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Cloning the *trpR* Gene*

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Summary. In *Escherichia coli*, the structural gene for purine nucleoside phosphorylase, *deoD*, is subject to insertional inactivation by prophage λ. From one such secondary site λ lysogen, strain SP265, one may isolate deletions that remove all or part of the *trpR* gene and other genes in the *deo-thr* sector of the *E. coli* chromosome. Specialized transducing phages harboring $serB^+$ and $trpR^+$ were liberated following induction of SP265. All such phages were N-defective, *bio*-type pseudolysogens whose DNA persisted in the form of plasmids. A collection of transducing phages, differing in their complement of bacterial DNA, was used to locate cleavage sites for *BamH*I, *Sal*I, and *Pvu*I within the *deoD-trpR* region of the *E. coli* genome. The *trpR* gene lies within a specific 950 base pair *BamH*I-*Pvu*I segment.

A 1250 base pair *BamH*I fragment carrying a functional *trpR* gene was cloned into the amplifiable plasmid pBR322. A single *Sal*I site in this fragment was shown to lie within the *trpR* gene.

In two situations where increased gene dosage might generate elevated amounts of Trp repressor (N-defective $trpR^+$ pseudolysogens and strains harboring pBR322 $trpR^+$ plasmids) neither tryptophan auxotrophy, enhanced sensitivity to DL-5-methyltryptophan, nor super repression of the tryptophan biosynthetic enzymes was observed.

Introduction

Work done during the past 15 years has confirmed and extended the transcriptional control model proposed by Jacob and Monod (1961) for the *lac*, λ and *trp* systems. Both the *lac* and λ repressor proteins have been purified and shown to bind specifically to their cognate operators (Gilbert and Müller-Hill, 1966; Ptashne, 1967). The complete sequences of both the Lac repressor protein and gene are known (Beyreuther et al. 1973; Farabaugh, 1978) as are those of λ repressor (Sauer and Anderegg, 1978; Sauer, 1978).

Analyses of the DNA binding parameters of the Lac and λ repressors have enhanced our understanding of the ways that gene expression is controlled by the binding of certain proteins to specific DNA sequences. Studies with chemically or mutationally altered Lac operator have defined arrays of base pairs important to the interaction of Lac repressor and operator (reviewed by Barkley and Bourgeois, 1978). Similar experiments (Ptashne et al., 1976); Walz et al. (1976) have shown that λ repressor binds cooperatively to three linked sites at both O_L and O_R. The binding of one mole of repressor to O_R inhibits transcription of the O and P genes but stimulates the synthesis of additional repressor (Walz et al., 1976).

Our understanding of Trp repressor-Trp operator interactions is neither as complete nor as detailed as that of the Lac or λ systems. Extensive purification of the Trp repressor protein has not been accomplished, probably because the repressor is present only at levels of 20–40 copies per cell (Rose and Yanofsky, 1974). Attempts at protein purification (Shimizu et al., 1973; Squires et al., 1973) have yielded preparations estimated to be only 1% pure after 500-fold purification. With such preparations tryptophan-dependent repression of in vitro Trp mRNA synthesis has been demonstrated and kinetic paramaters determined (Rose and Yanofsky, 1974). Squires et al. (1975) demonstrated that RNA polymerase and TRP repressor mutually exclude each other from binding to $trpO^+$ DNA and Bennett and Yanofsky (1976) showed that partially purified repressor protects Trp operator DNA from cleavage by the restriction endonuclease *Hpa* I.

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More detailed and definitive experiments in the *trpR* system require chemical characterization of the components. Our efforts have therefore, been directed toward the isolation of λ specialized transducing phages carrying the *trpR* gene and recombinant molecules bearing short restriction fragments defining the limits of the *trpR* gene.

The availability of DNA preparations (either λ specialized transducing phages or plasmids bearing short restriction fragments) has simplified the analysis of many systems. Nomura and coworkers (Jaskunas et al., 1977), using both λ specialized transducing phages and recombinant plasmids, were able to map numerous ribosomal protein genes and were even able to match proteins with genes in which mutations have never been found. The gene product of *recA* was identified by in vitro transcription and translation of DNA enriched in *recA* gene sequences (McEntee et al., 1976). Thus, in addition to their well-established usefulness as specific probes of transcription, λ specialized transducing phages have facilitated the study of systems where assays of the gene products is difficult or mutations defining the cistrons are not available.

The isolation and characterization of DNA fragments bearing *trpR* sequences opens the way to studies of *trpR* expression and its relation to the control of the Trp biosynthetic system in a fashion analogous to the studies cited above.

