JOURNAL OF BACTERIOLOGY, June 1976, p. 1082-1088
Vol. 126, No. 3

Printed in U.S.A.

Genetic Mapping of *xthA*, the Structural Gene for Exonuclease III in *Escherichia coli* K-12

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Received for publication 13 February 1976

The genes *xthA*, *pncA*, and *pabB* were ordered relative to others by two- and three-factor transductional crosses with bacteriophage P1. The genes studied span 2 min (2%) of the genetic map of *Escherichia coli* K-12 in the clockwise sequence *pheS-pfkB-xthA-pncA-gap-pabB-fadD*. Eleven independently derived *xth* mutations were examined; all were known to affect exonuclease III and its associated endonuclease II activity, and all were mapped in the *xthA* region. *pncA* mutations were found to confer resistance to 6-aminonicotinamide, whereas some *pheS* mutations are known to specify resistance to *p*-fluorophenylalanine. *xth* mutations were readily transferred into other strains by selecting for these co-transducible drug resistance markers.

Exonuclease III of *Escherichia coli* (19, 20) is a single polypeptide with several enzymatic activities (25a): its exonuclease activity removes 5'-mononucleotides from the 3' ends of deoxyribonucleic acid (DNA) duplexes, its DNA 3'-phosphatase activity releases 3' terminal phosphomonoesters from DNA, and its endonuclease II activity cleaves phosphodiester bonds in DNA that has been partially depurinated by treatment with acid or with methyl methanesulfonate. These activities are simultaneously affected by mutations in *xthA*, the structural gene for exonuclease III in *E. coli* K-12 (30).

In a preliminary study (15), *xthA* was found to be co-transducible with *aroD*, *aroH*, and *pps*, but its precise location remained unknown. In this paper, we continue the mapping of *xthA* by means of P1 transduction, and we order it relative to other genes in the region between *aroD* and *fadD*. We also locate within this region *pncA* and *pabB*, two genes for which no co-transducible markers have been previously demonstrated. In addition, we describe techniques for transducing *xthA* mutations into wild-type strains by selecting for linked markers for drug resistance.

(Many of these results have been incorporated into the most recent genetic map of *E. coli* [3].)

MATERIALS AND METHODS

Bacterial strains. The *xth* mutants used in this study were previously described (14, 30). Table 1 lists other strains, their parents, and intermediates in their construction.

Nomenclature. Gene symbols are those of Bachmann et al. (3). Phenotypic symbols are derived from the gene symbols with the following exceptions: F-phe^R, resistance to *p*-fluorophenylalanine as expressed by the *pheS11* allele; Ana^R, resistance to 6-aminonicotinamide, a property of *pncA* mutants; and ts, temperature sensitive.

Media and chemicals. TY medium was the nutrient (tryptone-yeast) medium, and 56/2 was the minimal medium used by Adelberg et al. (2). Tryptone broth contained 10 g of tryptone (Difco) and 5 g of sodium chloride per liter. Mannose-tetrazolium plates were prepared as described by Miller (16). VB medium was minimal medium E of Vogel and Bonner (24), supplemented with glucose (0.4%) and thiamine (1 μg/ml). When needed, minimal media were also supplemented with L-proline (200 μg/ml), other L-amino acids (100 μg/ml), nucleoside bases (20 μg/ml), and additional nutrients or drugs at concentrations described below under scoring of specific markers.

Chemicals were purchased from the following sources: nicotinamide, nicotinic acid, 6-aminonicotinamide, sodium palmitate, Brij-35, *p*-aminobenzoic acid, D-glucuronic acid, and DL-*p*-fluorophenylalanine from Sigma Chemical Co., St. Louis, Mo.; other amino acids and mannose from Calbiochem, La Jolla, Calif.; and sodium pyruvate from Schwarz/Mann, Orangeburg, N.Y. The following solutions were sterilized by passage through a membrane filter (Millipore Corp.) and added to autoclaved media: *p*-aminobenzoic acid, 1 mg/ml; nicotinamide, 3 mg/ml; 6-aminonicotinamide, 30 mg/ml in 0.2 M HCl; and DL-*p*-fluorophenylalanine, 50 mg/ml in 0.25 M NaOH. Sodium pyruvate, Brij-35, and sodium palmitate were added to media before autoclaving. Other supplements were autoclaved separately.

