Chloramphenicol Acetyltransferases Determined by R Plasmids from Gram-negative Bacteria

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The mechanism of resistance to chloramphenicol specified by 18 plasmids from Gram-negative bacteria representing different incompatibility groups was investigated. Most determined the drug-inactivating enzyme chloramphenicol acetyltransferase. The enzymes were purified and their properties were compared with those of the previously characterized enzyme types specified by R429 (type I), s-a (type II) and R387 (type III). The type I enzyme was determined by plasmids representing incompatibility groups FII, C, S, I, H, L, O and Com9. Plasmids from incompatibility groups K, I γ and the A-C complex specified the type III enzyme, while elements representing incompatibility groups V and W determined the type II variant.

INTRODUCTION

Chloramphenicol acetyltransferase (CAT) determined by R plasmids is responsible for the chloramphenicol resistance (Cm^r) of most Gram-negative bacteria (Fitton *et al.*, 1978). It catalyses the 3-*O*-acetylation of chloramphenicol in the presence of acetyl-coenzyme A (Shaw, 1967; Suzuki & Okamoto, 1967). An inducible Cm^r determinant which probably confers impermeability to the drug is specified by some Fi⁺ R plasmids (Nagai & Mitsuhashi, 1972). Gram-negative bacterial plasmids are now classified by incompatibility tests (Chabbert *et al.*, 1972; Datta & Hedges, 1972, 1973, Hedges, 1974, 1975; Hedges & Datta, 1971; Grindley *et al.*, 1973). Early studies on chloramphenicol resistance suggested that the determinant was mainly associated with Fi⁺ IncFII plasmids. Hedges & Datta (1971) described some Fi⁻ R plasmids that conferred resistance to chloramphenicol; each of these specified the production of CAT. The IncW plasmids and R387 (now classified as IncK; Hedges, 1975) determined novel types of CAT enzymes designated types II and III, respectively (Foster & Shaw, 1973). These were differentiated biochemically and immunologically from the classical type I CAT determined by IncFII and IncN plasmids.

Plasmids from different incompatibility groups usually have less than 10% DNA base sequence homology (Grindley *et al.*, 1973). However such plasmids can carry identical antibiotic resistance determinants. This is exemplified by the determinant for TEM β -lactamase which confers ampicillin resistance in many different bacterial hosts and is specified by several different plasmids (Hedges *et al.*, 1974; Heffron *et al.*, 1975*b*). The β -lactamase gene resides in a genetic element which can be translocated to other replicons independently of the host bacterial general (*recA*-controlled) recombination mechanism (Hedges & Jacob, 1974; Heffron *et al.*, 1975*a*; Heffron *et al.*, 1975*b*). The Cm^r determinant specifying type I CAT production which was derived from an Fi⁺ IncFII plasmid is also translocatable (Gottesman & Rosner, 1975; Rosner & Gottesman, 1977; MacHattie & Jackowski, 1977). If translocation of a chloramphenicol resistance determinant has occurred

Table 1. *Characteristics of the plasmids*

Plasmid	Incom- patibility group	CAT production	Resistance determinants*	Source or reference
R55	C	+	Cm Ap Gk Su	Chabbert <i>et al.</i> (1972)
R55-1†	C	—	Cm Su	Chabbert <i>et al.</i> (1972)
R57b	C	+	Cm Ap Gk Su	Datta & Hedges (1972)
R57b-1†	C	—	Cm Su	Datta & Hedges (1973)
RA1-1b	A	—	Cm Su	Datta & Hedges (1973)
R994	A-C	+	Cm Sm Tc	Hedges & Jacob (1975)
R429	FII	+	Cm Km Tc Ap	Shaw <i>et al.</i> (1972)
R16213	FII	+	Cm Sm Su	N. Datta
R726	H	+	Cm Sm Su Tc	Datta & Olarte (1974)
CS Col	I	+	Cm Sm Su	Richmond & Weidemann (1974)
R621ala	I γ	+	Cm Ap Km	Hedges (1974)
R387	K	+	Cm Sm	Hedges & Datta (1971); Hedges (1975)
R471b	L	+	Cm Ap Km	Hedges <i>et al.</i> (1975)
R478	S	+	Cm Km Tc	Hedges <i>et al.</i> (1975)
R724	O	+	Cm Sm Su Tc	Datta & Olarte (1974)
R753	V	+	Cm Ap Sm Su	Hedges (1975)
R901	V	+	Cm Km Sm Su	Hedges (1975)
s-a	W	+	Cm Km Sm Su	Hedges & Datta (1971)
R71a	Com9	+	Cm Ap Sm Su Tc	Chabbert <i>et al.</i> (1972)
R26	P	—	Cm Ap Km Sm Su	Stanisich <i>et al.</i> (1976)
R527	P	—	Cm Ap Km Sm Su	Stanisich <i>et al.</i> (1976)

* Abbreviations: Cm, chloramphenicol; Ap, ampicillin; Gk, gentamicin/kanamycin; Km, kanamycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline.

