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Genetic Mapping of *trxA*, a Gene Affecting Thioredoxin in *Escherichia coli* K12

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Summary. The DNA polymerase induced by bacteriophage T7 is composed of a phage-specified subunit, the gene 5 protein, and a host-specified subunit, the 12,000 dalton thioredoxin of *Escherichia coli*. *tsnC* mutants of *E. coli* B (Chamberlin, 1974) have no detectable thioredoxin, and thus cannot support the growth of phage T7, although they are killed by phage infection. A mutant of *E. coli* K12 affecting thioredoxin has been isolated by a modification of the procedure used by Chamberlin (1974) to isolate *tsnC* mutants of *E. coli* B. The gene affecting thioredoxin has been designated *trxA*. This mutant, *E. coli* JM109, shows the TsnC phenotype in that it is killed by, but cannot support the growth of, bacteriophage T7. T7 DNA replication does not occur in mutant-infected cells. These phenotypic expressions of the *tsnC* mutation have enabled us to screen recombinants for the *trxA* allele in Hfr × F^- crosses and F-ductants in episome transfer experiments. Extracts of transductants in generalized transduction by P1 phage were screened for their ability to complement partially purified phage T7 gene 5 protein to form T7 DNA polymerase. The *trxA* gene is located at 84 min on the *E. coli* linkage map, between *uvrE* and *metE*; *trxA* is 34% co-transducible with *metE*.

Introduction

Thioredoxin of *Escherichia coli* is a heat-stable, 12,000 dalton protein (Laurent et al., 1964). In vitro thioredoxin provides the reducing power, in the form of two cysteine residues, for the reduction of ribonucleoside diphosphates to deoxyribonucleoside diphosphates in a reaction catalyzed by ribonucleoside diphosphate reductase (Reichard, 1967). In the process the two cysteine residues of thoredoxin are oxidized to cystine, and the reduced form of thioredoxin is regenerated by the action of thioredoxin reductase at the expense of one molecule of TPNH. In recent years thioredoxin has been subjected to extensive structural studies (Holmgren, 1975). Mutants having defects in the B1 and B2 subunits of ribonucleoside diphosphate reductase have been isolated, and the structural genes, *nrdA* and *nrdB*, are located at 48 min on the *E. coli* map (Fuchs and Karlström, 1976). Inasmuch as *nrdA* mutants are temperature-sensitive for growth, this enzyme plays a key role in nucleotide reduction (Wechsler and Gross, 1971; Fuchs et al., 1972). However, a mutant having no detectable levels of thioredoxin reductase grows normally (Fuchs, 1977), raising the question of whether it serves as the obligatory cofactor of ribonucleotide reductase in vivo.

Recently we reported that the DNA polymerase of bacteriophage T7 is composed of two subunits: One is specified by gene 5 of the phage, and the other is thioredoxin of *E. coli* (Modrich and Richardson, 1975a, b; Mark and Richardson, 1976). A mutant of *E. coli* B, *E. coli* 7004, originally selected by its inability to support the growth of phage T7 although it is killed by the phage (Chamberlin, 1974), was subsequently found to lack thioredoxin (Mark and Richardson, 1976). Thus, after infection of the mutant by phage T7, no active T7 DNA polymerase is formed, and hence there is no T7 DNA replication. Interestingly, although there is no detectable thioredoxin protein present in extracts, the mutant grows normally (Chamberlin, 1974). These original thioredoxin mutants were isolated from an *E. coli* B strain, and were designated *tsnC* to describe their T7 negative phenotype. We now propose that *trx* be used to denote the more specific phenotype, the alteration in thioredoxin.

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More detailed studies on the actual role of thioredoxin in *E. coli* and in T7 DNA metabolism will be facilitated greatly by knowing the map position of the *trx* gene. With such information, deletions of the *trx* gene can be obtained in order to assess more accurately the essential nature of thioredoxin in *E. coli*. In addition, knowledge of the map position of *trx* will make possible construction of strains carrying specific mutations in conjunction with the *trx* mutation that will facilitate studies on T7 DNA replication. Finally, a well defined collection of thioredoxin mutants will be of great value in the further correlation of thioredoxin structure and function. This paper describes the isolation of a similar mutant from *E. coli* K12 and the mapping of the *trxA* gene.

