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DNA Polymerase I Function Is Required for the Utilization of Ethanolamine, 1,2-Propanediol, and Propionate by *Salmonella typhimurium* LT2

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Evidence documenting the requirement for a functional DNA polymerase I when *Salmonella typhimurium* LT2 uses ethanolamine (EA), 1,2-propanediol (1,2-PDL), or propionate (PRP) as the sole carbon and energy source is presented. Providing rat polymerase β in *trans* demonstrated that the growth phenotypes observed were due exclusively to the lack of DNA polymerase I functions. The location of the mutation (a MudI1734 insertion) that rendered cells unable to grow on EA, 1,2-PDL, or PRP was determined by DNA sequencing to be within the *polA* gene. *polA* mutants of this bacterium may be unable to repair the damage caused by reactive aldehydes generated during the catabolism of EA, 1,2-PDL, or PRP. Consistent with this hypothesis, the inhibitory effects of acetaldehyde and propionaldehyde on the growth of this *polA* mutant were demonstrated. A derivative of the *polA* mutant unable to synthesize glutathione (GSH) was markedly more sensitive to acetaldehyde and propionaldehyde than was the *polA* mutant proficient in GSH synthesis. This finding was in agreement with the recently proposed role of GSH as a mechanism for quenching reactive aldehydes generated during the catabolism of these compounds (M. R. Rondon, R. Kazmierczack, and J. C. Escalante-Semerena, J. Bacteriol. 177:5434–5439, 1995).

Detailed analysis of the metabolic network of any organism requires a basic understanding of the biochemical reactions that define the metabolic pathways of interest. Our interest in cobalamin biosynthesis and its regulation (18, 19, 23, 29, 32) in *Salmonella typhimurium* has brought into focus pathways that require adenosyl-cobalamin (Ado-CBL) to function. Among these are the ethanolamine (EA) utilization (*eut*) and 1,2-propanediol (1,2-PDL) utilization (*pdu*) pathways, which convert EA and 1,2-PDL to acetyl coenzyme A (acetyl-CoA) and propionyl-CoA, respectively (see reactions 1 and 2 below). At present, the catabolic pathway for propionate (PRP) in *S. typhimurium* remains speculative.

Reactions 1

$$\underset{\text{EA}}{H_2NCH_2CHOH} \xrightarrow{\text{EAL}} \underset{\text{acetaldehyde}}{CH_3CHO} + NH_3$$

$$\underset{\text{acetaldehyde}}{CH_3CHO} + \underset{\text{CoA}}{HS\text{-}CoA} \xrightarrow{\text{ADH}} \underset{\text{acetyl-CoA}}{CH_3COSCoA} + 2e^-$$

where EAL is EA ammonia-lyase (Ado-CBL dependent) and ADH is acetaldehyde dehydrogenase.

Reactions 2

$$\underset{\text{1,2-PDL}}{CH_3CH(OH)CH_2OH} \xrightarrow{\text{PDD}} \underset{\text{propionaldehyde}}{CH_3CH_2CHO} + H_2O$$

$$\underset{\text{propionaldehyde}}{CH_3CH_2CHO} + \underset{\text{CoA}}{HSCoA} \xrightarrow{\text{PDH}} \underset{\text{propionyl-CoA}}{CH_3CH_2CHOSCoA} + 2e^-$$

where PDD is 1,2-PDL dehydratase (Ado-CBL dependent) and PDH is propionaldehyde dehydrogenase.

The *eut* genes, required for EA utilization, have previously been identified and sequenced (6, 25, 28). Given the reduced number of reactions defining this pathway, it is surprising that the *eut* operon may contain ≥10 genes, 8 of which have been sequenced (6, 28). The deduced amino acid sequences of two of the proteins encoded by the *eut* operon, CchA and CchB, resemble those of carboxysome shell proteins in *Thiobacillus neapolitanus* and a *Synechococcus* sp. On the basis of these data, it was proposed that in *S. typhimurium* EA is catabolized within a microcompartment and it was postulated that containment of reactive acetaldehyde could be the reason for the existence of this compartmentalization (28).

The *pdu* genes of *S. typhimurium*, which are required for 1,2-PDL utilization, have previously been identified and partially sequenced (2, 8). The first gene of the *pdu* operon, *pduA*, showed 56% amino acid identity to CcmK (carbon dioxide concentrating mechanism protein) of a *Synechococcus* sp. (21). This finding prompted Chen et al. to suggest that the enzymes responsible for the breakdown of 1,2-PDL are contained in a microcompartment (2).

To understand transcriptional activation of the *cob*/*pdu* regulon, we isolated a number of *S. typhimurium* mutants unable to utilize 1,2-PDL or EA. One such mutant was found to be defective in glutathione synthesis (24), implying a role for this tripeptide in some aspect of the utilization of these compounds. In this communication, we describe the isolation and characterization of another mutant unable to utilize EA, 1,2-PDL, or PRP as the carbon source. This mutant has a insertion in the *polA* gene, which encodes DNA polymerase I (PolI).

