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Genetic Control of the Resistance to Phage C1 of *Escherichia coli* K-12

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***Escherichia coli* K-12 lytic phage C1 was earlier isolated in our laboratory. Its adsorption is controlled by at least three bacterial genes: *dcrA*, *dcrB*, and *btuB*. Our results provide evidence that the *dcrA* gene located at 60 min on the *E. coli* genetic map is identical to the *sdaC* gene. This gene product is an inner membrane protein recently identified as a putative specific serine transporter. The *dcrB* gene, located at 76.5 min, encodes a 20-kDa processed periplasmic protein, as determined by maxicell analysis, and corresponds to a recently determined open reading frame with a previously unknown function. The *btuB* gene product is known to be an outer membrane receptor protein responsible for adsorption of BF23 phage and vitamin B_{12} uptake. According to our data the DcrA and DcrB proteins are not involved in these processes. However, the DcrA protein probably participates in some cell division steps.**

Studying phage resistance is a common means of revealing nonessential bacterial genes controlling phage receptors, which can simultaneously execute other cellular functions, such as transport of specific substrates. In gram-negative bacteria, phage receptors are usually located in the outer membrane, which is the physical barrier between the external environment and the cell (6, 22, 32, 48). However, proteins of the cytoplasmic membrane are required for the irreversible adsorption of certain phages (12, 20), and recent experimental data imply that phage adsorption can be controlled by several genes (20, 31).

In a previous paper we described several *Escherichia coli* mutants deficient in the adsorption of phage C1. The mutations, originally called "*cor*," were located at three loci, at 60, 76.5, and 89 min of the genetic map (26). In the present work, these loci are renamed *dcrA*, *dcrB*, and *dcrC*, respectively. One locus, *dcrC*, is identical to the *btuB* gene, also responsible for phage BF23 adsorption and vitamin B_{12} transport (3).

In this article we present the cloning, physical mapping, and characterization of two *E. coli* genes responsible for C1 phage adsorption. According to sequence data, the *dcrA* locus is identical to the *sdaC* gene, recently identified as responsible for a putative serine transport protein located in the inner membrane (39). The second gene studied, *dcrB*, is located at 76.5 min on the genetic map and corresponds to an open reading frame in this region recently described by Sofia et al. (42). Here we present the identification and localization of the DcrB protein and some new properties of the *dcrA*/*sdaC* gene.

MATERIALS AND METHODS

Bacteria, phages, and plasmids. See Table 1.

Media and chemicals. Growth of bacteria, propagation and handling of phages, and plasmid manipulations were carried out as described elsewhere (28). Luria-Bertani broth was used for bacterial growth; minimal media M9 and M63 were also used, supplemented, as required, with 10 μg of vitamin B_1 per ml, vitamin B_{12} at 1 or 10 ng/ml, 0.4% glucose as carbon source, and required amino acids at 40 μg/ml (28). Antibiotics were used at the following concentrations (in micrograms per milliliter): tetracycline, 15; kanamycin, 30; ampicillin, 100. Restriction enzymes and ligase were from Fermentas. Proteins were labeled for 60 min with [^{35}S]Met (Obninsk) at 400 MBq/ml.

Genetic techniques. Standard techniques were used for P1*vir* (29) and T4gt7 (49) transduction.

Insertions in the *dcrB* and *dcrA* genes were constructed as described elsewhere (50), by insertion of the RSM (restriction site mobilizing) Km^r element from plasmid pUC4K (46) into the cloned *dcrB* gene and insertion of the chloramphenicol acetyltransferase (CAT) Cm^r element from pCM7 (43) into *dcrA*. The resulting plasmids, pBC764 with RSM in *dcrB* and pBC45 with CAT in gene *dcrA*, were introduced into strain JC623 (*recB recC sbcB*). C1-resistant clones were selected among Km^r Ap^s or Cm^r Ap^s transformants (in which plasmid loss was ensured). The resulting strains, B6630 and B6817, were used as donors for P1*vir* transduction of the *dcrB*::Km and *dcrA*::Cm alleles into strains N99 and W3350.

Vitamin B_{12} utilization. To study vitamin B_{12} utilization in *dcrA* and *dcrB* mutants, *metE*::Tn*10* strains B6621 and B6861 were constructed by T4gt7 transduction; *metE* mutants can grow on minimal plates containing 1 ng of vitamin B_{12} per ml (and lacking methionine) only if there is B_{12} transport, since vitamin B_{12} is not synthesized in aerobic conditions (9).

DNA manipulations. Extraction of plasmid DNA, restriction, ligation, DNA transformation, nick translation, and hybridization were carried out as described elsewhere (28).

