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MGG

cir, a Gene Conferring Resistance to Colicin I Maps Between *mgl* and *fpk* on the *Escherichia coli* Chromosome

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Summary. With the help of the tetracycline resistance transposon Tn*10* in and around the *mgl* genes the gene *cir* was mapped. *cir* is 80% cotransducible with *mgl* by P1 transduction. The sequence of the surrounding markers in clockwise order was established as: *cdd fpk cir mgl gyrA*. The direction of transcription in *cir* was determined as clockwise on the *Escherichia coli* chromosome. The gene product of *cir*, an outer membrane receptor for colicin I, is not part of the *mgl* operon. It is not regulated by D-fucose, the inducer of the *mgl* system and mutants defective in *cir* are unimpaired in the uptake of substrates of the *mgl*-dependent transport system.

Introduction

The *mgl* genes code for the galactose-binding protein (GBP) dependent transport system (Boos and Sarvas 1970). They are located at 45 min on the *E. coli* linkage map (Boos et al. 1981). This area of the map is not very well characterized (Bachmann and Low 1980). It is flanked by the *his* marker at 44 and *gyrA* at 48 min.

The isolation of insertions of the tetracycline resistance transposon next to *mgl* and *gatA* allowed the ordering of *his cdd fpk ptsF mgl gatA* in clockwise order (Boos et al. 1981). However, none of the Tn*10* insertions determined the linkage to *gyrA*, the next well established marker on the chromosome. Recently, we isolated Tn*10* insertions around *glpT*, another gene that is located next and clockwise to *gyrA*. One of these Tn*10*'s (*zeg-722*) was found to cotransduce with *mgl* (Ludtke et al. 1982). This opened the possibility of spanning the entire area between *cdd* and *gyrA* by cotransducing markers. In addition, well described mutants in *cir*, a gene coding for the colicin I receptor (Bowles et al. 1983), became available recently (Worsham and Konisky 1981; Hantke 1981). These mutants are supposedly also located in the *his-gyrA* region (Cardelli and Konisky 1974). Here we report the mapping of *cir* and the connection of the *cdd fpk cir mgl* sequence to *gyrA*. The availability of Mud (Ap *lac*)::*cir* insertions allowed the isolation of λ phages carrying *cir*::*lac* fusions. Using these phages the direction of transcription of *cir* could be determined.

Materials and Methods

Bacterial strains are listed in Table 1. They were constructed by P1 transduction according to standard procedures (Miller 1972).

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To determine *cir* by P1-mediated cotransduction we used nearby Tn*10* insertions (Boos et al. 1981; Ludtke et al. 1982) as selective markers. Selection was done on NB agar plates containing 5 μg/ml tetracycline and 20 mM citrate. The transductants were screened on agar plates containing 20 μg/ml tetracycline and 20 mM citrate. Screening for *cir-lac* was done on McConkey lactose; for *cir* by cross streaking against colicin Ia on NB plates; for *fpk* on minimal plates containing 0.2% glycerol and 1 mM fructose (Ferenci and Kornberg 1974); for *cdd* on minimal plates containing 0.4% cytidine (Tabor et al. 1980); for *gyrA* on NB plates containing 20 μg/ml nalidixic acid. Colicin Ia was prepared (Konisky 1971) from the colicinogenic strain JK16 (Cardelli and Konisky 1974) after growth in LB and 0.5 μg/ml mitomycin C. The cells were harvested and broken by passing through a French pressure cell. The cell free extract of a one liter culture contained in 30 ml 100 mM Tris-HCl, pH 7.0 and was used without further purification. The *cir*::Mud (Ap *lac*) fusion strain H1300 was obtained from K. Hantke (1981). The fusion was transferred onto λ using phage λp1 (209) (Casadaban 1976) by the procedure of Komeda and Iino (1979). λp *cir-lac* phages were plaque purified. Lysogens were obtained in MC4100 and their position in *cir* verified by cotransduction to *mgl-500*::Tn*10*. Introduction of a spontaneous mutation in *cir* was done by selection for resistance against colicin Ia (cross-streaking on NB plates). *lacY*::Tn*9* was introduced by P1 transduction using NK5579 as donor and chloramphenicol resistance as selection. Again the position of *cir* and *lacY*::Tn*9* was verified by P1 transduction with *mgl*-500::Tn*10*. Also, the presence of the lysogen was verified by cross-streaking against λcIh*80* and λ*vir*. In the crosses involving λ lysogens as donors as well as recipients (Table 3, Fig. 3 and 4) about 10% of the transductants had lost the fusion phage. These were disregarded in the transductional analysis. Also, about 8% of the transductants appeared Lac^+, even through they were still chloramphenicol resistant. These can only be the result of a Tn*9* transposition during the cross; they were counted as Lac^+ in the transductional analysis.

