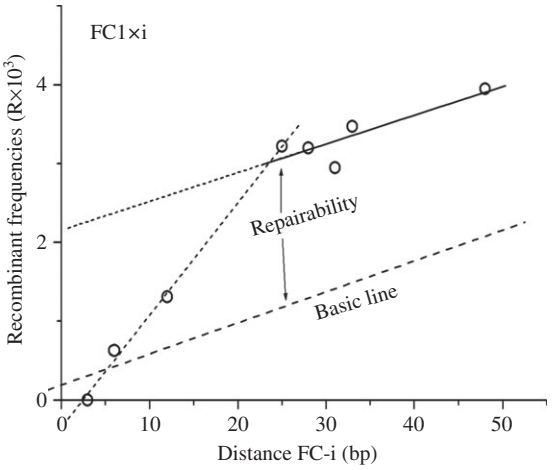

Figure 3 An example of measuring the repairability value and repair tract length.
Open circles show the recombinant frequencies obtained in crosses of HR marker FC1 against several LR markers i. Basic line, taken from Figure 2, shows frequency-distance relationship in the absence of MMR. The recombinant frequencies first rapidly grow with the distance, and then, at ~25 bp, the difference between the recombinant frequency and the basic line reaches maximal value, which is the MMR contribution to the recombinant frequency (repairability FC1 → +). The corresponding distance (~25 bp) is the repair tract.

of FC1×i crosses, the recombinant frequency shows a two-phase character. During the first phase, it grows rapidly with distance until a special point corresponding to the indicator distance. At this point, the difference between the observed recombinant frequencies and the basic line reaches its maximum value, which is the measured repairability. Because the contribution of MMR decreases with the distance FC1-i (see above), the slope of the FC1 line is a bit smaller than that of the basic line. Thus, it is more appropriate to calculate repairability as the difference between the points of intersection of the corresponding lines with the ordinate. The repairability determined in this way is a constant characterizing the given marker. For the data shown in Figure 3, the points of intersection for the basic line and FC1 line are 0.195×10^{-3} and 2.155×10^{-3}, respectively; hence, $\kappa_{FC1}=1.96\times10^{-3}$.

The data in Figure 3 also enable one to measure the length of the repair tract. The repair tract is equal to the distance corresponding to the end of the first phase of the frequency-distance relationship. The difference between the basic line and the FC1 line reaches its maximum value at this point. The repair tract length equals ~25 bp in this case.

An alternative way to measure the repairability is illustrated by three-factor crosses of the type i k×j. For example (Figure 4), one may compare crosses HE122 FC55×amUV357 and HE122 FC55×UV357 (46). The HR marker amUV357 and the LR marker UV357 occupy the same position. Both side markers HE122 and FC55 are of the LR type, and both intervals HE122-UV357 and UV357-FC55 correspond to indicator distances. In most cases, all three sites fall into the same hybrid region. In the cross HE122 FC55×UV357, the double exchanges leading to the formation of wild-type recombinants

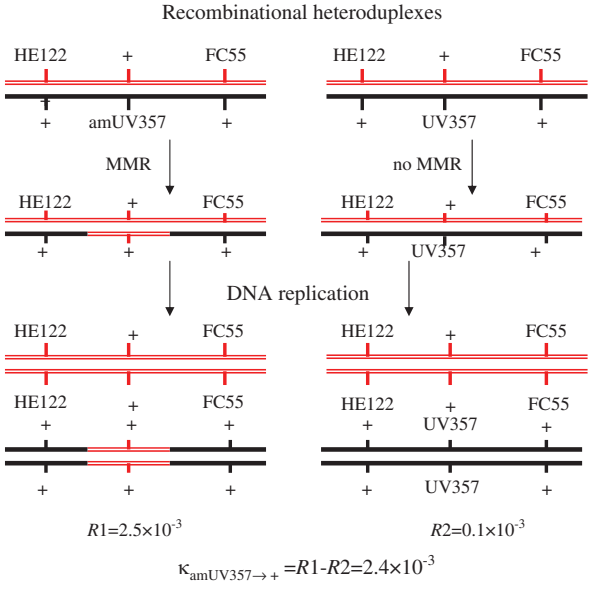

Figure 4 A way to estimate MMR contribution (κ) to the recombinant frequency (*R*) in homoallelic three-factor crosses HE122 FC55×amUV357 and HE122 FC55×UV357.
Heteroduplex intermediates and their repair and replication are shown. Repairability $\kappa_{amUV357\rightarrow +}=R1\text{-}R2=2.4\times10^{-3}$ (46).

are very rare events, whereas in the case of amUV357, double exchanges are produced via MMR. The observed frequency of wild-type recombinants minus that observed in the cross HE122 FC55×UV357 is the contribution of MMR to the wild-type recombinant frequency ($\kappa_{amUV357\rightarrow +}$). The values of repairability were estimated for many *rIIB* mutants, with the two different methods giving good congruence (46).