Transduction and conjugation. The procedure of Willetts et al. (28) was used for transductions with phage P1 *virA*, and that of Adelberg and Burns (1) was used for mating.

Patch testing. Unselected markers were scored

TABLE 1. *E. coli K-12 strains used*

Strain[a]	Relevant genotype[b]	Source and reference[c]
AB1360	*aroD6 his-4*	CGSC (25)
AB1871	*pps-2*	CGSC (5)
AB3303	*pabB3*	CGSC (12)
BW128	*pncA1 nadB30 pheS11 pps-2*	P1 (BW9053) × W3899-*nam11* → F-phe^R (*pheS11*)
BW172	*pabB3 pncA1 pheS5* (ts)	P1 (BWT152) × BWT159 → Pps$^+$
BW173	*pabB3 pncA1*	P1 (KL16) × BW172 → growth at 42 C (*pheS*$^+$)
BW177	*fadD88 pabB3*	P1 (BW172) × DF250 → Eda$^+$
BW182	*pheS11 pps-2*	P1 (NP21) × AB1871 → F-phe^R (*pheS11*)
BW183	*pabB3 nadB30 pheS11 pps-2*	P1 (AB3303) × BW128 → Pnc$^+$
BW9020	*xthA3 aroD6*	Conjugation: MR10/1 × AB1360 → His$^+$ Leu$^+$ Thr$^+$
BW9053	*xthA3 pheS11 pps-2*	P1 (BW182) × BW9093 → F-phe^R (*pheS11*)
BW9093	*xthA3*	(14)
BW9101	*xth-pncA* deletion	Spontaneous mutation of KL16 to Ana^R (*pncA*$^-$)
BWT152	*pncA1 pheS5*	P1 (W3899-*nam11*) × NP37 → Ana^R (*pncA*$^-$)
BWT159	*pabB3 pheS11 pps-2*	P1 (KL16) × BW183 → Nad$^+$
DF71	*eda-1*	CGSC (10)
DF220	*gap-1*	J. Hillman (11)
DF250	*eda-1 fadD88*	J. Hillman (11)
KL16	Wild type	CGSC (13)
MR10/1	F$^+$ *xthA3 leu-6 thr-1*	(15)
NP21	*pheS11* (F-phe^R)	F. Neidhardt (4)
NP37	*pheS5* (ts)	F. Neidhardt (4)
RT212	*pfkA1 pfkB101 aroD6*	D. Fraenkel (23)
W3899-*nam11*	*pncA1 nadB30*	A. B. Pardee (9, 18)

[a] For unlisted *xth* mutants, see references 14 and 30.

[b] Listed for each strain are all of its known mutations in the region from *aroD* to *eda* plus markers outside this region only if they affected the scoring of these mutations or if they were used in strain construction. Symbols and abbreviations: see under Nomenclature. Allele numbers are those registered with the Coli Genetic Stock Center, Yale University School of Medicine; *pheS11* was previously called *pheS1* (4) and *pfkB101* was *pfkB1* (23).

[c] CGSC, Obtained from the Coli Genetic Stock Center. P1 transductional crosses are described as follows: P1 (donor) × recipient → selected phenotype.

by growth of cells in confluent patches on appropriate media. The colonies were first purified by restreaking and grown to saturation in nutrient broth contained in microwells of a 96-well depression plate (Linbro Chemical Co., New Haven, Conn.). Then droplets of the cultures were transferred to the surface of agar plates with a 48-prong inoculator. Details were as previously described (26).