Isolation of outer membrane proteins was done according to Hantke (1981) after growth in LB in the presence and absence of 120 μg/ml α, α′ dipyridyl. Polyacrylamide gel electrophoresis was done as described by Lämmli (1970).

(2R)-glycerol-β-D-galactoside (Boos 1982) was used to select for mutations in *mgl* as described (Silhavy and Boos 1974). Mutagenesis by Tn*10* insertion was done with phage λNK55 (Kleckner et al. 1978). *mgl*-dependent transport activity of galactose was done as described (Parnes and Boos 1973).

Results and Discussion

Isolation of a Tn10 Insertion in mgl

(2R)-glycerol-β-D-galactoside (RGG) in a strain lacking the lactose permease (*lacY*) is exclusively transported via the *mgl*-dependent transport system. Once inside RGG is split

Table 1. Bacterial strains

Strain	Parent	Known phenotype	Reference
MC4100		F⁻ *araD139 Δ(argF-lac)U169 rpsL1150 flbB5301 deoC1 ptsF25 relA*	Casadaban 1976
AT2243.11^c.25	HfrC	Hfr *metB fpk pyrE uhp*^{c}	Ferenci and Kornberg 1974
H1300		*Δ(proAB-lac) aroB malT tsx thi cir*::Mud (Ap *lac*)	Hantke 1981
Sϕ1324	MC4100	*cdd cod*; otherwise as MC4100	A. Münch-Petersen
TL45	MC4100	*Δ(glpT-glpA)593 glpR gyrA*	Ludtke et al. 1982
AW550		F⁻ *thr1 leuB6 lacY1 phoA14 rpsL114 malA1 metB1 his gatA λ mglB550*	Haselbauer and Adler 1971
DL4	MC4100	*glpR zeg-722*::Tn*10 gyrA*	Ludtke et al. 1982
NK5587		F⁻ *Δ(lac-pro) thiA rha trkA trkB*/F′*lacZ*⁺ *lacY*::Tn*9*	N. Kleckner
LA5455	KL99 × M188-444	F⁻ *lacY galE arg ptsF*	Silhavy and Boos 1974
LA5539	LA5531 × LA5455	*zee-700*::Tn*10 fpk ptsF*⁺; otherwise as LA5455	This study
LA5606	LA5455 × λNK55	*mgl-500*::Tn*10*; otherwise as LA5455	This study
LA5641	LA5606 × TL45	*mgl-500*::Tn*10*; otherwise as TL45	This study
LA5531	AT2243.11^c.25	*zee-700*::Tn*10*; otherwise as AT2243.11^c.25	Boos et al. 1981
LA5642	LA5564 × TL45	*zef-704*::Tn*10*; otherwise as TL45	Boos et al. 1981
LA5645	LA5642 × AT2243.11^c.25	*zeg-722*::Tn*10*; otherwise as AT2243.11^c.25	This study
LA5644	LA5641 × AT2243.11^c.25	*mgl-500*::Tn*10*; otherwise as AT2243.11^c.25	This study
LA5651	DL4 × AW550	*zeg-722*::Tn*10 gyrA*; otherwise as AW550	This study
LA5652	HS203 × H1300	*malP,Q*::Tn*10 malT*⁺; otherwise as H1300	This study
LA5657	λp1 209 × LA5652	LA5652 ϕ (*cir-lacZ*)	This study
LA5461	MC4100	MC4100 *λp cir′lacZ*	This study
LA5466	LA5644 × LA5461	*mgl-500*::Tn*10*; otherwise as LA5461	This study
LA5473	LA5531 × LA5461	*zee-700*::Tn*10 fpk*; otherwise as LA5461	This study
LA5469	LA5461	*cir λpcir′lacZ lacY*::Tn*10*; otherwise as LA5461	This study
LA5471	LA5644	*mgl-500*::Tn*10*; otherwise as H1300	This study
LA5472	× H1300	*mgl-500*::Tn*10 cir*⁺; otherwise as H1300	