† R55-1 and R57b-1 are segregants of R55 and R57b, respectively; they still confer Cm^r but no longer produce CAT.

recently in natural populations, then the majority of CAT determinants from different plasmids and bacterial hosts might specify the same types of enzymes.

We have studied the Cm^r determinants of R plasmids from a variety of incompatibility groups to investigate the distribution of CAT enzyme types and the occurrence of other mechanisms of chloramphenicol resistance in Gram-negative bacteria. The enzymes were purified from the CAT-producing strains and were classified into types I, II and III, as described by Foster & Shaw (1973), on the basis of electrophoretic mobility, reaction with anti-CAT sera and susceptibility to inhibition by an inhibitor of thiol groups.

METHODS

Bacterial strains and plasmids. The chloramphenicol resistance-determining plasmids used in this study are listed in Table 1. The bacterial host strain was *Escherichia coli* K12 strain J53 Pro⁻ Met⁻ (Clowes & Hayes, 1968) or a spontaneous nalidixic acid resistant derivative of J53.

Chloramphenicol resistance level determinations. Chloramphenicol was added to Lemco nutrient agar to give concentrations between 20 and 400 $\mu\text{g ml}^{-1}$. Overnight broth cultures grown in the absence or presence of chloramphenicol (1 $\mu\text{g ml}^{-1}$) were diluted 10^{-4} in sterile saline and drops (about 0.01 ml) were applied to the surface of the plates. The resistance level was defined as the concentration of antibiotic which prevented the growth of single colonies after overnight incubation at 37 °C. The composition of the nutrient broth and agar was described previously (Foster & Walsh, 1974).

Preparation and assay of chloramphenicol acetyltransferase. Bacterial cultures inoculated from single colonies grown on drug-containing plates were grown overnight in 500 ml nutrient broth or in Difco Penassay broth containing 1% (v/v) glycerol with shaking (200 rev. min⁻¹) in a Gallenkamp orbital incubator at 37 °C. Strains carrying the two IncV plasmids, which were known to be unstable in *E. coli* hosts (Hedges, 1975), were inoculated into broth which initially contained 20 μg chloramphenicol ml⁻¹. The cells were harvested by centrifuging (10000 g, 10 min, 4 °C), washed in buffer 1 (0.05 M-Tris/HCl pH 7.8 containing 0.1 M-2-mercaptoethanol) and finally resuspended in 10 ml buffer 1. The cells were lysed either in an Aminco French press (420 kgf cm⁻²) or by sonic disruption with a 100 W MSE ultrasonic disintegrator at 0 °C

(4 × 30 s bursts at maximum output). The cell debris was removed by centrifuging (30000 g, 30 min) and the supernatant fluid was dialysed twice against 50 vol. buffer 1.

The CAT enzymes were purified by affinity chromatography, as described by Zaidenzaig & Shaw (1976), using Sepharose-bound 2-amino hexanoyl chloramphenicol. A Pasteur pipette column containing 1 ml of derivatized Sepharose was loaded with about 100 units of enzyme from crude lysates. The enzyme was eluted from the column by a series of salt and buffer washes as previously described, and each fraction was assayed for CAT activity. Those containing enzyme activity were pooled and dialysed twice against buffer 1. The purified CAT enzymes were concentrated by Amicon A-25 ultrafiltration. Agarose gel electrophoresis showed that most of the enzymes were purified to homogeneity by this method. A faint contaminating band running in front of the CAT enzyme band was detected in some preparations (e.g., R994 CAT).

Chloramphenicol acetyltransferase was assayed by the spectrophotometric method of Shaw & Brodsky (1968) using a Varian Techtron recording spectrophotometer. Protein concentrations were determined by the method of Lowry *et al.* (1951).

