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The *pcnB* Gene of *Escherichia coli*, Which Is Required for ColE1 Copy Number Maintenance, Is Dispensable

MILLICENT MASTERS,* MICHAEL D. COLLOMS, IAN R. OLIVER, LIN HE, EDWARD J. MACNAUGHTON, AND YVONNE CHARTERS

Institute of Cell & Molecular Biology, University of Edinburgh, Kings Buildings, Mayfield Road, Edinburgh EH9 3JR, United Kingdom

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The *pcnB* gene product of *Escherichia coli* is required for copy number maintenance of plasmids related to ColE1 and also for that of the IncFII plasmid R1. Because PcnB is similar to the tRNA-binding protein tRNA nucleotidyltransferase, we have suggested that the protein would be required only for processes in which an RNA is a prominent regulatory component. This appears to be so; strains deleted for *pcnB*, although defective in ColE1 and R1 plasmid maintenance, maintain the iteron-regulated plasmids F and P1 normally. We also find that strains deleted for *pcnB* grow normally, demonstrating that PcnB has no essential cellular role under the conditions tested and suggesting that regulation by antisense RNAs similar to RNAI has no critical role in any essential host process. We confirm by immunological tests that PcnB is likely to be the commercially available enzyme poly(A) polymerase.

The *Escherichia coli pcnB* gene product is required for normal copy number maintenance of plasmids related to either ColE1 or R1 (13, 14, 16, 17). Replication frequency in each of these plasmid groups is controlled, in part, by an antisense RNA (for a review, see reference 5). RNAI binding prevents productive folding of the ColE1 replication primer, while *copA* RNA prevents translation of the message for the essential R1 replication protein, RepA. PcnB shares homology with the product of the *E. coli cca* gene, tRNA nucleotidyltransferase, a protein which binds tRNA (17). It has therefore been suggested that PcnB may also be an RNA-binding protein and may expedite plasmid replication by interfering with the inhibitory sense-antisense RNA interactions which limit it. Although several host processes have been described in which RNA interactions are believed to affect gene expression (for reviews, see references 6 and 26), none of these appear to be of critical importance to the cell, and it is thus possible that the principal role of PcnB is the modulation of plasmid replication. To investigate this question further, we have constructed a strain from which the *pcnB* gene has been deleted and report its properties here. We find *pcnB* to be a dispensable gene.

MATERIALS AND METHODS

Bacterial strains, plasmids, and genetic techniques. The bacterial strains used are described in Table 1. Linear transformations were performed as described previously (25). The plasmids used in curing experiments, pBR325, the mini-R1 plasmid pGW71, the mini-F plasmids pSC138, pXX325, and pXX327, the mini-P1 plasmids pAX274 and pAX275, and pRE1-1, in which *pcnB* is expressed from a λ p_L, are also described in Table 1. pRE1-1 is maintained in MZ1, which is lysogenic for λ*c*I857. Plasmids used for fusion protein production are described below. Other transformations, transductions, and conjugations were performed by using standard techniques, and media used were as previously described (19). Antibiotic concentrations used for routine growth were as follows: ampicillin, 10 μg/ml (mini-F and mini-P1 plasmids) or 50 μg/ml (ColE1-based plasmids); tetracycline, 10 μg/ml; chloramphenicol, 10 μg/ml; and kanamycin, 25 or 50 μg/ml. Cell sizes were measured by using a Coulter Counter and Channelyzer.

Construction of *pcnB* deletion strains. pJM513 (pBR325 containing a 5.4-kb chromosomal DNA fragment which includes *pcnB* [15]) was fully digested with *Kpn*I and then partially digested with *Sph*I. The four fragments obtained were separated on an agarose gel, and the desired 7.6-kb band (Fig. 1) was extracted and purified by using Geneclean. pUC4-K (Pharmacia) was cut with *Pst*I, and the 2.3-kb Kan^r cassette was purified as described above. The 3′ overhangs of both fragments were trimmed with T4 DNA polymerase and blunt end ligated with T4 ligase. Products were transformed into ER1562, Amp^r Kan^r progeny were selected, and plasmid DNA was prepared from progeny clones. One of these plasmids, the structure of which was confirmed as correct by restriction analysis, was named pMD20. It was demonstrated to lack *pcnB*-complementing ability. NM621 was transformed with purified pMD20 DNA which had been digested with *Sph*I and *Eco*RI to separate the plasmid replication region from the insert DNA containing the Kan^r cassette and *pcnB* flanking DNA. Kan^r Amp^r progeny were selected. It was necessary to use kanamycin at 50 μg/ml to avoid selection of spontaneous Kan^r progeny. Colony hybridization was performed as described previously (8) on a selection of Kan^r Amp^r and Kan^r Amp^s transformants, using the *Sph*I-*Kpn*I and *Kpn*I-*Kpn*I fragments (Fig. 1) from pJM513 as the probe. Only three clones that did not hybridize with this probe were found, and all were from the initially Amp^r Kan^r class of transformant. Chromosomal DNA prepared (7, 16) from two of these presumed deletion strains and from NM621 was cut with restriction endonucleases, and Southern hybridizations were carried out as previously described (21). The *pcnB* fragments described above (probe I) or Kan^r cassette DNA (probe II) was used as the probe.