by β-galactosidase to glycerol and galactose. In a *galE* background the accumulating galactose is toxic. This is the basis of a selection procedure for *mgl* mutants (Silhavy and Boos 1974). Using this technique we isolated random insertions of the tetracycline resistance transposon Tn*10* (Kleckner et al. 1977) and screened for resistance against RGG. Strain LA5606 carries Tn*10* in *mgl*. P1 lysates grown on LA5606 transduce *mgl* 100% with the tetracycline resistance. The *mgl* operon consists of at least three genes (Rotman and Guzman 1982; Harayama et al. 1983) one of which (*mglB*) codes for the galactose-binding protein (GBP). Since Tn*10* insertions appear to be strongly polar and LA5606 still produces GBP normally the Tn*10* is inserted distal to *mglB* in the *mgl* operon.

Other Tn*10* insertions in the 45 min region were known previously. *zeg-722*::Tn*10* was isolated as a random insertion cotransducing *gyrA* and *glpT* (Ludtke et al. 1982), *zef-704*::Tn*10* as cotransducing *mgl* and *gatA*, and *zee-700*::Tn*10* as cotransducing *mgl* and *fpk* (Boos et al. 1981). The strains used and their construction are summarized in Table 1.

Mapping of Mud (Ap lac) Insertions in cir

The four Tn*10* insertions in and around the *mgl* region were used to define the location of a Mud (Ap *lac*) insertion in *cir* that had been isolated and characterized as colicin Ia resistant by K. Hantke (1981). Figure 1 shows the gel electrophoretic analysis of outer membrane proteins of an *mgl*::Tn*10* derivative (LA5471) and the isogenic *cir*⁺ strain LA5472 grown at high and low iron concentrations. The latter condition is known to derepress the synthesis of the colicin I receptor (Konisky et al. 1976). As can be seen, strain LA5471 lacks a protein that has previously been identified as the colicin I receptor (Hantke 1981), whereas its *cir*⁺ derivative synthesizes it in an iron concentration-dependent manner.

To map the *cir*::Mud (Ap *lac*) insertion we used the Tn*10* insertions of the different strains as P1 donors. Selection was always for tetracycline resistance followed by screening for the nearby markers. Where possible, three factor crosses were done. The results are shown in Table 2. Our conclusion from these results for the sequence of

Table 2. P1 cotransduction frequencies of Tn*10* insertion in the *gyrA-his* region with nearby markers