Transduction and scoring of *p*-fluorophenylalanine resistance (*pheS11*). The *pheS11* marker confers resistance to *p*-fluorophenylalanine (4), and it is useful in the transductional transfer of *xthA* mutations and other closely linked alleles. In transductions, positive selection for *pheS11* is complicated by a high rate of spontaneous mutation to drug resistance and by the recessive nature of the *pheS11* mutation. Therefore, we infected the cells with phage P1 at a high multiplicity (1.0 to 2.0) to ensure a high number of transductants relative to mutants. After adsorption, the cells were washed twice by centrifugation, and 10^9 cells were suspended in 50 ml of VB medium. The citrate in VB medium inhibited further cycles of calcium-dependent phage adsorption. After overnight growth to permit segregation and expression of the recessive *pheS11* marker, 0.1-ml samples were spread on VB agar plates containing 1, 2, and 3 mM DL-*p*-fluorophenylalanine. Several concentrations were used because of the varying sensitivities of different wild-type strains. After 2 to 3 days at 37 C, *pheS11* colonies were observed as large colonies upon a background of slower-growing *pheS*$^+$ cells. Numerous smaller drug-resistant colonies were also observed; they represented leaky *pheS* mutations and mutations at other sites. The larger colonies were purified by streaking on plates containing 1 mM DL-*p*-fluorophenylalanine. When *pheS11* was scored as an unselected marker, the cells were patch-tested on VB agar plates containing 1, 2, and 3 mM DL-*p*-fluorophenylalanine. Growth was always at 37 C in the presence of the drug; at 25 to 30 C many wild-type strains are resistant and produce mucoid colonies.

Scoring of *pncA* markers. The *pncA* gene specifies nicotinamide deamidase (9, 18), a nonessential salvage enzyme. It has been conventionally scored only in nicotinic acid auxotrophs (e.g., *nadB*$^-$ strains). A *pncA*$^+$ *nadB*$^-$ strain can grow on minimal medium containing either nicotinic acid or nicotinamide, whereas a *pncA*$^-$ *nadB*$^-$ strain cannot utilize nicotinamide and requires nicotinic acid. In transductions involving *pncA*$^-$ *nadB*$^-$ recipients, the *pncA*$^+$ transductants were selected on VB agar containing 0.1 mM nicotinamide. Although both *nadB*$^+$ and *pncA*$^+$ transductants grew on the selective medium, they were readily distinguished; unlike the *nadB*$^+$ cells, the *pncA*$^+$ colonies were sur-

rounded by a halo of cross-fed *pncA*$^-$ *nadB*$^-$ parent cells, and they were unable to grow on unsupplemented minimal medium.

Two methods were devised for testing *pncA* markers in *nadB*$^+$ strains: a cross-feeding test and a test for resistance to 6-aminonicotinamide. The cross-feeding assay used W3899-*nam*11 (*pncA*$^-$ *nadB*$^-$) as the indicator strain. Stationary-phase indicator cells were washed by centrifugation in minimal medium, and 5×10^8 cells were spread on a VB agar plate containing 15 μM nicotinamide. Cultures were patch tested on the surface of the plate and scored after 12 to 24 h at 37 C. *pnc*$^+$ cells broke down nicotinamide to nicotinic acid and cross-fed the lawn of *pncA*$^-$ *nadB*$^-$ cells that then grew in a halo around the patch, whereas *pncA*$^-$ patches failed to cross-feed the lawn.

The second assay was that for 6-aminonicotinamide resistance, and it always agreed with the cross-feeding assay. We discovered that *pncA*$^+$ *nadB*$^+$ cells were killed by the drug, whereas *pncA*$^-$ *nadB*$^+$ cells were resistant. Presumably the lethal agent is really the 6-aminonicotinic acid produced from the drug by the *pncA*$^+$ gene product. To score the *pncA* gene in *nadB*$^+$ cells, the strains were patch-tested on VB agar containing 2 mM 6-aminonicotinamide. Although the drug is inhibited by nicotinamide or nicotinic acid, droplets of TY broth transferred with the cells apparently did not contain enough of these compounds to interfere either with this test or with the cross-feeding assay.