Materials and Methods

Bacterial and Phage Strains

All strains used were derived from *E. coli* K12 except *E. coli* 7004 which is a derivative of *E. coli* B. *E. coli* JG138 *su7*$^+$, carrying a tandem duplication of one *su7*$^+$ allele and one *su7*$^-$ allele, has been previously described (Campbell et al., 1972). A list of the strains used and their properties are given in Table 1. Figure 1 is a map of the *E. coli* chromosome showing the position of relative markers (Bachmann et al., 1976) and the point of origin of the Hfr strains used (Low, 1972). Wild type phage T7, $T7_{3,6}$ and $T7_{3,5,6}$ were obtained from Studier (1969). For convenience, T7 mutants are designated by gene number only. The *amber* mutations used are gene 3, *am*29; gene 5, *am*28; gene 6, *am*147. Phage P1CM*clr*100 was used for generalized transductions (Rosner, 1972).

Media

The minimal medium used was that described by Vogel and Bonner (1956) supplemented with 0.5% of the desired sugar, 0.2 μg per ml of thiamine, and 40 μg per ml of any required amino acid except for proline which was present at a concentration of 200 μg per ml. Thymine was present at 50 μg per ml where required. L broth was prepared according to Lennox (1955) with 50 μg per ml thymine.

T broth contained 10 g of tryptone (Difco) and 5 g of sodium chloride per liter. L broth and T broth plates were prepared from the same medium containing 1.5% agar. L broth and T broth soft agar were prepared with L broth and T broth, respectively, containing 0.6% agar. L broth plates containing calcium and chloramphenicol used in transduction studies were prepared as described by Miller (1972). MacConkey Agar was prepared with

Table 1. Bacterial strains

Strain	Characteristics	Source
Hfr strains:		
Hfr KL14	argG — metE — rha	*Coli* Genetic Stock Center
Hfr KL16-99	thy — recAl	
Hfr KL209	rha — metE — thyA	
Hfr P72	rha — thr	
F′ strains	Episome F111	
KLF11/JC1553	malB — rha — metE — ilv — pyrE	
JC1553	*arg*G6, *met*B1, *his*-1, *leu*-6, *rec*A1, *mtl*-2, *xyl*-7, *mal*A1, *gal*-6, *lac*Y1, *str*-104, *ton*A2, *tsx*-1, λ^R, λ^-, *sup*E44	*Coli* Genetic Stock Center
F$^-$ strains:		
JG138 su7$^+$	F$^-$, *thy*A, *pol*A1, *rha*$^-$, *lac*Zam, *str*^{R}, su7$^+$/su7$^-$ This is the parent of JM109	Campbell et al. (1972)
JG138	F$^-$, *su7*$^-$ segregant of JG138 *su7*$^+$	This paper
JM109	F$^-$, *thy*A, *pol*A1, *rha*$^-$, *lac*Zam, *str*^{R}, *trx*A,	This paper
JM110	F$^-$, *thy*A, *pol*A1, *rha*$^-$, *lac*Zam, *str*^{R}, *trx*A, *thr*$^-$	This paper
JM115	F$^-$, *met*E, *thr*$^-$, *rha*$^-$, *lac*Zam, *str*^{R}, *trx*A	This paper
JM116	F$^-$, *met*E, *thr*$^-$, *rha*$^-$, *lac*Zam, *str*^{R}, *trx*A, *rec*A	This paper
AB2200	F$^-$, *thi*-1, *met*E46, *his*-4, *trp*-3, *mal*A1, *lac*Z-13 *mtl*-1, *ara*-9, *str*-8, *ton*-1, *tsx*-3, λ^-, λ^R	*Coli* Genetic Stock Center
ES245	F$^-$, *ilv*D188, *uvr*E	Siegel (1973)
Other strains:		
B/1	T1^R; parent of 7004	Chamberlin (1974)
7004	T1^R, *tsnC*	Chamberlin (1974)