	Relevant genotype		Unselected marker		Percentage of total recombinants	
	Donor	Recipient				
1	LA5531 *zee-700*::Tn*10 fpk*	H1300, *cir*	*fpk* *fpk* *fpk*$^+$ *fpk*$^+$	*cir* *cir*$^+$ *cir*$^+$ *cir*	48.7 31.3 11.7 8.1	(120/246) (77/246) (29/246) (20/246)
2	LA5641 *mgl*::Tn*10 gyrA*	AT2243.11°.25 *fpk*	fpk *fpk*$^+$ 	*gyrA*$^+$ *gyrA*$^+$ *gyrA*	52 48 0	(49/96) (46/96) (0/96)
3	LA5641 *mgl*::Tn*10*	H1300, *cir*	*cir*$^+$		77.5	(90/116)
4	LA5644 *mgl*::Tn*10 fpk*	H1300, *cir*	*cir*$^+$ *cir*$^+$ *cir* *cir*	*fpk*$^+$ *fpk* *fpk*$^+$ *fpk*	50 27.5 22.5 0	(40/80) (22/80) (18/80) (0/80)
5	LA5640 *mgl*::Tn*10 fpk*	Sφ1324, *cdd*	*fpk*$^+$ *fpk* *fpk* *fpk*$^+$	*cdd* *cdd* *cdd*$^+$ *cdd*$^+$	66,6 30 0.3 0	(40/60) (18/60) (2/60) (0/60)
6	LA5645 *zef-704*::Tn*10* *fpk*	H1300, *cir*	*cir* *cir*$^+$ *cir*$^+$ *cir*	*fpk*$^+$ *fpk* *fpk*$^+$ *fpk*	94.6 2.6 2.6 0	(142/150) (4/150) (4/150) (0/150)
7	LA5645 *zef-704*::Tn*10* *fpk*	AW550, *mglB*	*mglB* *mgl*$^+$ *mgl*$^+$ *mglB*	*fpk*$^+$ *fpk*$^+$ *fpk* *fpk*	92.6 5.3 2.0 0	(139/150) (8/150) (3/150) (0/150)
8	LA5642 *zef-704*::Tn*10* *gyrA*	AT2243.11°.25, *fpk*	*fpk* *fpk*$^+$ 	*gyrA*$^+$ *gyrA*$^+$ *gyrA*	93.3 6.6 0	(70/75) (5/75) (0/75)
9	LA5651 *zeg-722*::Tn*10* *gyrA*	H1300, *cir*	*cir* *cir* *cir*$^+$ *cir*$^+$	*gyrA*$^+$ *gyrA* *gyrA*$^+$ *gyrA*	84 8.5 7.4 0	(79/94) (8/94) (7/94) (0/94)
10	DL4 *zeg-722*::Tn*10* *gyrA*	AW550, *mglB*	*mglB* *mglB* *mgl*$^+$ *mgl*$^+$	*gyrA*$^+$ *gyrA* *gyrA*$^+$ *gyrA*	78.3 18 3.6 0	(235/300) (54/300) (11/300) (0/300)
11	DL4 *zeg-722*::Tn*10* *gyrA*	AT2243.11°.25, *fpk*	*fpk* *fpk* *fpk*$^+$	*gyrA*$^+$ *gyrA*	70.8 29.2 0	(68/96) (28/96) (0/96)
12	DL4 *zeg-722*::Tn*10* *gyrA*	Sφ1324, *cdd*	*gyrA*$^+$ *gyrA* *cdd*$^+$	*cdd* *cdd*	83.8 16.2 0	(72/86) (14/86) (0/86)

Selected phenotype was always Tet

markers (Fig. 2) is in clockwise order: *cdd fpk zee-700*::Tn*10 cir mgl zef-704*::Tn*10 zeg-722*::Tn*10 gyrA*. This conclusion is primarily based on cross 4 that places *mgl* outside *cir-fpk*, closer to *cir* than to *fpk*. The clockwise or counterclockwise order of the *mgl cir fpk* sequence on the chromosome is given by looking at the cotransduction range of *zeg-722*::Tn*10*. This Tn*10* insertion is located between *mgl* and *gyrA* (cross No. 10), can still cotransduce *cir* and *mgl* (cross No. 9), but not *fpk* (No. 11) or *cdd* (No. 12).

One irregularity that we came across during these studies was the observation that strains carrying the *fpk* marker originating from strain AT2243.11°.25 were growing very poorly on glucose. The phenotype of *fpk* mutants is sensitiv-