PolI has been characterized extensively (7, 10, 12). This enzyme has three activities, a 5′-to-3′ exonuclease activity, a 3′-to-5′ exonuclease activity, and polymerase activity (Klenow). PolI is involved in several repair pathways, including excision repair, processing of Okazaki fragments, replication initiation of some plasmids, and the MutY-dependent mis-

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TABLE 1. Strains and kit used

Strain or kit	Description	Reference or source
S. typhimurium Mud-P22 kit		N. Benson via K. E. Sanderson
TT12915	*leuA414*(Am) r$^-$ *fels2* F′ 114ts (*lacZZF20*::Tn*10 ZZF3551*::MudP)	2
TT12916	*leuA414*(Am) r$^-$ *fels2* F′ 114ts (*lacZZF20*::Tn*10 ZZF3553*::MudQ)	2
TR6583	*metE205 ara-9*	K. E. Sanderson via J. Roth
JE2996	*metE205 ara-9 polA71*::Mud1734	This study
JE2546	*metE205 ara-9 gshA101*::Tn*10*del*16*del*17*	24
JE2997	*metE205 ara-9 gshA101*::Tn*10d*(Tc) *polA71*::MudJ	This study
JE3581	*metE205 ara-9 polA71*::MudP	
JE3582	*metE205 ara-9 polA71*::MudQ	
JE3705	*metE205 ara-9* pβ1RBS	
JE3708	*metE205 ara-9 polA71*::MudQ *eutE18*::Mud1-8	
JE3709	*metE205 ara-9 polA71*::MudQ *pdu-8*::MudJ	
JE3710	*metE205 ara-9 polA71*::MudQ *cbi-24*::MudJ	
JE3712	*metE205 ara-9 polA71*::MudJ pβ1RBS	
JE3746	*metE205 ara-9 atrC20* (*polA*)::MudJ	
JE3755	*metE205 ara-9 polA101*::Tn*10d*(Cm)	
JE3756	*metE205 ara-9 polA102*::Tn*10d*(Tc)	
JE3757	*metE205 ara-9 polA103*::Tn*10d*(Tc)	
JF2464	*atrC20*(*polA*)::MudJ	J. Foster
TE5411	*putPA1303*::Kan^r-*hemA-lac* (pr) *polA101*::Tn*10d*(Cm)	T. Elliott
TE5412	*putPA1303*::Kan^r-*hemA-lac* (pr) *polA102*::Tn*10d*(Tc)	
TE5413	*putPA1303*::Kan^r-*hemA-lac* (pr) *polA103*::Tn*10d*(Tc)	
TE3626	*hemN732*::Tn*10d*(Tc)	
AG5	*trpD1 ilv uvrA331 rpsL*	K. E. Sanderson
TA100	*his* del *uvrB rfa*	
AA3007	*ara-9 polA2*	
TN2373	*ara-9 polA2*	C. Miller
E. coli		
CJ225	Δ*polA* Km^r/pCJ100 (F′ *polA*$^+$ Cm^r)	9
CJ231	Δ*polA* Km^r/pCJ102 (F′ 5′ Exo Cm^r)	
CJ233	Δ*polA* Km^r/pCJ103 (F′ Klenow Cm^r)	
CJ251	pCJ105 (pOX38-Cm^r)	
CM4772	*polA*$^+$	
JS250	F$^-$ *hsdS20* (rB$^-$mB$^-$)*recA13 leuB6 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 supE44* pβ1RBS	31

match repair pathway (22). *polA* mutants of *Escherichia coli* and *S. typhimurium* are sensitive to mutagens and unable to replicate some plasmids (5, 14, 15, 34). The phenotypes described below are novel in that they uncover a link between PolI activity and carbon metabolism in *S. typhimurium*.

MATERIALS AND METHODS

Strains, media, and growth conditions. The strains used in this study are listed in Table 1. Rich media (nutrient broth and Luria-Bertani broth) and minimal media (E and NCE) have been described previously (4, 33). Carbon sources were used at the following concentrations: EA at 30 mM, PRP at 30 mM, and 1,2-PDL at 50 mM. Cyanocobalamin was added to 15 nM when EA or 1,2-PDL was used as the carbon source, methionine was added to 0.5 mM, crystal violet (CV) was added to 24.5 mM in solid medium and to 12.25 mM in liquid medium, and nitrate was added to 20 mM. Growth was monitored at 650 nm with a Spectronic 20D spectrophotometer (Milton Roy Co., Rochester, N.Y.). When CV was included in the medium, the A_{400} was measured. UV light sensitivity was determined by exposing newly printed plates to 100 μW of UV light per cm^2 for 45 s and then allowing cells to grow overnight at 37°C in the dark.