Materials and Methods

1. Media. Cultures were grown in either L-broth (Lennox, 1955) or minimal E (Vogel and Bonner, 1956) supplemented with 0.2% glucose, 1 μg/ml thiamine, 0.1 μg/ml biotin and amino acids as required at 20 μg/ml. Solid media contained 1.5% Difco Bacto agar. T-agar, used to titrate λ lysates, is described by Miller (1972). Difco nutrient agar, supplemented with either ampicillin (25 μg/ml) or tetracycline (20 μg/ml) was used to score and select pBR322 markers.

2. Bacterial and Phage Strains. Strains used in this work are listed in Table 1.

3. Positioning of λ Near trpR by the Isolation of a Secondary-Site Lysogen. A gene cluster near *trpR* (Fig.1) codes for four enzymes involved in deoxynucleoside catabolism. The gene products of the *deo* cluster catalyze the following reactions: *deoD*, 2-deoxyadenosine + Pi → 2-dRib-1-P + adenine; *deoA*, thymidine + Pi → 2-dRib-1-P + thymine; *deoB*, 2dRib-1-P → 2-dRib-5-P; *deoC*, 2-dRib-5-P → acetaldehyde + glyceraldehyde-3-P. Since the accumulation of 2-dRib-5-P inhibits cell growth, *deoC* mutants are sensitive to the presence of deoxynucleosides. A mutation earlier in the pathway that blocks the production of 2-dRib-5-P overcomes this sensitivity (Lomax and Greenberg, 1968). Thus, *deoD, deoC* double mutants are resistant to 2-deoxyadenosine but sensitive to thymidine.

We first isolated a *thyA* derivative of KS302 by screening a set of trimethoprim resistant isolates for those requiring thymine (50 μg/ml) for growth. An additional mutation *(deoC)* that lowered the thymine requirement to 1–2 μg/ml was obtained after mutagenesis with ICR-191. This strain, KS302*deoC*, was made *thyA*$^+$ by Plkc transduction (Lennox, 1955). SP265 was selected as a 2-deoxyadenosine-resistant isolate from a pool of λc1857S7 secondary-site lysogens of KS302*deoC* prepared as described by Shimada et al. (1972). This strain does not grow on media containing thymidine but is completely resistant to 2-deoxyadenosine.

4. Selection and Screening of Deletion Strains. Temperature resistant derivatives of SP265, isolated on media containing 2-deoxyadenosine at 42° C, are often deletions that remove phage killing functions and variable amounts of adjoining bacterial DNA. The limits of these deletions were determined by scoring the presence of the phage genes O, P, Q, R, A, L, I and J by marker rescue (Shimada et al., 1972) and scoring the presence of the bacterial genes *deoB, serB, trpR*, and *thr, deoB* strains were resistant to thymidine; *trpR* strains were resistant to DL-5-methyl-tryptophan. Strains deleted for the *serB* or *thr* genes were scored as auxotrophs. T-agar containing 200 μg toluidine blue per ml was used to score *dye* (See below).

5. λ Lysate Preparation and Transduction Procedures. High titer transducing lysates were produced as outlined by Shimada et al. (1972). λ*serB*$^+$ transducing particles were detected after low multiplicity infection of either a *serB* deletion or point mutant. λ*spi* lysates and λ*spi* DNA were prepared as described by Blattner

Table 1a. Bacterial strains

Strain	Relevant genotype	Source or reference
KS302	*gal-attλ-bio* △	Shimada et al. (1972)
CSH25	*sup F*	Miller (1972)
SA1550	lysogen of λbio_{256}△HI	D. Court
61-1	KS302, *deoB-serB*△	this work
37-1	KS302, *deoB-trpR*△	this work
122-1	KS302, *deoB-dye*△	this work
1240	*serB*	Colson and Van Pel (1974)

Table 1b. Phage strains[a]

Phage	Source	Reference
λcI857S7	Induction of CSH45	Miller (1972)
λimm 434	M. Feiss	
λb2c	R. Weisberg	
λN7N53	E. Tessman	Signer (1969)
λcI857029	R. Weisberg	Shimada et al. (1972)
λcI857080	R Weisberg	Shimada et al (1972)
λcI857Q76Q501	R. Weisberg	Shimada et al (1972)
λcI857R5	R. Weisberg	Shimada et al. (1972)
λcI857A11	R. Weisberg	Shimada et al. (1972)
λcI857L63	R Weisberg	Shimada et al (1972)
λc$^+$I2	R. Weisberg	Shimada et al. (1972)
λcItsJ60	R. Weisberg	Shimada et al. (1972)
λcI857F423	R. Weisberg	Shimada et al. (1972)
λch80de19	H. Umbarger	Miller (1972)
∅80vir	R. Somerville	
λvir	R. Somerville	
Plkc	R. Somerville	Miller (1972), Lennox (1955)

[a] All markers, except c and cI857, are suppressible by *sup*E or *sup*F except for S7, which is suppressed by *supF* alone

