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Some features of base pair mismatch repair and its role in the formation of genetic recombinants

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Abstract. For the formation of recombinants involving closely linked markers, two distinct processes play a role. The recombinational interaction between homologous DNA molecules results in the presence of heteroduplex DNA joining the parental components of the recombinant. The presence of markers distinguishing the parents in the region of heteroduplex DNA can result in base pair mismatches. The post recombination repair of such mismatches can contribute to the separation of closely linked markers. The processes responsible for such repair also play roles in mutation avoidance. The specificities, functions and contribution to the formation of recombinants for closely linked markers of the processes in *Escherichia coli* are described.
Key words. Mismatch repair; heteroduplex DNA; heterozygous DNA.

The prominent models that describe homologous recombination rely on pairing of complementary DNA strands to provide fidelity. One class of models suggests a single strand transfer from one parental duplex to the other[35] driven by DNA synthesis to displace the transferred strand from the donor, with exonuclease digestion on the recipient to provide an expanding site for transfer. Such an intermediate could make a transition to the kind of structure suggested by Holliday[19], in which strands are reciprocally transferred[65].

A possibly distinct class of models was provoked by the evidence for double strand break repair of X-ray damaged DNA[47]. Double strand breaks in DNA, perhaps expanded to gaps, become substrates for strand invasion of a homologous sequence, forming Holliday junctions, that by synthesis from the strand break can reconstruct the continuity of the broken duplex[56]. There is abundant evidence accumulating for this route for formation of recombinants[22,55,56,57]. However a role for a single strand transfer mechanism remains viable. Both of these mechanisms include formation of heteroduplex DNA as a prominent feature. In what follows we will provide a narrow view of the role of processing of such heteroduplex structures in the formation of recombinants when participating markers are closely linked. The major emphasis will be on processing in *Escherichia coli.*

Heteroduplex DNA in recombination products

Substantial evidence has been assembled for the presence of heteroduplex DNA in the products of homologous recombination. Perhaps the first suggestion came from Levinthal[26] who provided evidence for the proposal that heterozygotes emerging from bacteriophage T2 crosses were products of recombination and made the proposal that the parental contributions to recombinant products were joined by heteroduplex DNA. The heterozygosity would thus be the consequence of the presence of markers distinguishing the two parents in the region of heteroduplex. Some of the evidence for the presence of heteroduplex DNA in the primary products of recombination came from the detection of aberrant tetrads in fungi[25,37], post meiotic segregation in fungi[7,41,48], the analysis of the DNA of meiotic cells of *Saccharomyces cerevisiae*[28], the characterization of the DNA products of pneumococcal transformation[16], analysis of the products of recombination with bacteriophage T4[60] and bacteriophage λ[21], the analysis of heterozygosity in phage T4[49], and the characterization of the DNA products of bacterial conjugation[52].

There are a few genetic systems in which an estimate of heteroduplex length has been made. There are many problems in making such estimates, among them the role of mismatch repair processes acting on the unreplicated products of recombination. For the case of *Escherichia coli* bacteriophage λ recombination products, the average polynucleotide length is about three or four kilobases[20,66]. In addition to including markers reflecting point mutations these heteroduplex regions may include substantial regions of heterology[27]. The heteroduplex regions can connect homoduplex DNA from both parents, a splice, or can be bounded by duplex DNA from one of the parents, a patch. Huisman's[20] observations suggest that among λ recombinants formed under wild type recombination conditions, patches are in substantial excess over splices. For the case of the heteroduplex DNA formed as a product of pneumococcal transformation, the average length of the patches is several kilobases[17].

Since they play a substantial role in the final outcome of DNA breakage and joining or breakage and repair

events, attention will be focused on the processing of such heteroduplex regions when they include sequences that distinguish the parental contributors. The discovery of the excision of UV irradiation induced thymine dimer products and the subsequent repair of the polynucleotide chain from which the dimer had been removed[4,50] suggested a model to account for the aberrant tetrads (conversion) observed among the products of meiosis in fungi[19]. The process of the repair of DNA damage, and the repair of mismatched bases establish a connection between mutation avoidance and the processing of heterozygous heteroduplex DNA products of recombination. Tiraby[58] showed that the functions responsible for characteristic marker discrimination observed in pneumococcal transformation[13,51,59], also played a role in mutation avoidance.

