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Genetics

Involvement of DNA-dependent RNA polymerase in a *recA*-independent pathway of genetic recombination in *Escherichia coli*

(recombinant DNA/DNA packaging/rifampin/localized crossover/RNA nucleotidyltransferase)

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ABSTRACT **Recombinant DNA molecules of phage λ formed in *Escherichia coli* in the presence of chloramphenicol and/or rifampin can be assayed by their biological activity. *recA*⁻ cells were found to be capable of forming recombinant λ phage DNA in the presence of chloramphenicol. The relatively high *recA*-independent recombination observed in this system contrasts with the relatively low *recA*-independent recombination when recombinant phage particles rather than recombinant DNA are titrated. Formation of the recombinant DNA was suppressed by the addition of rifampin. The introduction of the *rif*ʳ mutation into host bacteria made their recombination activity rifampin-resistant. These results show that DNA-dependent RNA polymerase (EC 2.7.7.6) is involved in this *recA*-independent pathway of recombination, which is named the "Rpo pathway." This is distinct from Red, Int, RecBC, RecE, or Der pathways of recombination. Crossover was much more frequent in the *N–p_L–cI* and *cI–p_R–O* regions than in the *A–D* and *O–S* regions. The crossover seems to occur in the regions that are transcribed actively. Some local change of DNA structure caused by transcription might be required for the Rpo pathway of recombination.**

In order to understand the roles of *Escherichia coli* functions in genetic recombination, we have developed a system that has enabled us to study recombination separated from DNA replication, transcription, translation, and progeny formation (1). *E. coli* was mixedly infected with two mutants of λ phage in the presence of chloramphenicol and rifampin, and the recombinant DNA formed within the cell was detected by *in vitro* packaging. In this system, the *recA* gene product was required for the formation of recombinant DNA molecules, in agreement with studies on *recA* mutations by other investigators (2). During the course of this study, we encountered an unexpected observation that *recA* mutations did not block completely the formation of recombinant λ DNA when mixed infection was carried out in the presence of chloramphenicol without addition of rifampin. This fact suggested that the recombinant DNA was formed in *recA*⁻ cells by a rifampin-sensitive mechanism. In the present study, we show that RNA polymerase (RNA nucleotidyltransferase, EC 2.7.7.6) is involved in this *recA*-independent pathway of recombination and that the transcription process *per se* plays an essential role in this recombination.

MATERIALS AND METHODS

Bacterial and Phage Strains. The *Escherichia coli* K-12 and bacteriophage λ strains used are listed in Tables 1 and 2, respectively.

Media and Chemicals. Media, buffer, and chemicals used are generally those described previously (1). The final concentrations of chloramphenicol (Sankyo) and rifampin (B grade, Calbiochem) are 150 and 200 μg/ml, respectively, unless otherwise stated.

Mixed Infection. The procedure was the same as that described previously (1). In brief, bacteria were incubated with inhibitor(s) (chloramphenicol only or chloramphenicol and rifampin) in λ dilution buffer [10 mM $MgSO_4$/10 mM Tris·HCl (pH 7.4)] for 5 min and then were infected with a pair of λ *imm434* amber mutants at a multiplicity of 20 for each phage. (One of the amber mutants was labeled with ^{32}P.) After 15 min of adsorption, the cells were aerated at 37° in λ broth containing the inhibitor(s). After 60 min, they were harvested for the extraction of DNA.

Preparation of DNA from Infected Cells. DNA was prepared from infected cells by the Pronase/phenol method, which consisted of successive treatments with lysozyme, sodium dodecyl sulfate, Pronase, and phenol (1).

***In Vitro* Packaging Assay of Recombinant DNA.** The packaging mixture was prepared from induced lysogens 594 (λ *cI857 Sam7 Dam15 F_1am96B*) and 594 (λ *cI857 Sam7 Eam4*), as described before (1). A DNA solution (10 μl) containing about 10^9 λ DNA molecules was added to the mixture (25 μl), which was then incubated at 28° for 90 min. The reaction was stopped by adding 1 ml of SMC-DNase (0.7% Na_2HPO_4/0.3% KH_2PO_4/0.05% NaCl/0.1% NH_4Cl/1 mM $MgCl_2$/0.1 mM $CaCl_2$/pancreatic DNase at 10 μg/ml) and a drop of $CHCl_3$. Portions were plated on an appropriate Su⁻ (nonsuppressing) λ lysogen for the *imm434 am*⁺ recombinant plaque formers.

