Recombination and complementation between R factors in *Escherichia coli* K 12

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SUMMARY

Recombination between chloramphenicol-sensitive (Cm^S) mutants of R 1, and R 100, has been demonstrated in *Escherichia coli* K12 rec^+; it occurs at reduced frequency in *recB* and *recC*, and is not detectable in *recA*, indicating that R factor recombination depends on host functions. Some mutants of R 1 also recombine with an R 100 mutant in a similar way. *recA* cells carrying an R 1 and an R 100 Cm^S mutant (hetero-R state) have a low level of chloramphenicol-resistance, and form a chloramphenicol acetyl transferase that has lower specific activity than enzyme from hosts carrying wild-type or recombinant factors. These results suggest the occurrence of interallelic complementation between mutant R factors.

1. INTRODUCTION

Two types of recombination between transferable drug resistance (R) factors have been described. The first, combination of two different R factors to form one linkage group, has been shown (1) between two fi^+ (fertility-inhibiting; Watanabe *et al.* 1964) factors (Mitsuhashi *et al.* 1962), (2) between an fi^+ and an fi^- factor (Yokota *et al.* 1969), and (3) between segregants of an fi^+ factor (Watanabe & Lyang, 1962; Nisioka, Mitani & Clowes, 1970); this last example is supported by measurements of DNA lengths of parental and recombinant molecules. The second type of recombination is between point-mutational sensitive alleles of drug-resistance genes carried on isogenic plasmids, and has been shown in the chloramphenicol-resistance (Hashimoto & Hirota, 1966) and tetracycline-resistance (Hashimoto & Mitsuhashi, 1966) loci of derivatives of R 100. This system is suitable for measuring recombination frequency since drug-resistant clones, which arise by recombination between sensitive mutants in a single gene, can be selected on agar medium incorporating the drug.

The object of this work was to find out whether the genes mediating recombination between R factors are borne on the host chromosome or the R factor itself. Three genes known to control chromosomal recombination in *Escherichia coli* K 12 are *recA*, *recB* and *recC*; $recB^+$ and $recC^+$ are thought to code for polypeptide components of an ATP-dependent nuclease involved in recombination, while $recA^+$ is believed to code for a function that reduces the activity of this nuclease (Willetts & Clark, 1969; Barbour & Clark, 1970) and controls septum formation

(Inouye, 1971). It has been reported that derivatives of 222 (Takano, 1966) and of R100 (Hashimoto, Iyobe & Mitsuhashi, 1970) recombine in a Rec$^-$ host, but Hoar (1970) has failed to confirm this finding in a *recA* host. We have isolated chloramphenicol-sensitive mutants of R1, and those which recombined in *rec$^+$* hosts were tested in *rec$^-$* hosts; R100 mutants which were known to recombine (Hashimoto & Hirota, 1966) were used for comparison. Our results indicate recombination between these mutants; they also indicate recombination, and possibly complementation, between mutants of R1 and R100.

2. MATERIALS AND METHODS

(i) *Nomenclature and materials*

R factor antibiotic resistance markers are designated Ka (kanamycin), Sm (streptomycin), Cm (chloramphenicol), Su (sulphonamide), Ap (ampicillin), and Tc (tetracycline); S = sensitive, R = resistant. t$^+$ indicates R factor transferability. The antibiotic content of nutrient agar was as follows: Ap agar, 20 μg/ml ampicillin; Cm, 20 μg/ml chloramphenicol; Ka, 40 μg/ml kanamycin; Tc, 20 μg/ml oxytetracycline; Ap–Ka, 20 μg/ml ampicillin + 40 μg/ml kanamycin; Cm–Nx, 20 μg/ml chloramphenicol + 20 μg/ml nalidixic acid; Ka–Tc, 40 μg/ml kanamycin + 20 μg/ml oxytetracycline. A transcipient cell (Fullbrook, Elson & Slocombe, 1970) harbouring two R factors (*hetero-R* state) is represented as e.g. R100–99 Cm^S Tc^R/R1 Cm$^S_7$ Ap^S.

The bacterial strains and R factors are listed in Table 1. Cm^S mutants of R100–1 (a derepressed mutant of R100; Egawa & Hirota, 1962) were kindly supplied by Dr S. Iyobe, Gunma University, Maebashi, Japan.