β-Galactosidase assays. Assays were done as described previously (23).

Genetic techniques. The mapping of the insertion was achieved with the Mud-P22 mapping kit of Benson and Goldman (1). Mitomycin-induced lysates were used as donors, and JE2996 (*polA71*::MudJ) was used as the recipient in crosses that selected for growth on EA as the carbon and energy source. F′ *polA* plasmids were moved by mixing donor and recipient cells on the same plate, incubating for 4 to 6 h at 37°C, and then replica printing to selective plates. Control cultures of donor and recipient cells (unmixed) were included on each plate.

Recombinant-DNA techniques. DNA to be used for sequencing was obtained from mitomycin-induced lysates of strains JE3581 (*polA71*::MudP) and JE3582 (*polA71*::MudQ). *polA71*::MudJ (JE2996) was converted to *polA71*::MudP (JE3581) and *polA71*::MudQ (JE3582) as previously described (1, 36). Sequencing was done by the dideoxy method (27) with a SequiTherm Cycle sequencing kit (Epicentre Technologies, Madison, Wis.). We used primer 5′ATCCCGAA TAATCCAATGTCC3′ for MudP DNA and primer 5′ACTTTCGCGTTTTT CGTG3′ for MudQ DNA. Transformations and plasmid preparations were performed as described elsewhere (29).

RESULTS AND DISCUSSION

Isolation and mapping of *polA71*::MudJ. The mutation in strain JE2996 was isolated from a pool of randomly located MudJ insertions. We used this pool to transduce a *gshA101*::Tn*10d*(Tc) strain to kanamycin resistance in an attempt to replace that marker. Km^r Tc^s colonies were then tested for growth on 1,2-PDL, which selected against *gshA* mutants (24). The insertion in strain JE2996 was shown by P22 cotransduction to be unlinked to *gshA101*::Tn*10d*(Tc) and to other loci required for 1,2-PDL utilization (*pdu*, *pocR*, etc.).

The Mud-P22 kit of Benson and Goldman (1) was used to locate the insertion by selecting for growth on 1,2-PDL. Lysates which gave a positive result were TT15268 through TT15273; these results placed the insertion in the region of the chromosome from 83 to 86.7 min. The insertion was found to be 67% linked to *hemN* by P22 transduction, which more accurately placed the insertion at 86.5 centisomes on the *S. typhimurium* chromosome (26).

Sequence analysis of the mutation. To define the chromosomal location of the insertion, we sequenced the regions flanking the insertion element. The MudJ insertion was converted to MudP and MudQ insertions, as described elsewhere (1); Mud-P22 phage DNA was isolated from mitomycin C-

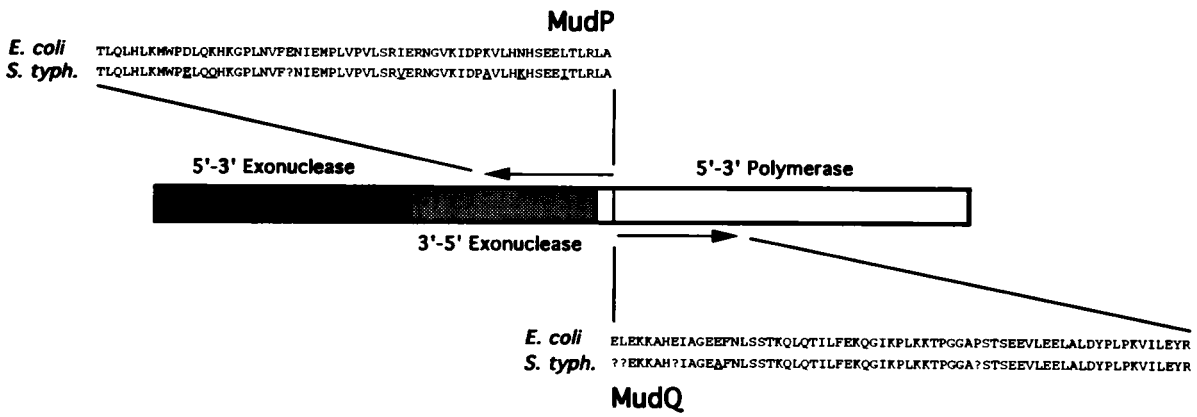

FIG. 1. Location of *polA71*::MudJ. The large bar is a schematic of the PolI primary structure, showing the different functional domains. The amino acid sequences shown were determined by sequence analysis, with the *E. coli* amino acid sequence shown for comparison. Amino acids that differ between *E. coli* and *S. typhimurium* (*S. typh.*) are underlined. Question marks indicate that the sequence was not determined at that position. The vertical line shows the site of insertion (alanine 564).

induced cultures; and DNA was sequenced with a primer specific for one end of the Mu element, as described in Materials and Methods. Comparison of this sequence with those in the GenBank database revealed homology to the *polA* gene from *E. coli* (GenBank accession number L19201 [10, 20]). Figure 1 shows the location of the MudJ insertion within the *polA* gene. On the basis of these data and the fact that strain JE2996 had all of the phenotypes previously described for *polA* mutants, we concluded that this insertion disrupted the *polA* gene of *S. typhimurium*. This allele is hereafter referred to as *polA71*::MudJ.