Inhibition of chloramphenicol acetyltransferases by 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). The crude lysates and the purified CAT enzymes were tested for sensitivity to DTNB (a reagent which modifies reactive thiol groups) by incubating 10 µl of a 5 to 10 units ml⁻¹ preparation of enzyme in reaction mixture with or without 1 mM-DTNB at 37 °C; 0.1 mM-chloramphenicol was added to portions of the mixture at 2, 4 and 6 min to start the CAT reaction. R901 CAT was also tested for inactivation by the acetyl-CoA component of the reaction mixture after it became apparent that this enzyme was being inactivated in the absence of DTNB.

The percentage reduction in CAT activity after incubation in DTNB for each specified time was calculated. The values were normalized to take into account any reduction in enzyme activity in the control sample (without DTNB) after the same incubation period.

Immunological studies. Crude antisera raised against purified CAT variants were used for all immunological experiments. The type I directed reagent was obtained from a goat immunized with electrophoretically pure type I enzyme specified by plasmid JR66b. This CAT variant has been the subject of protein chemistry studies and amino acid sequence analysis (Zaidenzaig & Shaw, 1978), and the R429-specified CAT has an identical peptide fingerprint and amino acid composition (W. V. Shaw, unpublished data). The antiserum specific for the type III variants of CAT was raised in a rabbit following immunization with pure CAT specified by plasmid R387. Double diffusion tests were performed in 1 % Difco Noble agar prepared in buffer 1. The purified CAT preparations (0.2 units) were tested for precipitin band formation with the antisera after incubation at 4 °C for 48 h.

The ability of the antisera to inhibit CAT activity was also tested. The concentrations of the crude lysates were adjusted to the same CAT activity (about 2.4 units ml⁻¹) and to the same protein concentration (3.7 mg ml⁻¹) by adding a combination of buffer 1 and lysate of the Cm^r R⁻ bacterial strains. A 25 µl sample of CAT was incubated with 5 to 25 µl of the antiserum, the concentration of which had been adjusted to give a gradation of inhibition with the homologous CAT over this range. Tris/HCl buffer (0.05 M, pH 7.8) was added to give a final volume of 50 µl. After incubation at 4 °C for 18 h the samples were assayed for CAT activity.

Electrophoretic mobilities. Purified enzyme preparations were subjected to agarose gel electrophoresis to ascertain the purity of the material and to measure the electrophoretic mobility of the enzyme proteins. About 20 µg of the purified proteins were applied to Universal agarose films (Corning ACI, Palo Alto, Calif., U.S.A.) or 1 % (w/v) agarose slab gels. The electrophoresis was performed at 80 V for 1.5 h in 0.05 M-sodium barbitol buffer pH 8.6. Protein was stained with naphthalene black [0.123 % (w/v) in 5 % (v/v) acetic acid] and destained with 5 % (v/v) acetic acid washes. CAT activity was located on the gels using the histochemical staining method described by Foster & Shaw (1973).

RESULTS

The mechanism of resistance to chloramphenicol specified by 18 Gram-negative bacterial R plasmids (Table 1) representing a variety of incompatibility groups was examined. Plasmids R429, s-a and R387 which were previously shown to determine types I, II and III CAT, respectively, (Foster & Shaw, 1973; Shaw *et al.*, 1972) were included for comparison. The level of resistance to chloramphenicol determined by the plasmids was measured and crude lysates of the strains were tested for the presence of CAT. In order to determine if the CAT enzymes corresponded to the three types described by Foster & Shaw (1973), the enzymes were purified and the following properties were examined: (i) the electrophoretic mobility of the proteins in agarose gels; (ii) the reaction of the enzyme with antisera prepared against

Table 2. *Comparison of the properties of chloramphenicol acetyltransferases*

Plasmid*	Chloramphenicol resistance level ($\mu\text{g ml}^{-1}$)	Type of CAT	Electrophoretic mobility at pH 8.6†	Precipitation by antisera‡		Inhibition by antisera§		Rate of inhibition by DTNB (k)
				Type I	Type III	Type I	Type III	
R471b	250	I	0.89	+	—	0.070	0.009	0.046
R478	200	I	0.92	+	—	0.124	0.005	0.023
(R429)	350	I	1.00	+	—	(0.054)	0.000	0.021
R16213	275	I	1.00	+	—	0.049	0.005	0.005
R55	300	I	1.00	+	—	0.059	0.000	0.013
R57b	300	I	1.00	+	—	0.076	0.005	0.000
R726	125	I	1.03	+	—	0.059	0.005	0.000
CS Col	300	I	1.02	+	—	0.059	0.005	0.048
R724	250	I	1.03	+	—	0.065	0.000	0.002
R71a	325	I	1.03	+	—	0.070	0.009	0.000
R901	75	II	1.44	—	—	0.022	0.000	0.133
(s-a)	150	II	1.46	—	—	0.022	0.009	0.074
R753	125	II	1.57	—	—	0.000	0.000	0.353
(R387)	200	III	1.20	—	+	0.043	(0.046)	0.035
R994	150	III	1.20	—	+	0.038	0.041	0.039
R621ala	150	III	1.20	—	+	0.043	0.041	0.027
R ⁻ host strains								
J53	4	None						
J53 Nal ^r	4	None						

