SHORT COMMUNICATION

Deletion Analysis of Essential Genes of *Escherichia coli*: Investigation of the *btuB-rpoBC* Interval

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A method is described for deletion mapping of essential genes in *Escherichia coli*. It involves the isolation of secondary-site insertions of λ cI857 $plac5$ into an F' plasmid. The transposed *lacZ* gene is useful both for the ready screening of plasmid-phage cointegrates and for rapid analysis of deletions that extend from the site of phage integration into the bacterial genes carried on the substituted plasmid. Such deletions may be used to 'hook-up' bacterial cistrons to the powerful *lac* promoter. We report the application of this technique to the study of the *btuB-rpoBC* interval, a cluster of genes encoding components of the transcription-translation apparatus.

INTRODUCTION

The isolation of transducing phages carrying bacterial sequences, and the use of cloning procedures have proved extremely useful for the deletion analysis of indispensable cistrons (see, for example, Fiil *et al.*, 1980; Newman & Hayward, 1980; Nomura & Post, 1979; Scaife *et al.*, 1980). Our approach to deletion analysis of essential genetic material has been to obtain secondary-site insertions of λ cI857 $plac5$ in an F' plasmid. The isolation of F' plasmid- λ cI857- $plac5$ cointegrates is made feasible by the ease of screening extra-chromosomal material. Moreover, the presence of the prophage *lacZ* gene both circumvents the necessity of selecting for insertional inactivation and aids the determination of deletion endpoints. We report the application of this method to the *btuB-rpoBC* interval (89.1 to 89.5 min), a region rich in genes encoding components of the transcription-translation apparatus (Bachmann & Low, 1980).

METHODS

Bacterial and phage strains. The starting strain for the isolation of plasmid- λ cointegrates, AJ249, was constructed from AJ217, a spontaneous Mal⁺ derivative of AJ7 [*argG metB lacZ53*(Am) *mal*(λ -r) *recA1 rpsL*(Str-r) *rpoB70*(Rif-r) (Boyd *et al.*, 1974)]. The following markers were first introduced into AJ217 by conjugation: Δ (*gal-att⁺-bio*) from KS302 [*thi* Δ (*gal-att⁺-bio*) HfrH (Shimada *et al.*, 1972)]; Δ (*lac-pro*)XIII from CA9005 [*thi* Δ (*proAB-lac*)XIII *tsx* HfrH]; and *recA56* from JC5088 (*ilv thr recA56 spc* Hfr KL16). A spontaneous phage BF23- and colicin E3-resistant derivative (*btuB*) was then obtained. Finally, the F' plasmid, F110, carrying the *metB-malB* region, was introduced from AJ39 (AJ7/F110). λ amber derivatives were kindly supplied by M. E. Gottesman and Noreen Murray; λ cI90c17 was obtained from R. Buxton.

Media and general methods. Minimal media were prepared with Meynell salts (Meynell & Meynell, 1970) supplemented with 0.2% (w/v) glucose or lactose, and amino acids at 20 μ g ml⁻¹. 5-Bromo-4-chloro-3-indolyl- β -D-galactoside (40 μ g ml⁻¹) and isopropyl- β -D-thiogalactopyranoside (10⁻³ M) were included in Lac indicator plates. Where necessary, rifampicin was added to a final concentration of 200 μ g ml⁻¹. Complex medium was 2.5% Oxoid nutrient broth no. 2; this was supplemented for phage λ work with 0.01 M-MgSO₄ and 0.2% maltose. Phage lysates were prepared by the standard plate technique (Miller, 1972). Colicin E3 extracts were obtained by a salt extraction procedure (Hunter & Glass, 1982).

Isolation of cointegrates of F110 and λ cI857 $plac5$. Starved AJ249 cells were treated with first λ cI857 $plac5$ and then λ h80c (Shimada *et al.*, 1972). Lysogens were isolated on minimal glucose plates at 30 °C. They were screened

for resistance to phage BF23 (and colicin E3) and rifampicin by dilution replica-plating to avoid false positives (Hunter & Glass, 1982). Met⁺ transconjugants of AJ252 (AJ7 *btuB* λ cI857) were tested for resistance against phage BF23/colicin E3 and rifampicin. Positive clones were checked for their ability to produce β -galactosidase on minimal Lac indicator plates. In no case was direct selection used to obtain cointegrates: putative *btuB* :: λ and *rpoB* :: λ insertions were obtained from the original lysogen plate.