Strains containing the *polA71*::MudJ insertion have novel growth phenotypes. In addition to its inability to grow on 1,2-PDL as the sole carbon and energy source, JE2996 failed to grow on EA and PRP (Fig. 2A to C). The growth of the *polA* mutant on solid medium with EA or 1,2-PDL as the carbon and energy source was drastically slower than the growth of the *polA*$^+$ strain on the same medium. The possible effects of oxygen on these phenotypes were not explored.

JE2996 (*polA71*::MudJ) grew on citrate, fucose, fumarate, gluconate, glucose, glycerol, lactate, malate, mannitol, proline, pyruvate, rhamnose, ribose, serine, sorbitol, trehalose, xylose, and rich medium as well as the *polA*$^+$ strain did (data not shown). Thus, the growth deficiency of JE2996 was specific for 1,2-PDL, EA, and PRP. We measured minor growth defects when JE2996 was grown on succinate, acetate, or alanine, but these effects were not as dramatic as those seen when EA, PRP, or 1,2-PDL was provided as the sole carbon and energy source. A number of nitrogen sources were also tested, but JE2996 grew as well as the *polA*$^+$ strain (TR6583) did on all of the compounds tested (data not shown). JE2996 was able to utilize EA as the sole nitrogen source as well as the *polA*$^+$ strain was, although it was unable to utilize EA as the carbon and energy source. This result was neither unexpected nor unprecedented. *gsh* mutants, which contain only 10% of the EA ammonia-lyase activity (see the introduction) found in *gsh*$^+$ strains, can use EA as a nitrogen source but are unable to use it as a carbon and energy source (24).

***polA71*::MudJ confers classical PolI phenotypes.** JE2996 displayed the phenotypes expected of a *polA* mutant (11, 13–15, 34), i.e., it was sensitive to UV irradiation and the dye CV (Fig. 2D) and was unable to maintain plasmids with the ColE1 replicon while being able to maintain plasmid R6K (data not shown).

Complementation by F′ *polA*$^+$ plasmids. Episomes containing all or part of the *polA* gene were introduced into JE2996, and the resulting exconjugants were tested for growth on EA, PRP, or 1,2-PDL, as well as for resistance to CV. All of the episomes containing the entire *polA*$^+$ gene, just the 5′-to-3′ exonuclease, or just the Klenow domain were able to complement all of the phenotypes of strain JE2996. This unusual pattern of complementation was similar to that originally reported for the F′ plasmids, in which either domain complemented the growth of a *polA* deletion mutant on rich medium

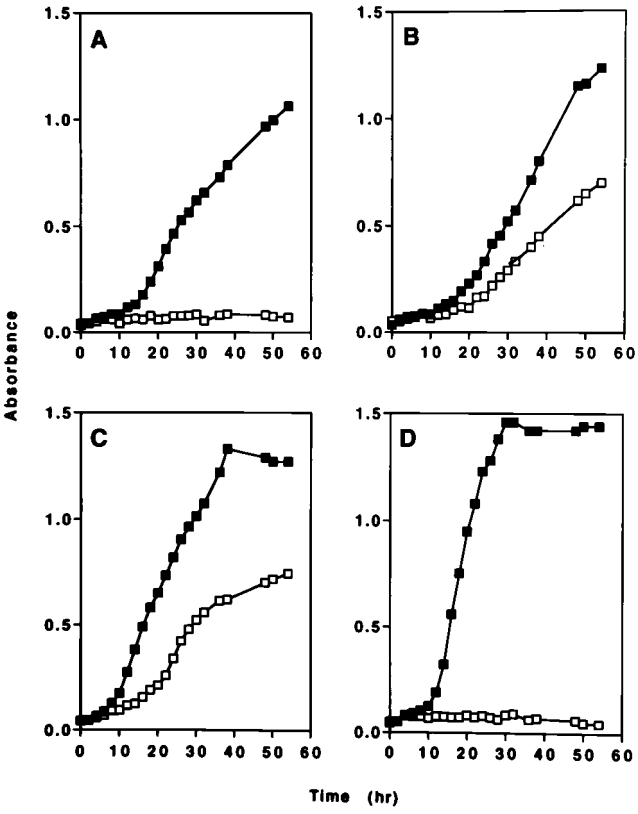

FIG. 2. Growth of *polA71*::MudJ. Growth of JE2996 (*polA71*::MudJ) (open squares) and TR6583 (*polA*$^+$) (filled squares) on PRP (A), 1,2-PDL (B), and EA (C) and in the presence of CV (D). Culture conditions are described in Materials and Methods. Growth was monitored at 650 (A to C) or 400 (D) nm.