In some experiments, the packaging mixture was prepared from *recA* lysogens HI111 and HI107, or *recB* lysogens HI112 and HI108, instead of *rec*⁺ lysogens. The properties of these mixtures have been described before (1).

In a cross between λ *imm434 Aam11* and λ *imm434 Dam15 F_1am96B*, titration of the resulting recombinant phages was expected to be disturbed by *imm*λ phages originating in the packaging mixture. These *imm*λ phages of endogenous origin were selectively killed by EDTA treatment as follows: the phage suspension was concentrated by high-speed centrifugation, diluted 50-fold in 20 mM EDTA, 28 mM Tris·HCl (pH 8.5), and incubated for 20 min at 33°. One-fifth volume of ice-cold 0.1 M $MgSO_4$/0.1 M Tris·HCl (pH 6.9) was added, and then the recombinant phage were titrated. The recovery of the *imm434 am*⁺ recombinant phages during this treatment was calibrated by that of total *imm434* phages and was about 50%, while that of the *imm*λ phages was less than 1%.

Other Methods. Preparations of unlabeled and ^{32}P-labeled phages have been described previously (1).

RESULTS

Detection of Recombinant λ DNA in *recA*⁻ Cells. *E. coli* HI121 *recA1* cells were jointly infected with λ amber mutants λ *imm434 Dam15 F_1am96B* and λ *imm434 Sam7 Ram5* in the

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Table 1. *Escherichia coli* strains

Strain	Properties	Ref. or source
594	Su⁻ *strA*	1
HI90	Su⁻ *strA endA*	1
HI121	Su⁻ *recA1 strA endA*	1
HI122	Su⁻ *recB21 strA endA*	1
AB1157	F⁻ *strA*	3
AB2463	F⁻ *recA13 strA*	4
QW22	Su⁻ *recA56 recB21 endA gal*	1
KY1340	*recA trpE tyr his arg metB*	T. Yura
KY7139	*recA* rif^r *trpE tyr his arg metB*	T. Yura
A8	*Su⁻ recA1 aroE his lac strA*	H. Uchida
Ymel	*supF58*	1
HI25	594 (λ *cI857 Sam7 Eam4*)	1
HI26	594 (λ *cI857 Sam7 Dam15* F_1*am96B*)	1
HI107	HI121 (λ *cI857 Sam7 Eam4*)	1
HI111	HI121 (λ *cI857 Sam7 Dam15* F_1*am96B*)	1
HI108	HI122 (λ *cI857 Sam7 Eam4*)	1
HI112	HI122 (λ *cI857 Sam7 Dam15* F_1*am96B*)	1
Ymel (λ)		1
HI96	HI121 (λ *Dam15* F_1*am96B Sam7 Ram5*)	1
HI212	A8 (λ *spc2*)	This work
HI236	HI121 (λ *ind⁻ Nam7 Oam8*)	This work
HI244	HI121 (λ *Nam7 Nam53 Sam7 Ram5*)	This work

Su⁻, nonsuppressing.

presence of chloramphenicol (see Fig. 1). After isolation of DNA from the cells, aliquots of the DNA preparation were packaged *in vitro*. The number of the resulting recombinant plaque formers was proportional to the amount of DNA added to the packaging mixture (Table 3). Therefore, the number of recombinant plaque formers is regarded as a measure of recombinant DNA molecules. Their number was 180 per 10^9 λ DNA molecules added to the mixture.

When pancreatic deoxyribonuclease was added to the packaging mixture, recombinant phages were not detected (Table 3). This indicates that the recombinant plaque formers originated from free DNA. When packaging mixture was prepared from *recA1* lysogens HI107 and HI111, the numbers of the resulting recombinant phages were similar to those in Table 3 (data not shown). This result excludes the possibility that the recombinant plaque formers produced from DNA of *recA1* cells are formed by a *recA*-dependent reaction in the packaging assay. Other control experiments already reported have excluded the possibility that the recombinant phages arose from recombination within indicator bacteria during titration (1).