Properties of cointegrates. Spontaneous and heat-pulse curing frequencies and prophage number were determined as described by Shimada *et al.* (1972). Revertants that carry a functional *rpoB* or *btuB* allele on the F' were selected from among a population of Ts⁺ Rec⁻ survivors. *rpoB*⁺ revertants were selected in AJ5022 [AJ7 *rpoB*12(Am)F100-13 *supE* (Nene & Glass, 1981)] on minimal Lac indicator plates; transconjugants of AJ5022 that carry F110*rpoB*⁺ should be Lac⁻. In the case of the *btuB* :: λ insertions, a *metE btuB recA* recipient [KL3000, *leu-6 metE70 proC32 lacI3 lacZ118*(Oc) *malA*(λ -r) *ara-14 mtl-1 xyl-5 thi-1 recA1 str-109 spc-15 gyrA btuB supD* (Birge & Low, 1974)] was employed since a functional *btuB* allele is necessary to enable the auxotroph to utilize exogenous vitamin B₁₂ as a co-factor for methionine biosynthesis. Ts⁺ survivors of *btuB* insertion derivatives were obtained in *recA btuB* and *recA*⁺ Δ (*btuB*) backgrounds; they were screened for sensitivity to colicin E3. The extent of loss of bacterial sequences carried on F110 was determined by complementation in KA2005 [*argE metB thr trp btuB lac rpoB*(Rif-r) *purH/D recA* (Armstrong & Herman, 1976)]: deletions of prophage material were detected by making use of λ amber derivatives (Shimada *et al.*, 1972).

RESULTS AND DISCUSSION

In the absence of a functional attachment site (Δatt^A), phage λ integrates at secondary sites that show some homology with the normal region (Shimada *et al.*, 1972, 1973). We have used this property to obtain stable secondary-site lysogens in which the prophage is present on an F' plasmid rather than the chromosome. The substituted plasmid employed in this study, F110, spans the *metB*-*malB* region (about 105 Mdal, data not shown).

Cointegrates of F110 and λ cI857*plac5* were identified from among homo-immune lysogens capable of complementing a *metB* Δlac λ ⁺ recipient (AJ252) in a genetic cross. [Zygotic induction, the killing of non-lysogenic recipients upon λ transfer, is not required for detecting the F110 :: λ insertions.] They were screened for insertion at *btuB* and *rpoB*, the genes for the B₁₂ multivalent receptor and the β subunit of RNA polymerase. Insertional inactivation of *btuB*⁺ (in a *btuB*⁺/*btuB*⁻ merodiploid) renders the secondary lysogen resistant to phage BF23 and E colicins (and unable to make use of the B₁₂-dependent homocysteine transmethylase: Jasper *et al.*, 1972; Kadner & Liggins, 1973). Similarly, integration of λ cI857*plac5* into the dominant, rifampicin-sensitive allele, *rpoB*⁺, on F110 allows growth in the presence of the antibiotic.

We have obtained a number of independent insertions at *btuB* and *rpoB*; their respective position is supported by deletion analysis (see below). The division of these lysogens into three main types according to their properties (Table 1) suggests that there are at least two secondary sites for λ cI857*plac5* in *btuB*: only a single class of *rpoB* insertions was found. Krueger *et al.* (1981) have recently reported that there are several insertion sites for λ cI857 in the *btuB* region.

Starting with two sets of cointegrates, *btuB* :: λ cI857*plac5* and *rpoB* :: λ cI857*plac5*, we have obtained a large number of different deletions that span the *btuB*-*rpoBC* region. This is made possible by the presence of the thermolabile phage repressor (the *cI857* gene product) and the fact that survivors carrying the *recA56* allele invariably lack both prophage and bacterial material (see Table 1). Loss of bacterial markers is readily ascertained by transfer of the F' deletion derivatives to KA2005 [*argE btuB lac rpoB*(Rif-r) *purH/D recA*]. The prophage *lacZ* gene is useful in determining deletion endpoints.

The results for deletions generated spontaneously and after heat-pulse curing in three independently isolated *btuB* and *rpoB* insertions are summarized in Table 2. Loss of adjacent bacterial DNA as well as removal of phage functions (data not shown), in conjunction with the known orientation of the prophage *lacZ* gene (Malamy *et al.*, 1972), allows an unambiguous positioning of the phage insertions. It can be seen that the transducing phage has apparently integrated in the same manner at the two loci (namely, *btuB*'-*lacPOZ*-L-*cI*'-*btuB* and *rpoB*'-*lacPOZ*-L-*cI*'-*rpoB*).

The plasmid- λ cointegrates have allowed the study of both spontaneous and phage-promoted deletions of a portion of the bacterial chromosome essential for cellular survival (Table 2). λ -dependent events remove, invariably, shorter regions of bacterial DNA. These events seem, as

Table 1. *Properties of secondary-site λ C1857plac5 lysogens*

Insertion site*	Plasmid class†	Curing frequency‡		Burst size	λ C190c17 test§
		Spontaneous	Heat-pulse		
F110 <i>btuB</i>	pQN10	4.6×10^{-6}	2.4×10^{-3}	0.001	sensitive
	pQN11	2.3×10^{-5}	2.4×10^{-3}	69	sensitive
F110 <i>rpoB</i>	pQN22	6.8×10^{-7}	1.9×10^{-2}	0.4	sensitive

* A total of 10835 secondary-site lysogens (of AJ249) were obtained at a frequency of 4.6×10^{-3} . Of these, 0.028% carried the phage integrated into *rpoB* on F110 and 1.72% (of 1048 lysogens screened) carried insertions into *btuB*.