The exact locations of the *dcrA* and *dcrB* genes on the *E. coli* physical map were determined by hybridization of plasmids bearing these genes with phages from the Kohara gene library (23), listed in Table 1. Phage plaques were transferred onto nylon filters (Hybond N; Amersham), and the DNA was fixed with UV light. The filters were prehybridized at 65°C for 12 h; they were then hybridized with nick-translated pBC25 or pBC763 DNA at 65°C.

DNA sequencing. DNA sequences were determined by the dideoxy chain termination method (38), using denatured double-stranded plasmid DNA as described in the Promega Protocols and Application Guide, 1993.

Complementation analysis. To test the ability of plasmids to complement the C1 adsorption defects, we transformed *dcrA* and *dcrB* mutants with plasmid subclones. Complementation was evaluated by adsorption assays and cross-streaking against C1.

C1 adsorption assay. Adsorption of phage C1 was measured as described elsewhere (35). Overnight cultures of bacteria were diluted in fresh Luria-Bertani broth and grown at 37°C to 5×10^8 cells per ml, centrifuged, and suspended in M63 medium prewarmed to 37°C, as for phage K10 (35). Phage C1, diluted to 10^6 phage per ml in M63 medium, was incubated 20 min at 37°C with an equal volume of cells. After incubation, a 0.1-ml sample was taken in cold broth with and without chloroform. Chloroform was added to kill the infected cells, permitting measurement of the unadsorbed phage particles remaining in the medium. The quantity of unadsorbed phage particles compared with the phage titer quantified the adsorption efficiencies of different strains.

Maxicell analysis. To identify plasmid-encoded proteins, we carried out a maxicell analysis as described elsewhere (37), developed for laboratory strain *E. coli* AB2463.

Gel electrophoresis and autoradiography. Radiolabeled proteins were analyzed by electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels as described elsewhere (11). After electrophoresis, the gels were treated with Amplify (Amersham), dried, and exposed to PM1 film at −70°C.

Cell fractionation. To extract membrane proteins, we used the method described by Zaror et al. (52). Bacterial cells were grown in 250 ml of Luria-Bertani broth and the appropriate antibiotic to late exponential growth phase. Cells were pelleted, resuspended in 3 ml of 10 mM Tris-HCl (pH 8.0), and sonicated. The cell lysate was separated from intact cells by centrifugation at 12,000 × *g* for 20 min. The supernatant was then ultracentrifuged (Beckman Ti65 rotor) at

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TABLE 1. *Escherichia coli* K-12 strains, phages, and plasmids

Strain, phage, or plasmid	Genotype or characteristic(s)	Source
Strains		
N99	F$^-$ *galKT rpsL* Sm^r	VKPM[a]
B3694	N99 *dcrA55* C1^r	VKPM
S455	*argA21 mut-53 lysA thi-1 mtl-2 xyl-7 tonA21 tsx-1*	VKPM
B6288	S455 *dcrA55* C1^r	This work
AB2463	*recA* Sm^r Rif^r *leu thr pro arg his*	VKPM
B4021	N99 *dcrB5* Tc^r C1^r	VKPM
AB1127	*recB21 recC22 sbcB15*	VKPM
B6556	N99 *dcrB20*::RSM (Km^r)	This work
KL209	*metE*::Tn*10* Hfr *sup53 malB* λ^r	VKPM
B2568	F$^-$ *btuB1* C1^r BF23^r *purD38 thi proA2 his4 rel1 lacY1 galK2 mtl1 xyl5 tsx29 sup48*	VKPM
B6619	KL209 *btuB1*	VKPM
B6622	B6288 *metE*::Tn*10*	VKPM
B6621	B6556 *metE*::Tn*10*	VKPM
B6630	AB1157 *dcrB20*::RSM (Km^r)	This work
BR 158	*tonB aroB thi malT tsx*	V. Braun (5)
HS 244	*tonB aroB thi malT tsx recA*	V. Braun (5)
W3350	*galKT*	VKPM
B6817	W3350 *dcrA60*::CAT	This work
B6884	*dcrB20*::RSM (Km^r)	This work
B6861	B6817 *metE*::Tn*10*	This work
B5395	*ptsG ptsM galK rpsL recA*::Tn*10* Δ(*srl-recA*)	VKPM
B6857	W3350 *recA*::Tn*10*	This work
B6858	B6817 *recA*::Tn*10*	This work
Phages		
P1*vir*		VKPM
T4gt7		VKPM
C1		VKPM
BF23		VKPM
λ		VKPM
ϕ80		VKPM
T2, T4, T5, T7, Tu1a, Tu1b, Tull*		VKPM
λpSL5	λcI 857 Ap^r ori ColE1	VKPM
λpSL36	λpSL5::*dcrA*	VKPM
λpSL43	λpSL5::*dcrB*	VKPM
9A10, 1B3, 8B9, 10B5, 8C5, 9A12, 10B6, 3G11, 5A10, 8H3	60 min	Y. Kohara (23)
12E4, 6A4, 10D10, 5F2, 7H7, 1B6, 5B10, 10F5, 16A5, E5B4, E4E4	76.5 min	Y. Kohara (23)
21D3, 6G3, 6G9, 3C5, 9B9, 7B7, E11C11, 4G11, 8H10	89 min	Y. Kohara (23)
Plasmids		
pBC36	pUC19::*dcrA* (15 kb)	This work
pBC15	pUC19::*dcrA* (11 kb)	This work
pBC2	pUC19::*dcrA* (9.0 kb)	This work
pBC25	pUC19::*dcrA* (5.3 kb)	This work
pBC45	pBC25::CAT (DcrA$^-$) (5.6 kb)	This work
pBC605	pUC19::*dcrB* (17 kb)	This work
pBC762	pUC19::*dcrB* (9.2 kb)	This work
pBC763	pUC19::*dcrB* (5.8 kb)	This work
pBC764	pBC763::RSM (DcrB$^-$) (6.9 kb)	This work
pAG1	pUC8::*btuB* (6 kb)	C. Bradbeer (3)
pUC4K	3.5 kb	VKPM
pUC19	2.7 kb	VKPM
pCM7	4.1 kb	VKPM