Processing of mismatch containing heteroduplex DNA in *Escherichia coli*

For *Escherichia coli*, three systems have been identified that play prominent roles in the excision and repair of mismatched basepairs in DNA. The methyl-directed or dam-directed mismatch repair system first proposed by Wagner and Meselson[63] is a mutation avoidance system that acts to eliminate replication errors by excising thousands of nucleotides of newly synthesized DNA if the sequence harbors a mismatched base pair[46,66]. The newly synthesized DNA is distinguished by virtue of the absence of adenine methylation of GATC sites that occurs as a post replication process. The methyl directed mismatch repair system has been well reviewed[8,38,39]. It has been suggested that this system can also act from a polynucleotide chain interruption[14] and evidence for such a GATC methylation independent activity has been reported[23,24].

Since the activity of this system results in such extensive repair, closely linked heterozygosities present in heteroduplex DNA are likely to be lost, diminishing a possible role of this pattern of mismatch repair in the formation of recombinants. Potentially more profound effects on the final products of homologous recombination can be anticipated by the action of localized mismatch repair systems. Two of them have been described in *Escherichia coli*.

The very short patch repair system (vsr)[30,32,53] results in excision of T in G:T mismatches, is independent of the methylation state of the DNA and removes very few nucleotides in the repair process. When *mutS* or *mutL*, components of the methyl directed repair system, are disabled, the *vsr* repair is severely reduced, perhaps eliminated[31,46]. The efficient correction of T in G:T mismatches appears also to require a sequence resembling the dcm site CC(A/T)GG in which mutation replaces the second C with T[32]. Such a system plays a role in the avoidance of mutations resulting from the spontaneous deamination of methylcytosine in DNA[29]. In recombination, this system plays a prominent role in events that separate closely linked markers[30].

A third *Escherichia coli* system, first identified as a mutation avoidance function for C:G to A:T transversions[40], was independently identified as a mismatch repair function capable of repairing the adenine in either C:A or G:A mismatches[44]. The gene responsible for this function was identified as *mutY* (*micA*)[62]. The mismatch repair activity of this function is independent of the methylation state of the DNA[33,34,44]. The repair tracts generated by this correction system are on average larger than 9 and shorter than 27 nucleotides[45] and as in the case of vsr[12], are mainly the product of action of DNA polymerase I acting on the products of the MutY activity[45]. In the absence of MutY, repair is reduced 10 to 20 fold. Preliminary evidence from the laboratory suggests that it can be reduced even further in the case of C:A mismatches, with the disabling of the function Fpg that was identified as a requirement for the repair of photodamages in DNA[3] and later as a mutation avoiding function for G:C to T:A transversions called MutM[5]. The repair activity of this function on C:A mismatches is modest and cannot be detected in the presence of Mut Y activity[44].

We have examined some features of the activity of the *mutY* system. An assay system has been devised that permits the examination of the repair of specific mismatches in the lacZ gene of bacteriophage λ *plac5*. The *lacZ* mutants used were CC101 to CC106, constructed by C. Cupples[9]. By isolating DNA from the appropriately marked phages, mixing equal amounts pairwise, denaturing, annealing and packaging, we can expect to find four products from the pair. For example, if one of the parents were *plac5* cc101 *Pam3* and the other *plac5 Pam80*, the packaged phage would include the following species in equal abundance.

The two parents

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-----Zcc101----/----------P3--------3' (l)
-----Zcc101----/----------P3--------5' (r)
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and

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-----Z+--------/----P80-------------3' (l)
-----Z+--------/----P80-------------5' (r)
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and the two heteroduplex types.

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a)  ----------Zcc101-----/----------P3--------3'
    ----------Z+---------/----P80-------------5'
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and

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b)  ----------Z+---------/----P80-------------3'
    ----------Zcc101-----/----------P3--------5'
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These can be rewritten in terms of nucleotide sequence as

a) -----Am----------C---------Tm-------3′
----G----------Am--------G--------5′

b) ---C----------Tm---------C--------3′
----Tm----------G----------Am-------5′

where m indicates a mutant nucleotide.