Preparation of GST-PCN fusion protein. To prepare antigen for making anti-PcnB antibody, it was decided to make a fusion protein in which PcnB would be combined with a protein moiety purifiable by affinity under nondenaturing conditions. Plasmid pGEX3X (29), in which glutathione

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or characteristics	Source or reference
Strains		
NM621	*recD*; recipient, linear DNA transformations	N. Murray (33)
MM38	*argG6 asnA31* or *asnB32 his-1 leuB6 metB1 pyrE gal-6 lacY1 xyl-7 supE44 bgl fhuA2 gyrA rpsL104 tsx-1 uhp*	Spontaneous Asn$^+$ revertant of MM18 (15)
IR8901, IR8903	NM621 Δ*pcnB* Kan^r (replacement of *pcnB* by the Kan^r cassette from pUC4-K)	This work
MM38K24, MM38K26	MM38 Δ*pcnB* Kan^r	P1 transduction from IR8901 and IR8903, respectively
JM38	MM38 *pcnB21*	This work
Mri84	*lacU169 araD139 thiA rpsL relA Δrbs-7 zad*::Tn*10*	J. Lopilato
Mri93	Mri84 *pcnB80*	J. Lopilato (14)
YA139	*panB6* (linked to *pcnB*)	15
NK7968	W3110 Str^r *nad*::Tn*10HH104*/pOX38::Tn*9*	N. Kleckner
NK7970	NK7968 *nad*$^+$ *his*::Tn*10HH104*	N. Kleckner
ER1562	*endA1 hsdR2 supE44 thi-1 mcrA1272*::Tn*10 mcrB1*	New England Biolabs
XL1Blue	*recA endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* (F′ *proAB lacI*^{q}Z*ΔM15*Tn*10*)	Stratagene Ltd.
Plasmids		
pBR325	Amp^r Tet^r Cmp^r pMB1-derived (ColE1-type) cloning vector, used to measure plasmid copy number	2
pGW71	Amp^r mini-R1	R. Bernander (1)
pSC138	Amp^r mini-F	T. Ogura (32)
pXX325	Amp^r pSC138 Δ*ccd*, stability reduced	T. Ogura (23)
pXX327	Amp^r pXX325 Δ*par*, stability further reduced	T. Ogura (23)
pAX274	Amp^r mini-P1	T. Ogura (24)
pAX275	Amp^r pAX274 Δ*par*, stability reduced	T. Ogura (24)
pJM513	pBR325 Cmp^s *pcnB*$^+$, 5.3-kb chromosomal insert	15
pUC4-K	Source of Kan^r cassette	Pharmacia Ltd.
pMD20	pBR325 in which the Kan^r cassette replaces *pcnB*	This work
pGEX3X	Amp^r fusion vector; product of cloned gene is fused to GST	29; Pharmacia Ltd.
pLH2d	pGEXEX derivative in which PcnB is expressed as a fusion with GST	This work
pLH1a	pBR322-based plasmid, intermediate in pLH2d construction	This work
pRE1-1	Amp^r; *pcnB* expressed from λ p_L	N. Sarkar (3)

S-transferase (GST) message is under *tac* promoter control, was selected, and the *pcnB* coding sequence was cloned downstream of the GST gene. The 26-kDa GST protein originates from the worm *Schistosoma japonicum* (28) and is thus normally absent from *E. coli*.

The construction was carried out as follows. The *Sph*I-*Hin*dIII fragment containing *pcnB* (Fig. 1) was cloned in pJF118EH (cut with *Sal*I and *Hin*dIII), using a linker to join the *Sph*I and *Sal*I overhangs. This plasmid, pLH1a, was then cut with *Bam*HI and *Hin*dIII, and the *pcnB* fragment was treated with the Klenow fragment of DNA polymerase I to obtain blunt ends. The purified fragment was then ligated to a *Sma*I digest of pGEX3X which had been dephosphorylated before ligation to avoid recyclization. This two-part procedure was adopted so as to obtain the necessary in-frame fusion. The ligated DNA was transformed into XL1Blue by electroporation, and progeny were selected on ampicillin plates. Restriction analysis was used to identify progeny in which the DNA insert had the correct orientation, and it was confirmed that a polypeptide of the expected size (74 kDa) was produced on induction. This plasmid was named pLH2d; its structure is shown in Fig. 2.

The hybrid protein, GST-PCN, was purified by affinity. This is made possible by the affinity of the GST moiety for glutathione, which can be immobilized on agarose beads. The protein can later be recovered from the washed beads by elution at neutral pH with free reduced glutathione. The procedure used was adapted from that of Smith et al. (28). To prepare fusion protein, an overnight culture of MM38, freshly transformed with pLH2d and grown with ampicillin, was diluted 10-fold into 2 liters of LB with ampicillin, and isopropylthiogalactoside (IPTG) was added to 0.2 mM. Incubation was continued for 2 to 4 h at 37°C with shaking. Cells were collected by centrifugation and resuspended in ice-cold phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM $NaHPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4). Cells were lysed by using an MSE probe sonicator (10-mm-diameter probe, 30 to 60 s). Triton X-100 was added to 1%, and the mixture was centrifuged at 10,000 × *g* for 5 min to remove insoluble material. The supernatant was mixed with 1 ml of a 50% slurry of glutathione-agarose beads (purchased from Sigma) and mixed gently for 2 min at room temperature. The beads were washed three times with 50 ml of PBS, suspended finally in 1.2 ml of PBS, transferred to a small tube, and centrifuged to collect the beads. The protein was eluted by adding 10 mM reduced glutathione in 1 ml of 50 mM Tris HCl (pH 8.0), mixing gently for 2 min, and collecting the supernatant by centrifugation. Elution was repeated several times; the protein content of each fraction was determined by A_{280}, and purity was verified by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Antibody preparation. Antibody was prepared by adapting methods described elsewhere (4, 9). Three hundred micrograms of purified GST-PCN was mixed with 3.5 ml of Freund's complete adjuvant in a total volume of 9 ml (0.12% NaCl, 0.1% Tween, 0.1% PBS); 5 ml was used for subcutaneous inoculation (rabbit 1), and 3 ml was used for intramuscular inoculation (rabbit 2). Final antiserum titers were the same for both animals. Boosts were given at 40 and 80 days and consisted of a mixture of 250 μg of protein, 0.5 ml of