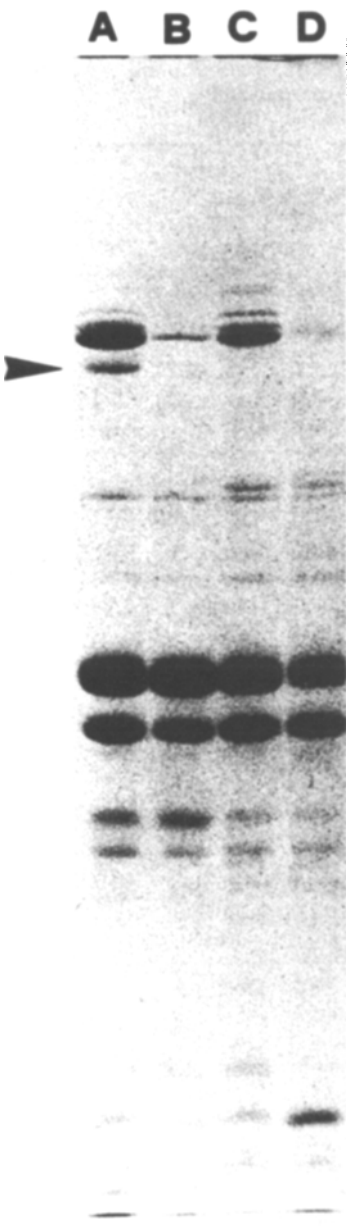

Fig. 1. SDS polyacrylamide gel electrophoresis of outer membrane proteins. Strains were grown in LB in the presence (A, C) and absence (B, D) of α, α' dipyridyl. A, B strain LA5472 (*cir*$^+$); C, D LA5471 (*cir'-lac*). Outer membranes were isolated as described by Hantke (1981). The arrow indicates the position of the *cir* gene product, the colicin I receptor

ity towards fructose due to the absence of fructose-1-phosphate kinase (Ferenci and Kornberg 1974). 'Revertants' can easily be isolated that are insensitive to fructose. They can be located in *ptsF* shutting off the transport of fructose (Ferenci and Kornberg 1974). These fructose resistant 'revertants' still have problems in growing on glucose. Thus, we believe the *fpk* marker in AT2243.11^c.25 is the result of a mutation effecting more than *fpk*. Possibly, it is a deletion starting in *fpk* and extending towards *cdd*. This may reduce the apparent distance of *zee-700*::Tn*10* to *his* or *cdd* when donors are used that were grown on a background carrying the *fpk* marker of AT2243.11^c.25.

We selected spontaneous mutations in strain LA5641 (*mgl-500*::Tn*10*) that became resistant to colicin Ia. To map these mutations we crossed them by P1 transduction into strain AT2243.11^c.25 (carrying the *fpk* mutation). Three of the mutations were located between *mgl* and *fpk* and are likely to be in *cir* while two were not cotransducible with the Tn*10* insertion and must be located outside the region. Mutations exhibiting a phenotype of colicin I tolerance have been reported and found to be located outside *cir* (Cardelli and Konisky 1974).

The Direction of Transcription in Cir

To determine the direction of transcription in *cir* relative to the nearby markers *mgl* and *fpk* we used the strategy employed previously by Eckhardt (1977) for analyzing the *argA* gene. Briefly, the *cir-lac* fusion is transferred onto λ (Komeda and Iino 1979). This transducing phage was then used to lysogenize a wild-type strain. Since this phage lacks the attachment site, it integrates primarily via *cir* homology which was verified by mapping against a nearby marker. The direction of integration depends on the orientation of *cir*. The two possible orientations are shown in Figs. 3A versus 3B and 4A versus 4B, respectively. To determine the order of genes in the lysogen, selectable markers were introduced and their position established by P1 transduction. Into the donor we introduced *mgl-500*::Tn*10*, into the recipient we introduced a spontaneous *cir* mutation (selection for colicin Ia resistance (Col^R)) as well as a *lacY*::Tn*9* mutation (Lac$^-$). The selection was for Tet. The results are given in Table 3. As can be seen