Rate of MMR

The measured values of repairability are the product of the probability for a marker to fall into a recombinational heteroduplex and the probability for it to be recognized and repaired before the heteroduplex DNA is replicated or packaged. These values can be used to estimate the conditional probability for the conversion a→A to occur once the a/A mismatch has been formed. The numerous calculations made in Ref. (46) gave values ranging from <2% for LR markers to more than 25% for the most effectively repaired HR markers.

Asymmetry of MMR

In the crosses shown in Figure 2, we scored recombinants of only one reciprocal class, namely, the rII$^+$ recombinants. A mismatched region is structurally asymmetric. Thus, it was reasonable to expect that repair of mismatches in two alternative directions could occur at different rates. Because the initial segment of the *rIIB* gene is not essential for T4 growth on the λ-lysogenic *E. coli*, the mutations producing frameshifts in opposite directions suppress each other with a high efficiency. The double mutants have completely wild-type phenotype, and both reciprocal recombinants in crosses between such mutants produce plaques on the λ lysogens. In some special cases, the reciprocal recombinants could be scored separately. For example, in the cross FC302×FCl (Figure 5A), two reciprocal recombinants arise: true wild type and the double mutant FC302-FC1. Both recombinants produce plaques on *E. coli* CA167(λ) bearing an ochre suppressor, whereas the double mutant cannot grow on *E. coli* 594(λ) because of the terminating ochre triplet TAA (barrier b_1) generated by the FC302 phase shift. The total frequency of two reciprocal recombinants measured on CA167(λ) was 4.9×10^{-3}, and the frequency of true wild-type recombinants measured on 594(λ) was 3.2×10^{-3}. Hence, FC302 FC1 double mutant is formed at a frequency of 1.5×10^{-3} in this cross. Importantly, this value does not differ from the corresponding value predicted in the absence of MMR (basic line in Figure 2).

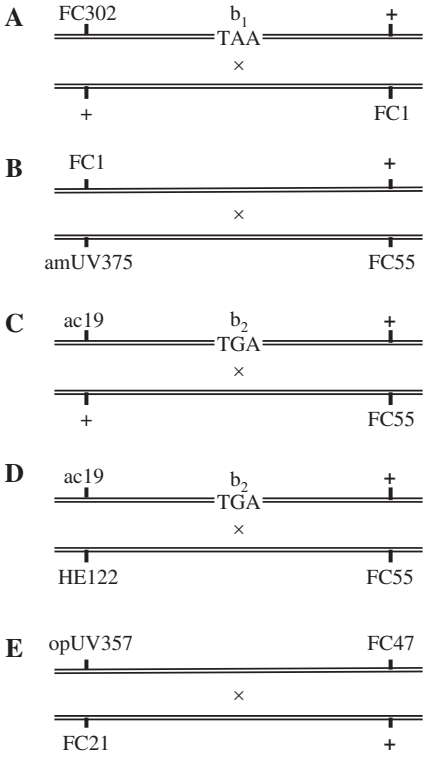

Figure 5 Examples of crosses used to separate measuring of frequencies of reciprocal recombinants.
(A) The method makes use of the fact that the reciprocal recombinants have different phenotypes and could be discriminated on proper *E. coli* strains: 594(λ) and CA244(λ) are wild-type (nonsuppressing) strains; the strains CA167(λ), CA265(λ), and K223(λ) use nonsense codons UAA (ochre), UAG (amber), and UGA (opal), respectively, as sense codons. (A) FC302 (one nucleotide insertion) shifts phase to the right, creating the ochre codon. Reciprocal recombinants are FC302 FC1 (ochre) and + + (rII$^+$). (B) Reciprocal recombinants are FC1 FC55 (rII$^+$ phenotype). (C) ac19 (two nucleotide insertions) makes a phase shift to the left. Reciprocal recombinants are ac19 FC55 (opal) and + + (rII$^+$). (D) Reciprocal recombinants are ac19 FC55 (opal) and HE122+(amber). (E) Reciprocal recombinants are opUV354+(opal) and FC21 FC47 (rII$^+$ phenotype) (47, 55). See text.