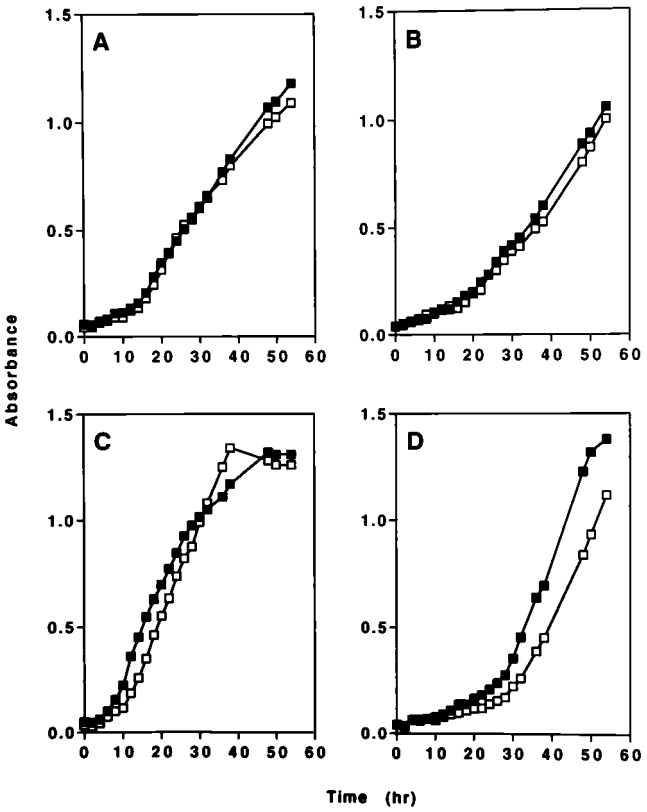

FIG. 3. Complementation of PolI phenotypes by pβ1RBS. Growth of JE3712 (*polA71*::MudJ)/pβ1RBS (open squares) and JE3705 (*polA*$^+$)/pβ1RBS (filled squares) on PRP (A), 1,2-PDL (B), and EA (C) and in the presence of CV (D). Culture conditions are described in Materials and Methods. Growth was monitored at 650 (A to C) and 400 (D) nm.

(9). Complementation could be due to the presence of the *spf* gene or another open reading frame (encoding a 22-kDa protein) on all of the complementing F′ plasmids.

Complementation by rat DNA polymerase β. To eliminate the possibility that the phenotypes associated with the *polA71*::MudJ mutation were due to a polar effect of this insertion on a downstream function, we sought to demonstrate complementation with a plasmid containing only *polA*$^+$. Although *polA* in *E. coli* is not part of an operon (10), *S. typhimurium* might be different in this regard. We introduced pβ1RBS, the plasmid which contains rat DNA polymerase β and has been shown to complement an *E. coli polA* mutant by Sweasy et al. (30, 31), into JE2996. As shown in Fig. 3, plasmid pβ1RBS complemented all of the growth phenotypes associated with *polA71*::MudJ.

Lack of PolI does not affect expression of the EA utilization (*eut*) or PDL utilization (*pdu*) genes. It was possible that transcription of the genes required for growth on EA, PRP, or 1,2-PDL would be affected in the *polA* mutant. To test this, we introduced *polA71*::MudP into strains containing *pdu*::Mu-*lacZ* or *eut*::Mu-*lacZ* operon fusions. We saw no effects of this *polA* mutation on the transcription of these genes in response to their effector molecules (1,2-PDL or EA-cyanocobalamin, respectively) (data not shown). These results suggested that the inability of *polA* mutants to grow on EA or 1,2-PDL was not due to an inability to express the gene products required for EA or 1,2-PDL degradation.

Strain JE2996 (*polA71*::MudJ) shows increased sensitivity to aldehydes. As seen in reactions 1 and 2, one common feature of EA and 1,2-PDL catabolism is that the degradative pathways proceed via aldehyde intermediates (8, 28). Aldehydes are known to be toxic and mutagenic and have been found to modify DNA (3, 16, 17, 35). Thus, perhaps the accumulation of aldehydes was responsible for the inability of JE2996 to grow on these substrates.

Growth of the *polA*$^+$ and *polA* mutant strains was tested in the presence of propionaldehyde or acetaldehyde, with glucose as the carbon source. Figure 4 shows that strain JE2996 (*polA71*::MudJ) was more sensitive to both compounds than was the *polA*$^+$ strain, since there was a lag of several hours before cultures started to grow. Once cells began to grow, they did so at a growth rate similar to that of the *polA*$^+$ strain, and the final yield was unaffected. Although the effects of externally supplied aldehydes are not directly comparable to those of internally produced molecules, these results are consistent with the hypothesis that *polA* mutants are impaired in handling aldehyde intermediates of EA, 1,2-PDL, and PRP degradation.