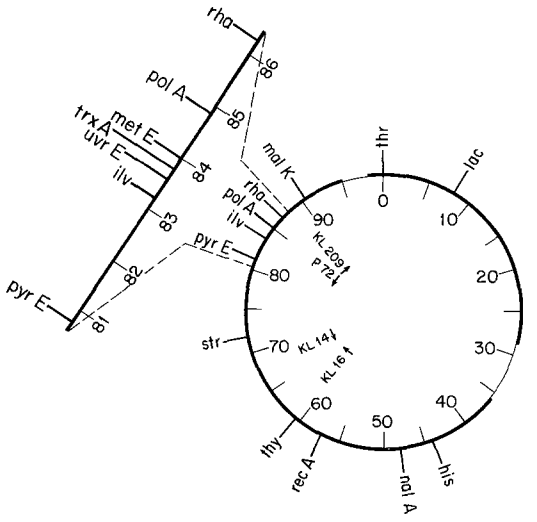

Fig. 1. Linkage map of *Escherichia coli* K12 showing the positions of the markers mentioned in the text and the approximate point of origin and direction of transfer of Hfr strains listed in Table 2. The expanded view of the *pyrE-rha* region of the chromosome shows the approximate position of the *trxA* locus. This map was adapted from Bachmann et al. (1976)

MacConkey agar base (Difco) and contained 1% of the desired sugar. Plates containing drugs or antibiotics contained the following concentrations: streptomycin, 200 μg per ml; methylmethane sulfonate, 0.04%; chloramphenicol, 1 mg per ml.

Mating and Transduction Procedures

All bacterial matings were performed as described by Miller (1972). Matings were interrupted by 40-fold dilution in 0.85% NaCl and mechanical shaking for 60 sec using the apparatus described by Low and Wood (1965). Transductions were carried out using P1CM*clr*100 as described by Miller (1972). Transductants were examined for lysogeny by sensitivity to chloramphenicol and phage T7.

Ampicillin Selection

Spontaneous auxotrophs were isolated by ampicillin enrichment as described by Miller (1972).

Preparation of Bacterial Strains

E. coli JM110 is a spontaneous *thr*$^-$ derivative of *E. coli* JM109 isolated by ampicillin selection. Strain JM116 *polA*$^+$ *metE recA* is a derivative of *E. coli* JM110 isolated by P1 transduction using *E. coli* AB2200 as donor. *polA*$^+$ transductants were selected on methyl-methane sulfonate plates and then scored for co-transduction of the *metE* locus. One isolate, strain JM110 *polA*$^+$ *metE*, was then mated with *E. coli* Hfr KL16-99 *recA*1 for 20 min. *thy*$^+$ recombinants were scored for sensitivity to UV-irradiation. The *thy*$^+$ UV^s isolates were checked for the retention of the *metE* and *trx* markers. Strain JM115 was a *thy*$^+$ UV^r recombinant obtained from this mating.

Isolation of trx Mutant

The procedure for the isolation of the *trx* mutant is a modification of that described by Chamberlin (1974) for the isolation of *tsn* mutants. *E. coli* JG138 *su7*$^+$ (Campbell et al., 1972) was mutagenized with N-methyl-N′-nitro-N-nitrosoguanidine, lmg per ml, according to the method of Adelberg et al. (1965) in Tris-maleate buffer (pH 6.1) for 30 min at 37° C. The mutagenized cells were washed twice with 0.85% NaCl, resuspended in L broth, and aliquots were diluted 10-fold into L broth for mutant segregation at 37° C overnight. After segregation approximately 2500 cells were spread on T broth agar plates seeded with 2×10^7 T7 phage. After 12–16 h, surviving colonies were grown overnight in L broth in 96-well microtiter plates and replica-plated into wells of microtiter plates containing 7×10^8 T7 phage per ml using procedures described by Milcarek and Weiss (1973). Cultures that failed to grow were purified from the master plate by streaking to obtain single colonies. These isolates were then scored by spot test for the ability to support the growth of, or to be killed by, phages T3, T4 and T7. Cultures which were killed by phages T3 and T7, but failed to produce bursts of phages T3 and T7 while supporting the normal growth of phage T4, were further tested in cell-free extracts for the ability to stimulate T7 gene 5 protein in a polymerase assay (see below). Cells whose extracts failed to stimulate the T7 gene 5 protein should carry a mutation in the *trx* gene. One such mutant, strain JM109, was isolated for further study.