It means that there is no significant MMR of the wild-type alleles to FC302 and to FCl, and virtually the entire observed repairability is explained by the repairability FC1→+. Note that the FC1 is an insertion of two nucleotides AC, so the observed asymmetry of repair of the mismatch FCl/+ suggests preferential removal of the longer DNA strand. This was confirmed in other crosses with FC1.

In three-factor cross FCl×amUV375-FC55 (Figure 5B), the allele FCl is situated just opposite the sequence amUV375 on the chromosome of the mating partner, which differs from that of wild type by two nucleotides (Figure 1). As a result, a rather complex mismatch is formed, which includes a bulge of two nucleotides along with two mismatched nucleotide pairs. The correction FCl→amUV375 contributes to the frequency of recombinants of the amber phenotype, whereas the opposite correction amUV375→FC1 would contribute to the frequency of double mutants FCl-FC55 of the wild-type phenotype. On the amber-suppressing *E. coli* strain CA265(λ), which is permissive for both recombinants, and on *E. coli* CA244(λ), which is permissive only for FCl-FC55, the recombinant frequencies were 4.7×l 0^{-3} and 1.4×l0^{-3}, respectively. The latter frequency did not exceed the corresponding basic frequency. Thus, there was no correction of amUV375 to FCl, whereas the opposite correction FCl → amUV375 occurred at a rate similar to that of FCl→+. It was evident that the longer strand was preferentially removed during MMR in this case as well. The results obtained with several other markers producing mismatches with unequal strands all agreed with the idea that a longer strand is removed during MMR (Figure 5 and Table 1). They also demonstrated the importance of both DNA strands in mismatch recognition (47, 55).

Table 1 Structures and repairability values for some of the mismatches formed in crosses of *rIIB* mutants (47).

Mismatch structure[a]	Repairability[b]	
J/j	j→J	J→j
wt -ATT***C***AAGTT- -TAA***A***TTCAA- UV357	Low	nm
wt -ATT***C***AAGTT- -TAA***C***TTCAA- opUV357	Low	Low
wt -ATTCA***A***GTT- -TAAGT_CAA- FC21	Low	Low
FC21 -ATT***C***A_GTT- -TAA***C***T***T***CAA- opUV357	1.0×10^{-3}	Low
wt -ATTCA_AGTT- -TAAGT***T***TCAA- FC40	Low	Low
wt -ATT***CA***AGTT- -TAA***AG***TCTT- amUV357	2.8×10^{-3}	nm
opUV357 -TAA***C***_TTCAA- -ATT***CA***AAGTT- FC40	4.1×10^{-3}	nm

[a]Mismatched and nonmatched nucleotides are in italic bold type.
[b]The term 'repairability' designates the contribution of MMR to the recombinant frequency; j→J or J→j shows direction of correction of the j/J mismatch. 'Low' means that the measured recombinant frequency did not differ significantly from the corresponding basic value; nm means not measured.

Factors affecting the mismatch repairability

Together, we analyzed the recombinational properties of all the markers shown in Figure 1 in their numerous combinations (46–48). The results enabled us to make several definitive conclusions on the nature of the marker discrimination by the MMR system operating during T4 crosses. First, heteroduplexes with one mismatched base pair, either transitions or transversions, as well as single-nucleotide mismatches of indel type are not repaired at significant frequency. Second, among the repairable mismatches, those with two or more contiguous mismatched nucleotides are the most effectively repaired, whereas insertion of one correct pair between two mismatched ones reduces the repairability. Third, heteroduplexes containing insertion mutations are repaired asymmetrically, the longer strand being preferentially removed. Fourth, the sequence environment is an important factor for the efficiency of repair. Inspection of the sequences flanking the mismatches (Figure 1) shows that runs of A:T pairs directly neighboring the mismatches greatly promote repair. Mutations ac19 and FC1 are AC insertions, but repairability of ac19 to the wild-type allele exceeds that of FCl twofold. However, the mismatch FCl/+ is surrounded by G:C pairs, whereas a rather long A:T run adjoins the acl9/+ mismatch. The structures of mismatches formed by mutations located near the UV357 site are especially demonstrative. The frameshift mutant FC40 presents a very interesting example. It is an insertion of A close to the UV357 site (Figure 1). In crosses with the wild-type parent, FC40 behaves as an LR marker: the mismatch FC40/+ is not repaired in either direction. However, FC40 behaves as an HR marker in crosses with opUV357, which is also an LR marker. The mismatch FC40/opUV357 (as compared to FC40/+) acquires two important structural peculiarities that transform it into the most favorable for repair: two contiguous mismatched nucleotides adjacent to a long A:T sequence. Accordingly, the repairability FC40 → opUV357 was the highest of all we have measured. This example visibly demonstrates the importance of both DNA strands of a mismatch for marker discrimination.

