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# Suppression of Promoter Mutations by the Pleiotropic *supX* Mutations*

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*Summary.* Mutations of the *supX* locus in *S. typhimurium* display multiple pleiotropy. The *supX* mutation, which can occur as a deletion or a point mutation, suppresses the promoter-like mutation *leu-500* and promoter mutations of the *lac* operon and increases the cell doubling time. The presence of the *supX* mutation in a strain infected with P22 phage increases the frequency of lysis relative to lysogeny. The *supX* mutation confers low-level resistance to streptomycin, kanamycin and neomycin and substantially increases the specific activity of alkaline phosphatase. Suppression by *supX* is unlike that of most known types of suppressor mutations. It may act by affecting the initiation mechanism of either transcription or translation.

## Introduction

The *supX* locus, (originally designated *su leu 500*) was first described by Mukai and Margolin (1963). Mutations at this locus act as suppressors of the leucine auxotrophy imposed by the *leu-500* mutation. At that time, the operator locus was considered to have two functions: to be the receptor site for the repressor, and to be the site of initiation of transcription. The *leu-500* mutation behaved like a classical 0° mutation although Mukai and Margolin (1963) suggested that it might act by altering the operator specificity. It is located at the extreme of one end of the leucine operon (Margolin, 1963; Calvo *et al.*, 1969), and it almost completely eliminates the expression of the entire leucine operon (Margolin, 1963; Burns *et al.*, 1966).

The chromosomal location of *supX* is between the tryptophan operon and the *cysB* locus (Mukai and Margolin, 1963), approximately 50 minutes away from the leucine operon on the *S. typhimurium* chromosome map (Sanderson, 1970). The *supX* locus is evidently not a leucine regulator gene, since leucine enzyme levels in *leu*$^+$ strains carrying a *supX* mutation continue to be regulated by leucine (Burns, unpublished observations quoted by Mukai and Margolin, 1963; Graf and Burns, 1971). One of the unique features of the *supX* suppressor mutations is that they can occur as deletions. In fact, some *supX* deletions eliminate the entire *trp-cysB* region of the chromosome (Mukai and Margolin, 1963). Therefore we must look for a mechanism of suppression which is based upon the elimination of a gene product. The *leu-500* mutation has attributes expected of a promoter mutation (Scaife and Beckwith, 1966; Ippen *et al.*, 1968), i. e. a location at the extreme operator end of the operon, with 0° mutations located between it and

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the first structural gene (Calvo *et al.*, 1969) and a low level expression of the operon, but continued response to regulation by leucine. It should be noted here that analysis of a large number of $0^c$ mutations of the lactose operon reveals that most of them have promoter-like effects, increasing or decreasing the induced level of expression of the lactose operon (Smith and Sadler, 1971).

No unlinked suppressors of the *lac P* mutations have been isolated (Arditti *et al.*, 1968). This report will characterize the properties of *supX*, an unlinked suppressor locus of the promoter-like mutation, *leu-500*.

## Materials and Methods

### *Strains*

All strains that were used are listed in Table 1.

### *Media*

Minimal medium in liquid (SSA) or agar form was prepared as previously described (Margolin, 1963). Glucose at 0.2% served as the carbon source unless otherwise indicated. Nutrient broth, used for growing cultures unless otherwise indicated, consisted of (w/v) 1% Difco tryptone, 0.5% yeast extract and 1% NaCl in distilled water. Nutrient agar was obtained from Baltimore Biological Laboratories.

### *Antibiotic Sensitivity Tests*

An overnight broth culture was diluted approximately 10 fold in fresh broth, incubated until growing exponentially, diluted appropriately for viable count assay and then plated on nutrient agar and on nutrient agar supplemented with the antibiotic to be tested. The antibiotic was added to molten agar after it had cooled somewhat, and the plates were poured the day before use. Following plating of the cells and 24 to 48 hours incubation at 37° C they were scored for ability to form colonies. The percent survival as well as the relative colony size was scored. Occasionally, antibiotic sensitivity was tested by streaking an exponentially growing culture on the antibiotic medium for single colonies and scoring colony size. Such tests were a less reliable measure of antibiotic resistance because of poor reproducibility.

### *Selection for supX Mutations*

#### a) Spontaneous Mutations

An overnight culture of a strain carrying the *leu-500* mutation was plated on minimal medium supplemented with all other required amino acids except leucine. After two days of incubation small $Leu^+$ colonies were selected as presumptive *supX* strains (Mukai and Margolin, 1963).

#### b) Mutagenesis

Treatment with 2-aminopurine in liquid culture was as described in Margolin and Mukai (1961) except that it was for 90 minutes.

### *Preparation of P22 Bacteriophage Lysates and Transduction*

Lysates were prepared and transductions carried out as described previously (Margolin, 1963) except that in order to obtain non-lysogenic transductants, an integration negative mutant of P22, *int-4* (Smith and Levine, 1967), was used.

### *Phenotypic Suppression*

Tests for phenotypic suppression by antibiotics were done according to Whitfield *et al.* (1966) except that the supplement was 1$\gamma$/ml L-leucine.

Table 1. *S. typhimurium* LT2 strain list

| Strain number | Genotype | Mode of origin[a] and/or reference |
|---|---|---|
| | *ara-9* | Demerec (see Margolin, 1963) |
| | *ara-9 leu-500* | Mutation: 5-bromodeoxyuridine treatment of *ara-9* (Margolin, 1963) |
| PM258 | *ara-9 leu-500 supX45* | Mutation: spontaneous (Margolin and Bauerle, 1966) |
| PM573 | *ara-9 cysB529* | Transduction: *ara-9 trpE200* × *ϕ cysB529* |
| PM572 | *ara-9 supX45* | Transduction: PM573 × *ϕsupX45* |
| PM570 | *ara-9 leu-500 supX45 ile-241* | Mutation: spontaneous of PM258 to isoleucine requirement |
| PM571 | *ara-9 leu-500 ile-241* | Transduction: PM570 × *ϕ*W.T. |
| PM447 | *ara-9 leu-500 str*$^r$ | Mutation: spontaneous of *ara-9 leu-500* to streptomycin resistance |
| PM574 | *ara-9 leu-500 supX45 nal*$^r$ | Mutation: spontaneous of PM258 to nalidixic acid resistance |
| PM575 | *ara-9 leu-500 nal*$^r$ | Transduction: PM574 + *ϕ*W.T. |
| PM576 | *ara-9 leu-500 nal*$^r$/F′13 (*pro*$^+$*lac*$^+$) | Conjugation: PM575 × E5019[b] |
| PM577 | *ara-9 leu-500 nal*$^r$/F′13 (*pro*$^+$*lacL1*) | Conjugation: PM575 × E8307[b] |
| PM580 | *ara-9 leu-500 supX45 nal*$^r$/ F′13 (*pro*$^+$*lac*$^+$) | Conjugation: PM574 × E5019 |
| PM581 | *ara-9 leu-500 supX45 nal*$^r$/ F′13 (*pro*$^+$*lacL1*) | Conjugation: PM574 × E8307 |
| PM488 | *leu-500 supQ1 trpA50* | Kemper and Margolin (1969) |
| PM530 | *leuD657 supQ1 trpA50 leuD804 sup*$^{amber}$ | Kemper and Margolin (1969) |
| PM563 | *leu-500 trpO-C101 cysB529* | Transduction: *leu-500 cysB529 pyrF146* × *ϕtrpO-C101* |
| | *leuA523* | Mutation: induced by 2-AP Parsons and Burns (1969) |
| | *leuA150* | Mutation: induced by 2-AP Parsons and Burns (1969) |
| | *leuA421* | Mutation: induced by X-ray Parsons and Burns (1969) |
| | *leuO-D447* | Mutation: induced by X-ray Margolin (1963) |
| PM92 | *leu-500 cysB529* | Transduction: PM573 × *ϕleu-500* |
| PM584 | *leu-500 trpA56* | Transduction: PM92 × *ϕtrpA56* |
| PM585 | *leu-500 trpA49* | Transduction: PM92 × *ϕtrpA49* |
| PM586 | *leu-500 trpA8* | Transduction: PM92 × *ϕtrpA8* |
| PM587 | *leu-500 trpO-E130* | Transduction: PM92 × *ϕtrpO-E130* |
| PM588 | *leu-500 trpA56 supX18* | Transduction: PM584 × *ϕara-9 leu-500 supX18* |