[a] VKPM, Russian National Collection of Industrial Microorganisms, GNIIGenetika, Moscow, Russia.

40,000 × *g* for 30 min. The pelleted membrane preparation was thoroughly resuspended in 10 mM Tris-HCl (pH 8.0), containing 10 mM $MgCl_2$ and 2% Triton X-100, and then incubated at 37°C for 45 min with continuous shaking. The suspension was centrifuged at 80,000 × *g* for 90 min. The outer membrane pellet was finally suspended in the sample buffer for electrophoresis. The inner membrane proteins from the supernatant were precipitated by 2.5 volumes of ethanol, and the pellet was dissolved in the sample buffer. We also used a simple procedure enabling simultaneous preparation of isotopically labeled membrane and periplasmic fraction proteins from many small-scale cultures (14).

RESULTS

Cloning of the *dcrA* and *dcrB* genes. To clone the *E. coli* genes responsible for C1 phage adsorption, we first used an *Eco*RI bacterial DNA library in the phasmid λpSL5 (51). Cloned bacterial DNA fragments containing such genes were detected as complementing mutation *dcrA55* (B3694) or *dcrB5* (B4021). Such clones were screened as C1 sensitive on Luria-

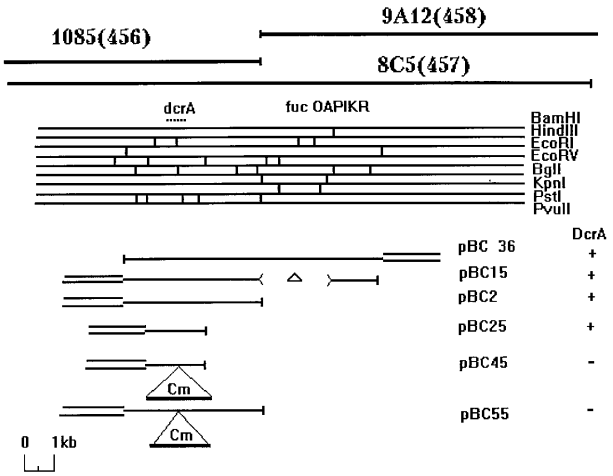

FIG. 1. Identification of the *dcrA* gene in the 60-min region of the *E. coli* chromosome. Locations of the bacterial DNA fragments from Kohara phages 10B5, 9A12, and 8C5 and the *fucOAPIKR* gene cluster on the physical map of the *E. coli* chromosome are shown at the top. Positions of the relevant restriction sites are given. The subcloning scheme of the *dcrA* gene is illustrated. The capabilities of the plasmids to complement the *dcrA60* mutation of strain B6817 are indicated on the right (+, complementation; −, no complementation).

Bertani agar petri dishes with 10^8 C1 phage. Among 800 Ap^r transformants of B3694, 5 Dcr^+ (C1-sensitive) clones were found. All contained the same bacterial DNA fragment with a restriction map identical to the fragment on the Kohara restriction map near 60 min of the *E. coli* chromosome, where the *dcrA* gene was genetically localized. This chromosome region is overlapped by the Kohara phages 10B5, 9A12, and 8C5. We propose that the *dcrA* gene is located in this fragment. For further characterization, phasmid λpSL36 was used.