* Plasmids R471b, R478, R429, R57b, R753, R387 and R621ala were harboured by the *E. coli* strain J53; the other plasmids were carried by a Nal^r mutant of J53. The plasmid numbers in parentheses denote the producers of the standard type I, type II and type III chloramphenicol acetyltransferases.

† Electrophoretic mobilities are expressed relative to that of the R429-determined enzyme.

‡ +, An unequivocal immunoprecipitate which gave a line of identity with the precipitate of the standard enzyme and antiserum. —, No immunoprecipitate.

§ Each enzyme was titrated with both of the antisera as described in Methods. The activity remaining after 18 h incubation at 4 °C was determined and the stoichiometry of the neutralization reaction was determined from the slope of the titration curve. The values obtained for the prototype enzymes against their respective antisera are shown in parentheses. The dimensions for the neutralization are units CAT activity neutralized per μl antiserum.

|| The rate of inactivation of each enzyme in the presence of DTNB was calculated graphically from the equation $\ln E/E_0 = -kt$, where E/E_0 is the fraction of the initial enzyme activity remaining after treatment with the inhibitor for time t (Zaidenzaig & Shaw, 1978).

type I and type III CAT; (iii) the loss of enzyme activity following incubation with 5,5'-dithiobis(2-nitrobenzoic acid).

Chloramphenicol resistance levels and the production of chloramphenicol acetyltransferase

The level of resistance to chloramphenicol specified by the plasmids in the *E. coli* K12 host strain J53 and the CAT activity in crude lysates were measured (Tables 2 and 3). With the exception of R726, the strains that were subsequently shown to produce type I CAT exhibited the highest chloramphenicol resistance levels (200 to 350 $\mu\text{g ml}^{-1}$). The three strains that produced type III CAT had intermediate levels of resistance (150 to 200 $\mu\text{g ml}^{-1}$), and those that formed type II CAT had the lowest resistance levels (75 to 150 $\mu\text{g ml}^{-1}$).

Five Cm^r strains were devoid of CAT activity, i.e. less than 0.0005 units activity per mg cell protein. (About 1.5 units of type I CAT per mg protein would be required to give a chloramphenicol resistance level of 100 $\mu\text{g ml}^{-1}$.) Furthermore, these strains exhibited a higher resistance level when grown in the presence of 1 μg chloramphenicol ml^{-1} , which suggests that the Cm^r determinant was inducible (Table 3). This has subsequently been confirmed by broth growth-and-challenge tests (D. F. Gaffney & T. J. Foster, unpublished experiments). Two of the plasmids, R55-1 and R57b-1, are segregants of plasmids R55 and R57b which determine type I CAT. These plasmids must therefore carry two determinants of chloramphenicol resistance.

Table 3. *Chloramphenicol resistance levels conferred by plasmids which do not specify chloramphenicol acetyltransferase*

Plasmid*	Chloramphenicol resistance level ($\mu\text{g ml}^{-1}$)	
	Uninduced	Induced†
R ⁻	4	4
R26	15	50
R527	15	50
R55-1	100	150
R57b-1	100	150
RA1-1b	25	100

* Plasmids were harboured by the NaI^r mutant derivative of the *E. coli* strain 153.

† Cultures were induced by growth in broth containing 1 μg chloramphenicol ml⁻¹.