Table 1 (continued)

| Strain number | Genotype | Mode of origin[a] and/or reference |
|---|---|---|
| PM589 | *leu-500 trpA49 supX18* | Transduction: PM585 × *φara-9 leu-500 supX18* |
| PM590 | *leu-500 trpA8 supX18* | Transduction: PM586 × *φara-9 leu-500 supX18* |
| PM591 | *leu-500 trpO-E130 supX18* | Transduction: PM587 × *φara-9 leu-500 supX18* |
| PM592 | *ara-9 leu-500 supX78* | Mutation: 2-AP induced mutation of *ara-9 leu-500* |
| PM593 | *ara-9 leu-500 supX79* | Mutation: 2-AP induced mutation of *ara-9 leu-500* |
| PM594 | *ara-9 leu-500 supX80* | Mutation: 2-AP induced mutation of *ara-9 leu-500* |
| PM595 | *ara-9 leu-500 supX81* | Mutation: 2-AP induced mutation of *ara-9 leu-500* |
| SB391 | *his644*/F′ (*lacX82*) | Berkowitz *et al.* (1968) |
| SB392 | *his644*/F′ (*lacU291*) | Berkowitz *et al.* (1968) |
| PM458 | *trpA512 leu-500 cysB529* | Transduction: *cysB29 leu-500 pyrF146* × *φtrpA512* |

[a] The transductions used to construct the strains are shown with the recipient bacterial strain on the left and the donor, preceded by a symbol for phage, (*φ*), on the right. The wild type LT2 strain is indicated by W.T.
[b] These *E. coli* strains were kindly supplied by Dr. J. Beckwith. E5019 carries F′13 (*pro*$^+$*lac*$^+$), E8307 carries F′13 (*pro*$^+$*lacL1*).

### *Transfer of F′lac*

The transfer was selected on agar medium with lactose as the sole carbon source. The *S. typhimurium* recipients were nalidixic acid resistant, and nalidixic acid was added to the agar medium (at 20*γ*/ml) to eliminate the donor, either *E. coli* or *S. typhimurium*.

### *Measurement of Enzyme Activity in Sonicated Preparations*

#### a) Growth of Cultures

The strains were grown overnight in minimal medium with glycerol (0.5%) as carbon source and supplemented with tryptophan (50*γ*/ml) and leucine (40*γ*/ml). For induction of *β*-galactosidase, IPTG ($2 \times 10^{-4}$M) was added. The cultures were diluted into 1 liter of the same medium and grown with shaking to a density of about $10^9$ cells/ml (100 Klett units). From this point on all operations were carried out in the cold. The culture was centrifuged and washed once with an equal volume of cold phosphate buffer (0.05 M, potassium phosphate, pH 7.2), centrifuged and washed again with 30 ml buffer and following final centrifugation the cell paste was frozen.

#### b) Preparation of Cell Extracts

The frozen cells were thawed and resuspended in about 16 ml cold phosphate buffer per liter equivalent of culture, divided into 2 samples of 8 ml each in Rosette cells and disrupted on ice with a Branson sonifier at a setting of 75 W. The sonication was for a total of 2.5 minutes,

and was applied in 30 sec pulses. The extracts were then centrifuged in the refrigerated Sorvall centrifuge for 15 minutes at 10000 rpm, the supernatant decanted and then centrifuged again to remove all cell debris. The clear extracts were stored in an ice bath in the cold room.

### c) Enzyme Assays

*β-Galactosidase.* The extract was diluted appropriately with phosphate buffer, pH 7.2. One ml of the dilution was incubated at 37° C with 0.6 ml ONPG (66.7 mg per 100 ml phosphate buffer, pH 7.2) until the yellow color developed. The reaction was terminated with 0.4 ml of 10% $Na_2CO_3$ and the optical density read in the Gilford spectrophotometer at 420 mμ. These readings were corrected for any turbidity by subtracting the OD at 550 mμ multiplied by 1.65. Each assay was done in duplicate. The enzyme units were calculated in mμM ONP/ml/min. One mμmole/ml ONP gives an OD of 0.004 at 420 mμ.

*β-Isopropylmalate Dehydrogenase.* The dehydrogenase activity was assayed according to the method of Burns *et al.* (1963). According to Burns (personal communication) the Klett reading × 0.0008 = number of μmoles α-ketoisocaproate. The enzyme units were calculated as μmoles α-ketoisocaproate formed/10 min/ml.

*DPNH Oxidase.* A 1 ml sample of an appropriate dilution (phosphate buffer) of the extract was added to a freshly prepared 0.1 ml aliquot of DPNH (1 mg/ml) and incubated at room temperature, in the Gilford spectrophotometer. The decrease in optical density at 340 mμ was followed with time on the automatic recorder. Enzyme units were calculated at OD units/min/ml extract. The blank contained no DPNH.

*Glucose-6-Phosphate Dehydrogenase.* The method of Fraenkel and Horecker (1964) was used. Enzyme units were calculated as OD units/min/ml.

*Alkaline Phosphatase.* The method of Echols *et al.* (1961), was used. Enzyme units were calculated as OD units/min/ml.

*Protein.* The protein determinations were done by the method of Lowry *et al.* (1951). A standard curve was run each time with bovine serum albumen.

## *Preparations of Ribosomes*

### a) Growth of Cells

Overnight broth cultures were diluted about 100-fold in broth and incubated at 37° C with shaking until the culture reached a Klett reading of 60 to 80, indicating 6 to 8 × $10^8$ cells/ml. At this point all further operations were carried out in the cold. The cells were centrifuged and washed once with Standard Buffer (0.01 M Tris, pH 7.8, 0.014 M magnesium acetate, 0.06 M KCl) and then frozen as a cell paste.

### b) Preparation of Ribosomes

The cells were resuspended in 8 ml Standard Buffer per liter equivalent of cells and 0.005 M mercaptoethanol was added. The cell suspension was sonicated in a Rosette cell on ice with the Branson sonicator for 5 minutes at a setting of 75 W, using 30 second pulses. The resulting extract was centrifuged in the Sorvall at 20000 rpm for 20 minutes. The supernatant was removed and centrifuged for 3 hours at 40000 rpm in the model L2-65B Beckman ultracentrifuge in the 50 Ti rotor. The ribosome pellet was then treated according to Kaji (1968) except that it was washed only once and that the ribosomes were stored in a Revco deep freezer.

## *Ribosome Streptomycin Binding Assays*

The method used was that of Kaji and Tanaka (1968). All reactions were run in duplicate.