Recombinant plaque formers produced *in vitro* from DNA of *recA1* cells were centrifuged in a CsCl density gradient. Their density (Fig. 2) was 1.503 g/cm³, in agreement with that of phage particles produced from DNA of *rec*⁺ cells, which was described previously (1). Therefore, the recombinant plaque

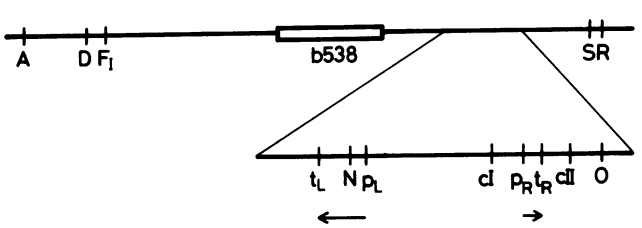

FIG. 1. Genetic map of phage λ showing the order and the approximate location of relevant genetic markers. Horizontal arrows indicate the regions transcribed in the presence of chloramphenicol.

Table 2. Phage λ strains

Strain	Properties	Ref. and/or source
λ *imm434cI Dam15* F_1*am96B b538 red3*	*b538* is a deletion (16% λ unit) extending through both sides of the *att* site	1
λ *imm434cI Sam7 Ram5 int6 red3*		1
λ *imm434 Sam7 Ram5 int6 red3*		Y. Sakakibara
λ *imm434cIam Nam7*		Y. Sakakibara
λ *imm434cI Oam8*		This work
λ *imm434cI Aam11*		5; H. Uchida
λ *spc2*	*A* through *b2* region substituted by *E. coli* DNA	This work
λ *imm434 Nam7 Nam53 cIIco1*		1
λ *papa*		1
λ *b538 cIsus34*		

former is a phage particle containing a normal size DNA molecule. The centrifugation experiment also showed that the fraction of the phages with the *b538* deletion was less than 2% in the recombinant *imm434 am*⁺ phages because of inefficient packaging of λ *b538* (1).

The time course of the recombinant DNA formation is shown in Fig. 3. The number of recombinant DNA molecules increased during the first hour of infection and then reached a plateau.

These results indicate that the recombinant DNA molecules were formed within jointly infected *recA*⁻ cells in the presence of chloramphenicol.

Specific Inhibition of the *recA*-Independent Recombination by Rifampin. When rifampin, in addition to chloramphenicol, was included to a *recA*⁻ culture during the mixed infection, the number of recombinant DNA molecules decreased greatly (Table 4, experiment 1). In contrast, rifampin

Table 3. *In vitro* packaging assay of recombinant λ DNA formed in *recA*⁻ bacteria

DNA solution added per tube, μl	*am*⁺ recombinant plaque formers per tube
0	0
1	11
2	54
5	154
10	231
10 + DNase	0

E. coli HI121 *recA1* cells were jointly infected with λ *imm434cI Dam15* F_1*am96B b538 red3* and λ *imm434cI Sam7 Ram5 int6 red3* in the presence of chloramphenicol as described in *Materials and Methods*. After incubation for 60 min at 37°, DNA was isolated by the Pronase/phenol method. The concentration of phage DNA calculated from ^{32}P radioactivity was 1.3×10^{11} λ *imm434* DNA molecules per ml. Various volumes of the DNA solution and 10 mM Tris/1 mM EDTA/50 mM KCl (TEK) buffer (total volume, 10 μl) were incubated with the packaging mixtures prepared from 594 (λ *cI857 Sam7 Eam4*) and 594 (λ *cI857 Sam7 Dam15* F_1*am96B*), as described in *Materials and Methods*. The resulting *imm434 am*⁺ recombinants were titrated on HI121 (λ *Dam15* F_1*am96B Sam7 Ram5*). In the last tube, pancreatic DNase was added to 100 μg/ml together with the DNA solution.