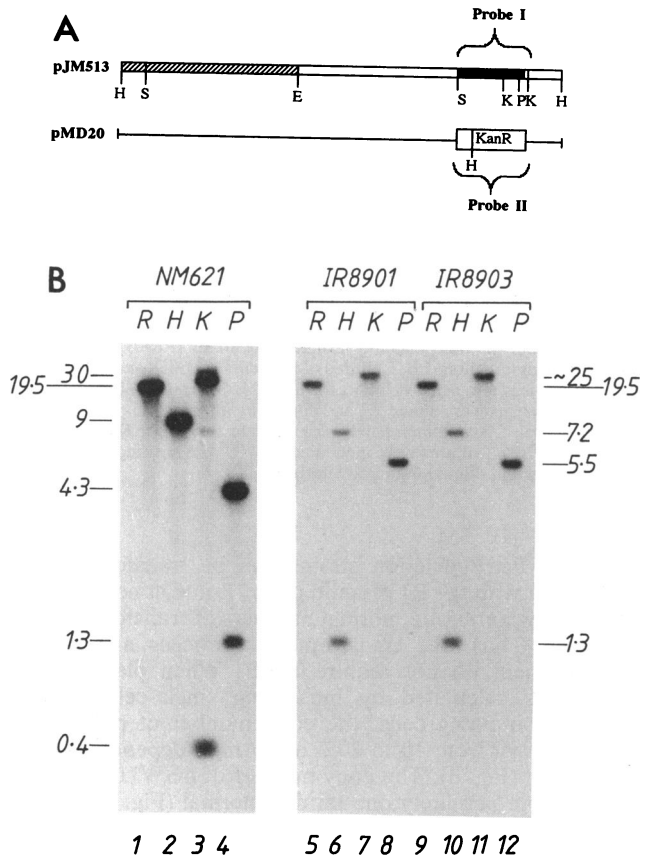

FIG. 1. Construction and verification of *pcnB* deletion strains. (A) Diagram of pJM513 (15) showing selected restriction endonuclease sites (H, *Hin*dIII; S, *Sph*I; E, *Eco*RI; K, *Kpn*I; P, *Pst*I). pMD20 is identical to pJM513 except that the DNA bracketed as probe I has been replaced by the DNA bracketed as probe II. The *Sph*I and *Kpn*I sites at the ends of probe I are not present on pMD20. Construction was as described in Materials and Methods. pJM513 is 8.9 kb long; the drawing is to scale. (▨) indicates vector sequence. The remainder of pJM513 is host sequence, with *pcnB* DNA shaded. The Kan^r cassette DNA in pMD20 is boxed. (B) Southern hybridizations to verify *pcnB* deletions from IR8901 and IR8903. DNA prepared from NM621, IR8901, and IR8903 was cut with the enzymes indicated (R, *Eco*RI; H, K, and P as defined above); fragments were separated on a single gel, transferred to a nylon membrane, and successively hybridized to probes I and II. Probe I hybridized to lanes 1 to 4 only, and probe II hybridized to lanes 5 to 12 only. Sizes are indicated in kilobases.

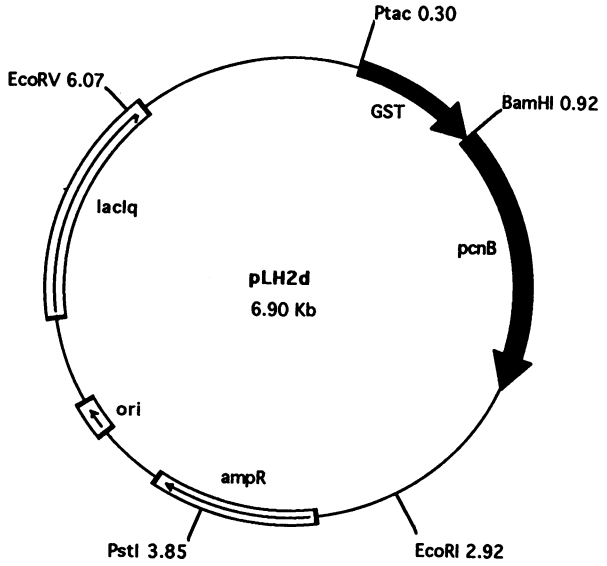

FIG. 2. Map of pLH2d. *pcnB* DNA was cloned into pGEX3X as described in the text. DNA encoding the fusion protein appears as a black bar. The *bla* gene (Amp^r) and *lac* repressor genes are indicated.

0.85% saline, 1.5 ml of Freund's incomplete adjuvant, and 1.5 ml of 1% Tween 80 in PBS in a total volume of 6 ml; 4 ml was used for rabbit 1, and 2 ml was used for rabbit 2. Blood was collected 9 or 10 days after inoculation, and serum was frozen in 0.2-ml aliquots at −70°C. Titers of antisera were measured by using ^{125}I-labeled anti-rabbit immunoglobulin G. GST-PCN was bound to etched polystyrene beads (Cell Culture Products Ltd.) by immersing the beads in a 50-μg/ml solution of antigen overnight in coating buffer (0.05 M $NaCO_3$ [pH 9.6]). Beads were washed twice with PBS and incubated (overnight at room temperature) in blocking buffer (3% bovine serum albumin [BSA] in PBS). Beads were again washed twice in PBS, incubated for 1 h at 37°C with serum, and washed four times with PBS. Each sample (one bead) was incubated with ^{125}I-labeled anti-rabbit immunoglobulin G (50,000 cpm) at 37°C for 1 h and washed four times with PBS. ^{125}I was measured in an LKB gamma counter.