## *Chemicals and Abbreviations Used*

Streptomycin sulfate and neomycin sulfate were purchased from Calbiochem. Lincomycin HCl and spectinomycin sulfate were gifts of the Upjohn Company. Erythromycin was a gift of the Lilly Research Laboratories and Kanamycin sulfate was a gift of Bristol Laboratories. IPTG (isopropyl-beta-D-Thiogalactopyranoside) was obtained from Mann Research Co.,

ONPG (0-nitrophenyl-$\beta$-D-galactopyranoside) was obtained from Calbiochem. $H^3$-dihydrostreptomycin was obtained from Amersham-Searle. 2-aminopurine (2-AP) was obtained from Calbiochem.

## Results

*Growth Rate of supX Strains.* The growth rates of all *supX* mutant strains tested are significantly slower than that of *supX*$^+$ strains (Table 2). The doubling time of most *supX* strains varied between 40–50 minutes, whereas the *supX*$^+$ parent had a doubling time of 29 minutes.

*Multiple Drug Resistance of supX Strains.* High-level streptomycin resistant clones in *supX* mutant strains appeared with a much higher frequency than in *supX*$^+$ strains, suggesting a possible mutator effect. Upon investigation this was found, like that of a similar effect described by Pierce and Meynell (1968), to be due to an initial low-level of streptomycin resistance apparently conferred by the *supX* mutation. Various other growth inhibitors and antibiotics were tested (Table 3) and it was found that the *supX45* mutation increased the level of resistance to kanamycin, neomycin and streptomycin. The increased level of resistance over that of the parent was not great, and the concentration of the antibiotic, the inoculum, and the time of incubation had to be controlled carefully

Table 2. Effect of *supX* mutations on growth rate

| Strain | Other genes affected by deletion | Genotype | Doubling time[a] (min) |
|---|---|---|---|
| PM199 | none[b] | *ara-9 leu-500 supX1* | 40 |
| PM204 | none[b] | *ara-9 leu-500 supX6* | 40 |
| PM206 | none[b] | *ara-9 leu-500 supX7* | 41 |
| PM208 | none[b] | *ara-9 leu-500 supX9* | 33 |
| PM212 | *cysB* | *ara-9 leu-500 supX11* | 45 |
| PM214 | *cysB* | *ara-9 leu-500 supX13* | 41 |
| PM216 | *cysB* | *ara-9 leu-500 supX14* | 38 |
| PM217 | *cysB* | *ara-9 leu-500 supX15* | 55 |
| PM220 | *cysB* | *ara-9 leu-500 supX17* | 45 |
| PM222 | *cysB* | *ara-9 leu-500 supX18* | 46 |
| PM224 | *cysB* | *ara-9 leu-500 supX19* | 48 |
| PM226 | *cysB* | *ara-9 leu-500 supX20* | 47 |
| PM245 | *trp* | *ara-9 leu-500 supX33* | 40 |
| PM248 | *trp* | *ara-9 leu-500 supX35* | 43 |
| PM255 | *trp* | *ara-9 leu-500 supX42* | 46 |
| PM258 | *trp* | *ara-9 leu-500 supX45* | 43 |
| PM259 | *trp* | *ara-9 leu-500 supX46* | 41 |
| Control (*supX*$^+$) | none | *ara-9 leu-500*[c] | 29 |

[a] Strains were grown in Luria broth. Doubling time was calculated from the change in Klett readings with time. Each value is the average from two independent growth curves.

[b] These *supX* mutations, selected for suppression of leucine auxotrophy imposed by the *leu-500* mutation, cause no other auxotrophy. They are probably point mutations in the *supX* locus or possibly short deletions which do not reach the *cysB* locus or the tryptophan operon.

[c] All *supX* strains in the Table were derived by spontaneous mutation from this strain.

Table 3. Effect of *supX* mutation on the level of antibiotic resistance[a]

| Antibiotic[b] | Concentration | 37° incubation | % Survival *ara-9 leu-500* | % Survival *ara-9 leu-500 supX45* |
|---|---|---|---|---|
| Streptomycin | 20 γ/ml | 2 days | 1 | 50 |
| Kanamycin | 5 γ/ml | 3 days | 1 | 61 |
| Neomycin | 20 γ/ml | 2 days | 1 | 51 |

[a] Resistance was measured by plating exponentially growing broth cultures on nutrient agar with and without the indicated antibiotic. Antibiotics were added to molten agar just before pouring, and care was taken to pour plates to fairly constant depth, and to use fresh plates.
[b] Other antibiotics and growth inhibitors to which *supX* mutations did not confer increased resistance were: penicillin, tetracycline, nalidixic acid, chloramphenicol, mitomycin, ethionine, trimethoprin, rifampin, aminopterin, streptovaricin and azetidine.

in order to get reproducible results. In the strain carrying the *supX45* mutation, we found no increased resistance to penicillin, tetracycline, nalidixic acid, chloramphenicol, mitomycin, ethionine, trimethoprin, aminopterin, rifampin, streptovaricin and azetidine. Results obtained with spectinomycin and erythromycin were variable from test to test and therefore unclear.

The *supX45* mutation is a deletion extending from the *supX* locus into the tryptophan operon (Margolin and Bauerle, 1966). Therefore strains with *supX* point mutations induced by 2-aminopurine were isolated to determine if the multiple drug resistance of *supX45* was due to the *supX* mutation itself or to the deletion of a previously undetected gene located between the *supX* locus and the tryptophan operon. The *ara-9 leu-500* strain was treated with 2-aminopurine and Leu$^+$ revertant colonies selected. About 10 times more small Leu$^+$ colonies and about 3 times more large Leu$^+$ colonies were obtained from the 2-aminopurine treated culture than from the untreated control.

Forty-nine small colony Leu$^+$ revertants were selected, purified and tested by transduction for linkage of the mutations responsible for the Leu$^+$ phenotype to either the *leu-ara* or the *cysB-trp* regions of the chromosome. Each clone was also tested for low-level resistance to streptomycin, kanamycin, spectinomycin and neomycin. With eight of the clones linkage was found with the *leu-ara* region and not with the *cysB-trp* region. These strains, therefore, probably represent reversion mutations at or near the *leu-500* site. All eight of these strains showed normal sensitivity to the four drugs. With 39 of the strains linkage to the *cysB-trp* chromosome region was found, indicating that they represented *supX* mutations. Of these, 20 showed low-level resistance to all four drugs. Seven were resistant to streptomycin, kanamycin and neomycin but normally sensitive to spectinomycin. Four were resistant to streptomycin, kanamycin and spectinomycin but not to neomycin. One was resistant only to kanamycin, spectinomycin and neomycin but not to streptomycin and another was resistant only to neomycin. Six of the presumed *supX* mutant strains were normally sensitive to all four drugs.

The remaining two clones exhibited no linkage to either the *leu-ara* or *cysB-trp* regions of the chromosome and showed normal drug sensitivity. These may

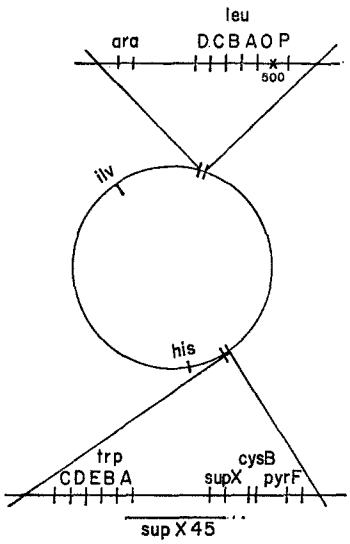


Fig. 1. Linkage map of *S. typhimurium* LT2 showing in detail the *trp* — *supX* — *cysB* and the *leu* regions. The *leu-500* mutation is indicated in the leucine operator-promoter region by an x. The *supX45* deletion is shown below the chromosome line and extends about half-way into the *trpA* gene. Chromosome map positions are according to Sanderson (1970)

represent a class of *supX* mutations which very weakly suppress the leucine auxotrophy and have only recently been recognized, using new methods.