Electrophoretic mobilities

The prototype CAT variants identified by Foster & Shaw (1973) had characteristic electrophoretic mobilities in polyacrylamide gels. The purified CAT proteins were therefore compared by agarose gel electrophoresis (Table 2). The putative type I CAT proteins had the lowest anodic electrophoretic mobilities, in accordance with previous findings (Foster & Shaw, 1973). However, very slight but reproducible differences in migration necessitated subdivision of the group. Thus R471b and R478 determined the slowest moving enzymes, having electrophoretic mobilities relative to the R429 enzyme of 0.89 and 0.92, respectively. The CAT proteins specified by R16213, R57b and R55 migrated at the same rate as the prototype type I enzyme specified by R429. The fastest type I CAT migration was shown by the enzymes directed by R726, R724, R71a and CS Col, with mobilities relative to R429 CAT of 1.02 to 1.03.

The putative type II CAT variants determined by R753, s-a and R901 showed the fastest anodic electrophoretic mobilities, although small differences in relative mobilities were noted. Thus the rate of migration towards the anode for R753 CAT was greater than that for s-a CAT, which in turn migrated marginally faster than R901 CAT. Their mobilities relative to that of the R429 enzyme were 1.57, 1.46 and 1.44, respectively.

The type III CAT variants determined by R621ala, R994 and R387 had identical mobilities between the type I and type II CAT values. Their mobilities relative to R429 CAT were 1.20.

The observed electrophoretic mobilities of the type II and type III CAT enzymes differed from those reported by Foster & Shaw (1973) who showed that R387 (type III) CAT migrated faster than the type II CAT variants specified by s-a, RA3 and RA4. Thus the electrophoretic mobility of type II CAT was originally reported to lie between that of the type I and type III enzymes. This discrepancy can only be attributed to the different electrophoretic techniques employed in the two studies. To confirm this, crude lysates of strains producing the previously identified RA3 and RA4 type II CAT were subjected to agarose gel electrophoresis. These CAT bands, identified *in situ* with a histochemical stain, were found to migrate at the same fast rate as s-a CAT.

Reactions with anti-chloramphenicol acetyltransferase sera

The ability of antisera against a type I CAT and a type III CAT to form precipitin bands in Ouchterlony gels and to inhibit the catalytic activity of the enzymes was determined. The results are summarized in Table 2.

The putative type I CAT enzymes were rapidly inactivated by the anti-I serum but were unaffected in tests with the anti-III serum. They formed precipitin bands which gave reactions of identity with the immunoprecipitate formed by the serum and the prototype

CAT specified by R429. No reaction occurred between any of the type I CAT enzymes and the anti-III serum.

The catalytic activity of the R387, R994 and R621ala CAT variants was rapidly lost after incubation with the anti-III serum. These enzymes were also inhibited by the anti-I serum, but less markedly than the type I CATs. This effect is characteristic of the standard type III R387 CAT and suggests that some surface antigenic determinants may be shared by the type I and type III enzymes. In addition, the R994 and R621ala CAT proteins formed immunoprecipitates with the anti-III serum but none were formed in tests with the anti-I serum. R994 formed two precipitin bands with the anti-III serum, one of which gave a cross-reaction of partial identity with the single R387 and R621ala immunoprecipitates. The second band may represent a contaminating protein which was common to the R387 preparation used to raise the serum and the R994 CAT prepared in this study. It is noteworthy that the R994 CAT preparation exhibited two protein bands on agarose gel electrophoresis; the faster moving band was devoid of catalytic activity as shown by the histochemical staining method.

The catalytic activity of the putative type II CAT variants was not affected by the anti-III CAT serum. However, each of the three enzymes behaved differently in tests with the anti-I serum. Thus, R753 CAT activity was unaffected by the anti-I serum whereas the R901 and s-a enzymes were slightly inactivated. None of the type II CAT preparations reacted significantly with either the anti-I or the anti-III CAT serum in double diffusion tests.

Inhibition by 5,5'-dithiobis(2-nitrobenzoic acid)

The CAT enzymes which were classified as type II by Foster & Shaw (1973) were unusual in that their catalytic activity was rapidly lost during pre-incubation with DTNB, a thiol reagent used in the CAT enzyme assay. The DTNB sensitivity of the 13 new CAT variants and the standard type I, II and III enzymes was therefore examined. Some of the type I CAT enzymes and each of the type III variants were slightly inhibited by DTNB (Table 2). The type II enzymes (specified by plasmids R753, R901 and s-a) were rapidly inactivated. Preliminary experiments with R901 CAT indicated that this enzyme was being inactivated in the control sample containing reaction mixture without DTNB. It was demonstrated that R901 CAT was inactivated by pre-incubation with either the acetyl-CoA or the DTNB component of the reaction mixture but was not affected by incubation at 37 °C in buffer alone. The acetyl-CoA effect has also been observed in another context. Zaidenzaig & Shaw (1978) have shown that at least one type I variant undergoes a very slow inactivation in the presence of acetyl-CoA, although the rate constant for this process is very much lower than that observed for the R901 enzyme. The augmentation of the acetyl-CoA effect observed in the latter case may ultimately prove to be of importance in studies of the mechanism of enzymic chloramphenicol acetylation.