Immunoblots. Proteins were first separated on SDS–10% polyacrylamide gels. For whole cell samples, 200 μl of overnight or induced cultures was spun down, and pellets were resuspended in 30 μl of 1× loading buffer (12). Samples were boiled for 3 min, and 10 to 15 μl was loaded into wells. Purified GST-PCN or purchased poly(A) polymerase was diluted into loading buffer and boiled for 2 min before loading. Pairs of gels were run together so that one could be stained with Coomassie blue and the second could be immunoblotted.

To carry out immunoblotting, SDS-polyacrylamide gels were transferred to BioBlot nitrocellulose blotting membranes by using a Bio-Rad Trans-Blot semidry transfer cell. The blotting buffer was 20 mM Tris–150 mM glycine. Membranes were blocked with 3% BSA in PBS, incubated with 100 μl of antiserum (preabsorbed with 500 μl of sonicated *pcnB* deletion strain extract) in 50 ml of PBS–1% BSA, and washed. Immune complexes were detected by adding 10 μl of anti-rabbit immunoglobulin G-alkaline phosphatase conjugate (Sigma) in 50 ml of PBS–1% BSA. For each step, membranes were incubated for 1 h at room temperature on a rotary shaker. Blots were developed as described previously (9).

RESULTS

Construction of a *pcnB* deletion strain. A strain from which the *pcnB* gene has been deleted was constructed by first replacing a plasmid-borne copy of *pcnB* with a Kan^r cassette (see Materials and Methods). This plasmid construct, pMD20, was cut with two restriction endonucleases such that the plasmid replication origin and the *bla* gene were contained on a fragment separate from that containing the cloned chromosomal DNA flanking the Kan^r cassette. The mixture was used to transform NM621 (*recD*), as homologous recombination between the chromosome and linear

fragments has been reported to be enhanced in *recD* strains (25). We expected, using this method, to achieve homologous replacement of the chromosomal *pcnB* gene with the Kan^r fragment. Colony hybridization was used to screen both Amp^r Kan^r and Amp^s Kan^r transformants for retention of *pcnB* DNA. We found, surprisingly, that the *pcnB* gene was absent only from transformants which had initially been Amp^r. This result suggests that the deletants may have arisen by resolution of duplications created by integration into the chromosome of intact plasmid molecules that had failed to be cut, rather than by the double crossover expected.

DNA was extracted from, and Southern hybridization was performed on, two putative *pcnB*-deleted progeny strains, IR8901 and IR8903, using as probes the DNA which had been replaced by the Kan^r cassette and the cassette DNA itself (Fig. 1). No hybridization occurred between the *pcnB* probe and the putative deletion strain DNA, verifying that the *pcnB* DNA had indeed been deleted. The sizes of parental DNA fragments that hybridized to the *pcnB* probe were consistent with the Kohara map predictions for three of the four enzymes tested. *Kpn*I digests contained a small extra fragment, supporting previous conclusions that the *Kpn*I site at KB160 is in fact a doublet. Hybridizations of the Kan^r cassette DNA probe with deletion strain DNA showed loss of the *Pst*I and *Kpn*I sites within *pcnB* as anticipated. The new *Hin*dIII bands result from cutting at a site within the Kan^r cassette.

P1 lysates were prepared from IR8901 and IR8903 and used in cotransductional crosses to show genetically that the Kan^r marker was indeed located at 3.5 min, at the *pcnB* locus. When Mri84, in which Tn*10* is closely linked to *pcnB*, was transduced with these lysates, approximately 98% of Kan^r progeny became Tet^s, consistent with the close linkage that would be expected if the Kan^r cassette were at the position of *pcnB*. Similarly, Kan^r YA139 progeny were 73% Pan$^+$, confirming the degree of linkage previously reported for *pcnB* and *pan*.

PcnB deletion produces little change of phenotype in plasmid-free cells. P1 lysates prepared on IR8901 were used to transduce Kan^r to several recipient strains that were not closely related. The PcnB$^-$ phenotype of transductants was confirmed by demonstrating reduced plasmid copy number (see below). No other phenotype could be shown to be consistently associated with the lack of PcnB during growth on broth. Isogenic pairs grow at the same rates at 30, 37, and 42°C and reach the same maximum optical densities. Cells are of the same size in otherwise isogenic pairs and are equally UV resistant. Viabilities, both during exponential growth and after 5 days in standing stationary culture, are similar. However, some isolates of MM38K (the suffix K will be used to designate Kan^r deletion strains) formed colonies of reduced size. Furthermore, when MM38 and MM38K or Mri84 and Mri84K were mixed and cocultured in LB and the ratio of the two strains was measured at intervals (over 50 generations), *pcnB*$^+$ cells eventually came to dominate the cultures (Table 2). These data indicate that *pcnB* strains can have a growth disadvantage; this disadvantage is more marked in fresh transductants, suggesting the later accumulation of ameliorating mutations. We have not eliminated the possibility that the growth disadvantage exhibited in coculture is due to disruption of the DNA sequence at the *pcnB* locus, which could affect downstream transcription, or indeed to the addition of the Kan^r cassette, rather than to the absence of PcnB product.

PcnB is not essential for replication of ColE1- or R1-related plasmids. PcnB deletion strains can be transformed with pBR325 or with the R1 plasmid pGW71, and at normal levels of selecting antibiotic, normal numbers of transformants are obtained. Thus, the transformation process and plasmid establishment do not require PcnB. When plasmid copy number is calculated by measuring single-cell Amp^r of plasmid-containing cells, the copy number of pBR325 appears to vary from 10 to 20% of normal, depending on the host strain (Fig. 3). The copy number of pGW71 is reduced less, to approximately one-third of normal (Fig. 4). In each case, copy number in the deletion strain is lower than in the corresponding point mutant *pcnB21* or *pcnB80*; thus, these point mutants retain some *pcnB* function. The fact that plasmid replication continues in the absence of any PcnB product confirms that PcnB is not positively required for plasmid replication but is consistent with its proposed role as a modulator of copy number control.