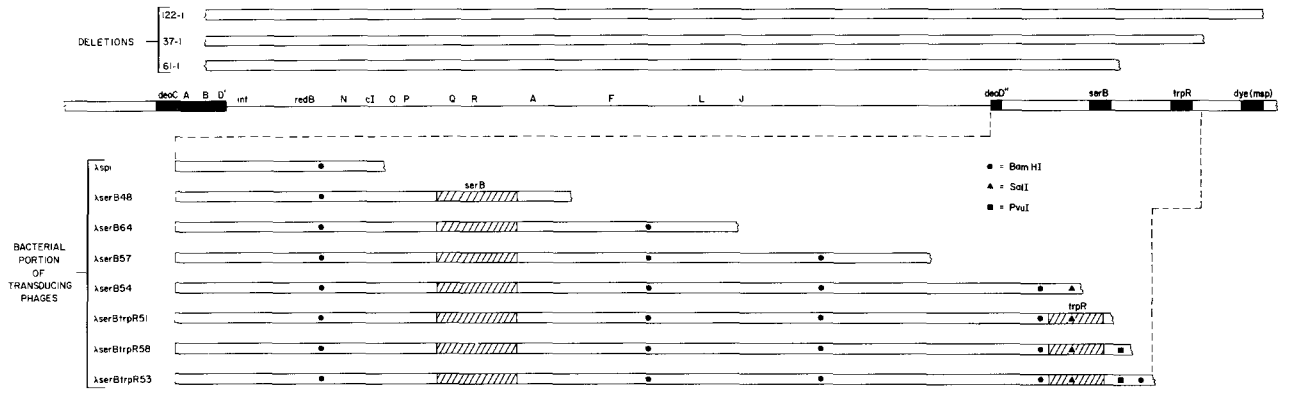

Fig. 1. General structures of the *deoD*::λ lysogen SP265, *serB* deletions derived from SP265 and the bacterial portions of λ*serB* and λ*serBtrpR* transducing phages. Cleavage sites for restriction endonucleases *BamHI* (●), *SalI* (▲) and PvuI (■) are shown Drawn approximately to scale; the distance from *deoD* to *trpR* is about 12 kb

et al. (1977). Transducing DNA from λ*serB*$^+$ transductants was obtained by the cleared lysate – CsCl/ethidium bromide centrifugation procedure developed for the isolation of closed circular plasmids (Guerry et al., 1973).

6. Recombinant DNA Techniques. Plasmids were isolated by the procedure of Guerry et al. (1973). Ligations were performed in reaction mixtures containing 50 mM Tris-Cl, pH 7.4, 10 mM dithiothreitol, 5 mM $MgCl_2 \cdot 6\ H_2O$, 2 mM ATP, and DNA ligase. Ligations were allowed to proceed for 24 to 36 h at 16.5° C. Ligation mixtures were used directly to transform strain 122–1 (See Fig. 1) by the procedure of Mandel and Higa (1970). Restriction enzymes were obtained from New England Biolabs and Bethesda Research Labs. DNA digestions were carried out in 10 mM Tris-Cl, 5 mM $MgCl_2$, and 150 mM NaCl. The construction and handling of recombinant molecules was done in accordance with the NIH guidelines governing recombinant DNA research.

7. Enzyme Assays. Anthranilate synthase activity in dialyzed crude extracts prepared from cells grown under repressing conditions was determined as described previously (Kuhn et al., 1972). Cells were grown to late log phase in minimal medium containing 0.25% acid hydrolyzed casein (Sigma), 1.0 μg thiamine per ml, 0.1 μg biotin per ml, and 0.2% glucose.

Results

Characterization of SP265 by Deletion Analysis

The first step in the isolation of a transducing phage carrying the *trpR*$^+$ gene involved positioning prophage λ near *trpR*. The *deo* cluster is closely linked to *trpR* and *deo* mutations are easily selected (Lomax and Greenberg, 1968; Buxton et al., 1978). To position λ within *deoD* we exploited the sensitivity of *deoC, deoD*$^+$ strains to exogenous 2-deoxynucleosides. The sensitivity of KS302*deoC* to 2-deoxyadenosine was relieved by the insertion of λcI857S7 into *deoD*. Strain SP265 (Table 1) is resistant to 2-deoxyadenosine, sensitive to thymidine, and is killed at 42° C by induction of the λ prophage (the cI857 gene codes for a temperature-sensitive λ repressor).

The deoxyadenosine resistance and temperature sensitivity of SP265 are directly attributable to λ insertion into *deoD*. Eighty temperature-resistant derivatives isolated on L-agar at 42° C had all reacquired sensitivity to 2-deoxyadenosine, indicating that the event leading to survival at 42° C had also restored the *deoD*$^+$ gene sequence. When temperature resistant clones were selected on plates containing 0.1% 2-deoxyadenosine (to maintain selection for *deoD*), survival was decreased by a factor of 100. Many of the latter strains were deletions that removed λ genes coding for cell killing functions.