Growth of Cells and Preparation of Cell Extracts

Overnight cultures of *E. coli* grown in L broth were diluted 100-fold into 500 ml of L broth and incubated at 37° C with virgorous shaking. At $A_{590}=2.0$ (10^9 cells per ml) the cultures were infected with T7 phage at a multiplicity of 5, incubated at 37° C for another 12–15 min with shaking, and then cooled by swirling in an ice bath. The cells were collected by centrifugation in a Sorvall GS3 rotor and resuspended in 4 ml of 0.05 M Tris-HCl buffer (pH 7.6) −10% sucrose, and then frozen in liquid nitrogen. Extracts were prepared as described by Hinkle and Richardson (1974). Extracts of uninfected cells were prepared in an identical manner.

Enzymes and Proteins

T7 DNA polymerase was the DNA-cellulose pool (Fraction VI) of Modrich and Richardson (1975a). *E. coli* thioredoxin (Laurent et al., 1964) was a gift from Dr. Arne Holmgren. Both thioredoxin and T7 DNA polymerase were more than 90% pure as judged by gel electrophoresis in the presence of sodium dodecylsulfate (Weber and Osborne, 1969). T7 gene 5 protein was the phosphocellulose pass-through fraction previously described (Modrich and Richardson, 1975b).

Enzyme Assays

T7 DNA polymerase was assayed as previously described (Grippo and Richardson, 1971). In vitro replication of T7 DNA was measured according to the method of Hinkle and Richardson (1974).

Thioredoxin was assayed by measuring its ability to complement partially purified gene 5 protein to form an active DNA polymerase as previously described (Modrich and Richardson, 1975b). When a large number of extracts were assayed for thioredoxin, as in the screening of transductants, the following procedure was used: Cells were grown in 96-well microtiter plates in the appropriate medium, collected by centrifugation (Milcarek and Weiss, 1973), and resuspended in 50 μl of 33 mM Tris-HCl (pH 8.0) − 2.5 mM EDTA − 0.1 μg per ml lysozyme. The cells were then lysed by freeze-thawing three times, and cell debris was removed by centrifugation. A reaction mixture containing 130 mM potassium phosphate buffer (pH 7.4), 9.5 mM $MgCl_2$, 7.5 mM 2-

mercaptoethanol, 64 nmol of heat-denatured salmon sperm DNA, 22.5 nmol each of dTTP, dCTP, dGTP, 2.5 nmol of [α-^{32}P] dATP and 50 µg of gene 5 protein was added to each well in the microtiter plate (150 µl final volume). After 30 min at 37° C, the reaction was terminated by cooling on ice, and the cell debris was removed by centrifugation. An aliquot of each reaction mixture (25 µl) was transferred, 12 at a time, to a sheet of DEAE-cellulose paper (Whatman DE81), and the paper was washed twice for 10 min in 500 ml of ice-cold 0.5 M NaCl and then for 10 min in 95% ethanol as described by Mead (1964) to remove non-polymerized nucleotides. The paper was dried and exposed to X-ray film (Kodak No-Screen NS54T). Under the conditions given, the [^{32}P] labeled DNA synthesized in the reaction absorbs strongly to the DEAE paper so that a small exposed area is observed on the X-ray film wherever DNA polymerase activity, and hence thioredoxin, was present in an incubation well. When polymerase I was present in extracts, it was inactivated by heating the extract at 65° C for 10 min.

Protein Determination

Protein was determined by the method of Lowry et al. (1951).

Chemicals

N-Methyl-N′-nitro-N-nitrosoguanidine was purchased from Aldrich Chemical Co. Chloramphenicol was obtained from Calbiochem. Unlabeled nucleotides were obtained from Schwarz Bioresearch. [^{3}H]thymidine, [^{3}H]-deoxythymidine triphosphate and [α-^{32}P]deoxyadenosine triphosphate were from New England Nuclear. Salmon sperm DNA was purchased from Sigma and heat-denatured according to Grippo and Richardson (1971). T7 [^{3}H] DNA was prepared as previously described (Hinkle and Richardson, 1974).