A *gshA* mutant known to be unable to utilize EA, 1,2-PDL, or PRP as the sole carbon source was also sensitive to the presence of aldehydes in the medium, although it did not appear to be as sensitive to propionaldehyde as was the *polA* mutant (Fig. 4). The *gshA polA* double mutant was extremely sensitive to both compounds, suggesting that such a mutant was further compromised in its ability to grow in the presence of aldehydes.

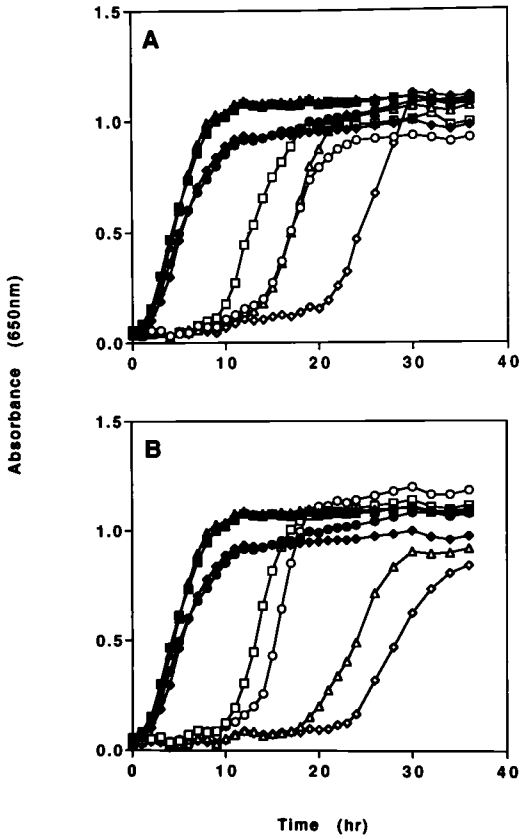

FIG. 4. Sensitivities of *polA* and *gshA* mutants to aldehydes. Growth in the presence (open symbols) or absence (closed symbols) of 10 mM acetaldehyde (A) or propionaldehyde (B) is shown. Strains: TR6583 (*polA*$^+$; squares), JE2996 (*polA71*::MudJ; triangles), JE2546 [*gshA101*::Tn*10d*(Tc); circles], and JE2997 [*gshA101*::Tn*10d*(Tc) *polA71*::MudJ; diamonds].

TABLE 2. Phenotypes of *polA* strains[a]

Strain	*polA* allele	Growth on[b]:			Sensitivity to[c]:	
		EA	1,2-PDL	PRP	CV	UV
JE3755	*polA101*::Tn*10d*(Cm)	−	−	−	S	S
JE3756	*polA102*::Tn*10d*(Tc)	−	−	−	S	S
JE3757	*polA103*::Tn*10d*(Tc)	−	−	−	S	S
JE3746	*atrC20*(*polA*)::MudJ	+	+	+	R	S
AA3007	*polA2*	+	+	+	R	S
TN2373	*polA2*	+	+	+	R	S
JE2996	*polA71*::MudJ	−	−	−	S	S
TR6583	*polA*$^+$	+	+	+	R	R

[a] Phenotypes were tested by patching strains on a nutrient agar plate and replica printing to the appropriate medium.
[b] +, growth similar to that of the *polA*$^+$ strain; −, no growth.
[c] S, sensitive; R, resistant.

***polA* mutants have variable phenotypes.** We tested whether the phenotypes of strain JE2996 were common to *polA* mutants. The results are shown in Table 2. Strains TN2373 (*polA2*) and JE3746 [*atrC20*(*polA*)::MudJ] did not have the phenotypes of strain JE2996 (*polA71*::MudJ), although they were sensitive to UV light. However, several *polA* insertions isolated by T. Elliott and coworkers (5a) [*polA101*::Tn*10d* (Cm), *polA102*::Tn*10d*(Tc), and *polA103*::Tn*10d*(Tc)] had the same phenotypes as those of JE2996 (*polA71*::MudJ), i.e., lack of growth on EA, PRP, or 1,2-PDL and sensitivity to CV. This suggested that our mutant was not unique; perhaps only certain disruptions of the *polA* gene result in the inability to grow on EA, 1,2-PDL, or PRP.

Because PolI is required in the excision repair pathway, other mutants defective in excision repair might be defective in growth on EA, PRP, or 1,2-PDL. We found that *uvrA* and *uvrB* mutants were able to grow on these compounds (data not shown), although both of them were sensitive to CV and UV light. These phenotypes suggest that the inability of JE2996 (*polA71*::MudJ) to grow on these carbon sources is due to a disruption of a function(s) distinct from the one involved in the excision repair pathway.