From 800 Ap^r transformants of B4021, 5 independently isolated *Eco*RI DNA fragments were found to complement the *dcrB5* mutation. Unexpectedly, these were of two different types. The first corresponded to the *Eco*RI fragment carrying the *dcrA* gene; the second had a fragment with a restriction map identical to the fragment of the Kohara restriction map near 76.5 min of the *E. coli* chromosome, where the *dcrB* locus was genetically localized. This chromosome region is overlapped by the Kohara phages 10D10, 5F2, and 7H7. In this case, we propose that the *dcrB5* mutation can be indirectly complemented with multiple copies of *dcrA* or directly by gene *dcrB* in the second-class cloned fragments. For further characterization of this class, phasmid λpSL43 was used.

To subclone *dcrA* and *dcrB* in plasmid pUC19, the phasmids λpSL36 and λpSL43 were treated with *Bam*HI and ligase, and then *E. coli dcrA55* and *dcrB5* cells, respectively, were transformed. The resulting plasmids were named pBC36 (*dcrA*) and pBC605 (*dcrB*).

Localization of the *dcrA* and *dcrB* genes within the cloned DNA fragments and on the *E. coli* physical map. First, detailed restriction maps were made of the bacterial DNA fragments cloned in pBC36 and pBC605, by using the restriction enzymes *Bam*HI, *Eco*RV, *Hin*dIII, *Bgl*II, *Pvu*II, *Eco*RI, *Kpn*I, and *Pst*I. The resulting maps are in full agreement with the Kohara restriction map of the regions 60 and 76.5 min (Fig. 1 and 2). To identify the minimal DNA fragments coding for the *dcrA* and *dcrB* genes, deletions were made in plasmids pBC36 and pBC605 (Fig. 1 and 2) with restriction endonucleases.

For *dcrA*, plasmids pBC15 (constructed from pBC36 by deleting a 4-kb *Kpn*I fragment of the insert), pBC2 (pBC15 truncated at the *Eco*RI site in the insert), and pBC25 (constructed by inserting the 2.5-kb *Eco*RV fragment from pBC2 into the vector pUC19 digested by *Sma*I) were able to complement the *dcrA55* mutation, whereas plasmid pBC45 (constructed by inserting the CAT element from pCM7 into a *Hin*dIII site of pBC25) did not (Fig. 1). Plasmid pBC55 was constructed in vivo by double recombination between plasmid pBC2 and the chromosome of strain B6817; this plasmid did not complement the *dcrA55* mutation and conferred a Cm^r phenotype (Fig. 1).

For *dcrB*, plasmids pBC762 (constructed by deleting an *Eco*RV fragment from pBC605) and pBC763 (constructed by deleting a *Sma*I-*Eco*105 fragment from pBC605) were able to complement the *dcrB5* mutation, whereas plasmid pBC764 (constructed by inserting the *kan* gene from pUC4K into the *Ava*III site of pBC763) did not (Fig. 2).

Localization of the *dcrA* and *dcrB* genes on the *E. coli* physical map was confirmed by hybridizing the 2.6-kb DNA frag-

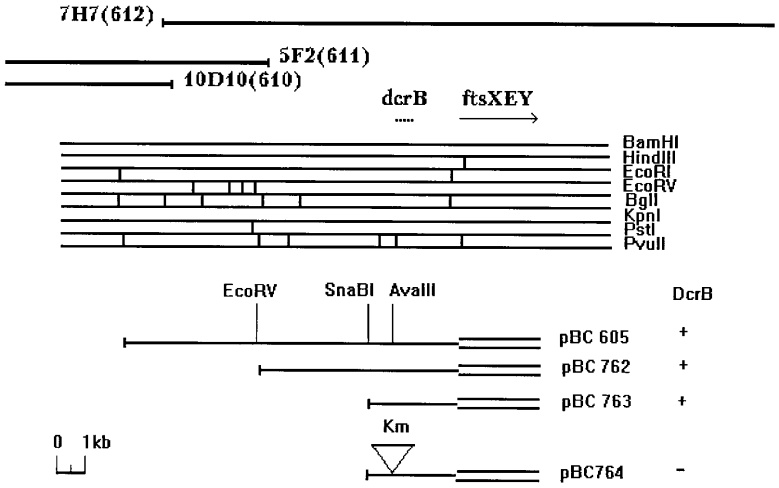

FIG. 2. Identification of the *dcrB* gene in the 76.5-min region of the *E. coli* chromosome. Locations of the bacterial DNA fragments from Kohara phages 7H7, 5F2, and 8C5 and the *ftsXEY* gene cluster on the physical map of the *E. coli* chromosome are shown at the top. Positions of the relevant restriction sites are given. The subcloning scheme of the *dcrB* gene is illustrated. The capabilities of the plasmids to complement the *dcrB20* mutation of strain B6556 are indicated on the right (+, complementation; −, no complementation).