DISCUSSION

The results presented in this paper demonstrate that the most common mechanism of chloramphenicol resistance determined by plasmids from different incompatibility groups is the inactivation of the drug by CAT. Two IncP plasmids and segregants derived from the CAT-producing IncC plasmids R55 and R57b exhibited a lower level of chloramphenicol resistance which was inducible and did not involve CAT. Plasmids R57b and R55 must therefore carry two Cm^r determinants since they also specify the production of type I CAT. It is possible that the mechanism of chloramphenicol resistance in these strains without detectable CAT is a barrier to the uptake of the drug as described for some other non-CAT-producing Cm^r strains by Nagai & Mitsuhashi (1972). This is currently being investigated.

The CAT variants specified by R471b, R478, R55, R57b, R16213, R724, R71a, CS Col and R726 were assigned to type I. Each enzyme was inactivated by anti-type I CAT serum with the formation of an immunoprecipitate which gave a reaction of identity with that

obtained in the standard type I CAT antigen-antibody system. Furthermore, each enzyme had a slow electrophoretic mobility and most were insensitive to DTNB. The electrophoretic mobilities of some of the enzymes differed slightly from the prototype R429 CAT. In addition, some of the purified type I enzymes were slightly inactivated by pre-incubation with DTNB. This suggests that type I CAT variants from different sources are not absolutely identical. The heterogeneity could be due to single amino acid changes which do not alter either catalytic activity or the majority of the surface antigenic determinants. The specific details of the interaction of DTNB with the type I variants of CAT have been studied by Zaidenzaig & Shaw (1978). The four half-cysteine residues present in the CAT monomer vary in their reactivity towards DTNB and other anti-thiol reagents.

The putative type III CAT variants specified by R994 and R621ala were closely related to R387 CAT. They were rapidly inactivated by antiserum to R387 CAT and also gave reactions of identity in agar double diffusion tests with the R387 CAT and its antiserum. Each CAT was also partially inactivated by anti-type I CAT serum but failed to form visible immunoprecipitates in Ouchterlony double diffusion tests. This could indicate that the type III CAT enzymes share some surface antigenic determinants with the type I enzyme. An evolutionary relationship between the type I and type III CAT variants is also suggested by the fact that the subunits of the two types of enzymes can form hybrids *in vivo* and *in vitro* (Shaw *et al.*, 1972).

The R753 and R901 CAT enzymes were assigned to CAT class II primarily on the basis of their sensitivity to DTNB, a characteristic of the s-a, RA3 and RA4 specified enzymes described by Foster & Shaw (1973). However, this CAT group is probably heterogeneous, as each of the putative type II enzymes differed in a number of other properties. The enzymes determined by R901 and s-a were partially inactivated by anti-type I CAT serum, whereas the R753-specified CAT was unaffected. The R901 enzyme differed from the other two in that it was inactivated by pre-incubation with acetyl-CoA as well as DTNB. The variation in electrophoretic mobility of the enzymes also indicates heterogeneity within the type II group.

Type I CAT is specified by plasmids from incompatibility groups FII, C, S, I, H, L, O and Com9 suggesting that the same ancestral Cm^r determinant may have been disseminated to different bacterial hosts by a transposition mechanism similar to that proposed for the TEM β -lactamase gene (Hedges *et al.*, 1974). Indeed a type I CAT determinant, termed Tn9, is known to be transposable (Gottesman & Rosner, 1975). However, Tn9 does not exist as such on R plasmids but was constructed from a F-like R plasmid by P1 transduction. Iida & Arber (1977) have suggested that the entire resistance-determinant region of R100 was transposed on to the P1 phage genome to yield a P1CmSmSu recombinant. This was too large to be accommodated in the phage head and the selection of plaque-forming derivatives which retained chloramphenicol resistance resulted from deletion of the Sm^r and Su^r genes to yield the P1Cm phage. This may explain the failure of attempts to demonstrate the transposability of the Cm^r determinants carried by the type I CAT-determining plasmids by the method of Shapiro & Sporn (1977) (T. J. Foster, unpublished data).

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