Transductions to 6-aminonicotinamide resistance (*pncA*$^-$). Positive selection for the *pncA*$^-$ marker required the same precautions as described above for the *pheS11* marker: namely, a high multiplicity of infection and overnight growth to permit phenotypic expression of the recessive trait. In addition, to reduce background growth and hence spontaneous mutants, the cells were washed several times in VB medium before plating on VB agar containing 2 mM 6-aminonicotinamide. Spreading no more than 5×10^7 cells on a plate further reduced background growth, perhaps by diminishing levels of excreted metabolites that interfere with 6-aminonicotinamide. Larger colonies were recloned on the same medium and then tested for the Pnc$^-$ trait by the cross-feeding assay. Most spontaneous mutants were leaky and were detected by this sensitive assay. Tight Pnc$^-$ clones were retained and confirmed as *pncA*$^-$ by co-transduction of nearby markers.

Scoring of *pabB*. The nonselective scoring of *pabB* in transductants was complicated by extensive cross-feeding of Pab$^-$ strains by Pab$^+$ strains (12). Therefore, we modified the patch-testing procedure described above. The cells were grown in a depression plate containing tryptone broth and then diluted about 70-fold by transfer with the multipronged inoculator into a second depression plate containing 50 μl of medium 56/2 per well. The diluted cultures were patch-tested on plates of 56/2 agar covered with 5×10^8 cells of AB3303 (*pabB*$^-$) that had been grown in tryptone broth and washed twice by centrifugation with medium 56/2. Free *p*-aminobenzoic acid was either complexed by the iron in medium 56/2 or consumed by the lawn of Pab$^-$ cells, and there was no significant cross-feeding between patches.

Scoring and selection of other markers. *aroD*$^-$ strains were unable to grow on VB minimal medium unless supplemented with (per ml) 100 μg each of L-phenylalanine, L-tryptophan, and L-tyrosine (25). *pps*$^-$ cells were unable to grow on minimal medium 56/2 in which the glucose was replaced by 20 mM sodium pyruvate (5). *pheS5* mutants were unable to grow on TY agar plates at 42 C, but could grow at 30 C (4). *pfkB101* was scored in *pfkA*$^-$ cells by growth on mannitol-minimal medium (22, 23); results were confirmed by mannose fermentation on mannose-tetrazolium indicator plates. *xth*$^-$ was scored by a depression plate microassay for the deoxyribonuclease (DNase) activity of exonuclease III (14, 26). *gap* mutants were unable to grow on VB minimal medium with glucose as the carbon source; they were also propagated in a glucose-free enriched broth (11). *pabB* mutants were unable to grow on VB minimal medium unless supplemented with *p*-aminobenzoic acid (1 μg/ml) (12). *fadD*$^-$ strains were unable to grow on VB medium when the glucose was replaced by 0.1% sodium palmitate and 1% Brij-35 (17). *eda*$^-$ cells were unable to grow on VB medium when the glucose was replaced by 0.4% glucuronic acid (neutralized by NaOH) (10).

RESULTS

Transductional mapping of *xthA3*, *pabB3*, and *pncA1*. The genes under study are shown in Fig. 1. Earlier work by Vinopal and Fraenkel (23) and by Hillman and Fraenkel (11) had established the following overall order: *aroD-pps-pheS-pfkB-gap-fadD-eda*. We now place the genes *xthA*, *pncA*, and *pabB* within this region. As described below, Fig. 1 shows their locations and provides supportive data from two-factor linkage analyses of phage P1 transductions. Table 2 provides data from three-factor transductional crosses that confirm the results in Fig. 1 and that resolve its ambiguities.