(ii) *Isolation of mutants of R1*

Two derivatives of R1, an Ap^S mutant and a Ka^S segregant, were isolated. The Ap mutant was isolated by treatment with N-methyl-N-nitro-N'-nitrosoguanidine followed by plating out on drug-free agar for single colonies, which were replicaplated on to Ap agar. One colony in the 10000 screened was Ap^S. The R factor carrying this mutation did not revert spontaneously to Ap^R, was lost at a frequency of 1–5 % in the absence of selective pressure, and was transfer-defective (t$^-$). The Ka^S segregant was isolated by transferring R1 into *Salmonella typhimurium*, where R *fi$^+$* factors are known to be unstable (Watanabe & Lyang, 1962), and replicaplating colonies from drug-free medium on to Ka agar. This segregant was transferred back to *E. coli* J5–3 *nal^r*.

Cm^S mutants of Ap^S and Ka^S derivatives of R1 were induced by mutagenesis with ethyl methane sulphonate followed by penicillin screening in presence of chloramphenicol (20 μg/ml) and ampicillin (1 mg/ml) in minimal salts medium; Cm^S mutants were isolated by replica plating colonies from drug-free agar on to Cm agar. Because of difficulty in using ampicillin as a screening agent for the Ap^R Ka^S segregant, D-cycloserine and cephalexin (Glaxo), a cephalosporin moderately resistant to hydrolysis by R1 β-lactamase, were used together at a

concentration of 1 mg/ml in minimal salts medium. Eight $Cm^S Ap^S Ka^R t^-$ mutants and four $Cm^S Ap^R Ka^S t^+$ mutants were found to revert to Cm^R spontaneously at low frequency ($< 10^{-7}$) and were screened for recombination activity; three of the former recombined with one of the latter.

Table 1. *Bacterial strains and R factors*

(i) Bacterial strains

Strain	Source
E. coli J 5–3 *pro₁ met₂* F⁻	N. Datta
E. coli J 5–3 *pro₁ met₂ nalʳ* F⁻	Isolated from J 5–3 *pro₁ met₂* F⁻
E. coli J 5–3 *pro₁ met₂* (R 1)	N. Datta
E. coli J 5–3 *pro₁ met₂* (222)	N. Datta
E. coli JC 5744 (SR 66) Tᵣᶜ *strʳ thr leu thi arg pro his lac ara gal mtl xyl rec⁺ λˢ* F⁻	E. Lederberg
E. coli JC 2926 (SR 70) *recA 13*	E. Lederberg
E. coli JC 5743 (SR 69) *recB 21*	E. Lederberg
E. coli JC 5489 (SR 68) *recC 22*	E. Lederberg
E. coli HfrH *thi λ⁺*	Lab. collection
E. coli HfrC 58–161	Lab. collection
S. typhimurium LT-2 wild-type	Lab. collection

Strains JC 5744, JC 2926, JC 5743 and JC 5489 are isogenic except for the *rec* marker.

(ii) R factors

R factor	Markers	Source
R 1	$Cm^R Ap^R Sm^R Su^R Ka^R fi^+ t^+$	J 5–3 *pro₁ met₂* (R 1)
222	$Cm^R Tc^R Sm^R Su^R fi^+ t^+$	J 5–3 *pro₁ met₂* (222)
R 1 $Cm_7^S Ap^S$		
R 1 $Cm_{12}^S Ap^S$	$Cm^S Ap^S Sm^R Su^R Ka^R t^-$	R 1 $Cm^R Ap^S Sm^R Su^R Ka^R t^-$
R 1 $Cm_{20}^S Ap^S$		
R 1 $Cm_{107}^S Ap^S$	$Cm^S Ap^R Sm^R Su^R Ka^S t^+$	R 1 $Cm^R Ap^R Sm^R Su^R Ka^S t^+$
R 100–84	$Cm_2^S Tc^R Sm^R Su^R t^-$	
R 100–99	$Cm_1^S Tc_1^S Sm^R Su^R t^+$	Hashimoto & Hirota (1966)
R 100–127	$Cm_9^S Tc^R Sm^S Su^S t^+$	
R 100–99 Tc^R	$Cm_1^S Tc^R Sm^R Su^R t^+$	Spontaneous Tc^R revertant of R 100–99

(iii) *Transduction*

The procedure for transduction with phage P 1 *kc* (Lennox, 1955) was applied to R factors as in Watanabe & Fukasawa (1961). The multiplicity of infection was *c.* 0·5.