The packaged phage are absorbed to an *Escherichia coli* host, Su^-, that is unable to suppress the amber mutations in the *P* gene of lambda and defective in both the methyl directed mismatch repair system and the T:G repair system (*mutL*). Since the *P* gene product is required for phage DNA replication, the phage with parental homoduplex DNA cannot grow on this host. The phage with heteroduplex DNA cannot grow either – unless they experience repair of the mutant A in the 5′ transcribed strand of the *P* gene. During the interval required for correction of the *Pam* mutation in the transcribed DNA strand the mismatch in the distant (20 kb) *lac* gene is a substrate for repair. A single cycle of growth of a phage that has experienced the appropriate *P* gene repair gives rise to a burst of phage which includes the P^+ phage and the *Pam* phage products from the complementary strands, each harboring their respective *lac* genes. If the *lac* mutant adenine is corrected then all of the phage in the burst are lac^+. If the guanine is corrected all of the phage in the burst are lac^-, if the *lac* mismatch escapes correction the burst will contain a mixture of lac^+ and lac^- phage. Since the *Pam3* is suppressed by suppressor *SuII*, while *Pam80* is suppressed by either *SuII* or *SuIII*, the corrected heteroduplex, a or b, of any given burst can be identified.

Using the set of *lacZ* mutants constructed by C. Cupples[9] we have examined the efficiency of repair of several mismatches (table 1). Two different mutants, each creating an A:G mismatch at *lacZ* amino acid position 461, but at adjacent positions and in inverted orientation, both show repair. Under conditions of repression of the *lacZ* gene for one A:G, less than 15% of the bursts show that the A:G has escaped repair while for the other about 50% of the A:G mismatches escape repair, suggesting a context dependence of the repair process. The T:C mismatch is not repaired in either orientation. The products of A:G repair can be either A:T or C:G. For the A:G mismatches examined, repair of G occurs with about 30% of the likelihood of repair of A. We have shown that both the A and the G repair require the MutY function (Yamamoto and Fox, unpubl. observ.). One G:G and its complementary C:C mismatch have been examined. The G:G is repaired and the C:C is not.

Table 1. Repair by MutY of A:G mismatches.

mismatch in	Lac Z induction	Lac Z repression
lacZ (l) $\frac{A}{G}$ mutant* (cc101) (r) $\frac{G}{G}$		
uncorrected	60	14
correction $\frac{A}{G} \rightarrow \frac{C}{G}$	40	59
correction $\frac{A}{G} \rightarrow \frac{A}{T}$	11	21
Average number of correction events	0.6	2.0
mismatch in *lacZ* (l) $\frac{G}{A}$ mutant* (cc104) (r) $\frac{A}{A}$		
uncorrected	52	31
correction $\frac{G}{A} \rightarrow \frac{G}{C}$	8	20
correction $\frac{G}{A} \rightarrow \frac{T}{A}$	3	7
Average number of correction events	0.2	0.6

*(l) and (r) refer to the DNA strands in bacteriophage lambda.

The screen for mutator mutants from which the MutY function was identified[40] is consistent with a role in the repair of A in G:A mismatches[1]. The biochemistry displays evidence of repair of the A in a G:A mismatch, including glycosylase activity[2,61] and evidence of subsequent endonuclease cutting of the A containing strand[61], but no evidence has been reported of cutting of the G containing strand. The paradoxical observation of either G or A correction of G:A mismatches by a function playing a role in mutation avoidance suggests that two distinct activities may be involved[61].

The biochemical result suggests the possibility of an as yet unidentified additional component for the full biological activity. The MutM function was also identified as a function that avoids G:C to T:A transversions[5]. In our assay, a mutator mutation in this gene does not have a detectable impact on the A removal in either G:A or C:A mismatches.

The possibility of examining repair of G:A mismatches in the *lacZ* gene has provided us with an opportunity to examine the impact of transcription on repair during the interval required for correction of the *Pam* site in heteroduplex lambda DNA molecules that are described above. With a multicopy plasmid encoding LacIq, the infected bacteria do not transcribe the *lacZ* gene, while in the presence of the inducer IPTG transcription is active.