Deletions that remove part of the prophage and extend into adjacent bacterial DNA enable on to establish prophage orientation (Shapiro and Adyha, 1969). Strains 9-2, 11-4, and 23-2 (Table 2) all lack *deoB* and variable amounts of prophage DNA as determined by marker rescue. Strain 19-4 lacks *serB* and is able to support the growth of N mutants but not of λ strains carrying defects in genes O, P, R, A, F, L or J. The prophage in SP265 is thus oriented within *deoD* such that N is distal and J proximal to *serB* (Table 2).

Other deletions have been obtained which lack all of the genes between *deoA* and *thr*. There are, therefore, no non-supplemental functions encoded by this sector of the chromosome.

Deletions with endpoints in the *deoD – thr* region (Fig. 1) not only confirm the order of *serB* and *trpR* but also define and position a recessive gene whose product is important for cellular resistance to methylene blue (a component of EMB media, commonly used to score fermentation of carbohydrates) and toluidine blue, a methylene blue analogue. This gene(s) lies between *trpR* and *thr*: deletion 37-1 re-

Table 2. Genotypes of temperature resistant, 2-deoxyadenosine resistant derivatives of SP265

	deoB	*N*	*Q*	*R*	*A*	*F*	*L*	*J*	*serB*	*trpR*
9-2	−	−	+	+	+	+	+	+	+	+
11-4	−	−	−	−	−	+	+	+	+	+
23-2	−	−	−	−	−	−	+	+	+	+
19-4	+	+	−	−	−	−	−	−	−	+

moves *trpR* and is Dye^R while 122-1 is both *trpR* and Dye^S. This gene may be related to *msp* or *cet*. Preliminary evidence (W. Roeder, Ph.D. thesis, Purdue University, 1978) indicates that *dye* specifies a function related to the cell envelope, as do *msp* (Buxton et al., 1978) and *cet* (Samson and Holland, 1970).

Isolation of λserB$^+$ and λserB$^+$ trpR$^+$ Specialized Transducing Phages

When strain SP265 was thermally induced the resulting lysates contained only 10^{-3} pfu per induced cell. This low yield is presumably attributable to inefficient prophage excision from the secondary attachment site within *deoD* (Shimada et al., 1972). However, *λserB$^+$* transducing particles were found at frequencies of $10^{-4}-10^{-5}$ per pfu. UV induction of Ser$^+$ transductants of 1240 (λimm434) (a *serB* point mutant) yielded lysates containing almost equal numbers of *λserB$^+$* and λimm434 particles, but no plaque forming *λserB$^+$* phages could be found. Moreover, Ser$^+$ transductants of 1240 (λimm434) were neither λ-immune nor thermosensitive. Ser$^+$ transductants also segregated Ser$^-$ clones at high rates; after overnight growth in L-broth only 5–14% of the population remained Ser$^+$.

These properties resemble those of *λN$^-$gal$^+$* (Signer, 1969) and λN$^-$ pseudolysogens (Lieb, 1970). To test the hypothesis that our *λserB$^+$* phages are N-defective, *λserB$^+$*, λimm434 mixed lysates were propagated on a strain (SA 1550) that synthesizes N-protein from a cryptic prophage, thereby allowing N-defective phages to form plaques. Phage stocks sequentially cloned three times on SA 1550 transduced *serB$^+$* at or near the plaque forming titer but failed to form plaques on another indicator (CSH 25) lacking a cryptic N$^+$ prophage. The introduction of nin-5, a deletion that removes a termination signal in the rightward operon of λ (Court and Sato, 1969) also enables *λserB$^+$* phages to form plaques, albeit poorly, in the absence of N protein.

N-defective phages lysogenize by replicating in the cell as plasmids (Lieb, 1970; Signer, 1969), maintenance of the phage genome proceeding via low level, N-independent production of the λ replication factors coded by the O and P genes. The use of a "helper" phage to supplement *λN$^-$serB$^+$* phage is therefore unnecessary for the propagation of the defective phage genome in the plasmid state. Additional *λserB$^+$* isolates, obtained after low multiplicity infection of the *serB-trpR-dye* deletion 122-1, were maintained as plasmids by nutritional selective pressure, thereby simplifying further manipulations and analysis.

In 13 of 190 *λserB$^+$* strains tested, *trpR$^+$* was cotransduced with *serB$^+$*. The segregation and immunity properties of the *trpR$^+$* containing strains were identical to those observed earlier. Covalently closed, circular duplex DNA was isolated from *λserB$^+$* and *λserB$^+$ trpR$^+$* transductants by the techniques employed in the isolation of small plasmids (Guerry et al., 1973).). A series of DNA preparations was cleaved with restriction endonucleases to define the restriction fragments carrying the *trpR$^+$* gene.