(iv) *Recombination between R factors*

R 1 $Cm_7^S Ap^S$, R 1 $Cm_{12}^{S,} Ap^S$, R 1 $Cm_{20}^S Ap^S$ and R 100–84 were transferred to *E. coli* K 12 *rec⁺* and *rec⁻* mutants by transduction with phage P 1 *kc*.

The hetero-R state, which is a prerequisite of recombination, was formed by mixing together log phase broth cultures of donor (t⁺) R factor-carrying strains and recipient (t⁻) R factor-carrying strains in equal volumes and incubating in a shaking water bath (37 °C for 2 h). Matings were stopped by placing the tubes in an ice bath, and suitable dilutions were plated on Cm agar for Cm^R recombinants

and also on agar selecting for hetero-R transcipients (Ap–Ka for R1/R1; Ka–Tc for R1/R100). It was not possible to select for the hetero-R state in R100/R100 crosses in the rec^- hosts because of lack of a suitable selective marker. Recombination between R1 mutants could be detected only by replica plating hetero-R colonies on Cm agar. No recombinants were detected when mating mixtures were plated directly on Cm agar, unlike R100/R100 and R1/R100 crosses.

R1/R1 hetero-R colonies were picked with a sterile Pasteur pipette and emulsified in sterile distilled water. Tenfold dilutions were plated on Cm agar for recombinants, and on drug-free agar for total viable counts, in order to estimate the proportion of Cm^R cells in the hetero-R colony.

(v) *Level of resistance to chloramphenicol*

Doubling dilutions of chloramphenicol from 1000 to 1 μg/ml were made in molten nutrient agar. Cultures were streaked on the agar and the plates were examined after 1 and 3 days incubation. The level of resistance was taken as the lowest dilution of chloramphenicol on which colonial growth was visible.

(vi) *Preparation and assay of chloramphenicol acetyl transferase (CAT)*

CAT is responsible for R factor mediated resistance to chloramphenicol (Shaw, 1966, 1967; Shaw & Brodsky, 1967; Suzuki & Okamoto, 1967; Mise & Suzuki, 1968).

Crude sonicate extracts were used as a source of enzyme. To prevent segregation of mutant R factors in the $recA$ host, cells were grown on the surface of Ka–Tc agar and harvested in 0·01 M Tris–HCl (20 ml, pH 7·8) after overnight incubation. Cell suspensions were sonicated for 2 min using a 'Soniprobe 1130A' (Dawe) at 2 A followed by centrifugation (30000 g for 30 min). The supernatant was dialysed overnight against 0·01 M Tris–HCl (pH 7·8) at 4 °C. The enzyme preparations were stored at -20 °C without further purification.

The enzyme activity of some extracts was increased by packing the dialysis tubing with Carbowax (Gurr) to facilitate loss of water.

CAT was assayed by the method of Shaw & Brodsky (1968). Reactions were performed in a Unicam SP 500 spectrophotometer, using the Direct Readout Mode, and a Unicam SP 22 recorder. Specific enzyme activities were calculated as μmoles acetyl coenzyme A utilized/min/mg protein. Protein was assayed by the method of Lowry *et al.* (1951).

3. RESULTS

(i) *Recombination between chloramphenicol-sensitive mutants of R1*

J5–3 (R1 Cm^S_{107} Ka^S) was mated with rec^+ and rec^- strains carrying R1 Cm^S_7 Ap^S, R1 Cm^S_{12} Ap^S and R1 Cm^S_{20} Ap^S. Between 100 and 500 hetero-R colonies, obtained by selection on Ap–Ka agar, were replica-plated on Cm agar. The proportion of Cm^R to total ($Cm^R + Cm^S$) hetero-R colonies was calculated (Table 2(i)). No Cm^R hetero-R colonies were detected with a $recA$ host, and less were obtained with $recB$ and $recC$ hosts than with rec^+. Hetero-R colonies that grew

when replica-plated on Cm agar were tested for their proportion of Cm^R to total cells (Table 2(ii)). No Cm^R cells were formed by *recA* hosts; the proportion of Cm^R to total cells in the hetero-R colony fluctuated in *rec*+, *recB* and *recC* hosts. The Cm^R marker in *rec*+ hosts was co-transducible with other R factor markers by phage P1: on testing ten clones from the $Cm_{107}^S \times Cm_{20}^S$ cross, Cm^R was 99% co-transduced with Ka^R and 1% with Ap^R. It was also transferred with Ap^R by conjugation in 30 min matings. It is concluded that the Cm^R allele arose by recombination between the two R factors.