Mismatches with strands of equal length could also be repaired asymmetrically. For example, the mismatch am360/op360 is repaired exclusively to op360 (48). The principles of the strand preference in these cases are not yet understood. The mismatch FC47/+ is poorly repaired in both directions

despite the fact that it includes one base substitution and one inserted nucleotide (Figure 1). The presence of one complementary base pair between two mispaired ones may be a factor that hampers recognition of the mismatch by the repair system. Such discontinuity of mismatches always seems to reduce repairability. Thus, the mismatches amUV375/+ and am360/+ are probably repaired rather infrequently for this reason. The mismatch FC21/opUV357 (Table 1) is repaired much less efficiently than the similar but continuous mismatch FC40/opUV357. An A:T run adjoining the mismatch should have a minimum length of more than three nucleotides to be able to promote repair.

Enzymes of MMR

Endonuclease VII: recognition and cutting

The observed virtual absence of repair of single-base substitutions and single-nucleotide indels along with the structural features of the best repaired mismatches suggested a rather simple principle of marker discrimination by the MMR mechanism. It was evident that local single-strandedness of DNA must play the main role in the mismatch recognition. If so, a single-strand-specific endonuclease should be a key enzyme in this process. Endonucleases that recognize minor distortions in the DNA double-helix structure were known, and they were thought to be involved in MMR in fungi (62–64). The most thoroughly studied enzymes, S1 nuclease from *Aspergillus oryzae* and DNase I from *Ustilago maydis* demonstrated activities very suitable for MMR of the sort we found in T4 phage. They recognize single-base mismatches poorly, but recognize distortions in the DNA helix produced by supercoiling or by more extended mismatches (65). Moreover, the DNA cleavage reaction was shown to be promoted at AT-rich regions, facilitating local DNA melting (64). Among known T4 endonucleases, endonuclease VII (endo VII) encoded by gene *49* was suggested as a plausible nickase that recognizes and incises the mismatches (47).

Endo VII is a resolvase responsible for clearing branched replicative DNA prior to packaging. It has been well characterized *in vitro*. The purified enzyme cleaves specifically at secondary structures in double-stranded DNA. These structures include branched DNAs, such as HJs (66, 67), Y structures (68), single-stranded overhangs (69, 70), base mispairings and heteroduplex loops (71–73), and bulky adducts (74). The enzyme does not attack the looping single strand (71).

The cleavage of single-base mismatches as well as heteroduplex loops by endo VII can initiate restoration for perfect double-strandedness in the presence of T4 DNA polymerase and T4 DNA ligase *in vitro* (72, 73). Loops of 8 and 20 nucleotides were repaired efficiently. The enzyme introduces double-strand breaks by placing delayed staggered nicks in the 3′-flanking area of the DNA secondary structure distortion. *In vitro*, the ability of endo VII to cleave single-base mismatches in double-stranded oligonucleotides has also been demonstrated (75). Our observation that single-base mismatches are not repaired could reflect the situation *in vivo*. The heteroduplex recombinational intermediates must have a very short lifetime, so the repair of single-base mismatches *in vivo* could proceed too slowly to make a noticeable contribution to the recombinant frequency.

We tried to check the involvement of endo VII in the MMR *in vivo* directly (76, 77). The recombinant frequencies were measured in two- and three-factor crosses of *rIIB* mutants under *49*$^-$ conditions (amber mutation E727 in gene *49*). We observed no difference between the HR and LR markers located in the same point. They gave identical, low recombinant frequencies, which was expected if endo VII were a mismatch-recognizing component of the phage MMR system. There, however, remained some reservation because the *49*$^-$ mutants are virtually lethal. They produce just a few phage particles per infected cell in nonpermissive conditions. One could wonder if those particles were a random and representative excerpt from the phage T4 DNA pool.