TABLE 2. Efficiency of adsorption of C1 phage

Strain	Allele	% Adsorption
W3350	Wild type	95
B6817	*dcrA60*::CAT (Cm^r)	0
B6884	*dcrB20*::RSM (Km^r)	0

ment cloned in pdcrA$^+$ (pBC25) and the 3.1-kb fragment cloned in pdcrB$^+$ (pBC763) with Kohara phages covering the corresponding regions of the *E. coli* chromosome (Table 1). Plasmid pdcrA$^+$ (pBC25) hybridized with Kohara phages 10B5(456) and 8C5(457) but not with the other phages. Plasmid pdcrB$^+$ (pBC763) DNA hybridized with phage 7H7(612) but not with the others (Fig. 1 and 2).

The mutations *dcrA20*::CAT (Cm^r) and *dcrB60*::RSM (Km^r) from the pdcrA::CAT (pBC45) and pdcrB::RSM (pBC763) plasmids were introduced into the *E. coli* chromosome, and resistance of the resulting mutants to C1 was confirmed and shown to result from an adsorption defect (Table 2). Complementation analysis using cloned *dcrA*, *dcrB*, and *btuB* genes showed that the *dcrA20*::CAT and *dcrB60*::RSM mutants could be complemented only by *dcrA*- and *dcrB*-containing plasmids, respectively, although, as shown above, the original *dcrB5* mutation was complemented by both *dcrA*- and *dcrB*-containing plasmids. Nevertheless, the *dcrB5* and *dcrB60*::RSM mutations are closely linked genetically and seem to affect the same gene. The different results with the *dcrB5* and *dcrB60*::RSM mutations may reflect partial (*dcrB5*) versus total (*dcrB60*::RSM) inactivation of the *dcrB* gene.

The data place *dcrA* in a 2.6-kb fragment between *metZ* and the *fucOADIKR* cluster and *dcrB* in a 3-kb fragment between the *ftsYEX* and *nikA* genes.

Nucleotide sequence analysis of the *dcrA* and *dcrB* genes. The nucleotide sequences of the *dcrA* and *dcrB* genes were determined. Comparison of the results with data in EMBL/GenBank/DDBJ DNA-SUN (VNIIGENETIKA, Moscow 1994, version 3.30) showed identity between the *dcrA* gene and *sdaC* (EMBL, EC 233) and identity between the *dcrB* gene and an open reading frame between the *ftsY* and *nikA* genes (EMBL, YHHR-ECOLI), coding for a hypothetical 19.8-kDa protein. These sequences are physically located in exactly the same regions as the *dcrA* and *dcrB* genes. The amino acid sequence analysis predicts that *dcrB* contains an amino-terminal signal peptide of 19 amino acid residues which is cleaved during export through the inner membrane. The proposed signal peptide contains three typical features: (i) the amino acid sequence has two positively charged amino acid residues, R-2 and K-6; (ii) the amino terminus is followed by a hydrophobic core, amino acid residues Y-7 to A-19; and (iii) the signal peptide is predicted to form alpha-helix and beta-sheet secondary structures.

Identification of the *dcrB* gene product. To identify the *dcrB* gene product, we carried out maxicell analysis (37), using pdcrB$^+$ (pBC763) and the analogous pdcrB::RSM (pBC764) mutant plasmid carrying an insertion in *dcrB*. Strain AB2463 was transformed by these plasmids. A 20.0-kDa protein and an 18.0-kDa proteins were detected in cells carrying pdcrB$^+$ (pBC763); these proteins were not detected in cells carrying the plasmid with an insertion in the *dcrB* gene (Fig. 3). The 20-kDa doublet suggested that *dcrB* may code for a processed protein, with the two bands representing the unprocessed precursor and the mature protein, in agreement with the prediction from the sequence.

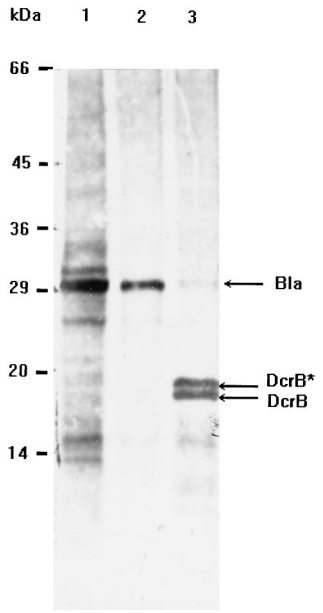

FIG. 3. Maxicell analysis of the *dcrB* gene product. Lane 1, pUC19 vector without insert; lane 2, pBC764 containing the indicated *dcrB20*::Km mutation; lane 3, pBC763. An SDS–11% polyacrylamide Laemmli gel (24) was used. DcrB*, unprocessed precursor; DcrB, mature protein.