† The properties of strains carrying F110 *btuB* : λ C1857plac5 and F110 *rpoB* : λ C1857plac5 (burst size, λ C190c17-sensitivity, and deletion profiles; Table 2) suggest that there are three main insertion sites, two in *btuB* and one in *rpoB*. Results for representative plasmid derivatives are given.

‡ Although heat-pulse curing is an effective method for obtaining λ^- derivatives, curing rarely stems from exact excision in a *recA* background. We have been unable to isolate revertants encoding the wild-type function, despite positive selection (*btuB*⁺ is regenerated in a *recA*⁺ strain). Thus, all Ts⁺ survivors obtained in a *recA* background carry extensive deletions (Table 2). This is consistent with the observation that most *recA* mutants do not support spontaneous induction (Roberts & Roberts, 1981).

§ In early studies on *recA*⁺ strains (Shimada *et al.*, 1972), there appeared to be two classes of secondary lysogens: those giving burst sizes > 100 (putative polylysogens), and those with a burst size ≤ 0.1 (possible single lysogens). [In the case of single insertions into the R plasmid, R100, burst sizes were near to, or less than unity (Dempsey & Willetts, 1976)]. Although burst-size data suggest that the F110 : λ C1857plac5 cointegrates, pQN11 and pQN22, are multiple (tandem) lysogens, we have been unable to substantiate polylysogeny using λ C190c17, a phage that can plate upon single but not multiple lysogens. The lack of correlation between burst size and λ C190c17 sensitivity presumably reflects the low copy number of F110 (the wild-type F' appears to be present in about 0.5 copies per chromosome: Hayward *et al.*, 1974); copy number may decrease with plasmid size (Pritchard, 1978).

Table 2. *Deletion profile for *btuB* : λ C1857plac5 and *rpoB* : λ C1857plac5 secondary-site insertions*

Extent of deletion						Proportion of survivors*					
						<i>btuB</i> insertion		<i>rpoB</i> insertion			
						pQN10		pQN11		pQN20	
<i>arg</i>	<i>btuB</i>	<i>lac</i>	λ C1	<i>rpoB</i>	<i>purD</i>	S	HP	S	HP	S	HP
—	—	—	—	—	—	0.2	4.4	—	1.8	—	—
—	—	—	—	—	—	0.4	9.5	4.3	60.0	—	—
—	—	—	—	—	—	—	—	84.2	0.4	—	—
—	—	—	—	—	—	3.5	82.8	3.5	6.4	—	—
—	—	—	—	—	—	3.5	3.2	1.1	30.0	—	—
—	—	—	—	—	—	92.4	0.1	6.9	1.4	—	—
—	—	—	—	†	—	—	—	—	—	0.6	1.8
—	—	—	—	†	—	—	—	—	—	38.9	98.2
—	—	—	—	†	—	—	—	—	—	37.8	—
—	—	—	—	†	—	—	—	—	—	22.7	—

* The two types of secondary-site insertions in *btuB* (see Table 1) are represented by plasmids pQN10 and pQN11; a total of 1125 spontaneously occurring deletions (430 for pQN10 and 695 for pQN11), and 1117 heat-pulse induced deletions (897 for pQN10 and 220 for pQN11) have been analysed. In the case of the *rpoB* insertion, a total of 643 spontaneously occurring deletions and 1324 heat-pulse induced deletions have been studied. For both *btuB* and *rpoB* secondary-site lysogens, heat-pulse induced deletions were consistently shorter. S, Spontaneously occurring; HP, heat-pulse induced.

† pQN20 deletions that remove λ C1 and *purD* should result in loss of the promoter-distal portion of *rpoB*: this was not tested for.

would be expected (Pfeifer *et al.*, 1974), to arise from specific processes. In the case of spontaneously generated deletions, there appears to be a 'hotspot' beyond *purD* since the majority of deletions have one endpoint that removes this locus (see also Linn *et al.*, 1979). The large number of deletions isolated are useful for fine structure analysis of the transcription-translation gene

cluster at 89 min. They can, for example, be transferred to *λrpoB* transducing phage (for instance, *λdrif^d18*: Kirschbaum & Konrad, 1973) as described by Linn *et al.* (1979). Furthermore, the insertion of *lacPOZ* adjacent to (or within) *rpoB* is a potential starting point for the *in vivo* construction of fusions placing transcription-translation genes under *lac* control (and vice versa).

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