Table 2. *Recombination between Cm^S mutants of R1*

(i) Proportion of Cm^R to total ($Cm^R + Cm^S$) hetero-R colonies

Host strain	$R1\ Cm_{107}^S\ Ka^S \times$ $R1\ Cm_7^S\ Ap^S$	$R1\ Cm_{107}^S\ Ka^S \times$ $R1\ Cm_{12}^S\ Ap^S$	$R1\ Cm_{107}^S\ Ka^S \times$ $R1\ Cm_{20}^S\ Ap^S$
rec+	0·5	0·5	0·5
recA	< 0·002	< 0·002	< 0·002
recB	0·06	0·05	0·07
recC	0·2	0·2	0·2

(ii) Proportion of Cm^R to total cells in hetero-R Cm^R colonies*

Host strain	$R1\ Cm_{107}^S\ Ka^S \times$ $R1\ Cm_7^S\ Ap^S$	$R1\ Cm_{107}^S\ Ka_S \times$ $R1\ Cm_{12}^S\ Ap^S$	$R1\ Cm_{107}^S\ Ka^S \times$ $R1\ Cm_{20}^S\ Ap^S$
rec+	10–100	10–100	10–100
recA	< 1	< 1	< 1
recB	10–100	5–7	10–40
recC	10–100	30–60	10–100

Hetero-R colonies were selected on Ap-Ka agar (Results, section (i)).
* All figures × 10^{-7}

Table 3. *Frequency of recombination between Cm^S mutants of R100*

	Cm^R recombinants per donor cell	
Host strain	$R100-99 \times R100-84$	$R100-127 \times R100-84$
rec+	3×10^{-5}	6×10^{-7}
recA	$< 5 \times 10^{-8}$ ($< 0·002$)	$< 1 \times 10^{-8}$ ($< 0·01$)
recB	2×10^{-6} (0·07)	2×10^{-8} (0·03)
recC	5×10^{-6} (0·2)	9×10^{-8} (0·1)

Ratios of recombination frequencies in *rec*− relative to *rec*+ hosts are shown in parentheses.

(ii) *Recombination between chloramphenicol-sensitive mutants of R100*

J5–3 (R100–99) and J5–3 (R100–127) were mated for two hours with *rec*− hosts carrying R100–84; Cm^R recombinants were selected by plating the mating mixture on Cm agar (Table 3). A direct comparison with the R1 system could not be made because the hetero-R state could not be selected in *rec*− hosts, which are highly resistant to streptomycin. No recombination was detected in *recA* bacteria, and the level was reduced in *recB* and *recC* hosts.

These results were compared to figures for analogous experiments with chromo-

somal recombination (Low, 1968), which were repeated with HfrH and HfrC donors. It was confirmed that the frequencies of R factor and chromosomal recombination were similar in rec^- relative to rec^+ hosts.

(iii) *Recombination and complementation between chloramphenicol-sensitive mutants of R1 and R100–1*

J5–3 (R100–99 Tc^R) was mated for 2 h with rec^- recipients carrying R1 $Cm_7^S Ap^S$, R1 $Cm_{12}^S Ap^S$ and R1 $Cm_{20}^S Ap^S$; dilutions of the mating mixtures were plated on Cm agar for Cm^R recombinants and Ka–Tc agar for hetero-R colonies. A striking observation, in view of results with R1/R1 and R100/R100 recombination, was that the *recA* host allowed the formation of many Cm^R colonies (Table 4), which were smaller than recombinant colonies (diameter *c.* 1 mm after 2 days). The rec^+ host formed many more colonies after three days incubation than after one.

Table 4. *Frequencies of transcipients in crosses between R100–99 Tc^R and Cm^S mutants of R1*