In the experiments of Fig. 1 and Table 2, we used the *xthA3* allele exclusively. It specifies a temperature-sensitive exonuclease III and is therefore a mutated structural gene (14). *xthA3* had been shown to be co-transducible with *aroD*, *aroH* (closely linked to *aroD*), and *pps*. The first cross in Table 2 indicated that *xthA* is located clockwise of *pfkB*. Two-factor linkages (Fig. 1) place it between *pheS* and *pncA* because it was co-transducible 30% with *pheS* and 41% with *pncA*, whereas *pheS* and *pncA* were only 2 to 7% linked. The data in Fig. 1 are most consistent with the gene order *pncA-gap-pabB*, but map distances in this region did not appear to be additive. This gene order was therefore confirmed by a three-factor cross (Table 2). Finally, the relative order of two closely linked markers, *pabB* and *fadD*, was established by two three-factor crosses in Table 2.

***argS* amd *man* genes.** The *argS* gene and a

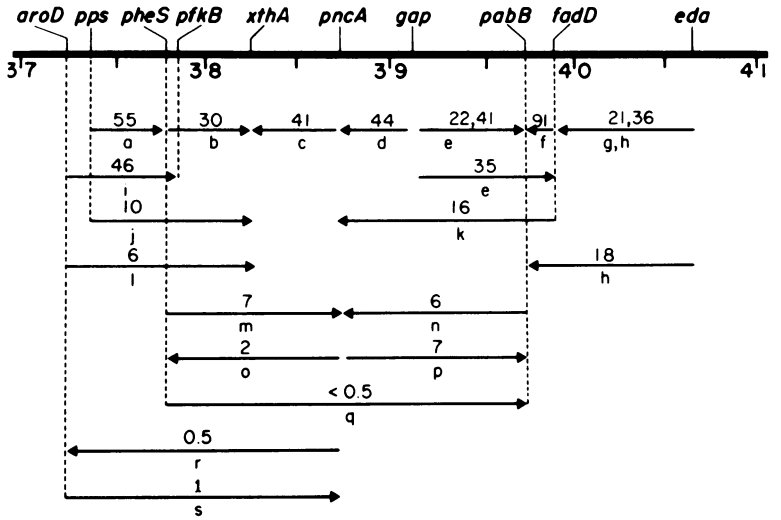

FIG. 1. *Transductional mapping of xthA and nearby genes in E. coli K-12. Gene locations (in minutes) correspond to those of the recalibrated map of Bachmann et al. (3), and intergenic distances are those calculated from co-transduction frequencies (29). Each P1 transductional cross is represented by an arrow extending from the selected to the unselected marker. Values are co-transduction frequencies (%). From 100 to 200 recombinants were scored in each cross. Crosses were as follows: (a) W3899-nam11 × BW182, (b) BW9020 × NP37, (c) BW9020 × W3899-nam11, (d) BW172 × DF220 (see Table 2), (e) BW177 × DF220 (see Table 2), (f) BW173 × DF250, (g) KL16 × DF250, (h) BW172 × DF250, (i) BW9093 × RT212 (see Table 2), (j) BW9093 × AB1871, (k) BW173 × DF250, (l) BW9093 × AB1360, (m) W3899-nam11 × NP37, (n) W3899-nam11 × AB1871, (o) NP37 × W3899-nam11, (p) AB3303 × W3899-nam11, (q) AB3303 × BWT152, (r) AB1360 × W3899-nam11, and (s) W3899-nam11 × AB1360. Note: F-phe^R (pheS11) and Ana^R (pncA$^-$) were not used as selected traits because of their recessive nature and frequent spontaneous occurrence; instead, heat resistance (pheS5 → pheS$^+$) and nicotinamide utilization (pncA$^-$ → pncA$^+$) were used selectively.*