The reactive-aldehyde hypothesis. The involvement of PolI and glutathione in the metabolism of EA and 1,2-PDL was in agreement with the hypothesis offered by others (2, 28) of the compartmentalization of the enzymes needed to catabolize these compounds. Compartments may be needed to contain acetaldehyde in the case of EA and propionaldehyde in the case of 1,2-PDL. The reason(s) for the lack of growth of *polA* and *gshA* mutants on PRP is unclear since the catabolic pathway for this compound in *S. typhimurium* has not been documented. We propose, however, that a reactive aldehyde generated during PRP catabolism is the likely cause.

On the basis of our data, it appears that aldehydes that escape quenching by glutathione exert their damaging effects by reacting with primary amine groups in the cytosine, adenine, and guanine present in deoxynucleoside triphosphates and/or DNA. The net result would be a requirement for a PolI editing function during DNA replication.

Alternative explanations. There are alternative explanations. Perhaps *polA* mutants are defective in the replication of some extrachromosomal element that is required for the utilization of these substrates. The maintenance of pSLT, the 90-kDa endogenous plasmid, has been reported to be unaffected by *polA* mutations (34). However, the *polA* mutant used in those studies was *polA2*, which behaves differently than does JE2996 (*polA71*::MudJ). Thus, the ability of *polA* mutants to maintain pSLT remains an open question.

Further analysis of this phenomenon is required to understand the link between carbon metabolism and PolI function.

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REFERENCES

1. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mu*d*-P22 prophages. J. Bacteriol. **174:**1673–1681.
2. **Chen, P., D. I. Andersson, and J. R. Roth.** 1994. The control region of the *pdu*/*cob* regulon in *Salmonella typhimurium*. J. Bacteriol. **176:**5474–5482.
3. **Cooper, K. O., G. Witz, and C. M. Witmer.** 1987. Mutagenicity and toxicity of several a,b-unsaturated aldehydes in the *Salmonella typhimurium* mutagenicity assay. Environ. Mutagen. **9:**289–295.
4. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **De Lucia, P., and J. Cairns.** 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224:**1164–1166.

5a. **Elliott, T.** Personal communication.

6. **Faust, L. P., J. A. Connor, D. M. Roof, J. A. Hoch, and B. M. Babior.** 1990. Cloning, sequencing and expression of the genes encoding the adenosylcobalamin-dependent ethanolamine ammonia-lyase of *Salmonella typhimurium*. J. Biol. Chem. **265:**12462–12466.
7. **Ishido, M., T. Hase, F. Ito, and Y. Masamune.** 1980. Comparative studies of the *Escherichia coli* DNA *polA* gene and primary structure of DNA polymerase. J. Gen. Appl. Microbiol. **26:**183–202.
8. **Jeter, R. M.** 1990. Cobalamin-dependent 1,2-propanediol utilization by *Salmonella typhimurium*. J. Gen. Microbiol. **136:**887–896.
9. **Joyce, C. M., and N. D. F. Grindley.** 1984. Method for determining whether a gene of *Escherichia coli* is essential: application of the *polA* gene. J. Bacteriol. **158:**636–643.
10. **Joyce, C. M., W. S. Kelley, and N. D. F. Grindley.** 1982. Nucleotide sequence of the *Escherichia coli* DNA *polA* gene and primary structure of DNA polymerase I. J. Biol. Chem. **257:**1958–1964.
11. **Kingsbury, D. T., and D. R. Helinski.** 1970. DNA polymerase as a requirement for the maintenance of the bacterial plasmid colicinogenic factor E1. Biochem. Biophys. Res. Commun. **41:**1538–1544.
12. **Kornberg, A., and T. A. Baker.** 1992. DNA replication, 2nd ed. W. H. Freeman & Co., New York.
13. **Levin, D. E., T. J. Lovely, and E. Klekowski.** 1982. Light-enhanced genetic toxicity of crystal violet. Mutat. Res. **103:**283–288.
14. **MacPhee, D. G., and M. R. Beazer.** 1973. Mutants of *Salmonella typhimurium* deficient in DNA polymerase I: detection by their failure to produce colicin E1. Mol. Gen. Genet. **127:**229–240.
15. **MacPhee, D. G., and M. R. Beazer.** 1975. Mutants of *Salmonella typhimurium* deficient in DNA polymerase I: further characterization and genetic analysis. Aust. J. Biol. Sci. **28:**559–565.
16. **Marinari, U. M., M. Ferro, L. Sciaba, and A. M. B. Bassi.** 1984. DNA-damaging activity of biotic and xenobiotic aldehydes in Chinese hamster ovary cells. Cell Biochem. Funct. **2:**243–248.
17. **Marnett, L. J., H. K. Hurd, M. C. Hollstein, D. E. Levin, H. Esterbauer, and B. N. Ames.** 1985. Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. Mutat. Res. **148:**25–34.
18. **O'Toole, G. A., and J. C. Escalante-Semerena.** 1995. Purification and characterization of the bifunctional CobU enzyme of *Salmonella typhimurium* LT2. Evidence for a CobU~GMP intermediate. J. Biol. Chem. **270:**23560–23569.
19. **O'Toole, G. A., J. R. Trzebiatowski, and J. C. Escalante-Semerena.** 1994. The *cobC* gene of *Salmonella typhimurium* codes for a novel phosphatase involved in the assembly of the nucleotide loop of cobalamin. J. Biol. Chem. **269:**26503–26511.
20. **Plunkett, G., III, V. Burland, D. L. Daniels, and F. R. Blattner.** 1993. Analysis of the *Escherichia coli* genome. III. DNA sequence of the region from 87.2 to 89.2 minutes. Nucleic Acids Res. **175:**6328–6336.
21. **Price, G. D., S. M. Howitt, K. Harrison, and M. R. Badger.** 1993. Analysis of a genomic DNA region from the cyanobacterium *Synechococcus* sp. strain PCC7942 involved in carboxysome assembly and function. J. Bacteriol. **175:** 2871–2879.