Restriction Analysis of the serB-trpR Region

The recombination events which generate *λserB$^+$* and *λserB$^+$ trpR$^+$* transducing phages are independent of the site-specific recombination functions of the phage. Thus, independent isolates of *λserB$^+$* transducing strains should carry varying amounts of bacterial DNA. We have compared the restriction patterns of *λserB$^+$ trpR$^+$*, *λserB$^+$* and λspi$^-$ phage DNA's and in a manner analogous to deletion mapping have located the *BamH*I, *Bgl*II, *Hind*III, *Sal*I and *Pvu*I sites which lie in this region. (λspi is a variant able to plate on a phage P2 lysogen (Lindahl et al., 1970) and carries bacterial DNA located clockwise from the *deoD* gene.

λwt is cleaved by *BamH*I at 5 sites (at 11.4, 46.7, 58.0, 71.3 and 86.1%; (Perricaudet and Tiollais, 1975) to generate six fragments. *λspi* substitutes bacterial DNA for phage N-operon DNA, deleting the *BamH*I site at 58%. However, another *BamH*I site of bacterial origin must lie to the right of the attachment site since two bands approximately 7 and 3 kb are seen rather than a fused C–D band. *λserB$_{48}$*, when cleaved by *BamH*I, produces only four fragments (Fig. 2). The B and C' fragments agree with those present in the *λspi* pattern. Since *λserB$^+$* and *λtrpR$^+$* phages were isolated as covalently closed, circular plasmids, the A and F fragments are linked to produce a *cos*-containing fragment of about 12kb. The fourth fragment contains the *serB$^+$* gene, the junction of bacterial and phage DNA, and the λ immunity region. The *BamH*I cleavage site at 71.3% is deleted in this N$^-$ phage.

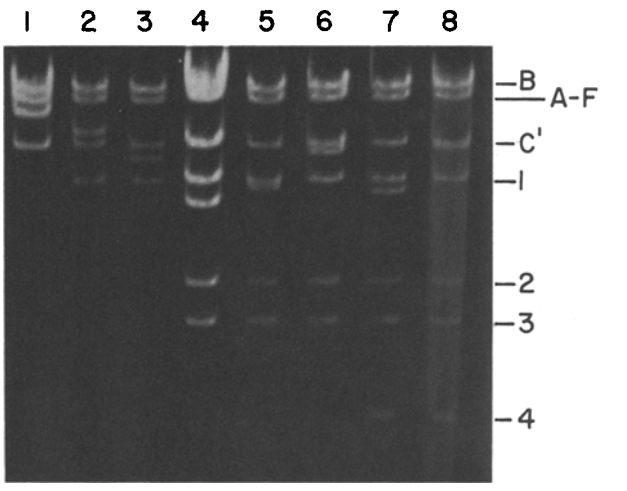

Fig. 2. *BamH*I digestion patterns of DNA from a series of $serB^+$ transducing phages. Fragments were separated on a 1 2% agarose slab gel in TBE (Tris-borate, 5 mM, EDTA, 1 mM EDTA, pH 8.3) at a constant voltage of 150 V Lane 1, $\lambda serB_{48}$; lane 2, $\lambda serB_{64}$; lane 3, $\lambda serB_{57}$; lane 4, $\lambda serB_{54}$; lane 5, $\lambda serB_{61}$; lane 6, $\lambda serBtrpR_{62}$; lane 7, $\lambda serBtrpR_{53}$; lane 8, $\lambda serBtrpR_{56}$; Phage fragments are indicated by letters A, B, C' and F; bacterial fragments by numbers 1–4