These data strongly support the interpretation that mutations of the *supX* locus itself can confer a low-level multiple drug resistance. These strains were examined for their drug resistance by the less accurate streak method. The evidence described above which suggests several patterns of multiple drug resistance will require confirmation by the much more reliable test of survival experiments or serial dilution tests.

*Linkage of the supX Locus to the cysB and trpA Genes.* Although, as shown in Fig. 1, it was established that the *supX* locus resided between *cysB* and the tryptophan operon (Mukai and Margolin, 1963) the relative distances had not been properly explored. Therefore, using *supX* mutations which were probably point mutations, we examined their linkage to *trpA512*, a deletion of most of *trpA* (Balbinder *et al.*, 1968), the closest gene of the tryptophan operon (Demerec and Demerec, 1956; Balbinder, 1962; Bauerle and Margolin 1966a). At the same time we also determined the linkage of these *supX* mutations to *cysB529*, a mutation of the *cysB* locus which does not revert and therefore may be a deletion.

Four of the 2-AP induced *supX* mutations were used as donors in transductions with the recipient strain, *trpA512 cysB529 leu-500*. On appropriate media Trp$^+$ Leu$^+$ as well as Cys$^+$ Leu$^+$ transductants were selected. These two types of transductants were then tested for the ability to grow on unsupplemented medium. With all four different *supX* mutant donors, the frequency with which *trp*$^+$*supX*

Table 4. Linkage of *supX* point mutations to *cysB529* and *trpA512* using PM458 as transduction recipient

| Donor phage grown on strain | Number $Cys^+Leu^+$ transductants tested | Number $Cys^+$ $Leu^+Trp^+$ | Cotransduction (%) | Number $Trp^+Leu^+$ transductants tested | Number $Trp^+$ $Leu^+Cys^+$ | Cotransduction (%) |
|---|---|---|---|---|---|---|
| *ara-9 leu-500 supX78* | 121 | 64 | 53 | 128 | 105 | 82 |
| *ara-9 leu-500 suoX79* | 157 | 100 | 63 | 236 | 229 | 97 |
| *ara-9 leu-500 supX80* | 79 | 48 | 61 | 164 | 150 | 92 |
| *ara-9 leu-500 supX81* | 101 | 70 | 69 | 135 | 112 | 83 |

transductants inherited the $cys^+$ from the donor was 82–97%, whereas the frequency with which $cys^+$ *supX* transductants inherited the $trp^+$ from the donor was 53–69% (Table 4). From these results, we conclude that the *supX* locus is more closely linked to *cysB* than to the *trp* operon, or at least, to the furthermost end of the *trpA512* deletion.

*Specificity of Suppression of leu-500.* If *leu-500* were a nonsense mutation it should be suppressed genetically by nonsense suppressors (Gorini and Beckwith, 1966) and perhaps phenotypically by drugs which cause ribosomal ambiguity (Gorini and Kataja, 1964). To test the first possibility an amber suppressor from strain PM530 (Kemper and Margolin, 1969) was introduced by transduction into the strain PM488 which bears the *leu-500* mutation and the amber *trpA50* mutation (Bauerle and Margolin, 1966b). The leucine auxotrophy was not suppressed.

As a further test, the *leu-500* mutation was introduced by transduction into a strain bearing the amber mutation *trpA50* as well as *hisC340*, an amber mutation in the histidine operon (Whitfield *et al.*, 1966). The resulting triple auxotroph ($Leu^-$, $Trp^-$, $His^-$), was then treated with diethylsulfate or nitrosoguanidine and selection made for mutants which became $His^+$ and $Trp^+$ simultaneously because of ochre and amber suppressor mutations. Of 255 $His^+$ $Trp^+$ colonies tested all still required leucine for growth, indicating that *leu-500* is not suppressed by any of these presumed ochre and amber suppressor mutations.

Phenotypic suppression of *leu-500* was tested by the disc method, using streptomycin, kanamycin and neomycin. Several nonsense mutations, *leuA523* *leuA150* and *leuA421* (Parsons and Burns, 1969) as well as *leuO-D447*, a deletion of the entire leucine operon (Margolin, 1963) were used as controls. The only strain which responded with apparent phenotypic suppression was *leuA523*, with kanamycin causing the strongest response. All the other strains, including *leuO-D447* and *leu-500*, exhibited a slightly heavier growth at the perimeter of the inhibition zone, whereas *leuA523* responded with a halo of growth at edge of the inhibition zone.

*Specificity of Suppression by supX.* Forty six different leucine auxotrophs (other than *leu-500*) were tested for suppression by *supX45*. To provide a common genetic background each *leu* mutation was introduced by transduction into a

Table 5. Specific activities[a] of enzymes in *supX*$^+$ and *supX45* strains[b] carrying F'*lac*$^+$ or F'*lacL1*

| Enzyme | *lac* and *supX genotype* | | | | | |
|---|---|---|---|---|---|---|
| | *lac*$^+$ *supX*$^+$ | *lac*$^+$ *supX45* | *lacL1* *supX*$^+$ | *lacL1* *supX45* | *lacL1*[c] *supX*$^+$ | *lacL1*[c] *supX45* |
| β-isopropylmalate dehydrogenase | 0.005 | 0.032 | 0.0042 | 0.023 | 0.0035 | 0.024 |
| β-galactosidase | 7100 | 5975 | 73 | 218 | 43 | 113 |
| DPNH oxidase | 7.9 | 8.6 | 15.1 | 15.5 | 10.0 | 8.3 |
| glucose-6-p-dehydrogenase | 29.1 | 29.3 | 27.1 | 31.8 | 31.9 | 29.9 |
| alkaline phosphatase | 27.8 | 315 | 17.3 | 572 | 29.4 | 522 |

[a] Units enzyme activity/mg protein.
[b] All strains also carried the *ara-9 leu-500* and *nal*$^r$ alleles.
[c] These cultures were not induced with IPTG.

recipient strain bearing *supX*$^+$. Subsequently the *supX45* mutation was introduced into each. All forty six of these strains remained leucine auxotrophs indicating lack of suppression by *supX45*. Of the 46 *leu* mutations, representing the four *leu* cistrons, twelve were spontaneous, 8 induced by 2-AP, 8 by BU, 4 by nitrous acid, 4 by nitrosoguanidine, 2 by UV, 2 by diethylsulfate and 6 by x-ray treatment. Two have been identified as frameshift mutations (Parsons and Burns, 1969). Some of these mutations, especially those induced by 2-AP (Whitfield *et al.*, 1966) are likely to be nonsense mutations, yet none are suppressed by *supX45*.

Two amber nonsense mutations *lacU281* and *lacX82* (Newton *et al.*, 1965) carried on F'*lac* episomes were tested for suppression by *supX45* with the tester strains and method of Berkowitz *et al.* (1968) and gave negative responses. In contrast, a strain bearing the known amber suppressor, *sup*$^{amber}$ (Kemper and Margolin, 1969), produced positive results. A *supX45* strain is quite capable of accepting F'*lac* episomes, as indicated below by the introduction of such episomes, bearing *lac* promoter mutations, into a *supX45* strain.