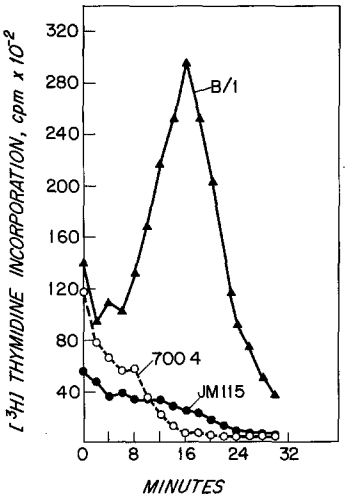

Fig. 2. DNA synthesis in *E. coli* B/1 (▲) strain 7004 *trxA*$^-$ (○) and strain JM115 *trxA*$^-$ (●) infected with T7 phage. Bacterial cultures were grown in minimal medium supplemented with 0.5% glucose, 0.2 µg/ml thiamine and 0.2% casamino acids to 2.5×10^8 cells/ml. For pulse labeling 5 ml volumes were incubated at 30° with vigorous shaking for 3 min and then infected with T7 phage at a multiplicity of 20. Samples of 0.2 ml were withdrawn at 2-min intervals and labeled with 5 µl of [^{3}H]thymidine (100 µCi/ml, 20 Ci/µmole). After 60 s incubation at 30°, 2.5 ml of ice cold 2.5% perchloric acid was added to each sample, and the total amount of acid-insoluble radioactivity was measured after filtration through Whatman GF/C filter discs

Results

Isolation and Characterization of the trxA Mutant

Using the selection procedure described by Chamberlin (1974) for the isolation of *tsn*$^-$ strains of *E. coli* B we isolated several *E. coli* K12 mutants following mutagenesis that had the same phenotype. They are killed by T7 phage, but do not support the growth of the phage. With the additional knowledge that *tsnC* mutants are lacking thioredoxin (Mark and Richardson, 1976) we were able to identify one of the *tsn*$^-$ mutants, JM109, as *tsnC*$^-$, i.e. extracts of the mutant cannot complement T7 gene 5 protein to form an active T7 DNA polymerase (Modrich and Richardson, 1975a, b).

Although the latter criteria should be adequate for the identification of a *trx*$^-$ strain, two additional experiments have been carried out in order to clearly establish that *E. coli* JM109 has a phenotype identical to that of the original *tsnC* mutants. First, we show that the *E. coli* K12 *trxA* mutant has a defect in T7 DNA replication, both in vivo and in vitro, and secondly we show that this defect is the results of a deficiency of T7 DNA polymerase activity.

T7 DNA Replication in the T7-infected trxA Mutant

As shown in Figure 2, the rate of DNA synthesis in wild type *E. coli* increases rapidly after phage T7 infection, reaching a maximum at approximately 16 min after infection. In contrast, DNA synthesis decreases after infection of either of the *trx*$^-$ strains, *E. coli* 7004 or *E. coli* JM115. These results are in agreement with those previously reported by Chamberlin for *E. coli* 7004 and its parent, *E. coli* B/1 (Chamberlin, 1974).

In order to show that the defect in DNA replication is due specifically to a lack of T7 DNA polymerase, T7 DNA replication was examined in an in vitro system (Hinkle and Richardson, 1974). Extracts of $T7_{3,6}-$infected *E. coli* JG138, the parent of the *trx*$^-$ strain, catalyzed extensive replication of duplex T7 DNA (Table 2). In contrast, extracts of the $T7_{3,6}-$infected *trx*$^-$ host JM110 were almost inactive, incorporating no more nucleotides than the uninfected or $T7_{3,5,6}-$infected parent lacking T7 DNA polymerase activity. However, synthetic activity of the infected mutant extracts on duplex T7 DNA was restored to

Table 2. In vitro defect in T7 DNA replication in extracts of infected *trx* hosts[a]

Extract		Addition	dNMP incorporated (nmoles)
Phage	Host		
$T7_{3,6}$	JG138 *trx*$^+$		0.57
	JG138 *trx*$^+$		<0.01
$T7_{3,5,6}$	JG138 *trx*$^+$		0.01
$T7_{3,6}$	JM110 *trx*−		0.04
$T7_{3,6}$	JM110 *trx*$^-$	120 μg JG138 extract	0.40
$T7_{3,6}$	JM110 *trx*−	0.065 μg T7 DNA polymerase	1.60
		0.065 μg T7 DNA polymerase	<0.01