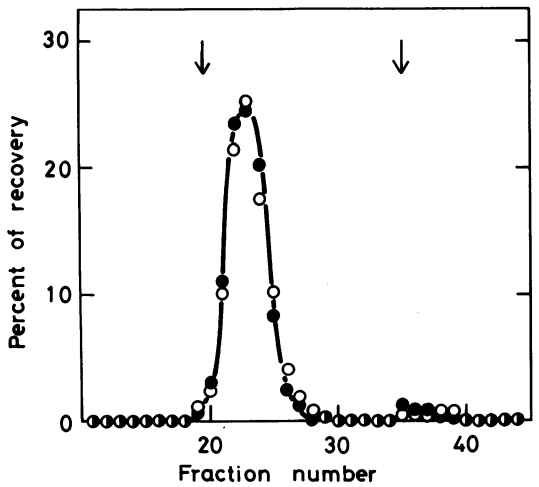

FIG. 2. CsCl density gradient centrifugation of the recombinant plaque formers produced by *in vitro* packaging. HI121 *recA1* cells were jointly infected with the $D^- F_I^-$ and $S^- R^-$ phages as for Table 3. DNA was isolated and incubated with the packaging mixture. The fraction of the am^+ recombinant phage in total *imm434* phage was 0.75%. The density of the phage suspension was adjusted to 1.490 g/cm^3 by the addition of solid CsCl. The solution was centrifuged at 23,000 rpm in the SW 50.1 rotor in a Spinco L ultracentrifuge for 20 hr at 12°. About 50 fractions were collected through the bottom of the tube, and plaque formers were determined on appropriate indicators. The left and right arrows indicate, respectively, the positions of λ *papa* (ρ = 1.508 g/cm^3) and λ *b538 cIsus34* (ρ = 1.484 g/cm^3) included in the sample. ●, Recombinant *imm434* am^+ plaque former; ○, *imm434* plaque former.

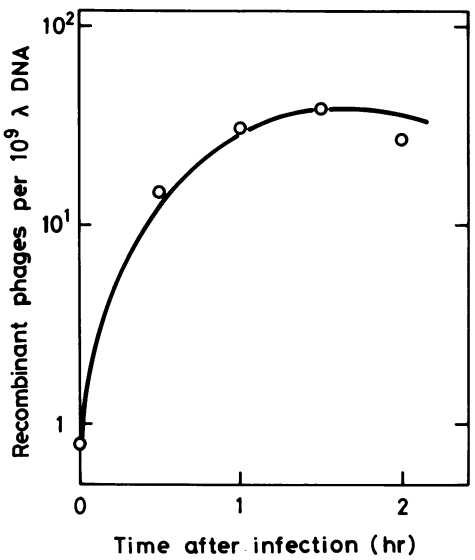

FIG. 3. Kinetics of formation of recombinant DNA. HI121 *recA1* was mixedly infected with the $D^- F_I^-$ and $S^- R^-$ phages as for Table 3. DNA was isolated at various times after infection, and 10 μl of the DNA preparation containing about 10^{11} λ *imm434* DNA molecules/ml was incubated with the packaging mixture. The number of *imm434* am^+ recombinant plaque formers per 10^9 λ *imm434* DNA molecules was determined.

did not influence the production of recombinant DNA in rec^+ hosts, as described previously (1). These results suggest that the recombinant DNA in $recA^-$ cells is formed by a *recA*-independent mechanism distinct from the *recA*-mediated recombination.

Relationship between the *recA*-Independent Recombination and Other Known Recombination Pathways. Next we asked what pathway is responsible for this *recA*-independent recombination. The Der pathway of λ *reverse* and the RecE pathway are known to mediate recombination of λ phage independent of *recA* function (6–8). Bacterial strains descended from AB1157 fail to generate the *sbcA* mutation, which defines the RecE pathway (8), and do not generate λ *reverse* (6). The *recA*-independent recombination occurred in AB2463, a *recA13* derivative of AB1157 (Table 4, experiment 2). This eliminates the role of the RecE or the Der pathway for the *recA*-independent recombination. Furthermore, this result shows that the occurrence of the *recA*-independent recombination is not specific for one *recA* allele or one genetic background. The role of *recB* function in the *recA*-independent recombination was examined by crosses in HI121 *recA1* cells and QW22 *recA56 recB21* cells. The frequencies of recombinant DNA from $recA^-$ and $recA^- recB^-$ hosts did not differ significantly, whether DNA was packaged in a rec^+ mixture (Table 4, experiment 3) or a $recB^-$ mixture (data not shown). It is inferred that *recB* function is dispensable to the *recA*-independent recombination.