*SupX does not suppress the Polarity Effects of Polar Mutations.* Scaife and Beckwith (1966) described a suppressor mutation, *suA*, originally isolated by Beckwith (1963), which relieves the polarity effect of nonsense mutations in *E. coli* without actually suppressing the nonsense mutation itself. It has been shown in *E. coli* (Morse and Primakoff, 1970), that strains with strongly polar mutations in *trpE*, the anthranilate synthetase gene, are inhibited by high concentrations of anthranilic acid. This is the result of the polarity effect and is reversed by *suA*. A *supX* mutation was tested for the ability to reverse anthranilate sensitivity in *S. typhimurium* polar mutations of *trpA* (anthranilate synthetase gene, equivalent to *E. coli trpE* mutations). A polar frameshift mutation, *trpA56* and a polar amber mutation, *trpA49*, as well as *trpA8*, a non-polar mutation (Bauerle and Margolin, 1966b) were each transferred into a *leu-500 cysB529* strain by cotransduction with *cys*$^+$. In accord with the results in *E. coli*, we found that growth of the resulting polar mutant strains *trpA56 leu-500* and *trpA49 leu-500* were strongly inhibited by anthranilic acid at 60 γ/ml, although they

grew well at 10 $\gamma$/ml. The non-polar *trpA8 leu-500* strain grew equally well at both concentrations of anthranilic acid. The *supX18* mutation, consisting of a deletion from the *supX* locus into the *cysB* locus, was introduced into the three *leu-500 trpA* strains by transduction. The resulting *supX18* transductants all exhibited the same degree of sensitivity to anthranilic acid as their *supX*$^+$ parents.

*Suppression of Lac Promoter Mutation L1 by supX45.* Since the *leu-500* mutation had many attributes of a promoter mutation, the effect of the *supX45* upon the lac promoter mutations was of interest. Dr. J. Beckwith kindly provided three F'*lac* strains of *E. coli* with episome borne *lac* promoter mutations. For these initial studies the *lacL1*, a deletion promoter mutation (Scaife and Beckwith, 1966) was utilized. The F'*lac* episome bearing the promoter mutation was transferred by conjugation from the *E. coli* host to strain *ara-9 leu-500 supX45 nal*$^r$ (nalidixic acid resistant) as well as to a *supX*$^+$ transductional derivative of this strain, *ara-9 leu-500 nal*$^r$. The F'*lac*$^+$ episome was also transferred to these two strains and the specific activities of isopropylmate dehydrogenase, $\beta$-galactosidase, DPNH oxidase, glucose-6-phosphate dehydrogenase and alkaline phosphatase were determined for all four strains (Table 5).

The isopropylmalate dehydrogenase enzyme is the product of the *leuB* (formerly *leuII*) cistron (Burns *et al.*, 1963) and served to monitor the suppressor action of *supX45* on the functioning of the leucine operon containing the *leu-500* mutation. As shown in Table 5 the $\beta$-isopropylmalate dehydrogenase specific activity of the strains with the *supX45* mutation was increased about six-fold over those with the *supX*$^+$ allele, in general agreement with the previous results of Friedman and Margolin (1968) who reported an approximately ten-fold increase in the enzyme level of the *supX* mutant strain. It should be noted that their enzyme levels were obtained with derepressing conditions of growth involving limiting leucine supplement whereas the data reported here is for repressed $\beta$-isopropylmalate dehydrogenase levels obtained following growth in the presence of excess leucine.

To determine the effect of *supX45* on the wild type leucine operon, the *supX45* deletion was transferred by transduction into a *leu*$^+$ *ara-9* strain. The repressed levels (growth in the presence of excess leucine) of $\beta$-isopropylymalate dehydrogenase were measured in the *ara-9 leu*$^+$ *supX45* strain and *ara-9 leu*$^+$ *supX*$^+$ strain. The specific activities in these two strains were not significantly different; 0.372 in the *supX*$^+$ strain and 0.527 in the *supX45* strain. These results agree with those of Burns (quoted in Mukai and Margolin, 1963).

In order to examine the levels of $\beta$-galactosidase all four strains were grown in the presence of IPTG to induce the *lac* operon. The two strains bearing the L1 promoter mutation were also grown without IPTG so as to determine the enzyme levels in uninduced cells. The presence of the *supX45* mutation increased the specific activity of $\beta$-galactosidase somewhat more than two-fold over that found with *supX*$^+$ in the strain bearing the L1 promoter mutation, both in the induced and uninduced cultures (Table 5). This increased enzyme level conformed to our previous observation (unpublished) that the *lacL1 supX45* strain produced a positive indication of lactose fermentation on MacConkey indicator agar whereas the *lacL1 supX*$^+$ strain did not. Recent experiments which included other *supX*

mutant alleles have indicated that the *lacL8* and *lacL29* promoter point mutations are suppressible by some mutations of the *supX* locus (unpublished results).

The β-galactosidase levels of uninduced cells with a wild type (*lac*$^+$) lactose operon were examined in order to determine if the effect of *supX45* was specific for mutant alleles of the *lac* promoter or if *supX45* generally affected a *lac* operon which was functioning at a low level. The two strains *ara-9 leu-500 nal*$^r$ *supX*$^+$/F'*lac*$^+$ and *ara-9 leu-500 nal*$^r$ *supX45*/F'*lac*$^+$ were grown in minimal medium with glycerol as carbon source and supplemented with leucine and tryptophan. Toluenized cells were assayed for β-galactosidase activity. The *supX*$^+$ strain produced a specific activity of 0.54/O.D. unit and the *supX45* strain produced a specific activity of 0.42/O.D. unit. A repeat of this experiment but calculating the uninduced β-galactosidase specific activities per $10^8$ cells (using a Coulter Counter) resulted in 1.53 for the *supX*$^+$ strain and 0.795 for the *supX45* strain. Finally the two strains were grown in Difco nutrient broth and the uninduced β-galactosidase activities/mg protein were determined from toluenized cells. The *supX*$^+$ strain produced 0.244 while that of the *supX45* strain was 0.320. All these results indicate that the *supX45* mutation has no detectable enhancing effect upon the low uninduced β-galactosidase levels in strains with a wild type *lac* operon.

The three other enzyme activities studied (Table 5) were intended to serve as controls for the specificity of suppression by *supX45*. There was no significant effect upon the specific activity levels of DPNH oxidase and glucose-6-phosphate dehydrogenase. An unexpected result was the very significant increase in the levels of alkaline phosphatase specific activity in those strains bearing the *supX45* mutation.

As a further check on the degree of specificity of the increased enzyme levels, the effect of the *supX45* deletion on the total protein per cell was determined. The cell numbers were measured with the Coulter Counter. The strains used were isogenic except for the alleles at the *supX* locus. The *supX*$^+$ strain had 246 γ protein/$10^9$ cells and the *supX45* strain had 212 γ protein/$10^9$ cells.