TABLE 2. *Ordering genes near xthA by three-factor transductional crosses*

Donor	Recipient	Selected marker	Unselected markers		Suggested gene order[a]
			Classes	No.	
BW9093 (*xthA3*)	RT212 (*pfkA1 pfkB101 aroD*$^-$)[b]	*aroD*$^+$	*pfkB101 xthA*$^+$	81	*aroD-pfkB-xthA*
			pfkB$^+$ *xthA*$^+$	64	
			pfkB$^+$ *xthA*$^-$	6	
			pfkB101 xthA$^-$	0	
BW172 (*pncA*$^-$ *pabB*$^-$)	DF220 (*gap*$^-$)	*gap*$^+$	*pncA*$^+$ *pabB*$^+$	76	*pncA-gap-pabB*
			pncA$^-$ *pabB*$^+$	65	
			pncA$^+$ *pabB*$^-$	25	
			pncA$^-$ *pabB*$^-$	14	
BW177 (*fadD*$^-$ *pabB*$^-$)	DF220 (*gap*$^-$)	*gap*$^+$	*pabB*$^+$ *fadD*$^+$	98	*gap-pabB-fadD*
			pabB$^-$ *fadD*$^-$	54	
			pabB$^-$ *fadD*$^+$	19	
			pabB$^+$ *fadD*$^-$	9	
BW172 (*pabB*$^-$)	DF250 (*eda*$^-$ *fadD*$^-$)	*eda*$^+$	*fadD*$^-$ *pabB*$^+$	55	*eda-fadD-pabB*
			fadD$^+$ *pabB*$^+$	16	
			fadD$^+$ *pabB*$^-$	15	
			fadD$^-$ *pabB*$^-$	1	

[a] In the second cross (BW172 × DF220), to derive the gene order, we assumed that if *gap* were an outside marker, the lowest-frequency class would have been generated by four crossovers, one of which must occur between *pabB* and *pncA*. For the gene orders suggested by the other crosses, we assumed the lowest-frequency class to be that produced by four crossovers.

[b] The *pfkB101* mutation suppresses the distantly located *pfkA1* (mannose-negative) mutation (23).

man⁻ allele were shown to be in this general region of the map by conjugation experiments (6, 21, 27). We were unable to map the *argS* gene because three mutants (27), supplied by L. Williams, appeared to be too unstable genetically. The *man*⁻ mutation in strain AT701 of Taylor and Trotter (21) was previously placed clockwise of *aroD* by three-factor conjugational crosses (15). We used a P1 lysate of AT701 (*man*⁻) to transduce AB3303 (*pabB*⁻) to Pab⁺ and DF71 (*eda*⁻) to Eda⁺. Fifty percent (40/80) Pab⁺ transductants were Man⁻ (on mannose-tetrazolium indicator plates), and 45% (80/176) of the Eda⁺ transductants were Man⁻. These results clearly distinguish the *man*⁻ allele of Taylor and Trotter from the *man* gene at 36 min (3) and suggest that the *man*⁻ mutation of Taylor and Trotter is at the *ptsM* locus (3), a gene affecting mannose utilization, between *fadD* and *eda*.

BW9101 (30) contains a deletion extending from the *pncA* gene either into or through the *xthA* gene. The mutant, to be further described in a subsequent communication, is Man⁺; it is of course viable, and it grows on glucose-minimal media. Thus, we can exclude from this region between *xthA* and *pncA* the *man* alleles, the vital *argS* (arginyl-transfer ribonucleic acid synthetase) gene, the *gap* gene, and any auxotrophic markers.

Other *xth* alleles. In addition to the *xthA3* and deletion mutations mentioned above, we studied other independently derived mutations affecting exonuclease III and endonuclease II activity. Some were originally identified as exonuclease III mutants (*xth-1* to *xth-9*) (14), and others were discovered as mutants with reduced endonuclease II activity (*xth-11* to *xth-14*) (30), but all were deficient in both activities. The results to be described below are consistent with all the mutations being in or near the *xthA* gene.

In Table 3, the results of three-factor transductional crosses performed with six different *xth* mutant alleles indicate that they are all located between the *pheS* and *pncA* genes. Other alleles were tested in two-factor crosses. P1 lysates were prepared from strains BW9094 (*xth-4*), BW9095 (*xth-5*), BW9097 (*xth-7*), and BW9098 (*xth-8*) and were used to transduce W3899-*nam*11 (*pncA*⁻) to Pnc⁺. In each cross, from 85 to 156 transductants were picked, recloned, and tested for exonuclease III activity. The *xth* mutations were 19 to 29% co-transducible with *pncA*. In previous experiments (15), these mutations were shown to be 8 to 18% co-transducible with *aroD*, whereas *aroD* and *pncA* are no more than 1% co-transducible (Fig. 1). Therefore, *xth-1*, *xth-4*, *xth-7*, and *xth-8* must be between *aroD* and *pncA*.