Table 4. Effect of rifampin on the *recA*-independent recombination in various hosts

Host	am^+ recombinant phages per 10^9 λ DNA	
	−rifampin	+rifampin
Experiment 1		
594 rec^+	1100	1400
HI121 *recA1*	190	6.3
Experiment 2		
AB1157 rec^+	580	1500
AB2463 *recA13*	55	3.7
Experiment 3		
HI90 rec^+	490	900
HI121 *recA1*	190	2.6
QW22 *recA56 recB21*	180	2.2

Bacteria were jointly infected with the $D^- F_I^-$ and $S^- R^-$ phages as for Table 3 and incubated for 60 min at 37° in the presence of chloramphenicol alone or chloramphenicol and rifampin. After isolation of DNA by the Pronase/phenol method, 10 μl of DNA preparation containing about 10^{11} λ DNA molecules/ml was incubated with the packaging mixture for the assay of recombinant DNA.

int or *red* function of phage λ could not be responsible for the *recA*-independent recombination, because parental phages carried *int* and *red* mutations and because the expression of phage functions was blocked by chloramphenicol during phage infection.

Therefore, it is suggested that this *recA*-independent recombination is mediated by a novel pathway distinct from other known recombination pathways.

Studies with rif^r Bacteria. The effect of rifampin on the *recA*-independent recombination and on RNA synthesis was examined with a $recA^-$ cell, KY1340, and its rifampin-resistant derivative, KY7139. Jointly infected bacteria were incubated in media containing [^{3}H]uridine, chloramphenicol, and increasing concentrations of rifampin. After 60 min, DNA was extracted and the am^+ recombinant DNA was measured by the packaging assay. The incorporation of [^{3}H]uridine into an acid-insoluble fraction was also measured with aliquots of the cultures. In rif^+ $recA^-$ bacteria, both the formation of recombinant DNA and ^{3}H incorporation started to decrease at a rifampin concentration of 0.1 μg/ml and they were severely depressed at concentrations above 1 μg/ml (Fig. 4). In rif^r

Table 5. Distribution of crossover points

Host	Rifampin	$A^- \times D^- F_I^-$	$N^- cIam \times O^- cI^-$		$N^- cII \times S^- R^-$	
		$A^+ D^+ F_I^+$ PFU*	$N^+ O^+$ PFU*	$N^+ O^+ cI^-$ PFU/ $N^+ O^+ cIam$ PFU	$N^+ S^+ R^+$ PFU*	$N^+ S^+ R^+ cII^+$ PFU/ $N^+ S^+ R^+ cII$ PFU
HI90 *rec*$^+$	−	250	350	ND	190	2.6
HI90 *rec*$^+$	+	390	120	2.1	170	4.4
HI121 *recA1*	−	3.4	140	0.85	77	0.38
HI121 *recA1*	+	9.9	3.2	ND	2.0	ND

Crosses were carried out as for Table 4, except that three pairs of λ strains, λ *imm434cI Aam11* with λ *imm434cI Dam15 F*$_I$*am96B b538 red3*, λ *imm434cIam Nam7* with λ *imm434cI Oam8*, and λ *imm434 Nam7 Nam53 cII* with λ *imm434 Sam7 Ram5 int6 red3*, were used. The resulting *imm434 am*$^+$ recombinant phages were titrated on appropriate indicator bacteria, A8 (λ *spc2*) for the $A^- \times D^- F_I^-$ cross, HI121 (λ *ind*$^-$ *Nam7 Oam8*) for the $N^- \times O^-$ cross, and HI121 (λ *Nam7 Nam53 Sam7 Ram5*) for the $N^- \times S^- R^-$ cross. In the $N^- \times O^-$ cross, the *cI* genotype of the $N^+ O^+$ recombinants was tested by picking up the resulting plaques and spotting on Ymel (λ). Several percent of the plaques originating from *rec*$^+$ and *recA1* hosts were of a "mixed type" for the *cI* allele, that is, they contained nearly equal numbers of *cI*$^-$ and *cIam* particles. These plaques were excluded from the calculation of the relative frequency of $N^+ O^+ cI^-$ PFU to $N^+ O^+ cIam$ PFU. ND, not determined.
* PFU, plaque-forming units. Data are given as PFU/10^9 λ DNA molecules.

recA$^-$ bacteria, both the recombination and ^{3}H incorporation were not decreased by rifampin up to 100 μg/ml (Fig. 4). That is, the *recA*-independent recombination became rifampin resistant by the introduction of a *rif*^{r} mutation. This fact was observed not only in the cross between $N^- \times O^-$ but also in the cross between $D^- F^- \times S^- R^-$. These results show that the