*Sensitivity to P22 Phage.* It had been generally observed in this laboratory that the transduction frequencies of *supX* mutations are extremely low and that strains carrying these mutations are very poor recipients for transduction. A pair of strains carrying the same *ile* mutation and isogenic except for the *supX* allele (*supX45* and *supX*$^+$) were used as recipients in transduction experiments. Aliquots from exponentially growing cultures of both strains were incubated for 15 minutes at 37° C with various multiplicities of P22 phage (which had been grown on *S. typhimurium* LT2) and then plated for survivors and for *ile*$^+$ transductants. The results (Fig. 2) show that the *supX45* strain is about ten times more sensitive to the killing action of P22, giving 5% survival at a multiplicity of 13 p. f. u./cell compared to 53% survival at a multiplicity of 12 p. f. u./cell for the *supX*$^+$ strain. The frequency of *ile*$^+$ transductants/survivor rises sharply with increasing multiplicity with the *supX45* recipient, whereas, with the *supX*$^+$ recipient the frequency rises and then levels off. The curves suggest that the strains bearing *supX* mutations are more dependent upon multiple infection for survival of transductants than *supX*$^+$ strains. The hypersensitivity of *supX* mutant strains to killing by P22 is also demonstrated by the P22 plaque morphology. Plaques on *supX* strains are

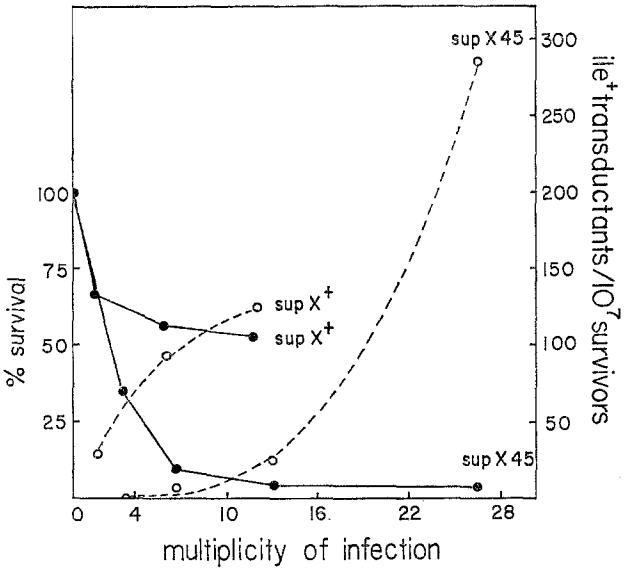


Fig. 2. Frequency of survival and of transduction after infection with P22 transducing phage. Strains *ara-9 leu-500 ile-241* carrying either the *supX*+ or *supX45* allele were infected with P22 phage grown on an *ile*+ strain. Solid lines represent frequency of survival and broken lines represent frequency of *ile*+ transduction

either completely clear or they have a small turbid center, whereas on *supX*+ strains, the plaques have a characteristically large turbid center.

The ability of a strain carrying the *supX45* mutation to be lysogenized by P22 was tested. Two strains, isogenic except for the *supX* alleles (*supX*+ and *supX45*), were infected with P22 phage (multiplicity of approximately 2 p. f. u./cell) and grown at 37° C overnight. Out of 5 *supX*+ survivors tested, all 5 were lysogenic; 3 out 5 *supX45* survivors tested were lysogenic and the other two were nonlysogenic. A nonlysogenic survivor of the *supX45* strain was found to be as sensitive as the original strain and a lysogenic survivor of the *supX45* strain was as resistant to superinfection killing as the lysogenic survivor of the *supX*+ strain.

*Catabolite Repression Sensitivity of supX Mutant Strains.* The mutation *cat* which in *E. coli* maps near the *trp-cysB* region of the chromosome decreases the sensitivity to catabolite repression (Tyler *et al.*, 1969). There is some suggestion that *supX* mutations can affect catabolite repression since a second mutation at the *leu-500* site which appeared to make the leucine operon sensitive to catabolite repression was suppressed by a deletion of the *supX* locus (Friedman and Margolin, 1968). If *supX45* were a *cat* type of mutation, the increased levels of certain enzymes could possibly be due to the removal of the repressing influence of a catabolite repression mechanism. Therefore we compared the sensitivity to catabolite repression of $\beta$-galactosidase induction in two F'*lac*+ strains, one carrying *supX*+, and the other, *supX45*, but otherwise isogenic. These strains, growing exponentially in minimal medium with glycerol, tryptophan and leucine were

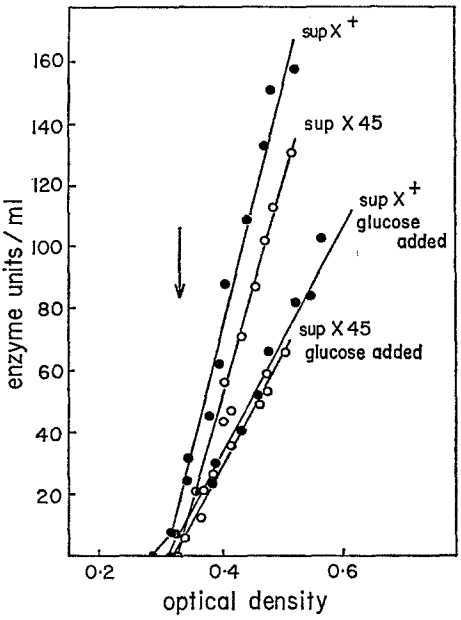


Fig. 3. Effect of glucose addition on the rate of β-galactosidase synthesis after induction in *ara-9 leu-500 nal*$^r$ (F'*lac*$^+$) carrying either the *supX*$^+$ or *supX45* allele. The arrow indicates the point of glucose addition to the cultures

induced with IPTG ($2 \times 10^{-4}$M), each with and without glucose (0.5%). Samples were removed at timed intervals for optical density (550 mμ in the Gilford spectrophotometer) measurements and β-galactosidase assays after toluenization. The results (Fig. 3) show that the *supX* mutation does not confer resistance to catabolite repression, at least not for the induction of β-galactosidase. Graf and Burns (personal communication) reached a similar conclusion studying another *supX* mutation (*supX24*). Friedman and Margolin (1968) noted that a different *supX* mutation (*supX20*) did not eliminate glucose caused catabolite repression of another inducible enzyme, histidase.

It has been postulated (Friedman and Margolin, 1968) that the *leu-500* mutation resulted in an altered *leu* operator which had become extremely sensitive to a specific catabolite repressor which was normally present in the cell. Since cyclic AMP can relieve catabolite repression of both the transient and permanent type, we tested the ability of cyclic AMP to suppress the *leu-500* phenotype. The induction of β-galactosidase was also followed to demonstrate that cyclic AMP would reverse catabolite repression in *S. typhimurium* under the conditions used. The strain (*ara-9 leu-500 nal*$^r$/F'*lac*$^+$) was grown to the exponential stage in minimal medium supplemented with glycerol and leucine, washed, treated with EDTA ($10^{-3}$M), and then diluted into two flasks of minimal medium supplemented with glycerol, one with and the other without cyclic AMP ($10^{-3}$M). Optical density of these cultures (followed with a Klett) did not increase in either case over a period of 5 hours, although in a control supplemented with leucine the strain