Localization of the *dcrB* protein in membrane fractions. Since the *dcrB* gene product is efficiently detected by maxicell analysis, we fractionated maxicells expressing *dcrB* from pdcrB$^+$ (pBC763) to determine its subcellular location. We confirmed that maxicells fractionate similarly to viable cells with this procedure by comparing the fractionation patterns of maxicells and viable cells on a Coomassie blue-stained gel, as described previously by Kiino and Rothman-Denes (20).

Visualization of the *dcrB* gene products by autoradiography of the electrophoresed maxicell samples showed that DcrB is associated with the inner membrane and periplasmic fractions (Fig. 4). DcrB protein was found in the inner membrane and periplasmic fraction (Fig. 4, lanes 4 and 3). In the inner membrane fraction there seemed to be some unprocessed protein (DcrB*) as well. The presence of DcrB in the periplasm is consistent with the idea that the *dcrB* gene product is a processed protein exported to the periplasm.

The *dcrA* gene product does not affect expression of *dcrB*. Using plasmid pdcrB$^+$ (pBC763), we showed directly that the chromosomal *dcrA60*::CAT mutation did not prevent the expression or processing of the cloned *dcrB* gene product in maxicells (Fig. 5).

Vitamin B_{12} utilization in *dcrA* and *dcrB* mutants. We found previously that the *btuB* gene is one of the loci required for C1 phage adsorption (26). The *E. coli* outer membrane protein BtuB is a multivalent protein, responsible both for active transport of vitamin B_{12} and for adsorption of phage BF23 and group E colicins (3, 10). It was therefore of interest to test whether the *dcrA* and *dcrB* genes, involved in phage C1 adsorption, are also involved in vitamin B_{12} transport.

It is known that *E. coli metE* auxotrophic mutants can grow aerobically in the absence of methionine only if at least 1 ng of vitamin B_{12} (which cannot be synthesized) per ml is present in the medium. This is because, of the two isoenzymes for this step in methionine biosynthesis, the one controlled by *metE* is B_{12} independent whereas the second, controlled by *metH*, requires B_{12} (27). *E. coli metE* mutants are thus convenient for

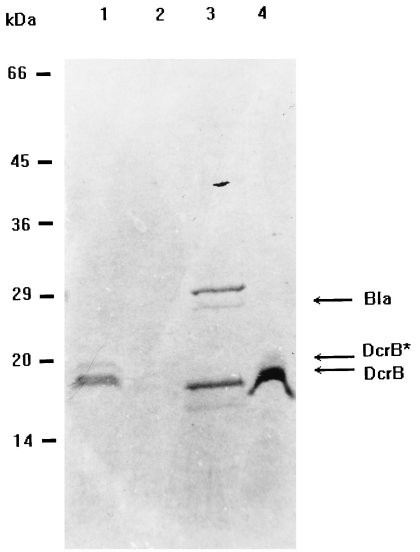

FIG. 4. Subcellular fractionation of pBC763 maxicell products. Labeling of plasmid-encoded proteins by the maxicell procedure was followed by fractionation of the cell extract. Lane 1, inner and outer membrane fractions; lane 2, outer membrane; lane 3, periplasm; lane 4, inner membrane. An SDS–11% polyacrylamide Laemmli gel (24) was used.

determining the influence of other mutations on vitamin B_{12} transport. We measured B_{12} utilization in *dcrA60*::Cm *metE*::Tn*10* and *dcrB20*::Km *metE*::Tn*10* strains. The *metE*::Tn*10* allele was introduced, by transduction with phage T4gt7, from donor strain KL209 into strains B6817 (*dcrA*), B6556 (*dcrB*), and B2568 (*btuB*), which is C1 resistant because of the *btuB1* mutation. As a control, we used strain B6619 *btuB metE*::Tn*10* with plasmid pAG1, carrying the *btuB* gene. It is known that *btuB* on a multicopy plasmid provides a significant increase of B_{12} binding to *E. coli* receptors; in such strains, the level of B_{12} uptake does not drop even when the cells are grown in media with low B_{12} concentrations (2, 16, 30). The growth of these *metE* derivatives, with mutations in three genes determining