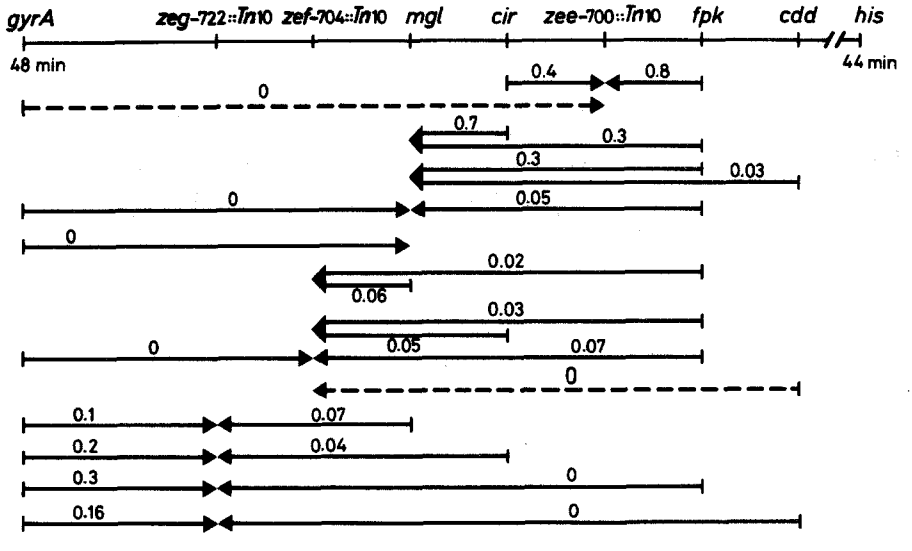

Fig. 2. Linkage of *cir* to nearby markers by P1 transduction. Cotransduction frequencies are expressed as fractions of 1 (=100%). The position of the arrow indicates the selected marker. Double arrows represent data from the same cross (three factor crosses). Dashed lines are taken from previously published data (Boos et al. 1981). Distances are not to scale