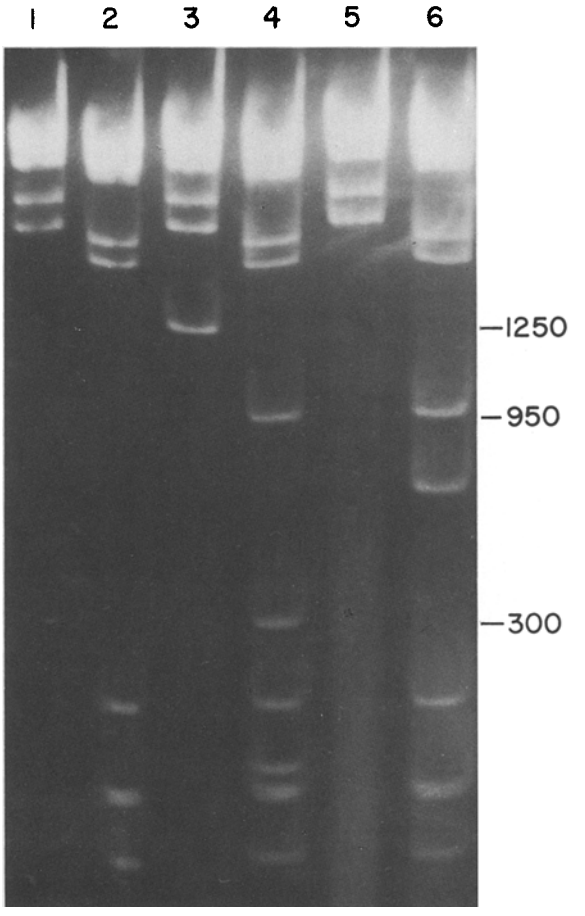

Fig. 3. *Pvu*I fragmentation patterns. After digestion, DNA samples were separated by electrophoresis on a 1 6% agarose slab gel in TBE buffer at constant voltage of 150V. Lane 1, $\lambda serBtrpR_{51}$ cleaved by *BamH*I; lane 2, $\lambda serBtrpR_{51}$ cleaved with *BamH*I and *Pvu*I; lane 3, $\lambda serBtrpR_{53}$ cleaved with *BamH*I; lane 4, $\lambda serBtrpR_{53}$ cleaved with *BamH*I and *Pvu*I: lane 5, $\lambda serBtrpR_{58}$ cleaved with *BamH*I; lane 6, $\lambda serBtrpR_{58}$ cleaved with *BamH*I and *Pvu*I. The sizes of selected fragments (in base pairs) is indicated

Additional bacterial DNA is carried on $\lambda serB^+ trpR^+$ and other $\lambda serB^+$ phages. Consider $\lambda serB^+ trpR^+{}_{53}$ and $\lambda serB^+ trpR^+{}_{56}$. Each is cleaved into eight fragments, four of which are identified easily as containing some phage DNA: bands B, A–F and C' agree with those seen in the λspi^- and $\lambda serB_{48}$ patterns. The fragment carrying the phage-bacterial junction is assigned easily because it differs in size in these two independent isolates. The other four fragments (1–4, Fig. 2) therefore must contain only bacterial DNA.

$\lambda serB^+ trpR^+{}_{62}$, $\lambda serB^+{}_{57}$ and $\lambda serB^+{}_{64}$ are cleaved into seven, six and five fragments, respectively. $\lambda serB^+ trpR^+{}_{62}$ does not produce fragment four (about 1250 bp) while $\lambda serB^+{}_{57}$ does not produce fragments two or four and $\lambda serB^+{}_{64}$ produces only fragment one. Clearly, the order of the fragments must be 1, 3, 2, 4.

$\lambda serB^+{}_{54}$ and $\lambda serB^+{}_{61}$ produce fragments one, three, and two, yet lysogens of these phages are resistant to 5MT. Thus, the DNA of these fragments is not sufficient to encode a functional $trpR^+$ gene. $\lambda serB^+ trpR^+{}_{62}$ does not carry the cleavage site which defines the right end of fragment four, therefore, the entire $trpR^+$ gene must lie to the left of this *BamH*I site. We thus conclude that the $trpR^+$ gene is carried, in whole or in part, in the 1250 bp fragment. These data do not exclude the posibility that the *BamH*I site between fragments two and four lies within the *trpR* gene.

A cleavage site for *Sal*I has been positioned and is depicted in Fig. 1. We find that *BamH*I fragment four is cleaved by *Sal*I into fragments of approximately 430 and 820 bp. Double digestions of $\lambda serB^+{}_{54}$, $\lambda serB^+ trpR^+{}_{62}$ and $\lambda serB^+ trpR^+{}_{53}$ enabled us to determine the order of these fragments in the prophage. All three phages produce the 430 bp fragment but only $\lambda serB^+ trpR_{53}$ produces the 820 bp fragment. Thus, the 430 bp fragment must lie on the *serB* side of the 1250 bp fragment. Since $\lambda serB^+{}_{54}$ contains the 430 bp *BamH*I-*Sal*I fragment but does not encode functional Trp repressor, at least part of the *trpR* gene must lie to the right of the *Sal*I site in the 830 *Sal*I-*BamH*I fragment.

In a similar manner a *Pvu*I site was positioned in *BamH*I fragment 4. $\lambda serB^+ trpR^+{}_{51}$, $\lambda serB^+ trpR^+{}_{58}$ and $\lambda serB^+ trpR^+{}_{53}$ were cleaved with