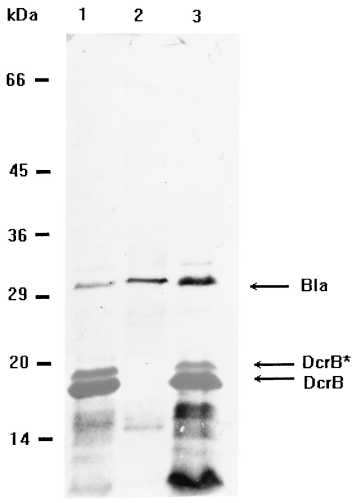

FIG. 5. Expression of *dcrB* in the *dcrA*::CAT mutant. Maxicell extracts were electrophoresed from the following strains. Lane 1, B6858 *dcrA60*::CAT/pBC763 *dcrB*$^+$; lane 2, B6858 *dcrA60*::CAT/pBC764 *dcrB20*::Km; lane 3, B6857 *dcr*$^+$/pBC763 *dcrB*$^+$. Electrophoresis was carried out as for Fig. 4.

TABLE 3. Effects of vitamin B_{12} concentration on growth of *metE* auxotrophs

Strain	Growth on M9 media with[a]:			
	No supplement	Met (20 μg/ml)	B_{12} 1 ng/ml	B_{12} 10 ng/ml
B6619 BtuB$^-$ *metE*::Tn*10*	−	+	−	−
B6620 BtuB$^-$ (pAG1) *metE*::Tn*10*	−	+	+	+
B6621 DcrB$^-$ *metE*::Tn*10*	−	+	+	+
B6622 DcrA$^-$ *metE*::Tn*10*	−	+	+	+
B6861 DcrA$^-$ *metE*::Tn*10*	−	+	+	+

[a] −, no growth after 48 h at 37°C.

the resistance to phage C1, was measured on M9 minimal medium with vitamin B_{12} at 1 or 10 ng/ml (Table 3). The *metE dcrA* and *metE dcrB* mutants grew well even at 1 ng of B_{12} per ml, indicating normal B_{12} transport. The *btuB metE*::Tn*10* mutant, on the contrary, could not grow even at 10 ng of B_{12} per ml, although it grew after transformation with plasmid pAG1, carrying the *btuB* gene (Table 3).

Since the *dcrA* and *dcrB* insertion mutations result in complete inactivation of the *dcrA* and *dcrB* genes without impairing vitamin B_{12} utilization, we conclude that the *dcrA* and *dcrB* gene products are not involved in vitamin B_{12} transport in *E. coli.*

DISCUSSION

Bacteriophage C1 is a virulent phage found in nature. It belongs to the morphotype B1 (34), related to phages BF23 and T5 (26, 36) as well as λ. These phages use different cellular receptors, which are controlled by several bacterial genes (3, 8, 22, 47).

We previously isolated and described mutants with mutations in three loci controlling phage C1 adsorption. One gene responsible for the adsorption of phages C1 and BF23, *btuB*, was already known. The protein specified by this gene also serves as a receptor for iron siderophores and for vitamin B_{12} (cobalamin) transport across the outer membrane (3). Two other genes, *dcrA* (60 min) and *dcrB* (76.5 min), were required for C1, but not BF23, adsorption.

In this article we present the cloning, physical localization, and characterization of the *dcrA* and *dcrB* genes. Comparison of the DNA sequences of these genes with nucleotide sequence data libraries identified the *dcrA* gene as the recently described *sdaC* gene, coding for a putative serine transporter probably located in the inner membrane (39). The *dcrB* gene, at 76.5 min, was found to be identical with an open reading frame coding for an approximately 20-kDa protein of unknown function, recently described by Sofia et al. (42). Maxicell analysis suggested that the *dcrB* gene product is a 20-kDa processed periplasmic protein, possibly anchored in the inner membrane. These results are in good agreement with the DNA sequence analysis.

From these data, we can say that C1 phage adsorption is controlled by at least three bacterial proteins: BtuB in the outer membrane, DcrB in the periplasm, and DcrA/SdaC in the inner membrane. Although DcrA/SdaC overproduction was able to restore C1 sensitivity in the *dcrB5* point mutant, it did not restore sensitivity in the *dcrB20*::RSM insertion mutant, indicating that both *dcrA* and *dcrB* are required for C1 adsorption. This shows that C1 resistance induced by partial but not complete inactivation of DcrB can be suppressed by a