Comparison of the composition of single bursts from bacteria in the presence or in the absence of IPTG results in no change in the frequency of repair of the *Pam* mutant site. For the G:A site in *lacZ* however, when transcription is active in the *lacZ* gene, the repair of the mismatch is substantially reduced with no change in the relative efficiency of repair of G versus A. In one case when *lacZ* is repressed, only 14% of mismatches

escape repair, while under conditions of induction (high level of transcription) 55% of the mismatches escape repair. The qualitative effects observed with another G:A mismatch and for a G:G mismatch are similar. On the basis of the frequency of mismatches that escape repair, we estimate that transcription reduces the likelihood of repair in *lacZ*, during the interval required for the *Pam* repair, by about a factor of three. This reduction is equally evident on both the transcribed and the non transcribed strands (table 1).

In addition to the observation of different levels of correction for the two G:A mismatches in *lacZ*, the two G:A mismatches in the *O* gene showed a four fold difference in the efficiency of repair[44]. The examination of the efficiencies of correction of seven different *Pam* mutants that give rise to C:A mismatches in hetroduplex DNA also suggests a strong context dependence (Clark, Chen and Fox, unpubl. results).

The presumed C:C repair system that we had reported[44], reflected misinterpretation of the evidence. The activity was inferred from the plating of a heteroduplex containing a particular *Oam* mutation in the transcribed strand. We have since shown that such *Oam* heteroduplexes with a *Pam* mutation in trans often give a burst on Su^- bacteria and that these bursts include both *Oam* and *Pam* phage. The location of the mutation is near the end of the *O* gene of lambda. It would appear that the incomplete *O* gene product displays enough activity to permit one round of replication of the *Oam*/*Pam* heteroduplex and the formation of homoduplex products that can provide O function and P function. By complementation, a full burst of the two parent phage and occasional recombinants is observed after a single cycle of growth on Su^- bacteria (Yamamoto and Fox, unpubl. observ.).

For the localized mismatch repair systems, we have now examined repair of all the possible correction fates of the various mismatches and the preliminary results indicate:

- G:A, correction to either G:C or T:A without apparent strand discrimination.
- T:C, no evidence of repair.
- A:C, correction to G:C without apparent strand discrimation.
- G:T, correction to G:C witout apparent strand[32,46] discrimination, and sensitive to context. No apparent correction to A:T.
- A:A, no evidence of correction.
- C:C, no evidence of correction.
- G:G, correction to G:C.
- T:T, no evidence of correction.

Processing of heterology containing heteroduplex DNA in *Escherichia coli*

In addition to mismatched base pairs, the methyl directed mismatch repair system acts on frame shift heterologies. Frame shift heterologies that are one, two or three nucleotides long are efficiently processed[42] but there is little or no processing of larger heterologies[6]. There seems to be no function in *Escherichia coli* that acts on larger heterologies[6,42,46]. There is one case evident on examination of the products of transfection with bacteriophage heteroduplex DNA, that harbours a heterology due to the presence of a transposition element TN10 in one of the strands[46]. The infectivity of these molecules is high, greater than one third that of homoduplex molecules. Alleles marking both strands are evident in the infective centers but the transposon has been lost. This loss could be the result of aberrant replication of such heteroduplex molecules or some as yet unidentified function that acts on such special structures. Another case in which large heterologies (>400 base loops) have been shown to be repaired is when a repairable mismatch was present nearby[6,46]. Here the dam-directed mismatch repair acting on the mismatch presumably cocorrects the heterology. The report of evidence for correction of a deletion heterology in bacteriophage λ heteroduplex molecules is likely to reflect the presence of a minority of contaminating homoduplex molecules resulting from incomplete strand separation[10].

Impact of mismatch repair on the ultimate recombination products

Localized repair of mismatched base pairs provides a mechanism to account for the observations of White and Fox[64] suggesting that a substantial fraction of recombination events in which closely linked markers are separated are the result of processing of non recombinant heterozygous heteroduplex products of DNA breakage and joining events.