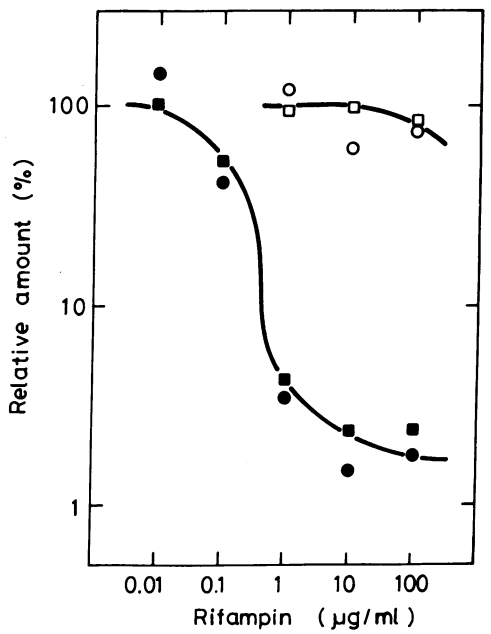

FIG. 4. Effect of rifampin on the *recA*-independent recombination and on RNA synthesis in *rif*$^+$ and *rif*^{r} bacteria. Five-milliliter cultures of KY1340 *rif*$^+$ *recA* and KY7139 *rif*^{r} *recA* were jointly infected with λ *imm434cIam Nam7* and λ *imm434cI Oam8* as described in *Materials and Methods*, and aerated in λ broth containing [^{3}H]uridine (1 μCi/ml), chloramphenicol, and various concentrations of rifampin. After 60 min, 100 μl of the cultures was mixed with 0.25 ml of bovine serum albumin solution (200 μg/ml) containing uridine (2 mg/ml) and then with 2.5 ml of 10% trichloroacetic acid at 0°. The mixture was filtered through Whatman GF/C filters and the radioactivity was measured by liquid scintillation counting. DNA was extracted from the remaining cultures and assayed for recombinant λ DNA as for Table 4. The resulting *am*$^+$ recombinant phages were titrated on HI121 (λ *ind*$^-$ *Nam7 Oam8*). The control cultures (− rifampin) contained 5.3 × 10^4 (KY1340) and 8.7 × 10^4 cpm/ml (KY7139) of acid-insoluble radioactive material and yielded 90 (KY1340) and 120 (KY7139) recombinant phages per 10^9 λ DNA molecules. The data are presented as % recombinant phage or as % radioactivity of the control cultures. Recombinant phages (●, ○); acid-insoluble radioactive material (■, □); KY1340 *rif*$^+$ (●, ■); KY7139 *rif*^{r} (○, □).

function of RNA polymerase, or at least its β subunit, is responsible for the *recA*-independent recombination. Moreover, a concentration of rifampin required for the inhibition of the recombination was comparable to that required for the inhibition of transcription. This suggests that the transcription process may participate in the recombination.

Distribution of Crossover Points in the λ Phage Genome Formed by the *recA*-Independent Recombination. In the presence of chloramphenicol, transcription of λ genome is known to take place in short regions to both sides of the *cI* gene, as shown in Fig. 1 (9, 10). Then, the *recA*-independent recombination might also take place in these but no other regions of λ genome when this drug is present. *rec*$^+$ or *recA1* bacteria were jointly infected with λ *imm434cIam Nam7* and λ *imm434cI Oam8* phages or with λ *imm434 Aam11* and λ *imm434 Dam15 F*$_I$*am96B* phages in the presence of chloramphenicol only or of chloramphenicol and rifampin together. DNA was isolated and subjected to the packaging assay for the *am*$^+$ recombinant DNA. In *recA*$^-$ cells, rifampin-sensitive crossovers occurred in the *N–O* interval, but those in the *A–D* interval were undetectable (Table 5). In *rec*$^+$ cells, both crossovers occurred at similar frequencies with or without rifampin.

Crossovers in the *N–O* interval were further resolved into two regions, the *N–cI* region and the *cI–O* region, by measuring the numbers of $N^+ O^+ cIam$ and $N^+ O^+ cI^-$ recombinant phages. The crossovers occurred in both of these regions in the *recA*$^-$ cross as well as in the *rec*$^+$ cross (Table 5). In this experiment, 6.0% of the recombinant phages were heterozygous for the *cI* allele in the *recA*$^-$ cross, while 3.3% were heterozygous in the *rec*$^+$ cross.