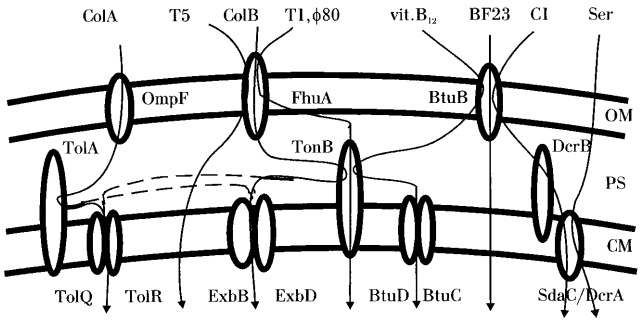

FIG. 6. Membrane proteins essential for phage T5, T1, ϕ80, BF23, and C1 and colicin ColA and ColB infections and vitamin (vit.) B_{12} and serin-specific uptake. Arrows, proposed pathways of the specific transport processes. OM, outer membrane; PS, periplasmic space; CM, cytoplasmic membrane.

high level of DcrA/SdaC and suggests that the two proteins interact.

Analysis of the *dcrA*/*sdaC* promoter region suggests complex transcriptional regulation, since there are many inverted repeats, and according to recent results, the *sdaC* gene is probably a member of the leucine/Lrp regulon (39).

Present results suggest that the role of the *dcrA*/*sdaC* gene product is pleiotropic. Cells carrying a multicopy plasmid with the *dcrA* gene stop dividing and form filaments on rich media at 42°C (data not shown), and insertional inactivation of the *dcrA* (*sdaC*) gene leads to increased ampicillin resistance: mutant B6817 *dcrA*::CAT is resistant to 4 μg of ampicillin per ml, whereas *dcrA*$^+$ cells are sensitive to 1.5 μg/ml. Localization of the DcrA/SdaC protein in the inner membrane and the specific resistance of *dcrA60*::CAT mutants to β-lactam antibiotics suggest a possible influence of the DcrA/SdaC protein on the activity of penicillin-binding proteins or on ampicillin–penicillin-binding protein complex formation. If the absence of DcrA decreases complex formation, an increase in DcrA may lead to increased penicillin-binding protein activity and, as a result, discoordination of peptidoglycan synthesis and other steps of cell division, possibly resulting in filamentation.

Involvement of an inner membrane protein in phage adsorption is unusual but not unprecedented. Analogous multigene adsorption control was recently described for phage N4 (20). The locations of the DcrA/SdaC, DcrB, and BtuB proteins provide some information about their possible roles in C1 phage adsorption. As an outer membrane protein, BtuB is probably the receptor for phage C1, as for BF23 (3). The function in C1 adsorption of DcrB, a periplasmic protein probably anchored in the inner membrane, may be similar to that of TonB, required for the adsorption of several phages, including T1 and ϕ80. The TonB protein is involved in many energy-dependent transport systems mediated by outer membrane receptors, including BtuB, and probably interacts with these proteins (13, 17, 33). DcrB may also be involved in some energy-dependent transport processes, although it does not seem to be involved in BtuB-dependent vitamin B_{12} transport. In this respect, it is of interest that the third protein responsible for C1 adsorption, DcrA/SdaC, which probably interacts with DcrB, has been proposed to be a highly specific serine transporter. As a working model, we suggest that DcrB affects the conformation of BtuB to make it competent for C1 adsorption, although both conformations are competent for other known BtuB functions.

In addition to TonB, a similar role as periplasmic transport protein is also played by the TolA protein, which can complement some TonB functions and is essential for cell sensitivity to ColA and certain filamentous phages (45, 47).

TonB and DcrB have different activities. TonB is not essential for C1 adsorption, and DcrB is not required for vitamin B_{12} transport, which is controlled by TonB and BtuB, or for the adsorption of the TonB-dependent phages ϕ80 and T1 (12, 15, 18). In Fig. 6, data illustrating the roles of TonB and TolA together with the outer membrane receptors OmpF, TonA, and BtuB in different processes, including ColA, ColM, and B_{12} uptake and infection by phages T1, T5, ϕ80, and BF23, are summarized. We also present the proposed pathway of C1 infection, determined by the BtuB, DcrB, and DcrA/SdaC proteins. TolA and TonB, periplasmic proteins anchored to the inner membrane, can interact in these processes with outer and inner membrane proteins (1, 4, 21, 25, 40, 45). Although TonB is known to interact with different outer membrane receptors (13, 17, 19, 41, 44), to our knowledge BtuB would be the first example of an outer membrane protein interacting with several periplasmic proteins for different transport pathways.

Several interesting questions remain to be answered. Is DcrB involved in serine transport? Is the function of DcrB analogous to that of TonB, activating or transducing energy to outer membrane receptors? Is DcrB involved in other transport processes, and does it interact with different outer membrane proteins, like TonB?

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