While these mapping studies were in progress, we obtained biochemical evidence that four of the mutations are within the same gene. The *xthA3*, *xth-4*, *xth-7*, *xth-12*, and *xth-13* mutations each produce altered (thermolabile) enzymes (14, 30) and are therefore located within structural genes for exonuclease III. The enzyme, however, contains only one structural component, i.e, a single polypeptide (25a). Therefore, these mutations must all be within the same gene, the *xthA* gene.

A general method for the genetic transfer of *xth*⁻ markers. P1 transduction is an ideal method for gene transfer because the transductant is ≥98% isogenic with the recipient parent strain, which can then serve as a suitable control in biological experiments. Unfortunately, in transductional transfers there are currently no techniques of direct selection for the Xth⁻ or Xth⁺ phenotype. Even as unselected traits, they are scored with relative difficulty, i.e., by enzyme assay. The *xthA* gene, however, is co-transducible with two markers for drug resistance, *pncA*⁻ (Ana^R) and *pheS11* (F-phe^R). *pncA*⁻ strains lack nicotinamide deamidase and are resistant to 6-aminonicotinamide. *pheS*⁻ strains, having an altered phenylalanyl-transfer ribonucleic acid synthetase, may be temperature sensitive (e.g., *pheS5*) or resistant to *p*-fluorophenylalanine (e.g., *pheS11*). As described below, we first constructed drug-resistant derivatives of our *xth* mutants, and we then used them as donors to transduce the *xth* muta-

TABLE 3. *Three-point cross analysis of xth mutant alleles*[a]

Donor[b]	No. of *pheS*⁺ recombinants selected	Recombinant classes (%)			
		xth⁺ *pnc*⁻	*xth*⁻ *pnc*⁻	*xth*⁻ *pnc*⁺	*xth*⁺ *pnc*⁺[c]
BW9091 (*xth-1*)	180	64	23	10	3
BW9099 (*xth-9*)	179	64	22	12	2
BW2001 (*xth-11*)	180	61	25	13	1
BW2002 (*xth-12*)	178	74	13	11	2
BW2003 (*xth-13*)	149	73	17	10	0
AB3027 (*xth-14*)	191	64	26	9	1

[a] The recipient in these transductional crosses with BW172 (*pheS5 pncA1*), and selection was for growth at 42 C (*pheS*⁺).

[b] Donor strains were previously described (12a, 14, 30).

[c] If the gene order is *pheS-xth-pncA*, this class would be generated by four crossovers and is therefore expected to occur with lowest frequency.

tions into other strains by selecting for drug-resistant transductants. This approach has two advantages: (i) almost any strain may be used as a recipient—it need not have negative markers near the *xthA* gene; and (ii) it requires only a small number of exonuclease III assays that can therefore be performed feasibly in test tubes (14, 19, 20) rather than by the more elaborate microwell assays used in our mapping studies.

Donor strains having the genotype *xthA*$^-$ *pheS11 pps-2* were constructed as exemplified by BW9053 (Table 1); i.e., *pheS11* and *pps-2* markers were transferred together from BW182 into an *xth*$^-$ strain by selecting for F-phe^R recombinants. Subsequent transfer of the *xth* marker into other strains is accomplished in three steps: (i) a phage P1 lysate of the donor strain *(xth*$^-$ *pheS11 pps-2)* is used to infect the recipient strain *(xth*$^+$ *pheS*$^+$ *pps*$^+$*)*, and after intermediate growth to permit the expression of the recessive *pheS11* allele, F-phe^R colonies are selected (see Materials and Methods); (ii) F-phe^R transductants are scored for *pps;* and (iii) about 10 to 20 *pps*$^-$ clones are assayed for exonuclease III activity, and an *xth*$^-$ clone is retained. In the transductional selection for F-phe^R, spontaneous mutants may outnumber the transductants, but by screening out Pps$^+$ colonies, we discard these mutants by a simple scoring technique, thereby reducing the number of more difficult exonuclease III assays to be performed. Applying the formulas of Wu (29) to our data (Table 1), we expect that 21% of the F-phe^R Pps$^-$ colonies should also be Xth$^-$; this calculation, however, assumes an equal growth rate for all transductants and segregants during the intermediate incubation prior to the selection for F-phe^R. Our results have generally met this expectation.