Recently (78), we made use of the fact that mutations in *uvsX* gene encoding T4 recombinase (79) suppress mutations in gene *49* (80, 81). The lethality of gene *49* mutants results from their inability to resolve highly branched DNA formed by recombination into linear duplex DNA packageable into a phage capsid. In the absence of UvsX function, recombination runs via a replication resolution pathway that does not lead to formation of the branched DNA (82).

We compared the recombination of homoallelic HR and LR markers in S17 and S17 E727 background (S17 and E727 are amber mutations in genes *uvsX* and *49*, respectively). The data obtained clearly demonstrated absolute dependence of MMR in T4 on the endo VII activity. In S17 crosses, the HR and LR markers retained their respective HR and LR characteristics. However, in S17 E727 crosses, the HR and LR markers demonstrated no difference in the recombination frequency, and both behaved as LR markers. We concluded that endo VII is the enzyme that recognizes mismatches in recombinational heteroduplexes and performs their incision.

T4 DNA polymerase

Because endo VII cuts a DNA strand of the heteroduplex on the 3′ side of the mismatch (71), we expected the cut strand to be attacked by a 3′-specific exonuclease. T4 DNA polymerase has an active proofreading 3′→5′ exonuclease activity (83, 84). The antimutator enzymes differ from the normal gene product by a heightened activity of 3′→5′ exonuclease (85). We regarded the proofreading 3′→5′ exonuclease of T4 DNA polymerase (gp43) to be a likely candidate for promoting the second step of the process because the DNA intermediate with a nonpaired single-stranded 3′ end resulting from cutting a mismatch is a very good substrate for the 3′→5′ exonuclease of the DNA polymerase (83, 84). In fact, we postulated 3′→5′ exonuclease of T4 DNA polymerase as the enzyme performing the second step in MMR when it has not been known yet that endo VII performs the first step. Our suggestion was based on the observation that antimutator mutation tsL42 in T4 DNA polymerase (86) affected the length of the MMR tract, whereas the repairability values remained the same as those under 43$^+$ conditions (56). This was confirmed later by

detailed analysis of recombinational effects of the tsL42 DNA polymerase mutant (48).

An example illustrating the tsL42 effects is shown in Figure 6. A series of FC1×i crosses identical to those illustrated in Figure 3 were performed in the tsL42 background. The basic line (dashed) shows the frequency-distance relationship observed in tsL42 crosses between markers of the LR type, i.e., in the absence of MMR. Open circles show the recombinant frequencies obtained in crosses of HR marker FC1 against several i markers. In FC1 crosses, the recombinant frequencies first grow rapidly with the distance. The difference between the recombinant frequency and the basic line reaches its maximum value at ~15 bp. This value is the MMR contribution to the recombinant frequency (repairability FC1→+). The points of intersection for the basic line and the FC1 line are 1.15×10^{-3} and 3.30×10^{-3}, respectively. The κ_{FC1} of 2.15×10^{-3} does not differ significantly from the κ_{FC1} of 1.96×10^{-3} in the wild-type crosses. The ordinate intersection for the basic line is nearly six times higher than in normal crosses. The nature of this parameter was discussed in Ref. (48).

The length of the repair tract, determined as the distance where the difference between the basic line and the FC1 line reach maximum, was significantly shorter than in normal crosses (15 bp vs. 25 bp). The length of the repair tract most likely depends on the properties of the nuclease performing the excision step. We therefore concluded that T4 DNA polymerase directly participates in MMR *in vivo*. The observed length of the repair tract implies that the 3′→5′ exonuclease of T4 DNA polymerase removes mismatched strands in recombination intermediates along with some complementary section, ~25 bp and ~15 bp in the wild-type and tsL42 background, respectively. Note that this observation must be related to proofreading during T4 DNA replication as well.

To explain the effect of tsL42 on the length of the repair tract, we suggested that tsL42 polymerase switches from nucleotide excision to polymerization faster than the wild-type enzyme. Because the 3′→5′ exonuclease activity on double-stranded DNA is inhibited by deoxyribonucleotide triphosphates (dNTPs) (84, 85), the quicker switch to polymerization may not be an intrinsic property of the tsL42 enzyme but a result of the much higher pool of dNTPs in the antimutator DNA polymerase background compared to that in a wild-type T4 infection (87).