TABLE 2. Competition between *pcnB*$^+$ and *pcnB* deletion strains[a]

Cycles of coculture	% *pcnB* deletion in mixture			
	MM38		Mri84	
	MM38K24	MM38K26	Mri84K24	Mri84K26
0	38	56	92	92
1	53	12	73	82
2	56	1	47	53
3	4	1	19	45

[a] Six strains, MM38, MM38K24, MM38K26, Mri84, Mri84K24, and Mri84K26 (where K denotes a *pcnB* deletion and 24 or 26 indicates that it originates from the independently isolated IR8901 or IR8903, respectively), were grown overnight in LB. Cultures of the *pcnB*$^+$ (MM38 and Mri84) and of each of the isogenic *pcnB* deletion (MM38K24, MM38K26, Mri84K24, and Mri84K26) strains were mixed 1:1 and diluted by a factor of 5×10^4. Aliquots were plated on LB, and individual colonies were scored for Kan^r (deletions) or Kan^s (*pcnB*$^+$). Cultures were incubated at 37°C with shaking overnight and then sampled and diluted for regrowth daily.

PcnB is not required for maintenance of plasmid F or P1. The large plasmids F and P1 are strictly maintained at a copy number of about one per chromosome by an incompletely understood mechanism involving iterated DNA and proteins which bind to these repeated sequences (22). No antisense RNA has been implicated in this process, and we would therefore not expect PcnB to be required for F or P1 plasmid maintenance. In an initial experiment, MM38 and MM38K were mated with NK7968 or NK7970, and Nal^r Cmp^r progeny were selected. Numbers of progeny per donor or per recipient were similar in all crosses, suggesting that initial establishment of F plasmid is not dependent on PcnB. It is possible, however, that continued maintenance of F plasmid could involve PcnB but that this is not apparent in transfer proficient F′ strains, as repeated conjugational transfer could replace lost plasmid, and the F-plasmid-encoded *par* and *ccd* systems maximize the numbers of plasmid-containing daughters and ensure that only plasmid-containing cells survive.

To eliminate these possibilities, we obtained miniplasmid derivatives of both F and P1 plasmids which, as well as lacking the ability to transfer (or infect), lacked either the postsegregational killing (*ccd*) or partitioning (*par*) system or both. We then compared the maintenance of these F and P1 derivatives in wild-type and *pcnB*-deleted hosts. The mini-F plasmid pXX325 is derived from pSC138 and lacks the F plasmid *ccd* system. pXX327 lacks the partitioning system (*par*) in addition. Copy numbers of these plasmids in MM38 and MM38K were compared by measuring the single-cell

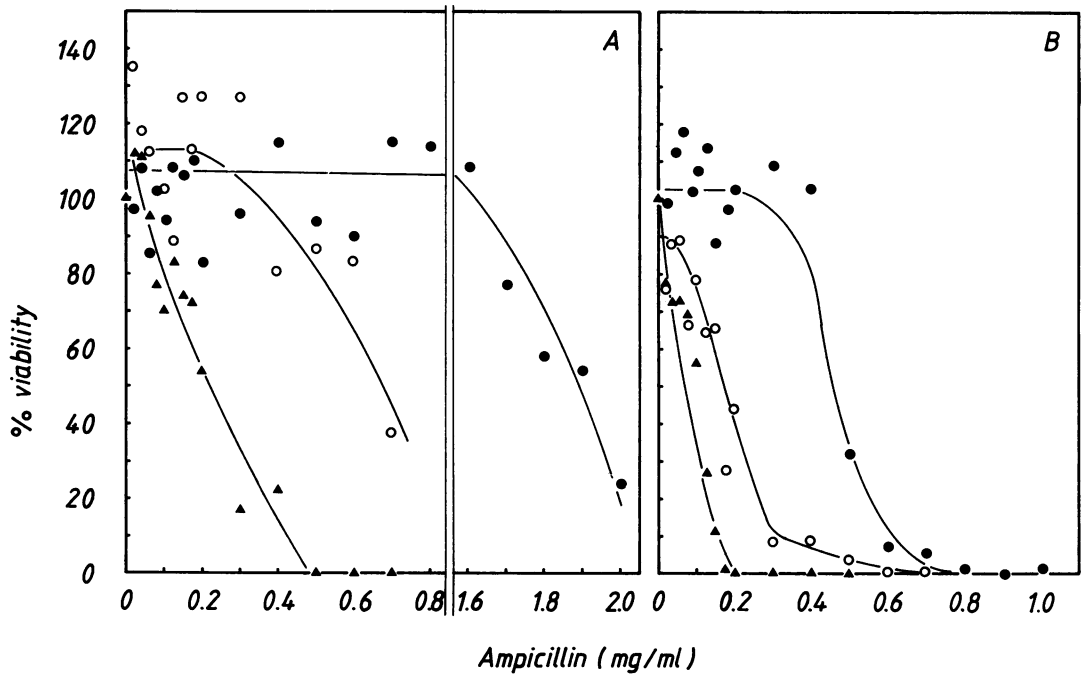

FIG. 3. Single-cell Amp^r of pBR325 in *pcnB* strains. Cultures were grown overnight in LB with ampicillin (50 μg/ml), diluted in broth so as to obtain 200 colonies per plate on antibiotic-free plates, and plated on LB containing the indicated concentrations of ampicillin as described previously (15). Points for MM38K and Mri84K are the averages of two independent experiments. (A) MM38 (*pcnB*$^+$) (●), JM38 (*pcnB21*) (○), and MM38K (Δ*pcnB*) (▲); (B) Mri84 (*pcnB*$^+$) (●), Mri93 (*pcnB80*) (○), and Mri84 (Δ*pcnB*) (▲).