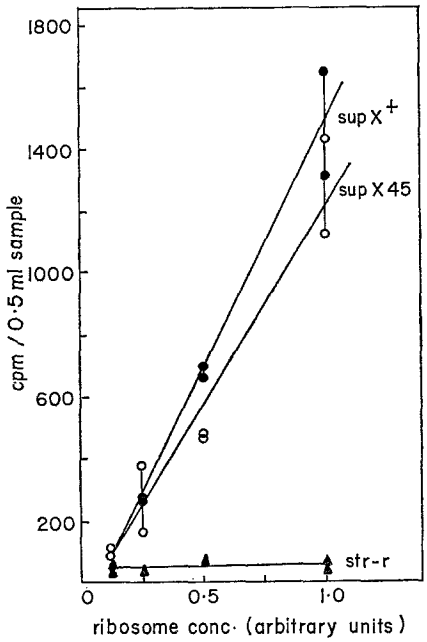


Fig. 4. The binding of $H^3$-labelled dihydrostreptomycin to 70S ribosomes prepared from *ara-9 leu-500* strains carrying either *supX*$^+$ *str*$^+$ (●), *supX45 str*$^+$ (○) or *supX*$^+$ *str*$^r$ (▲). The range in values for each ribosome concentration is shown by connecting the points obtained from duplicate samples

grew normally. In a parallel experiment the same strain was diluted into minimal media supplemented with glycerol, leucine and TMG ($5 \times 10^{-3}$M) and divided into 3 flasks. The induction of β-galactosidase was assayed in this medium in the presence of 1) glucose (22mM), 2) glucose + cyclic AMP, and 3) no further supplements. The rate of β-galactosidase synthesis in the culture with no further supplements was 0.88 enzyme units/ml/min, the rate in the culture to which glucose was added was 0.274 enzyme units/ml/min and the rate in the culture to which both glucose and cyclic AMP were added was 1.18 enzyme units/ml/min. It has been reported that cyclic AMP stimulates β-galactosidase induction even in the absence of glucose (Perlman and Pastan, 1968).

Apparently cyclic AMP does not reverse the leucine auxotrophy imposed by *leu-500*, although it effectively reverses catabolite repression of β-galactosidase induction in the *leu-500* strain. These results agree with the findings of Graf and Burns (personal communication) that cyclic AMP does not increase β-isopropylmalate dehydrogenase levels in a strain bearing the *leu-500* mutation.

*Studies of the Ribosomes of supX45 Strains.* The streptomycin, kanamycin and neomycin resistance conferred by the *supX* mutation led us to examine the ribosomes. The binding of $H^3$-dihydrostreptomycin was compared in 70S ribosomes isolated from three strains: PM447 (*ara-9 leu-500 supX*$^+$ *str*$^r$), PM258 (*ara-9 leu-500 supX45*) and *ara-9 leu-500 supX*$^+$ (Fig. 4). As described by Kaji and

Tanaki (1968), no binding occurred with the ribosomes from the streptomycin resistant (*str*$^r$) strain. From the data (Fig. 4), we cannot distinguish any difference in the dihydrostreptomycin binding between the ribosomes from the *ara-9 leu-500 supX*$^+$ and *ara-9 leu-500 supX45* strains. Similar results were obtained with the 30S subunits of ribosomes from these strains. The ribosomal protein of both the 30S and 50S subunits from strains *ara-9 leu-500 supX45* and *ara-9 leu500 supX*$^+$ were examined on acrilimide gels in Dr. I. Smith's laboratory and no differences were found. Similar results were obtained by Graf and Burns (1971) with gel electrophoresis of the ribosomal proteins.

## Discussion

The results from tests with selected specific nonsense suppressors and antibiotics affecting ribosome function have provided only negative evidence that *leu-500* is not a nonsense mutation. However, conforming to this interpretation is the additional evidence presented here and previously (Mukai and Margolin, 1963) concerning the locations of the sites of the Leu$^+$ revertants of *leu-500*. Essentially all such sites map only in the *leu-ara* chromosome region (presumably back mutations at or near the *leu-500* site) or between *cysB* and the tryptophan operon (*supX* mutations). If *leu-500* were suppressible by nonsense suppressors, then a fair number of the Leu$^+$ revertants would be expected to result from mutations at nonsense suppressor loci scattered around the chromosome (Sanderson, 1970) rather than only at the *supX* locus and at the leucine operon. It seems unlikely that *leu-500* is a nonsense mutation.

Although *supX45* suppresses *leu-500*, it does not suppress any of 46 other leucine auxotrophs tested although it seems likely that some of these leucine auxotrophs are due to nonsense mutations (Whitfield *et al.*, 1966). Two known *lacZ* nonsense mutations are not suppressed by *supX*. Furthermore *supX* suppression is recessive, as shown by dominance tests carried out with an F'*trp*$^+$ *supX*$^+$ episome (Sandip Basu, unpublished observations) whereas most known nonsense suppressors are dominant (Gorini and Beckwith, 1966). It seems unlikely that *supX* is a nonsense suppressor.

We can effectively eliminate the possibility that *supX* is a *ram* (ribosomal ambiguity) mutation. The *ram* mutations map at the *strA* locus. They suppress all nonsense mutations, and ribosomes from *ram* mutants misread extensively, (Rosset and Gorini, 1969). *SupX* strains have none of these characteristics (unpublished preliminary results suggest that in the presence of streptomycin, ribosomes from *supX* strains, if anything, misread somewhat less than wild-type ribosomes).

The *supX* suppressor mutations differ from the *suA* suppressor reported for *E. coli*, both with respect to location and to function. The *suA* suppressor is close to the *ilv* region of the chromosome (Beckwith, 1963; Morse and Primakoff, 1970) whereas *supX* is located almost half the chromosome map length away, closely linked to the tryptophan operon and *cysB* (Fig. 1). The *suA* suppressor does not suppress the *lacL1* promoter mutation (Scaife and Beckwith, 1966) but does suppress polarity effects in the lactose operon (Scaife and Beckwith, 1966) and

the tryptophan operon (Morse and Primakoff, 1970) of *E. coli*. As described here, the *supX* mutations suppress the *lacL1* promoter mutation and do not relieve the polarity effects of a strongly polar nonsense mutation in *trpA*, the first tryptophan structural gene.

One conceivable mechanism for suppression by *supX* mutations would be by means of an increased lifetime for mRNA. Most biosynthetic pathways would compensate for this by means of endproduct regulation so as to prevent overproduction. As noted, the *supX45* deletion has no overall effect on the amount of protein produced per cell. However, a number of indications of such an extended mRNA lifetime would likely have been detected in *supX* mutant strains. The specific activity of the fully repressed level of β-isopropylmalate dehydrogenase from the wild type leucine operon should have become significantly higher. The late tryptophan pathway enzyme levels might have increased enough to overcome the inhibition caused by high-level anthranilate supplementation in the presence of a *trpA* nonsense mutation. The uninduced β-galactosidase levels from the wild type lactose operon should have increased significantly. As described in this report none of these effects were found in *supX* mutant strains. It therefore seems unlikely that *supX* mutations increase the lifetime of mRNA in general although they might conceivably suppress by extending the lifetime of mRNA produced by operons bearing specific types of mutations such as *leu-500* or *lacL1*.

Mutations of the *supX* locus exhibit multiple pleiotropy. Many of the studies described here were done with strains bearing the *supX45* deletion which extends into the nearby tryptophan operon. Some of the effects ascribed to deletion of the *supX* locus might, in fact, be due to the deletion of previously undetected loci between the *supX* locus and the tryptophan operon. However, the 2-AP induced *supX* mutations which are likely to be point mutations also exhibit the low-level antibiotic resistance previously found with *supX45*. Studies are underway to examine their ability to suppress *lac* promoter mutations, their level of alkaline phosphatase activities and their degree of sensitivity to killing by P22 phage.