[a] In vitro T7 DNA replication was measured as described in "Materials and Methods"

Table 3. Deficiency of phage DNA polymerase activity in extracts of phage infected trx mutant[a]

Extract[b]		Addition	dNMP incorporated (nmoles)
Phage	Host		
$T7_{3,6}$	JG138		3.31
$T7_{3,6}$	JG138	0.160 μg extract JM110	3.23
$T7_{3,5,6}$	JG138		0.06
$T7_{3,6}$	JM110		0.08
$T7_{3,6}$	JM110	0.167 μg extract JG138	0.84
$T7_{3,6}$	JM110	0.4 μg thioredoxin	1.78

[a] The assay for DNA polymerase activity is described in "Materials and Methods"

[b] 160–170 μg of phage infected extracts were used in these assays

almost normal levels by the addition of an extract from uninfected *E. coli* JG138 *trx*$^+$ (Table 2). Furthermore, the addition of small amounts of homogeneous T7 DNA polymerase to the mutant extracts resulted in a marked stimulation of synthesis on the duplex DNA; T7 DNA polymerase alone cannot use duplex DNA as a template. These results are identical to those previously reported for *E. coli* 7004 *tsnC*$^-$ (Modrich and Richardson, 1975a). Thus the T7−infected *trx*$^-$ mutant does not synthesize an active T7 DNA polymerase. On the other hand, the T7 proteins required for DNA replication are synthesized after infection, and the gene 5 protein can be activated by the addition of uninfected wild type extract.

Deficiency in Thioredoxin and T7 DNA Polymerase Activity

When assayed for repair synthesis activity on denatured salmon sperm DNA, extracts of T7−infected *E. coli* JM110 contained less than 3% of the DNA polymerase activity found in extracts of T7−infected *E. coli* JG138 (Table 3) and, in fact, is similar to the residual activity found in $T7_{3,5,6}$−infected JG138. Homogeneous thioredoxin, however, restores DNA polymerase activity to the phage-infected mutant extracts as does an extract of uninfected JG138 *trx*$^+$. Mixing extracts of $T7_{3,6}$−infected *E. coli* JG138 *trx*$^+$ and uninfected *E. coli* JM110 *trx*$^-$ did not reveal the presence of an inhibitor (Table 3). Again, these results are in agreement with those published for the *E. coli* B *tsnC* mutant (Modrich and Richardson, 1975a; Mark and Richardson, 1976).

Mapping of the trxA Locus. Approximate Map Position

Hfr mating experiments were carried out to determine the approximate position of the *trxA* locus on the *E. coli* K12 chromosome (Fig. 1, Table 1). *E. coli* Hfr KL209 was first mated with *E. coli* JM110 *trx*$^-$ *rha*$^-$ *thy*$^-$ and Rha$^+$ and Thy$^+$ recombinants were picked at the first time point at which they appeared and they were tested for their resistance to phage T7. Of 94 Rha$^+$ recombinants tested, 80 were Trx$^+$, while only 3 of 22 Thy$^+$ recombinants were Trx$^+$. A second mating experiment was performed with strain Hfr P72. Selection was for Rha$^+$ recombinants, but all were Trx$^-$. Finally, *E. coli* Hfr KL14 was mated with strain JM110, and again Rha$^+$ recombinants were selected; of 84 Rha$^+$ colonies, 77 were Trx$^+$. These results fixed the position of the mutation in strain JM110 between the origin of Hfr KL14 and *rha*.

Effects of Merodiploid on the trx Mutation

In order to map the *trx* locus more precisely, a partial diploid was constructed. F′111 was introduced into *E. coli* JM116 *pol*$^+$ *metE*$^-$ *recA* from the donor strain, KLF11/JC1553 (Table 1, Fig. 1) by bacterial conjugation. F′ derivatives of strain JM116 were selected as Met$^+$. Extracts of 4 Met$^+$ isolates contained thioredoxin activity, suggesting that the *trx*$^+$ allele was on the episome. These derivatives were able to donate *metE*$^+$ and *ilv*$^+$ alleles and were also sensitive to the male-specific phage fd. Since they were all recombination defective, carrying a mutation in the *recA* gene, it was reasonable to assume that they were trx$^+$/trx$^-$ merodiploids. Since all four trx$^+$/trx$^-$ merodiploids tested had thioredoxin activity in their cell-free extracts, we concluded that the *trx* mutation was recessive to the wild type allele.