Host	R100–99 Tc^R × R1 $Cm_7^S Ap^S$	R100–99 Tc^R × R1 $Cm_{12}^S Ap^S$	R100–99 Tc^R × R1 $Cm_{20}^S Ap^S$
rec^+			
Cm*	$5 \cdot 6 \times 10^3$	$1 \cdot 1 \times 10^5$	$1 \cdot 3 \times 10^6$
Ka-Tc*	$2 \cdot 7 \times 10^6$	$9 \cdot 7 \times 10^5$	$1 \cdot 2 \times 10^7$
recA			
Cm*	$1 \cdot 0 \times 10^3$	$4 \cdot 9 \times 10^3$	$2 \cdot 4 \times 10^5$
Ka-Tc*	$1 \cdot 8 \times 10^6$	$6 \cdot 4 \times 10^5$	$5 \cdot 3 \times 10^6$
recB			
Cm*	$7 \cdot 8 \times 10^2$	$8 \cdot 8 \times 10^3$	$2 \cdot 4 \times 10^5$
Ka-Tc*	$1 \cdot 2 \times 10^6$	$1 \cdot 9 \times 10^5$	$6 \cdot 0 \times 10^6$
recC			
Cm*	$3 \cdot 1 \times 10^2$	$< 1 \times 10^1$	$2 \cdot 0 \times 10^1$
Ka-Tc*	$5 \cdot 5 \times 10^5$	$3 \cdot 0 \times 10^5$	$1 \cdot 5 \times 10^6$
J5–3 (R100–99 Tc^R) cell count†			
	$2 \cdot 5 \times 10^8$	$1 \cdot 7 \times 10^8$	$1 \cdot 2 \times 10^8$

Colonies were counted after 3 days incubation.
* Cm, Ka–Tc: colonies per ml of mating mixture on Cm agar and on Ka–Tc agar respectively.
† Number of colonies per ml on antibiotic-free agar.

Hetero-R colonies from each host were streaked on to Cm agar and incubated for 2 days. The rec^+, *recB* and *recC* matings yielded a mixture of uniformly sized small and of large colonies, whereas the *recA* matings yielded only small slow-growing colonies. The rec^+, *recB* and *recC* small and large colonies, and the *recA* small colonies, were re-streaked on Cm agar. The large colonies bred true, but the small colonies, except for those from *recA*, gave a mixture of small and large colonies.

The following experiments were now performed to test the hypothesis that both recombination and complementation occurred in rec^+, *recB* and *recC* hosts, and that

complementation alone occurred in the *recA* host: transfer by transduction and conjugation, stability of Cm^R, the level of chloramphenicol-resistance, and the specific activity of chloramphenicol acetyl transferase (CAT) in crude lysates.

Large *rec*⁺ ($R\,100–99\ Cm^S\ Tc^R/R\,1\ Cm^S_{20}\ Ap^S$) Cm^R colonies and small *recA* ($R\,100–99\ Cm^S\ Tc^R/R\,1\ Cm^S_{20}\ Ap^S$) Cm^R colonies were grown in L-broth (Lennox, 1955); portions were used to prepare P1 transducing phage lysates and as donors in conjugation to a *nal*ʳ recipient (Table 5(i)). Since transducing phage lysates could not be prepared on *rec*⁻ hosts, further experiments were performed as in Table 5(ii). Clones containing transduced R factors from *rec*⁺ donors were selected on Cm agar and replica-plated on Ka agar and Tc agar to determine which markers

Table 5. *Tests on R 100–99 $Tc^R/R\,1\ Cm^S_{20}\ Ap^S$ transcipients*

(i) Transfer of the Cm^R marker by transduction and conjugation

Trancipient*	Transductants on Cm agar†	Colonies growing on replica plating transductants on		Conjugational transfer‡
		Ka agar	Tc agar	
rec⁺ ($R\,100–99\ Cm^S\ Tc^R/R\,1\ Cm^S_{20}\ Ap^S$)				
1	456	67 (14·6)	0	—
2	375	53 (14·1)	2 (0·5)	+
3	438	436 (99·5)	0	—
4	326	243 (74·5)	129 (39·5)	+
recA ($R\,100–99\ Cm^S\ Tc^R/R\,1\ Cm^S_{20}\ Ap^S$)				
1–4				—

(ii) Segregation of markers

Transcipient*	Colonies on Cm agar	Colonies growing on replica plating colonies from Cm agar to	
		Ka agar	Tc agar
rec⁺ ($R\,100–99\ Cm^S\ Tc^R/R\,1\ Cm^S_{20}\ Ap^S$)			
1	97	97 (100)	6 (6)
2	62	1 (1)	62 (100)
3	48	0 (2)	48 (100)
4	73	40 (54)	68 (93)
recA ($R\,100–99\ Cm^S\ Tc^R/R\,1\ Cm^S_{20}\ Ap^S$)			
1	100	100 (100)	100 (100)
2	50	50 (100)	50 (100)
3	500	500 (100)	500 (100)
4	100	100 (100)	100 (100)