Thus far we have found that *supX* mutations suppress promoter-like mutations of the leucine operon as well as *lac* promoter mutations. Preliminary data indicate that certain *supX* mutations suppress the point *lac* promoter mutations *L8* and *L29* (unpublished observations). The *supX* mutations also suppress another mutation of the leucine operon, *GD-1* (Friedman and Margolin, 1968) which is located at or very near the *leu-500* site and causes the leucine operon to act as though responding to catabolite repression control. The *GD-1* mutation causes leucine auxotrophy in the presence of glucose but not when citrate serves as the carbon source. In a glucose medium a *supX* deletion mutation in a *GD-1* mutant strain eliminated leucine auxotrophy and caused a five-to-six-fold increase in the level of β-isopropylmalate dehydrogenase (Friedman and Margolin, 1968).

The association of a catabolite repression type of response with mutations in this region of the leucine operon (Fig. 1) reinforces the suggestion (Calvo, Margolin and Umbarger, 1969) that *leu-500* may be a promoter mutation. Silverstone *et al.* (1969, 1970) have provided evidence suggesting that the condition of the promoter determines the ability of the *lac* operon to respond to catabolite repression control. Although the *supX* mutation eliminates the glucose caused repression of the *GD-1*

leucine operon, the data presented here indicate that it does not eliminate catabolite repression of the lactose operon nor does it eliminate catabolite repression of histidase synthesis (Friedman and Margolin, 1968). It is therefore difficult, at present, to simply equate the catabolite sensitive response of the *GD-1* leucine operon to that of normal catabolite sensitive genes.

The presence of the *supX* mutation does not change the induction or repression characteristics of the mutant operons it suppresses. The induction ratio of $\beta$-galactosidase in the *lacL1* mutant is the same in the presence and absence of *supX45*; induced and uninduced specific activity of $\beta$-galactosidase is approximately doubled in the presence of *supX45*. Similarly, in *leu-500* strains, both the repressed (Table 5) and derepressed (Friedman and Margolin, 1968) specific activity of $\beta$-isopropylmalate dehydrogenase are suppressed to about the same extent (increased 6–10 fold) by the *supX45* deletion. Graf and Burns (1971) reported similar results.

The original hypothesis (Mukai and Margolin, 1963) proposed that the *supX* mutations eliminate a "foreign repressor" which had been binding to the *leu-500* "xenesthetic" leucine operator because of the latter's altered specificity. We must now try to take into account the *supX* suppression of the *lac* promoter mutation *L1*, the effects on control of the P22 phage genes leading to lysis and on the synthesis of alkaline phosphatase, as well as the low-level antibiotic resistance. It seems unlikely that both the *leu-500* and *lacL1* mutations would cause their respective operons to become sensitive to the same "foreign" repressor unless an acquired sensitivity to this hypothetical repressor is the basis for mutations with promoter-like characteristics. Furthermore, both the leucine and lactose mutant operons continue to respond to their normal regulatory controls, albeit somewhat abnormally. It seems reasonable to consider the possibility that the *supX* gene product is a factor affecting the efficiency and specificity of the initiation of either the transcription or translation process. The reduced efficiency of transcription of mutant promoters might be overcome, for example, by a decreased specificity of the transcription initiating mechanism leading to increased transcription.

Recent work on *in vitro* transcription reveals that many factors are required for accurate, high level repressor sensitive transcription. Thus far it has been shown that for proper transcription of the *lac* operon, at least the following elements are necessary: a normal promoter sequence, cyclic AMP, cyclic AMP receptor protein, RNA polymerase containing sigma factor, possibly ppGpp (Arditti *et al.*, 1970; de Crombrugghe *et al.*, 1971). From *in vitro* studies on the regulation of transcription, we know there are a number of factors which interact with RNA polymerase in the initiation process, such as $\sigma$ and the M factor (Davison *et al.*, 1970; Travers, 1971). It has been postulated that certain phage promoters can be transcribed as a result of changing the specificity of the RNA polymerase complex (Travers, 1971).

Recently it has been shown (Hong *et al.*, 1971) that P22 phage forms clear plaques on *S. typhimurium* mutant strains defective in adenylate cyclase, or cyclic AMP receptor protein, or with mutant RNA polymerase. These workers showed that such hosts could maintain lysogeny, but were deficient in the estab-

lishment of lysogeny. They concluded that the transcription of certain phage genes requires cyclic AMP which activates a host RNA polymerase system.

If these phage genes are not transcribed,l ysogeny cannot be established. Similar results have been reported for $\lambda$ phage in *E. coli* (Grodzicker *et al.*, 1972). P22 phage also produces clear plaques on *supX* strains.

It is not clear at present how such a postulated deficiency in the transcriptional apparatus of *supX* strains would cause the 5–20 fold increased levels of alkaline phosphatase, in high phosphate medium. Recent work (A. Wilkins, personal communication) indicates that adenine nucleotides are involved in the regulation of alkaline phosphatase. Starvation for thymine, uracil or guanine, resulting in high adenine nucleotides pools derepresses alkaline phosphatase, even in high phosphate medium. There may be adenine nucleotides besides cyclic AMP which function in the transcription process. It may be of interest to compare the pool levels of adenine nucleotides in *supX*$^+$ and *supX* mutant strains.

Another possible *supX* suppressor mechanism would be a defect which decreased proper termination of transcription causing "read-through" from the preceding operon. Studies on the *in vitro* lactose operon transcription have demonstrated "read-through" when the rho factor was missing (Arditti *et al.*, 1970). This seems somewhat unlikely in this case because there is no evidence of "read-through" of the *trpB* gene in the many *supX* deletion strains which lack the tryptophan operon promoter, *P1* (Bauerle and Margolin, 1966a, 1967; Margolin and Bauerle, 1966).

The mechanism for *supX* suppression could also be on the level of translation. Mutant messages (derived from promoter and promoter-like mutations) might be translated by an altered translation complex at a higher rate than normal. The translation initiation complex involves the AUG or GUG codon on the messenger RNA, fmet-tRNA, ribosomes, GTP, and at least three initiation factors (cf. The Cold Spring Harbor Symposium of 1969). The *supX* mutation could involve changes in initiation factors, in the fmet-tRNA, in the ribosomes, or in the secondary structure of the messenger RNA. When the secondary structure of f2 RNA is altered by formaldehyde, there is a change in the type of polypeptides synthesized (Lodish, 1970a). Ribosomes themselves also have specificity for the initiation of translation (Lodish, 1970b); thus ribosomes from *B. stearothermophilus* translate only one cistron of the f2 messenger RNA whereas *E. coli* ribosomes translate all three cistrons. Dube and Rudland (1970) and Steitz *et al.*, (1970) have shown that changes occur in the initiation factors after T4 infection of *E. coli* which in turn change the specificity of initiation complexes formed with various messenger RNAs.

A translational mechanism of suppression would require that the mRNA transcribed from operons with promoter mutations be different from mRNA transcribed from operons with normal promoter genes. To explain the increased frequency of lytic development of phage P22 in *supX* strains by a translational mechanism would require that genes involved in causing lysis be usually transcribed in *supX*$^+$ strains at a very low level (below a metabolically active threshold) rather than being completely shut off. This mRNA would then be translated at a

greater frequency in *supX* mutant strains, more often leading to lysis than in *supX*$^+$ strains.

*Acknowledgements.* We gratefully acknowledge the fine work of Mr. Tom Shenk who studied the drug resistance of strains carrying the *supX* mutation and also compared the streptomycin binding to the ribosomes of *supX*$^+$ and *supX45* strains. We also would like to thank Ms. Pat Santanello for excellent technical assistance. We owe special thanks to Ms. J. Tamayo for incredible patience and excellent secretarial assistance.

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