The observations of Lieb[32] have shown that vsr plays a substantial role in determining the frequency of fine structure bacteriophage λ recombinants observed, when one of the participating markers permits the formation of a G:T mismatch in heteroduplex products of recombination. An appraisal of the roles of the prominent localized repair functions of *E. coli* in the formation of bacteriophage λ recombinants has been made by examining crosses related to those carried out earlier under both DNA replication inhibited[64] and replication permissive conditions. The hosts were disabled with regard to methyl directed mismatch repair and G:T repair (*mutL*), as well as localized repair (*mutY*). Phage emerging from crosses carried out under conditions severely restricting DNA synthesis may harbor heteroduplex regions that are considerably longer[15] than those carried out under permissive conditions.

The alleles used in the crosses are as follows

nucleotide position	38914	39759	39786
wildtype	--G--------C--------C----3′		
	--C--------G--------G----5′		
Oam 29	--T--------C--------C----3′		
	--A--------G--------G----5′		
Pam80	--G--------T--------C----3′		
	--C--------A--------G----5′		
Pam3	--G--------C--------T----3′		
	--C--------G--------A----5′		

For example, the *Oam29* × *Pam80* cross has the potential of resulting in heteroduplex DNA with the following mismatches

----G--------Tm----3′ or -----Tm-----C-----3′
----Am------G------5′ -----C------Am----5′

separated by 845 nucleotides. Such a heteroduplex heterozygote plated on an Su⁻ host cannot grow unless the mutant A nucleotide in the message producing strand is corrected.

The recombinants from each cross were assayed on various Su⁻ hosts, wildtype, *mutL*, *mutL mutY* and *mutL* harboring a plasmid (pMutY) that overproduces MutY. From previous experiments[44] it was known that *P3/P80* artificially constructed heteroduplex phage give infective centers with about 20% efficiency on the Su⁻ *mutL* indicator, 60–70% on the Su⁻ *mutL* pMutY and about 1–2% on the Su⁻ *mutL mutY* indicator. For the *Oam29/Pam80*, when *Pam80* is on the transcribed strand the result is similar to the *Pam3/Pam80* heteroduplex. When the *Oam29* is present on the transcribed strand the phage plate with a high efficiency, about 60% or more, on either *mutL* or on the pMutY strain. On the *mutY* strain the plating efficiency is about 5%. In table 2 are displayed the results of crosses carried out on Su⁺ *mutL mutY* host.

From these data in table 2 it can be seen that a substantial portion of the fine structure recombinants include mismatches that are subject to repair by the dam directed repair system. Substantially more recombinants are evident when this repair is disabled (*mutL*) in the Su⁻ selective indicator. It would appear that heterozygous substrates for MutY action are eliminated by dam directed repair. This suggests that a very substantial fraction of the recombinants that emerge from a permissive cross harbor heteroduplex regions in which at least one of the strands is unmethylated or undermethylated at GATC sites, presumably having been newly synthesized. This observation suggest that many recombinants are packaged immediately after their formation[20].

From the examination of the recombinant yields on the various indicators it is clear that the activity of the methyl directed mismatch repair system is responsible for the loss of precursors of fine structure recombination. It would appear that many heteroduplex products of recombination are packaged immediately after formation. The recombinant yields on the various *mutL* indicators show that some of the fine structure recombinants result from the activity of MutY in the indicator. For the 27 nucleotide interval between *Pam3* and *Pam80* about four times as many recombinants are realized when MutY is overproduced as compared to disabled *mutY*. With the wildtype level of MutY about twice as many recombinants are realized. For the 845 nucleotide interval between *Oam29* and *Pam80*, these markers have already been separated (recombined) in most of the phage that emerge from the cross. Residual vsr activity in the host for the cross could contribute to the recombinants that occur in the absence of MutY. Other possibilities include low levels of repair by Fpg or some other repair system with modest activity capable of removing the mutant adenine in heteroduplex DNA. Double strand break repair could also contribute recombinants insensitive to the intervention of localized mismatch repair.

Table 3 displays the relative numbers of recombinants emerging from a cross in which DNA replication is prevented by virtue of the Su⁻ and *dnaB* phenotypes of the host and the *Oam* and *Pam* mutations in the phage. From the data in table 3 it is clear that among phage emerging from crosses carried out under conditions preventing DNA replication the impact of the localized repair capacity of the indicator used to detect the fine structure recombinants becomes very prominent. When the dam directed repair system is disabled, *mutL*, there is some increase in the number of recombinants that are realized, smaller than the increase evident with the dna⁺ cross. One might expect that the products of the dna⁻

Table 2. Relative numbers of recombinants from *dna*⁺ *mutL mutY* crosses detected on various Su⁻ indicators.