22. **Radicella, J. P., E. A. Clark, S. Chen, and M. Fox.** 1993. Patch length of localized repair events: role of DNA polymerase I in *mutY*-dependent mismatch repair. J. Bacteriol. **175:**7732–7736.
23. **Rondon, M. R., and J. C. Escalante-Semerena.** 1992. The *poc* locus is required for 1,2-propanediol-dependent transcription of the cobalamin biosynthetic (*cob*) and propanediol utilization (*pdu*) genes of *Salmonella typhimurium*. J. Bacteriol. **174:**2267–2272.
24. **Rondon, M. R., R. Kazmierczack, and J. C. Escalante-Semerena.** 1995. Glutathione is required for maximal transcription of the cobalamin biosynthetic and 1,2-propanediol utilization (*cob*/*pdu*) regulon and for the catabolism of ethanolamine, 1,2-propanediol, and propionate in *Salmonella typhimurium* LT2. J. Bacteriol. **177:**5434–5439.
25. **Roof, D. M., and J. R. Roth.** 1988. Ethanolamine utilization in *Salmonella typhimurium*. J. Bacteriol. **170:**3855–3863.
26. **Sanderson, K. E., A. Hessel, and K. E. Rudd.** 1995. Genetic map of *Salmonella typhimurium*, edition VIII. Microbiol. Rev. **59:**241–303.
27. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
28. **Stojiljkovic, I., A. J. Bäumler, and F. Heffron.** 1995. Ethanolamine utilization in *Salmonella typhimurium*: nucleotide sequence, protein expression, and mutational analysis of the *cchA cchB eutE eutJ eutG eutH* gene cluster. J. Bacteriol. **177:**1357–1366.
29. **Suh, S.-J., and J. C. Escalante-Semerena.** 1995. Purification and initial characterization of the ATP:corrinoid adenosyltransferase encoded by the *cobA* gene of *Salmonella typhimurium*. J. Bacteriol. **177:**921–925.
30. **Sweasy, J. B., M. Chen, and L. A. Loeb.** 1995. DNA polymerase β can substitute for DNA polymerase I in the initiation of plasmid DNA replication. J. Bacteriol. **177:**2923–2925.
31. **Sweasy, J. B., and L. A. Loeb.** 1992. Mammalian DNA polymerase β can substitute for DNA polymerase I during DNA replication in *Escherichia coli*. J. Biol. Chem. **267:**1407–1410.
32. **Trzebiatowski, J. R., G. A. O'Toole, and J. C. Escalante-Semerena.** 1994. The *cobT* gene of *Salmonella typhimurium* encodes the NaMN:5,6-dimethylbenzimidazole phosphoribosyltransferase responsible for the synthesis of N^1-(5-phospho-α-D-ribosyl)-5,6-dimethylbenzimidazole, an intermediate in the synthesis of the nucleotide loop of cobalamin. J. Bacteriol. **176:**3568–3575.
33. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification, and some properties. J. Biol. Chem. **218:**97–106.
34. **Whitfield, H. J., and G. Levine.** 1973. Isolation and characterization of a mutant of *Salmonella typhimurium* deficient in a major deoxyribonucleic acid polymerase activity. J. Bacteriol. **116:**54–58.
35. **Yamaguchi, T.** 1982. Mutagenicity of trioses and methyl glyoxal on *Salmonella typhimurium*. Agric. Biol. Chem. **46:**849–851.
36. **Youderian, P., P. Sugiono, K. L. Brewer, N. P. Higgins, and T. Elliott.** 1988. Packaging specific segments of the salmonella chromosome with locked-in Mud-P22 prophages. Genetics **118:**581–592.