To see crossovers in the *cII–S* interval, a cross was carried out between λ *imm434 Nam7 Nam53 cII* and λ *imm434 c*$^+$ *Sam7 Ram5* in *rec*$^+$ and *recA1* bacteria. The relative frequency of crossovers in the *cII–S* interval with respect to those in the *N–cII* interval was estimated by the ratio of the numbers of *am*$^+$ *cII*$^+$ to *am*$^+$ *cII* recombinant phages (Table 5). The ratio in *rec*$^+$ cells with rifampin (*recA*-dependent recombination) was consistent with the value expected from map distance, while that in *recA1* cells without rifampin (*recA*-independent recombination) was much lower than the expected value. Therefore, *recA*-independent crossovers in the *cII–S* interval are less frequent than those in the *N–cII* region.

These results indicate that crossover points of the *recA*-independent recombination are localized in two specific regions of λ genome, *N–cI* and and *cI–O*. These regions include p_L and p_R, the promoter sites essential to the early λ transcription. It

seems, therefore, that the region of λ DNA active for transcription is also competent for this recombination.

DISCUSSION

The *in vitro* packaging assay revealed that recombinant λ DNA was formed in *recA*$^-$ bacteria in the presence of chloramphenicol. This recombination is suppressed greatly by rifampin, but its sensitivity to rifampin is reduced by the introduction of *rif*^{r} mutation into host cell. These results indicate that bacterial RNA polymerase is involved in the recombination. This fact is in contrast to *recA*-mediated recombination which is insensitive to rifampin. Hence the recombinant DNA molecule forming in the *recA*$^-$ cross can be neither an intermediate of the *recA*-mediated recombination nor a product originating from the leakiness of the *recA* mutation. The possibility that the Red, Int, RecBC, RecE, or Der pathway is responsible for this recombination was also ruled out. Therefore, the recombination is mediated by a novel pathway distinct from other known pathways. We designate this the "Rpo (*R*NA *po*lymerase) pathway" of recombination.

When various concentrations of rifampin were present in the *recA*$^-$ cross, the extent of inhibition of recombination was correlated with that of inhibition of RNA synthesis. The distribution of the crossover points was also correlated with the distribution of the transcribed region of the λ genome. Therefore, the transcription process *per se* seems to be required for the recombination mediated by the Rpo pathway. When transcription occurs, DNA is locally unwound in a short region within or close to the RNA polymerase, as evidenced by electron microscopic observation (11) and by sensitivity to single-strand-specific endonuclease from *Neurospora crassa* (12). Such an unwound region of DNA might be active in synapsis with a complementary strand of another DNA molecule. Alternatively, unwinding of DNA might be effective for the formation of single-stranded ends, which lead to the initiation of recombination. In any case, it is conceivable that some local change of DNA structure caused by transcription is required for this recombination.

In recent years, a group of specific DNA segments, so-called insertion sequences (IS), have been found to play an important role in some genetic recombination. F′ factors, ampicillin resistance, and tetracycline resistance genetic elements carrying an IS can integrate, excise, or translocate independently of the function of *recA* gene (13–16). It has also been proposed that palindromic sequences at promoter regions or at other specific sites of DNA are capable of forming a characteristic structure (palindromic loop), which promotes recombination (17–19). However it remains to be worked out whether or not these phenomena and models are related to the Rpo-mediated recombination.

A high frequency of recombinant DNA detected in *recA*$^-$ cells by our method appears to be in contrast to the occurrence of few recombinant phages in a *recA*$^-$ host with λ *red*$^-$ *int*$^-$ by ordinary crosses (20) or by crosses under conditions of depressed DNA replication (21). An explanation for this discrepancy would be that the recombinant DNA forming in the *recA*$^-$ cross is incapable of being packaged into a phage particle in the ordinary cross and in the replication-minus cross. Alternatively, the Rpo-mediated recombination may require the absence of translation.

Because the recombinant phages packaged *in vitro* contain a normal amount of DNA, the Rpo-mediated recombination appears to occur between homologous sites of two DNA molecules. Some of the recombinant phages from the $N \times O$ cross in *recA*$^-$ host were heterozygous for the *cI* locus. Heteroduplex DNA, presumably present in these phages, may be an intermediate in the recombination. The molecular form of the recombinant DNA and reciprocity of the Rpo-mediated recombination remain to be clarified.

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