Figure 7 illustrates the proposed pathway for MMR in T4 phage: 1) A mismatch is recognized by endo VII making a nick at the 3′ side of the mismatch with 3′OH and 5′PO_4 ends. 2) The 3′→5′ exonuclease of T4 DNA polymerase (gp43) attacks the nonmatched 3′OH end and removes noncomplementary nucleotides along with several (~25) complementary ones. The removed sequence determines the length of the repair tract. 3) The DNA polymerase switches from hydrolysis to DNA synthesis and fills the gap. 4) DNA ligase repairs the nick. Our conclusions on the direct participation of endo VII and DNA polymerase in MMR are fairly self-consistent. Endo VII cuts loop-containing heteroduplexes exclusively at the 3′ side of the loop (71–73), whereas 3′→5′ exonuclease

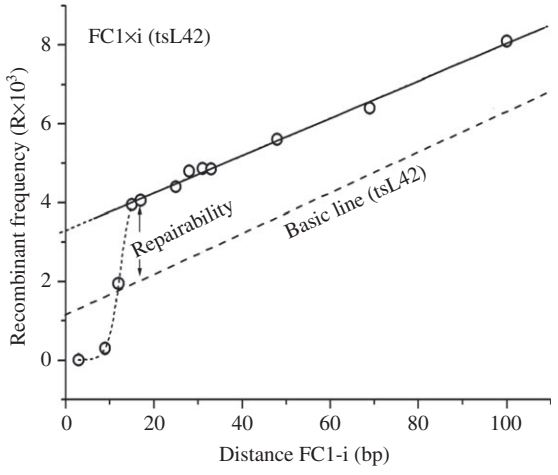

Figure 6 Measuring the repairability value and repair tract length in tsL42 background (48).
Open circles show the recombinant frequencies obtained in crosses of HR marker FC1 against several LR markers i. Basic line (dashed) shows frequency-distance relationship observed in tsL42 crosses between the markers of LR type, i.e., in the absence of MMR. In FC1 crosses, the recombinant frequencies first rapidly grow with the distance, and then, at ~15 bp, the difference between the recombinant frequency and the basic line reaches maximum value, which is the MMR contribution to the recombinant frequency (repairability FC1 →+). The corresponding distance (~15 bp) is the repairability tract. The relationship is similar to that in the wild-type crosses (Figure 3) except the repair tract in the tsL42 background is significantly shorter, 15 bp vs. 25 bp in the wild-type crosses.

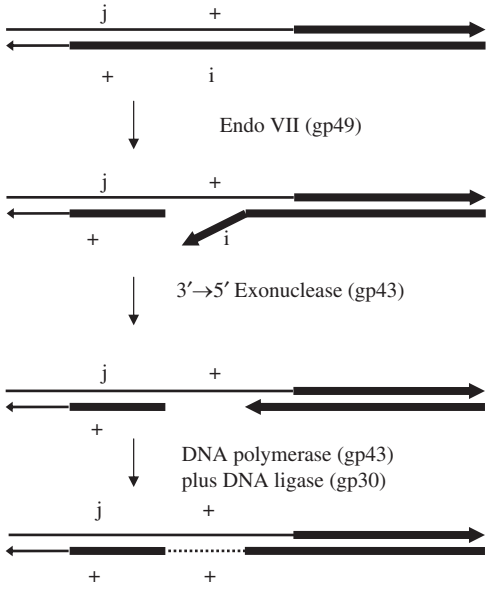

Figure 7 Schematic of the MMR pathway in bacteriophage T4 (76–78).
The i/+ mismatch in the heteroduplex DNA intermediate arisen in the cross j×i is recognized by endo VII and nicked on the 3′ side of the mismatch. The nonpaired 3′ terminus is attacked by the 3′→5′ exonuclease of T4 DNA polymerase that removes mismatched nucleotides along with several (~25) complementary nucleotides (the repair tract) and then switches to polymerization. The resulting nick is ligated by DNA ligase (gp30).

of DNA polymerase specifically attacks single-stranded 3′OH ends of DNA (88). The second nicking on the opposite strand observed *in vitro* could be forbidden *in vivo*. The postulated behavior of T4 DNA polymerase in this pathway is the same as in DNA synthesis when the proofreading exonuclease removes erroneously included nucleotides, and then polymerization is resumed (immediate MMR).