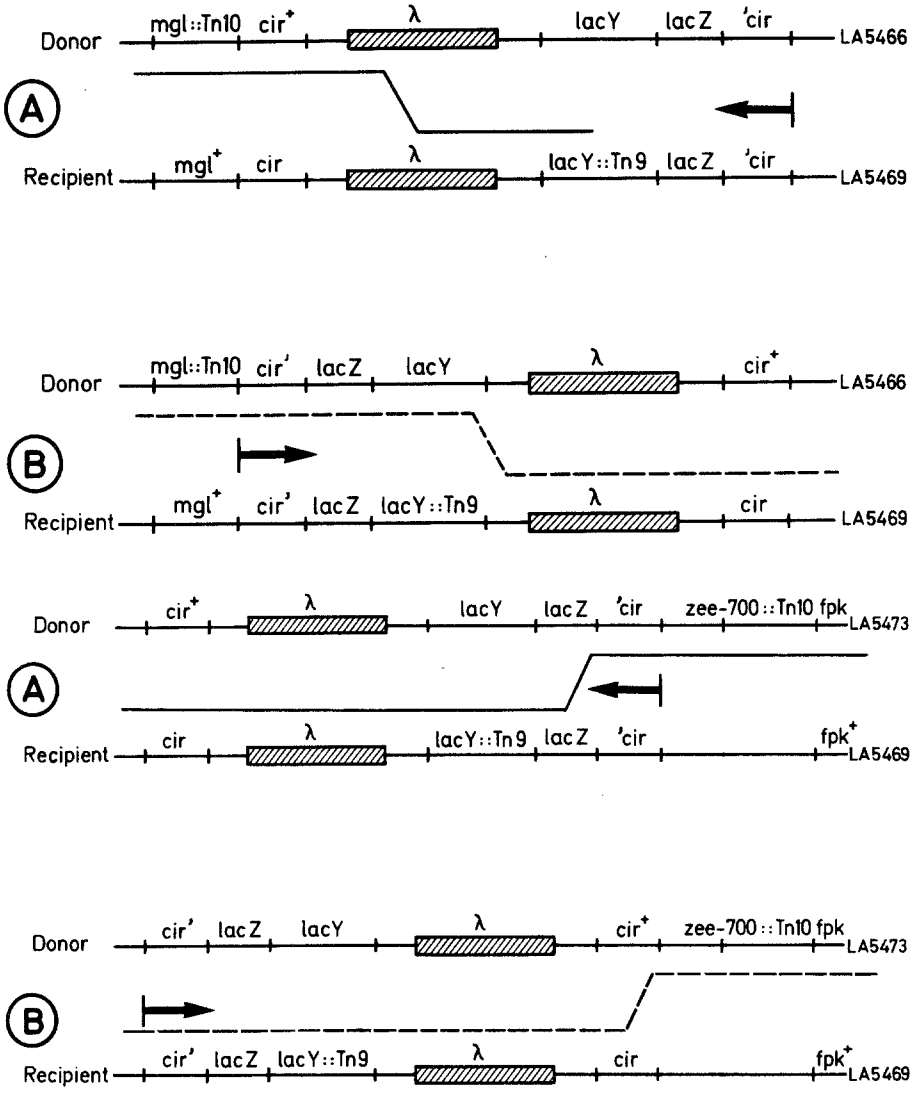

Fig. 3. Direction of transcription of *cir* as determined by P1 transduction of strains carrying *cir-lac* fusions. Depending on the direction of transcription in *cir*, *cir-lac* fusions carried on λ integrate by homology as in A or B. Markers were introduced into the donor (*mgl*::Tn*10*) and the recipient (*cir* and *lacY*::Tn*9*). After P1 transduction Tet resistance was selected and the order of markers determined. From the data given in Table 3 the alternative depicted in A was found to be the correct one

Fig. 4. Direction of transcription of *cir* as determined by P1 transduction of strains carrying *cir-lac* fusions. Depending on the direction of transcription in *cir*, *cir-lac* fusions carried on λ integrate by homology as in A or B. Markers were introduced into the donor (*zee-700*::Tn*10*) and the recipient (*cir* and *lacY*::Tn*9*). After P1 transduction Tet resistance was selected and the order of markers determined. From the data given in Table 3 the alternative depicted in A was found to be the correct one

Table 3. Determination of direction of transcription in *cir* by P1 transduction of *λpcir'lac* lysogens

Donor	Recipient	Unselected marker	Percentage of total recombinants
1 LA5466, *mgl*::Tn*10 cir*$^+$ *λpcir'lac*	LA5469, *cir λpcir'lacY*::Tn*9*	Col^S Lac$^-$	59 (96/162)
		Col^S Lac$^+$	13 (22/162)
		Col^R Lac$^-$	24 (40/162)
		Col^R Lac$^+$	2 (4/162)
2 LA5473, *zee-700*::Tn*10 fpk λpcir'lac cir*$^+$	LA5469, *cir λpcir'lacY*::Tn*9*	Col^R Lac$^-$ Fpk$^-$	61 (102/168)
		Col^R Lac$^-$ Fpk$^+$	20 (34/168)
		Col^R Lac$^+$ Fpk$^-$	8 (14/168)
		Col^R Lac$^+$ Fpk$^+$	9 (15/168)
		Col^S Lac$^-$ Fpk$^+$	0.5 (1/168)
		Col^S Lac$^+$ Fpk$^+$	0.5 (1/168)
		Col^S Lac$^+$ Fpk$^-$	0.5 (1/168)

Selection was for Tet

(Fig. 3) the combination Col^S Lac$^-$ was more frequent than Col^R Lac$^+$, indicating *mgl*::Tn*10-cir*$^+$*-λ-lac'cir*. In addition, among the Col^S transductants the majority were Lac$^-$, again supporting the above sequence. This demonstrates that of the two possibilities depicted in Fig. 3, A is the correct one and the direction of transcription of *cir* is from right to left or toward *mgl*. As a control, the experiment was repeated with *zee-700*::Tn*10* and *fpk* as markers in the donor strain. The two possible orientations are shown in Fig. 4. The results of the cross are given in Table 3. Here, the most frequent combination was Fpk$^-$ Col^R Lac$^-$ indicating the order *fpk-zee-700*::Tn*10-cir'lac-λ-cir*$^+$. Both

crosses gave the same order of markers and therefore the direction of transcription of *cir* is from *fpk* toward *mgl* or clockwise on the *E. coli* chromosome.

The Colicin I Receptor is not part of the mgl-Dependent Transport System

There are several outer membrane phage receptors that are involved in the passive diffusion of small polar molecules through the outer membrane. They belong to specific transport systems and thus are coordinately regulated. Typical examples are the λ receptor (Ferenci and Boos 1980), the PhoE protein (Argast and Boos 1980) and the T6 receptor (Hantke 1976). The close vicinity of the gene for the colicin I receptor to the *mgl* genes suggested to us that it might be a specific porin of the *mgl*-dependent galactose transport system. However, this was not the case: the β-galactosidase activity of the *cir' lacZ* fusion in strain H1300 was not inducible by D-fucose, the inducer of the *mgl* genes. In addition, its *mgl*-dependent transport activity in 0.1 μM galactose was identical to that of an isogenic cir^+ derivative (not shown).

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