Table 4. Co-transduction frequencies of *trxA*[a]

Donor	Recipient	No. transductants with selected marker			No. transductants with unselected marker		Co-transduction (%)
		metE	*ilvD*$^+$	*polA*$^+$	*trx*$^-$	*trx*$^+$	
(A) JM110 *metE*$^+$ *trxA*$^-$	AB2200 *metE*$^-$ *trx*$^+$	79			27		34
(B) JM110 *ilvD*$^+$ *trxA*$^-$	ES245 *ilvD*$^-$ *trx*$^+$		83		10		12
(C) AB2200 *polA*$^+$ *trxA*$^+$	JM110 *polA1 trxA*$^-$			300		0	0

[a] Phage P1CM*clr*100 was grown on strain JM110 *trx*$^-$, and transduction was performed as described in "Materials and Methods". (A) Selection was made on plates containing minimal medium supplemented with glucose, 0.5% histidine, 20 μg/ml; tryptophan, 20 μg/ml; and thiamine, 0.2 μg/ml. (B) Selection plates contained minimal medium supplemented with glucose, 0.5% and thiamine, 0.2 μg/ml. (C) Selection plates contained minimal medium supplemented with 0.5% glucose; threonine, 20 μg/ml; thymine, 50 μg/ml; thiamine, 0.2 μg/ml; and 0.04% MMS. The transductants were scored for the *trx* marker by assay of extracts for thioredoxin in microtiter plates as described in "Materials and Methods"

Table 5. Ordering of the genes in *ilv* region by 3-factor cross[a]

Selected marker	Unselected markers	Number of transductants	Transductants with unselected markers (%)
ilv$^+$	*uvrE*$^-$ *trxA*$^+$	116	42
	uvrE$^+$ *trxA*$^+$	98	35
	uvrE$^+$ *trxA*$^-$	38	14
	uvrE$^-$ *trxA*$^-$	26	9

Donor *ilvD*$^+$ *uvrE*$^+$ *trxA*$^-$

Recipient *ilvD*$^-$ *uvrE*$^-$ *trxA*$^+$

[a] 3-Factor cross was performed by transduction with PlCM*clr*100 grown in strain JM110 *trxA*$^-$, *uvrE*$^+$, *ilv*$^+$; the recipient strain was ES245 *trxA*$^+$, *uvrE*$^-$, ilvD$^-$. Selective plates contained minimal medium supplemented with glucose, 0.5% and thiamine, 0.2 μg/ml. *ilv*$^+$ colonies were grown in microtiter plates containing minimal medium supplemented with glucose, 0.5% and thiamine, 0.2 μg/ml. Transductants were scored for the *trxA* marker by preparing extracts and assay for thioredoxin as described in "Materials and Methods." Transductants were scored for the *uvrE* marker by replica plating from a microtiter plate onto two T-agar plates using 48-prong devices. The replicas were then placed 60 cm from a GE germicidal lamp and subjected to UV irradiation for 45 s

P1 Transduction

Since the *trxA* locus appeared to lie in a small region between *pyrE* and *rha* (Fig. 1), co-transduction frequencies were determined with several markers in this region. Transduction was initiated by phage P1CM*clr*100. The donor was strain JM110 *met*$^+$ *ilv*$^+$ *trx*$^-$. Selection was for the auxotrophic marker indicated (Table 4), and the extracts of the transductants were assayed for thioredoxin. *trxA* is 34% co-transducible with *metE* and 12% co-transducible with *ilvD*. We have also performed transductions with *E. coli* JM110 *polA1trxA*$^-$ as recipient and AB2200 *polA*$^+$ *trxA*$^+$ as donor to determine the co-transduction of *trxA* with *polA1* but out of 300 *polA*$^+$ JM110 transductants screened, none were *trxA*$^+$. Therefore *trxA* did not co-transduce with *polA1*. These co-transduction frequencies suggest that *trxA* is located between *ilv* and *metE*, but closer to the latter.