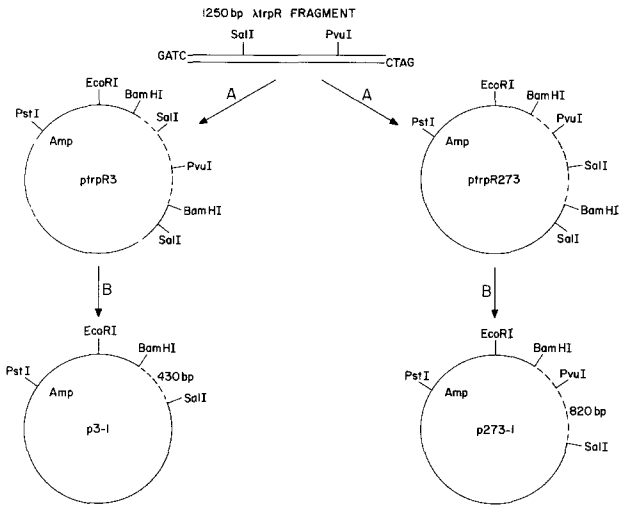

Fig. 4. Cloning of the 1250 bp *BamHI* fragment into pBR322. First, $trpR^+$ plasmids ($ptrpR_3$ and $ptrpR_{273}$) containing the *BamHI* fragment in each orientation were constructed (reaction A above). Cleavage of these plasmids by *SalI*, followed by ligation (reactions B) deletes either 820 bp (p3-1) or 430 bp (p273-1) of the 1250 bp fragment. Neither of these "trimmed" plasmids encodes a functional *trpR* gene (see text)

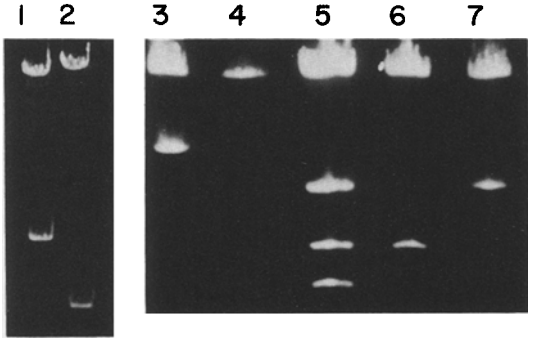

Fig. 5. Proof of structure for *SalI* "trimmed" plasmids (See Legend to Fig. 4) Lane 1, p*trpR3* cleaved by *SalI*; lane 2, $ptrpR_{273}$ cleaved with *SalI*; lane 3, p*trpR3* cleaved with *BamHI*; lane 4, pBR322 cleaved with *BamHI* + *SalI*; lane 5, p*trpR* cleaved with *BamHI* + *SalI*; lane 6, p3-1 cleaved with *BamHI* + *SalI*; lane 7, p273-1 cleaved with *BamHI* + *SalI*

Table 3. Anthranilate synthase specific activities of extracts prepared after growth in media containing 50 µg tryptophan/ml

	AS'ase
KS302 *deoC*	0.27
122-1	5.51
122-1 (λ*serB*48)	9.54
122-1 (λ*trpR*59)	0.14
122-1/p*trpR3*	0.85
122-1/p*trpR*273	0.32
122-1/p3-1	8.76
122-1/p273-1	8.40

BamHI and with *BamHI* + *PvuI* (Fig. 4). $\lambda serB^+trpR^+_{51}$ and $\lambda serBtrpR_{58}$ do not carry the *BamHI* site defining the right side of the 1250 bp, *BamHI* fragment. Double digestion of $\lambda serB^+trpR^+_{53}$ shows that the 1250 bp fragment is cleaved by *PvuI* into fragments of 950 and 300 bp (lane 4, Fig. 3). Digestion of $\lambda serBtrpR_{58}$ with *BamHI* and *PvuI* demonstrate that the 950 bp fragment lies on the *serB* side since the 950 bp fragment is present and the 300 bp fragment is not. The double digestion pattern of $\lambda serBtrpR_{51}$ does not display the 950 bp fragment. Since this phage carries the $trpR^+$ gene, *trpR* must be wholly encoded to the left of the *PvuI* site (Fig. 1).

Cloning of $trpR^+$ Into an Amplifiable Plasmid

Does the 1250 bp *BamHI* fragment carry the entire $trpR^+$ gene? To answer this question, we have cloned the *BamHI* fragments of $\lambda serB^+trpR^+_{59}$ (a phage whose cleavage pattern is similar to those of $\lambda serB^+trpR^+_{53}$ and $\lambda serB^+trpR^+_{56}$) into the *BamHI* site of the plasmid pBR322 (Fig. 4). Ampicillin resistant transformants of strain 122-1 were selected and plasmid DNA was prepared from ampicillin resistant, tetracycline sensitive, 5MT sensitive clones. Invariably, 5MT sensitive clones carry plasmids which contained the 1250 bp fragment (Fig. 4). Enzyme assays, performed on extracts of cells grown under repressing conditions, confirm that the expression of anthranilate synthase is repressed in strains carrying the recombinant plasmid (Table 2).