Figures in parentheses are % co-segregation of Ka^R or Tc^R with Cm^R.
* 1–4: four independent transcient colonies (see text for explanation).
† Transductants per *c.* 5×10^7 cells plated.
‡ Transfer of Cm^R to *nal*ʳ recipient in 30 min.

segregated with Cm^R. Transcipients were selected from conjugation experiments on Cm–Nx agar after 30 min mating. Table 5(i) shows that Cm^R is transducible from *rec*⁺ hosts, which is further evidence for its having arisen by recombination;

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there is wide variation in the linkage of Cm^R with other markers. Further rec^+ ($R\,100–99\;Cm^S\;Tc^R/R\,1\;Cm_{20}^S\;Ap^S$) and $recA$ ($R\,100–99\;Cm^S\;Tc^R/R\,1\;Cm_{20}^S\;Ap^S$) Cm^R colonies were grown in broth, plated on Cm agar, and replica-plated on Ka agar and Tc agar. Table 5(ii) shows that there is a variation in the association of Cm^R with other R factor markers in the rec^+ host, and that there is no requirement for both markers to be present for Cm^R to be expressed. Both Ka^R and Tc^R are present in every Cm^R cell of the $recA$ host.
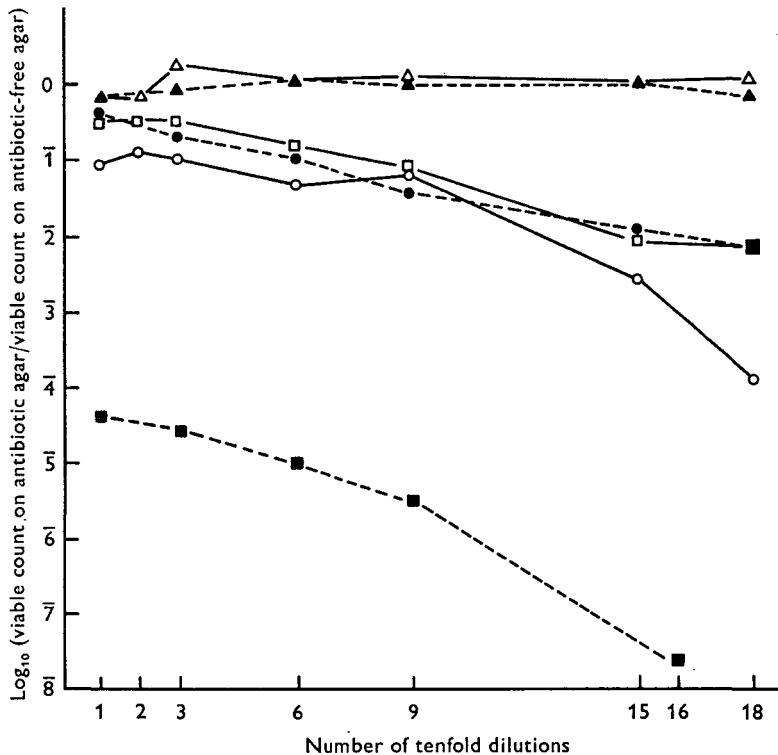
Fig. 1. Loss of antibiotic-resistance markers from rec^+ and $recA$ ($R\,100–99\;Cm^S$ $Tc^R/R1\;Cm_{20}^S\;Ap_S$) Cm^R hetero-R cultures during serial tenfold dilutions; $\triangle$, $\square$ and $\bigcirc$ indicate Tc^R, Ka^R and Cm^R respectively (open symbols for $recA$, black symbols for rec^+).

To confirm that both R factors must be present for Cm^R to be expressed in the $recA$ host, broth cultures of rec^+ ($R\,100–99\;Cm^S\;Tc^R/R\,1\;Cm_{20}^S\;Ap^S$) and $recA$ ($R\,100–99\;Cm^S\;Tc^R/R\,1\;Cm_{20}^S\;Ap^S$) Cm^R were serially subcultured in broth; the cultures were diluted at intervals and plated on nutrient agar, Cm agar, Ka agar and Tc agar. Colonies were counted after 3 days. The logarithm of the ratio of viable counts on antibiotic agar to viable counts on nutrient agar was plotted against an arbitrary time scale. Fig. 1 shows that in the $recA$ hetero-R culture Cm^R depends on both R factors being present and falls off as R 1 (Ka^R and unstable) is lost from the culture. In the rec^+ host, however, Cm^R does not depend on both R factors being present, although it is still lost from the population in the absence

of selection. It is concluded that the R100–99 mutant remains unaltered in the *recA* hetero-R state, since when it is transferred by conjugation to a *nal*^{r} cell carrying a R1 Cm^S mutant as recipient Cm^R colonies are formed on Cm–Nx agar.