Ordering of the Genes in the ilvD-uvrE region

To order the genes in the *ilvD-uvrE* region, we performed a three-factor cross, by P1 transduction, between *E. coli* JM110 *ilv*$^+$ *uvr*$^+$ *trxA*$^-$ and strain ES245 *ilvD*$^-$ *uvrE*$^-$ *trxA*$^+$ (Table 5). *ilv*$^+$ colonies were selected and scored as UV-sensitive (*uvrE*$^-$) or UV-resistant (*uvrE*$^+$). All *ilv*$^+$ transductants were then assayed separately for thioredoxin in cell-free extracts. Table 5 lists the co-transduction frequencies of the unselected markers and a possible ordering of the three gene loci. Such an ordering of the genes would predict that the recombinant *ilv*$^+$ *trxA*$^-$ *uvrE*$^-$ should appear at the lowest frequency because it involved two recombination events, while the other three possible recombinants would require only one cross-over. Furthermore, the recombinants which would appear most frequently should be *ilv*$^+$ *uvrE*$^-$ *trxA*$^+$ because it involved the shortest segment of the chromosome and would require only a single cross-over. These predictions were born out by our findings listed in Table 5. In the previous section we had concluded that the *trxA* locus could not be located between *polA* and *metE* since *trxA* did not co-transduce with *polA*. Therefore, together with the information derived from the three-factor cross, we can now conclude that the *trxA* locus is located between *metE* and *uvrE* (Fig. 1).

Discussion

The role of thioredoxin in *E. coli* has long been thought to be that of hydrogen donor in the reduction

of ribonucleotides to deoxyribonucleotides in the reaction catalyzed by nucleoside diphosphate reductase (Laurent et al., 1964; Reichard, 1967). There is little doubt that the reductase is an essential component of this reduction system in vivo in that conditionally lethal mutants, *nrdA* mutants (Wechsler and Gross, 1971; Fuchs and Karlström, 1976), defective in this enzyme, have been isolated. On the other hand, the *tsnC* mutants of *E. coli* B (Chamberlin, 1974) and the *trxA* mutant of *E. coli* K12 described in this paper grow normally, although they are clearly defective in thioredoxin. In fact *E. coli* 7004 *tsnC*$^-$ and the *E. coli* JM110 have no detectable thioredoxin by assay with ribonucleotide reductase or by radioimmune assay (Arne Holmgren, personal communication). The nonessential nature of thioredoxin is also supported by the lack of a detectable defect in recently isolated thioredoxin reductase mutants (Fuchs, 1977).

Using *E. coli* 7004 *tsnC*$^-$ lacking thioredoxin, Holmgren (1976) has identified a hydrogen transport system for ribonucleotide reduction that is dependent on physiological concentrations of reduced glutathione. In this regard it is interesting to note that a mutant, defective in glutathione synthetase, has been isolated and shown to have greatly reduced amounts of glutathione, although it had no obvious physiological defect (Fuchs and Warner, 1975). Possibly either the thioredoxin or glutathione reduction system alone is sufficient for ribonucleotide reduction. Now that the *trxA* locus is known, it is possible to construct thioredoxin-glutathione synthetase double mutants in order to investigate this possibility. In addition, the availability of *E. coli* K12 mutants lacking thioredoxin will facilitate the identification of alternative systems for reduction and the isolation of mutants defective in these alternate processes. Thioredoxin may also be involved in other pathways of metabolism. In yeast, thioredoxin has been postulated to be involved in the reduction of sulfates (Porque et al., 1970), and some evidence of thioredoxin involvement with an intermediate of sulfate reduction in *E. coli* has been advanced recently (Tsang and Schiff, 1976).

It must be stressed that the existing thioredoxin mutants may contain undetectable, but residual, amounts of thioredoxin. Thioredoxin is present in *E. coli* in relative abundance and clearly these large amounts are not essential to the cell. However, the ultimate question of whether thioredoxin is essential cannot be answered until a deletion mutant can be isolated.

We are currently studying the interaction of thioredoxin with purified T7 gene 5 protein to form an active DNA polymerase. By selective mutagnesis of the *trx* gene we hope to generate a number of altered thioredoxins that will be useful in investigating the structural features of thioredoxin that are important in this reaction. These altered thioredoxins should also prove useful in the detailed structural studies being carried out with thioredoxin as a reducing agent (Holmgren et al., 1975).

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