Amp^r conferred by the plasmids to their hosts. This is proportional to plasmid copy number. The data in Table 3 show that the *pcnB*$^+$ and *pcnB* strains are equally Amp^r. Copy number was also determined by comparing plasmid stability, measured as the rate of plasmid loss during growth in the absence of selection (Fig. 5). pSC138 (*par*$^+$ *ccd*$^+$) is stably maintained in both *pcnB*$^+$ and *pcnB* derivatives of MM38 as would be anticipated for a *par*$^+$ *ccd*$^+$ plasmid, even if the replication rate were reduced. This is because inhibiting replication of *ccd*$^+$ plasmids, such as pSC138, activates postsegregational killing systems, leading to death of plasmid-free segregants. The result is an apparent increase in generation time of cultures in which this is occurring, rather than plasmid loss. MM38 and MM38K harboring pSC138, however, grow with equal generation times, suggesting that *ccd* function is not differentially activated in the two types of host.

Curing rate of the Δ*ccd* plasmids from the *pcn*$^+$ and *pcn*-deleted strains was also measured in order to compare copy numbers. For pXX325, four fresh transformants of each strain were monitored for up to 60 generations. For

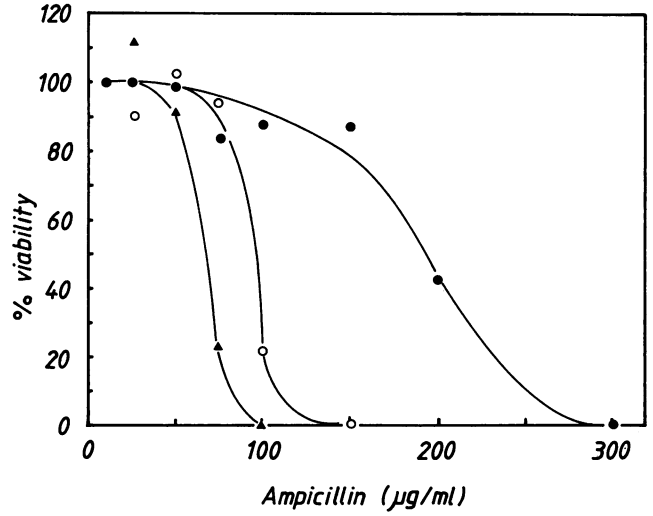

FIG. 4. Single-cell Amp^r of pGW71 in *pcnB* strains. Overnight cultures of MM38 (●), JM38 (○), and MM38K (▲) were prepared and plated as for Fig. 3. See the legend to Fig. 3 for *pcn* genotypes of these strains.

TABLE 3. Ampicillin resistance of *pcnB*$^+$ and *pcnB* deletion strains containing mini-F plasmids[a]

Plasmid	No. of colonies at ampicillin concn (μg/ml) of:					*pcnB* genotype
	10	15	20	30	50	
pSC138	105	49	14	15	7	+
	154	140	40	40	12	Deletion
pXX325	101	89	4	3	0.1	+
	138	65	40	26	31	Deletion
	94	80	40	43	42	Deletion
	116	93	49	5	<0.1	Deletion
pXX327	30	27	<0.1	0.5	<0.1	+
	21	9	3	4	0.7	Deletion

[a] Fresh transformants of MM38 or MM38K were grown overnight in LB-ampicillin (10 μg/ml), diluted appropriately, and spread on LB plates containing ampicillin at the concentrations indicated. The numbers of colonies on plates without ampicillin (between 150 and 2,000) were defined as 100; the figures in each horizontal row are percentages of these initial values.