	P3 × *P80*	*029* × *P80*	*029 P3* × *P80*
wildtype	1	1	1
mutL	3.7	1.8	4.8
mutL mutY	1.9	1.7	1.6
mutL pMutY	8.0	2.1	13.4

Table 3. Relative numbers of recombinants from *dna*⁻ *mutL mutY* crosses detected on various Su⁻ indicators.

	P3 × *P80*	*029* × *P80*	*029 P3* × *P80*
wildtype	1	1	1
mutL	1.6	1.5	2.0
mutL mutY	0.4	0.5	0.2
mutL pMutY	3.0	1.5	4.7

cross would be more fully or fully methylated at the GATC sites. Examination of the recombination products on various *mutL* indicators displays a prominent role for localized repair on the number that are evident. Among the products of a *Pam3* by *Pam80* cross only about thirteen percent of the recombinants that are evident in the MutY overproducing strain are detected on a *mutY* disabled strain. For the *Oam29* by *Pam80* cross about two thirds of the recombinants may be lost when *mutY* is disabled. The products of the three factor cross display an even more striking impact of the role of MutY in the formation of recombinants from the matured products of the cross, an impact not very different from what might be expected from the cumulative effects that are evident in the individual two factor crosses.

All in all localized mismatch repair functions encoded by *mutY* and vsr play a prominent role in the formation of fine structure recombinants. However, it becomes increasingly clear that we cannot yet provide a quantitative accounting of localized negative interference in bacteriophage λ recombination. It is also clear that there are other contributors. These could include for example double strand break repair, and/or mismatch repair functions whose activities on mismatches include normal nucleotides and have not yet been recognized. The residual A removal activity on artificially constructed heteroduplex molecules containing C:A mismatches in *mutY* disabled host is about 10% of that observed when MutY is present and is reduced further in *mutY*, *fpg* double mutant strains. The search in eukaryotes for functions that play a role in mismatch repair has produced a plethora of candidates[54].

Nevertheless there are some features of the processing of mismatches present in heteroduplex products of recombination that can be described. In *E. coli* the methyl directed system corrects mismatches in newly synthesized strands of DNA that are distinguished because of post replication adenine methylation at GATC sites. Evidence is accumulating for repair by this system in strands of DNA harboring a single strand interruption, independent of GATC methylation. This process is likely to contribute little to fine structure recombination. However it appears to eliminate candidates for fine structure recombination.

The prominent localized repair systems are MutY and vsr. They do not display strand specificity. The vsr system removes T's in G:T mismatches within certain contexts, and can enhance the formation of recombinants involving markers separated by as few as six nucleotides[32]. The paradoxical observation that a mutation avoidance function like MutY displays activity capable of correcting either the A or the G on A:G mismatches suggests that this correction does not play a role in mutation avoidance. Recent observations indicate that the mutation avoidance results from the removal of A's that have been paired opposite incorporated 8 oxyguanine residues[36]. The activity of this gene product in the repair of A:G and A:C mismatches would therefore constitute another feature of the specificity of this glycosylase. Similarly the Fpg (MutM) function whose reported activity in the removal of photoproducts and of 8 oxyguanine that was paired with cytosine[36], may also display activity in the repair of A's in A:C and perhaps A:G mismatches. How many other functions with a role in repair of aberrant bases display activity on mismatches containing normal nucleotides remains to be seen.

The *MutY* glycosylase is capable of removing either the A or the G of A:G mismatches, removing the A of A:C mismatches and one of the G's of G:G mismatches. The subsequent repair results in the loss of fewer than 27 nucleotides. This system shows evidence of context specificity. It is active on either the transcribed or the untranscribed strand and active transcription reduces its activity on either of the strands. This latter observation suggests a possible way to account for the reported 'effect of gene induction on frequency of intragenic recombination of chromosome and F merogenote in *Escherichia coli* K-12'[18].

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