We have used this method to transfer many of the *xth*$^-$ alleles to other strains. In a few cases, a transduction yielded only a few *pheS11 pps-2* derivatives, all of which were *xth*$^+$; however, these latter strains could then be used successfully as transductional recipients for *xth*$^-$ markers in transductions selective for Pps$^+$ in which spontaneous mutations are not a significant problem. We have been able to transfer *xthA3* and *xth-1* alleles into the DNA polymerase I mutant P3478 *(polA1)* (8) and into the multiple DNase mutant M0611 *(recB*$^-$ *recC*$^-$ *sbcB*$^-$ *end*$^-$*)* (7). The strains were viable, even at 42 C, and even though derivatives of the latter strain were deficient in five DNase activities, namely, exonucleases I, III, and V and endonucleases I and II.

xth mutations may also be linked with *pncA* mutations and then transferred to other strains by transductional selection for resistance to 6-aminonicotinamide. We have had limited experience with this technique, but we have used it to transfer an *xthA-pncA* deletion (30) into other strains.

DISCUSSION

Eleven independently derived mutations affecting exonuclease III were mapped at the *xthA* locus, between the *pfkB* and *pncA* genes of *E. coli*. Five of the mutations are known to result in physically altered proteins and are therefore within the *xthA* gene, the single structural gene for this enzyme. The others may be either within this gene or within nearby regions affecting its expression. Some of the mutants were originally isolated as endonuclease II mutants. The mapping data reported here contributed to other genetic evidence on the association of exonuclease III with an endonuclease II activity (30) and led to biochemical studies in which the two activities were shown to be properties of the same polypeptide (Weiss, in press).

These mapping studies are an important preliminary to future experiments on the biological roles of exonuclease III. Although the *xth* mutants were originally isolated for this ultimate purpose, only one mutant was shown to have biological abnormalities clearly attributable to the same mutation that caused the enzyme deficiency; it grew slowly and was sensitive to alkylating agents (30). None of the mutants, however, was conditionally lethal. It is possible, therefore, that exonuclease III is not a vital enzyme or that, if it has a vital function, other DNases may be able to substitute for it. It is also possible that all of our mutants may be leaky and that even immeasurably low levels of residual enzyme activity may be enough to ensure survival. To study possible redundancy of biological roles among the DNases of *E. coli*, we should have to construct mutants lacking various combinations of DNases. To overcome the possibility of leakiness, we might obtain mutants in which the entire gene is deleted. Because there are currently no feasible techniques for directly selecting or enriching for the Xth$^-$ phenotype, a knowledge of closely linked selectable markers enables us to begin these studies. We alluded to our early attempts under Results. Thus, we isolated an *xthA-pncA* deletion mutant by screening spontaneous Ana^R mutants, and we transferred *xth*$^-$ mutations into strains lacking other DNases by selecting for co-transduced markers.

ACKNOWLEDGMENTS

Supporting research grants were from the American Cancer Society (NP-126) and from the Public Health Service's National Cancer Institute (1 PO1 CA16519). B. Weiss was supported by a Public Health Service research career development award (5 KO4 GM29562), and S. J. Hochhauser and N. M. Cintrón were supported by a Public Health Service predoctoral training grant (5 TO1 GM00184), both from the National Institute of General Medical Sciences.

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