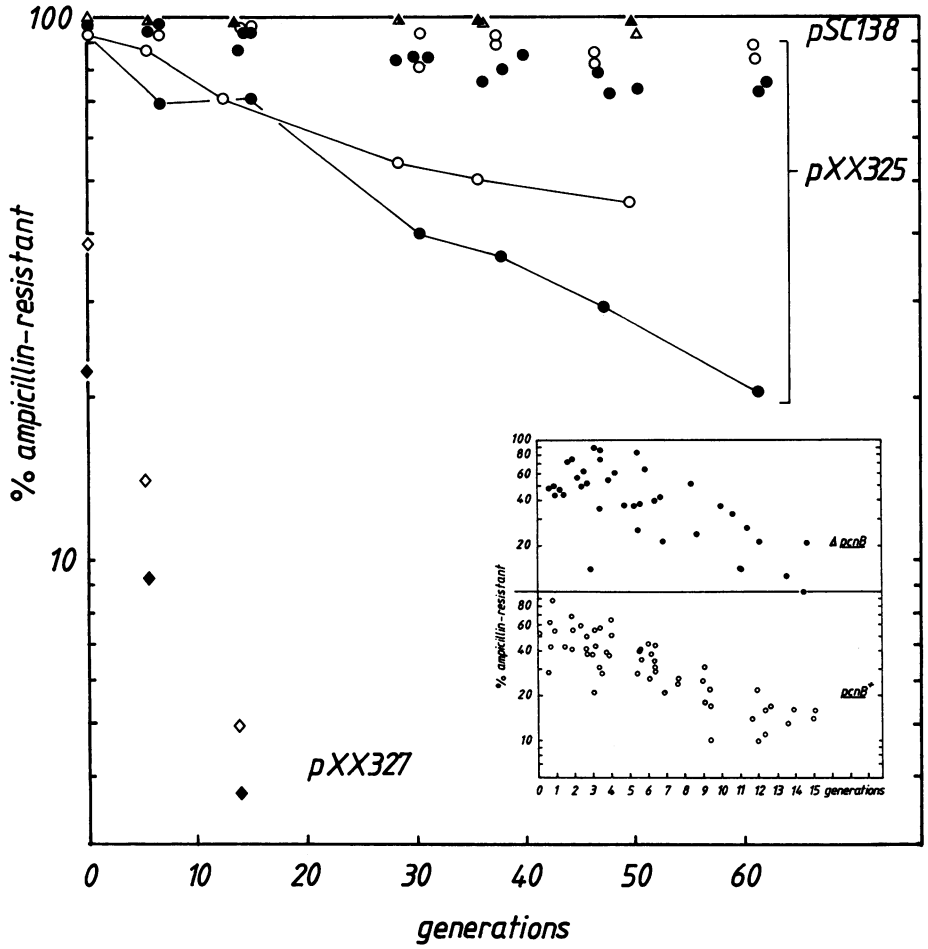

FIG. 5. Curing curves for F plasmids. Overnight cultures of plasmids were grown in LB with ampicillin (10 μg/ml) and diluted 250 times into LB. After 2 h, optical density at 540 nm was monitored to measure generation time. Cultures were maintained at optical densities below 0.35 by dilution into prewarmed medium. Overnight cultures were diluted 500 times for growth on successive days. Samples were taken at intervals, and dilutions were plated on LB; 100 colonies were picked and patched with and without ampicillin for each point. Results are averages of several experiments, each using independently isolated transformants. Open symbols, MM38 (*pcnB*$^+$); closed symbols, MM38K (Δ*pcnB*). The inset shows a measurement of pXX327 curing over a shorter time scale.

each strain, three of the four transformants behaved identically, with approximately 70% of cells retaining plasmid for 50 to 60 generations in the absence of selection. For each host, the remaining strain showed accelerated plasmid loss (20% plasmid retention in MM38 and 37% in MM38K); we have no explanation for this heterogeneity. pXX327 was highly unstable (Fig. 5, inset) but was, again, lost at the same rate from each strain. The P1 miniplasmid pAX274 and its Par$^-$ derivative pAX275 behaved similarly (Fig. 6). We can conclude from these data that PcnB is not required for F or P1 plasmid replication or maintenance.

***E. coli* poly(A) polymerase reacts with anti-PcnB antibody.** It has recently been found that PcnB has poly(A) polymerase activity (3). Poly(A) polymerase purified from *E. coli* according to a procedure described by Sippel in 1973 (27) is commercially available from several suppliers. We prepared an anti-PcnB antiserum and find that this antiserum, after preabsorption against MM38K to increase its specificity, reacts with the principal, 50-kDa component of one of these commercially available *E. coli* poly(A) polymerases as well as with PcnB and a PcnB fusion protein (Fig. 7). Since the product encoded by *pcnB* is also 50 kDa in size (3, 13, 15), it appears highly likely that PcnB is identical to the 50-kDa poly(A) polymerase described by Sippel in 1973 (27).

DISCUSSION

We report here experiments with strains from which we have deleted the chromosomal *pcnB* gene. Deleted strains are phenotypically similar to their *pcnB*$^+$ parents, demonstrating that the gene product plays no essential role in the host, although fresh transductants to *pcnB* cannot successfully compete with their *pcnB*$^+$ sisters. Our conclusions differ from those reached by Liu and Parkinson (13), who found that a strain which harbored a *pcnB* gene with an internal inversion grew poorly. The effects of this mutation were recessive, causing these authors to conclude that they were due to the absence of the PcnB protein rather than to any abnormal product which might have been produced or to

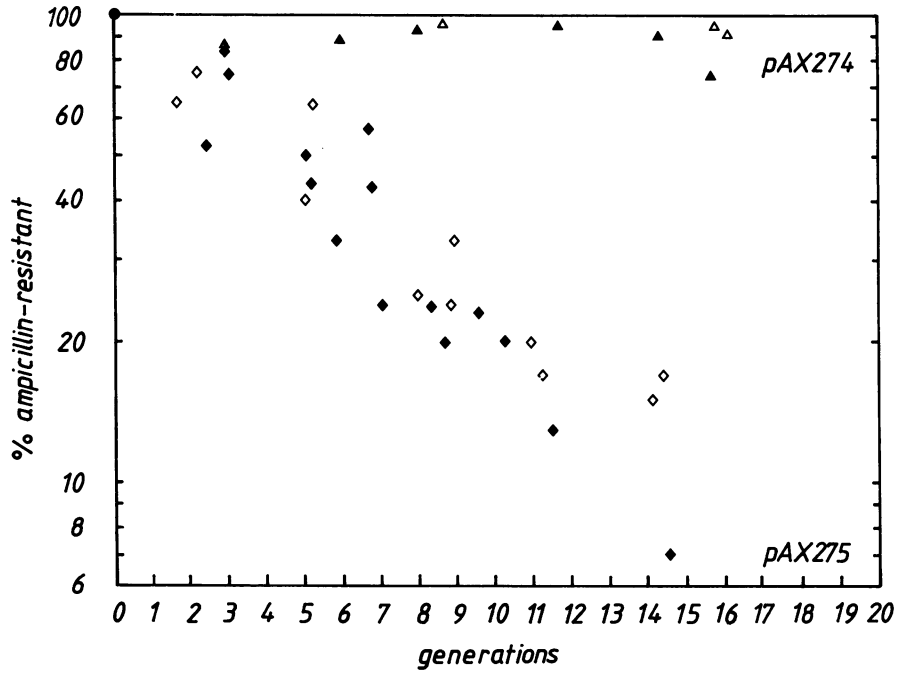

FIG. 6. Curing curve of P1 plasmids. The procedure was as for Fig. 5. Open symbols, MM38 (*pcnB*$^+$); closed symbols, MM38K (Δ*pcnB*).