Because the enzyme *SalI* cleaves the *trpR* fragment asymmetrically (into 430 and 820 bp fragments), we were able to assess the orientation of the *trpR* fragment relative to the single *SalI* site of pBR322. The *SalI* and *BamHI* sites of pBR322 are located 275 bp from each other (Sutcliffe, 1978). Cleavage of *ptrpR3* with *SalI* generates a fragment of about 1100 bp (820+275), while cleavage of $ptrpR_{273}$ produces a 700 bp (430+275) fragment (Fig. 5). Thus, p*trpR3* and p*trpR*273 each carry the *trpR* fragment in opposite orientations. Since strains bearing either p*trpR3* or p*trpR*273 show sensitivity to 5MT, it is reasonable to suppose that native pBR322 promoters are unnecessary for $trpR^+$ gene expression in these recombinant molecules.

We have established that the *SalI* site lies within the *trpR* gene by subcloning the 430 bp and 820 bp fragments. *SalI*-cleaved p*trpR3* and p*trpR*273 were ligated at low DNA concentrations (less than 10 µg/ml) and transformed into strain 122-1 (Fig. 4). Most of the ampicillin resistant clones obtained contain

plasmids which delete the DNA between the *Sal*I site of pBR322 and the site in the 1250 bp fragment (Fig. 5). p*trpR*3 derivatives are missing approximately 1100 bp (including 820 bp of the *trpR* fragment); p*trpR*273 derivatives have lost approximately 700 bp (including 430 bp of the *trpR* fragment). In neither case were strains carrying these "trimmed" plasmids sensitive to 5MT. The repressed anthranilate synthase levels of strains harboring these plasmids are reported in Table 3. It appears, therefore, that the *Sal*I site lies within the *trpR* gene.

Discussion

Our selection of a λcI857S7 lysogen whose point of insertion lies within *deoD*, the structural gene for purine nucleoside phosphorylase, has facilitated in two ways the study of the Trp repressor system. First, such lysogens constitute useful starting strains for the isolation of deletions having a variety of endpoints within the *deo-thr* sector of the *E. coli* genome. Descendants of *deoD*::λ insertions which survived exposure to elevated temperature on media containing deoxyadenosine were often deletions which had suffered the concomitant loss of prophage genes and some adjacent bacterial DNA. In many cases the deleted genetic material included the *serB* and *trpR* genes, resulting in a nutritional requirement for serine and resistance to 5-methyl-tryptophan. The relative proximity of the *trpR* locus to the inserted prophage is a second noteworthy feature of our system. About 7% of the $serB^+$ transducing particles obtained from our *deoD*::λ insertion incorporated a functional *trpR* gene. Restriction analysis allowed us to determine that the *serB* and *trpR* genes are about 6 kb and 12 kb, respectively, from the insertion site.

By subcloning a 1250 bp, *BamH*I fragment from a $\lambda serB^+trpR^+$ transducing phage into the plasmid vector pBR322, we have demonstrated that all of the information necessary for the expression of the *trpR* gene resides within this short segment of DNA. Restriction analysis of $\lambda serB^+trpR^+$ transducing phage genomes lead us to conclude that at most 950 of these base pairs are sufficient to encode the repressor protein.

In vitro experiments of Rose and Yanofsky (1974) indicate that the dissociation constant of repressor-operator complex to be 200–400 pM. They also estimate the intracellular concentration of repressor to be about 30 mM. One would predict from these data that the *trp* operon of $trpR^+$ haploids is repressed to only 1/75 of the constitutive level and that an increase in repressor concentration would more thoroughly repress the *trp* biosynthetic enzymes, i.e., the amount of repressor in the cell limits the extent of repression. Strains with twice as much operator (trp^+/F'trp^+) have been shown to produce about two fold more tryptophan synthase (Stetson and Somerville, 1971) as expected if the concentration of repressor limits the degree of repression.

What limits repressor concentration? If *trpR* is expressed at a low constitutive rate, then an increase in $trpR^+$ copy number ought to cause a proportionate increase in Trp repressor protein concentration, with a consequent reduction in Trp biosynthetic enzyme levels. On the other hand, if the expression of the *trpR* gene is in some way regulated, then strains multiploid for the $trpR^+$ gene might exhibit normal repression.

Manson and Yanofsky (1976) reported that repressed $trpR^+$/F'$trpR^+$ strains have Trp biosynthetic enzyme levels about twofold lower than $trpR^+$ haploids. In two cases we have studied where increased $trpR^+$ copy number may lead to increased repressor concentration, "super repression" of the Trp enzymes was not observed (Table 2). $\lambda serB^+trpR^+N^-$ pseudolysogens carry the λ genome as plasmids and contain 10–20 copies per cell; relaxed plasmids related to pBR322 replicate in the cell in similarly high copy number. It is tempting to speculate, therefore, that one or more mechanisms for controlling the level of functional Trp repressor exist in *E. coli*. Several models for this control are currently under investigation.

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Note Added in Proof

DNA sequencing studies on the 1250 b.p. BamHI fragment (Fig. 4), in conjunction with other data, have established that Trp repressor monomer is a polypeptide containing either 78 or 88 amino acids (Singleton, Roeder, Somerville and Weith, in preparation).