(iv) *Chloramphenicol acetyl transferase (CAT) activity determinations*

The levels of chloramphenicol-resistance of mutants, recombinants, hetero-R and wild-type cells were determined, together with specific enzyme activities of CAT from crude sonicates (µmoles acetyl coenzyme A utilized/min/mg protein; Table 6). The *rec*$^+$ Cm^R recombinants have the same resistance level as wild-type, and the enzyme activity is similar. The *recA* Cm^R hetero-R strains are about ten

Table 6. *Levels of chloramphenicol-resistance and specific activities of chloramphenicol acetyl transferase (CAT) determined by R factors in* rec$^+$ *and* recA *hosts*

Strain	Level of chloramphenicol- resistance (µg/ml)	Specific Activity of CAT*
rec$^+$ (R$^-$)	4	< 0·002†
recA (R$^-$)	4	< 0·002†
rec$^+$ (R1)	500	2·2
recA (R1)	500	2·0
rec$^+$ (222)	500	2·25
recA (222)	500	2·9
recA (R1 Cm$_7^S$ Ap^S)	8	< 0·002†
recA (R1 Cm$_{12}^S$ Ap^S)	4	< 0·002†
recA (R1 Cm$_{20}^S$ Ap^S)	8	< 0·002†
recA (R100–99)	4	0·022
rec$^+$ (R100–99/R1 Cm$_7^S$ Ap^S)	500	1·9
rec$^+$ (R100–99/R1 Cm$_{12}^S$ Ap^S)	500	1·5
rec$^+$ (R100–99/R1 Cm$_{20}^S$ Ap^S)	500	2·5
recA (R100–99/R1 Cm$_7^S$ Ap^S)	60	0·05
recA (R100–99/R1 Cm$_{12}^S$ Ap^S)	60	0·06
recA (R100–99/R1 Cm$_{20}^S$ Ap^S)	60	0·14

* µmoles acetyl coenzyme A utilized per minute per mg protein.
† Enzyme concentrated with Carbowax (see Materials and Methods, section (vi)).

times less chloramphenicol-resistant than the wild-type after 3 days incubation, and the specific enzyme activities are more than tenfold lower than that of wild-type. The chloramphenicol-resistance of hetero-R cultures (Table 6) differs from that of clones arising in mating experiments, in which more colonies were found to grow on Ka–Tc agar than on agar containing 20 µg/ml chloramphenicol (Table 4), as a result of the more stringent selection imposed in the latter experiment. No activity was detected in R$^-$ extracts; a very low activity was sometimes detected in Cm^S mutant controls after concentration by treatment with Carbowax.

4. DISCUSSION

Experiments on Cm^S mutants of R 1 and R 100 have shown that no recombinants are detectable in $recA$ hetero-R cells and that the number is reduced in $recB$ and $recC$ hosts. It appears that R factor recombination depends on the host bacterial recombination system.

Recombination between R 1 Cm^S and R 100–99 Tc^R Cm^S occurs in conjunction with what is thought to be complementation in rec^+, $recB$ and $recC$ hosts, while complementation alone occurs in $recA$ hosts. Complementation between these heterologous R factors could increase the probability of their recombining by allowing some growth on the selective medium, Cm agar. The evidence for complementation is that chloramphenicol-resistance *in vivo*, and chloramphenicol acetyl transferase activity *in vitro*, were at least tenfold lower than that of the recombinants or wild-type. Further, chloramphenicol-resistance in the $recA$ host depends on both R factors being present. It is interesting that complementation and recombination can occur between R factors of different origin.

R factor chloramphenicol acetyl transferase has a molecular weight of about 78 000 (Shaw & Brodsky, 1968), so it is quite likely that it is composed of subunits; there is evidence that *Staphylococcus* chloramphenicol acetyl transferase consists of four identical subunits each of molecular weight 20 000 (Shaw, Bentley & Sands, 1970). Interallelic complementation (Fincham, 1966) is therefore a plausible explanation for the low level of activity that we have found in $recA$ hetero-R cells.

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