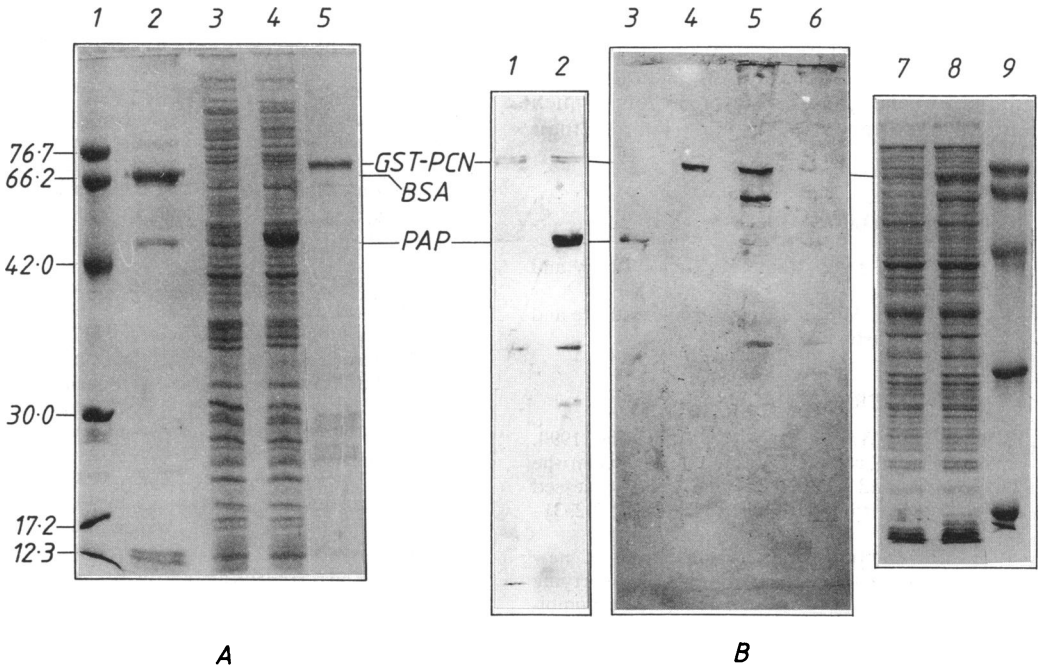

FIG. 7. Evidence that PcnB is immunologically related to poly(A) polymerase (PAP). (A) SDS–10% polyacrylamide gel stained with Coomassie blue. Lanes: 1, size markers; 2, poly(A) polymerase purchased from Pharmacia Ltd.; 3 and 4, MZ1pRE1-1 at 30°C (lane 3) and after 1 h at 39°C (lane 4); 5, purified GST-PCN fusion protein. Lanes 1 to 5 are from a single gel. Lanes 1 and 2 in panel B are from an identical gel run simultaneously. (B) Lanes: 1 and 2, immunoblot of lanes 3 and 4 of panel A, using antiserum prepared against GST-PCN as described in Materials and Methods; 3, Pharmacia poly(A) polymerase; 4, purified fusion protein; 5 and 6, extracts of MM38pLH2d grown with (lane 5) and without (lane 6) 0.2 mM IPTG; 7 and 8, stained samples of MM38pLH2d grown without (lane 7) and with (lane 8) IPTG. Sizes are indicated in kilodaltons. The intense band in panel A, lane 2, is BSA added by the manufacturer of the commercial product. The bands at 75 kDa in lanes 1 and 2 and at 35 kDa in lanes 1, 2, and 5 of panel B contain unidentified cross-reacting material.

effects on possible downstream genes. These conclusions will need to be reassessed in the light of the results reported here; perhaps strain differences are responsible.

PcnB-deleted strains maintain ColE1-related plasmids at greatly reduced copy number, leading to plasmid instability. In contrast to the unpublished observations of Liu and Parkinson (cited in reference 13), we find that both F and P1 plasmids and their partition-defective derivatives are maintained equally well in *pcnB*$^+$ and deleted hosts, indicating that the *pcnB* product is not involved in maintenance of these plasmids.

PcnB appears likely to be the 50-kDa *E. coli* poly(A) polymerase. If this is the only enzymatic activity which the PcnB protein possesses, it is necessary to reconcile this activity with its role in plasmid copy number maintenance. If RNAI is the substrate for PcnB, polyadenylated RNAI might be unable to bind RNAII; alternatively, it might have increased susceptibility to degradation. Either of these alternatives would account for the phenotype of *pcnB* mutants. Work in progress in our laboratory (10) suggests that the latter is the case.

PcnB can be added to the diverse group of host proteins co-opted by plasmids for a role in their replication or maintenance. Many such co-opted proteins appear to provide an activity distinct from that used in host cell metabolism or one of more critical importance to plasmid survival than to the cellular economy. The polymerizing activity of DNA polymerase I is normally required for ColE1 replication yet can be dispensed with by the host (11). XerA and XerB, each of which participates in the resolution of plasmid multimers and contributes to the stable maintenance of ColE1, appear to have very different roles in the host (30, 31). RNase H, an enzyme which prevents the formation of undesirable RNA-DNA hybrids, is required for ColE1 replication in vitro (20) but does not appear to be essential for host viability and growth (18). PcnB thus appears to be the most recent addition to the growing list of proteins which are of more critical importance to plasmids than to their hosts.

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