Relation to other MMR systems

The inferred MMR mechanism related to marker-dependent recombination in T4 phage differs qualitatively from the known bacterial and eukaryotic MMR (1–6, 37). The Mut system of *E. coli* and the Hex system of *S. pneumoniae* are the systems of postreplicative repair. They require the functions of *mutL*, *mutS*, *mutH*, and *mutU* genes, as well as the single-strand binding protein. They are aimed at the most frequent errors of replication: mismatches of transition type with minimal distortions of the double-helix structure. They also readily recognize single-base indels. Both systems produce long (~3000 bp) repair tracts. These systems discriminate the parental and newly made DNA strands so that only the latter is corrected. The discrimination depends on the transient absence of adenine methylation at GATC sequences in the daughter DNA strand.

A sequence-specific, very short patch (VSP) repair system observed in *E. coli* (33, 89) specifically corrects G/T mismatches resulting from transitions CAGG→TAGG or CCAG→CTAG. The biological sense of such refined repair lies in the observation that the sequences CCAGG or CCTGG are modified by the dcm methylase at the internal cytosine, and such sequences are hot spots for mutations due to deamination of the methylated cytosine (90). The length of the repair tracts for the VSP system is less than 10 nucleotides. This system crucially depends on the function of the *E. coli* gene *vsr* and, to a lesser extent, on *mutL* and *mutS* (89). Localized, sequence-specific MMR very similar to VSP repair of *E. coli* was observed also in *S. pneumoniae* (91).

None of these bacterial repair systems is similar in specificity to that in bacteriophage T4. Whereas the Mut, Hex, or VSP repair systems may manifest themselves in general recombination, their direct function is clearly related to the reduction of mutagenesis. In contrast, the MMR system of phage T4 does not discriminate between the parental and daughter DNA strands and does not recognize mismatches related to the most frequent spontaneous mutations (single-base substitutions and single-base insertions or deletions), so an antimutagenic role is unlikely.

One may wonder whether *E. coli* MMR systems make any contribution to the repair of recombinational heteroduplexes during T4 multiplication. The low repairability of single-base substitutions in T4 suggests that short patch repair systems of the host did not contribute to recombination in T4 crosses. The rII mutations we used did not have the context sequences 5′-CCAGG-3′ or 5′-CCTGG-3′, recognized by the VSP system. The effects of the systems with long repair tracts (either host or phage) would not be detected in our crosses because of joint repair.

The biological significance of MMR in phage T4

The MMR system described above operates on recombinational heteroduplexes, so it is unlikely to change the fidelity of DNA reproduction substantially. The average mutation rate in T4 phage, ~10^{-8} per base pair, can be fully accounted for by the observed fidelity of T4 DNA polymerase without additional accuracy-enhancing steps (92). The MMR in T4 is rather a manifestation of the process aimed at the repair of DNA secondary structure. T4 DNA undergoes a complex processing before packaging, which includes resolution or repair of branched and loop-containing structures (66, 93). The T4 marker-dependent recombination must be related to such processing aimed at restoring distorted areas of the double helix. Similar to other systems of recombinational MMR, the T4 MMR possibly contributes to the fidelity of phage reproduction by preventing DNA misalignments and imperfect complementarity. This may have evolutionary sense via sustaining reproductive isolation.

However, such an activity could be mutagenic and lead to mutations via sequence conversion. Studies of T4 *rIIB* mutants (94, 95) provided evidence for *in vivo* production of mutations via metabolic processing of quasipalindromic DNA sequences. Because mismatched regions in the stem-loop or hairpin structures formed by quasipalindromic sequences are similar to those in recombination heteroduplexes, it is reasonable to think that they are processed by the same mechanism. The mutagenic processing of secondary structures was shown to be similarly asymmetric: in mismatches with strands of unequal length, the longer one is preferentially removed. In addition, the size of the region involved in the ‘repair’ of quasipalindromes is also similar to that in the recombination-related MMR. DeBoer and Ripley (94) observed both joint and separate processing of multiple mismatches located only several base pairs apart in the same stem-loop structure, which argues in favor of a short-patch character of the process. The same MMR system may also operate in mutagenesis via sequence conversion that is related to direct sequence repeats with partial homology (95). Interestingly, the MMR in T4 is a biased process: it always removes a longer DNA strand. The evolutionary consequences of such trend to shortening of the phage genome are not quite clear. One may speculate that in the long run, it could operate as a stabilizing selection against increasing the genome size.

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