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Identification of a Genetic Locus Affecting Chromosome Stability and Cellular Survival in a *dnaB* Mutant

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A mutation has been identified in an *Escherichia coli* K-12 strain carrying *dnaB42*. This mutation potentiates both deoxyribonucleic acid degradation and cell death at nonpermissive temperatures. It is located 2 min away from *dnaB* between *malB* and *metA*.

Mutations within the *dnaB* gene of *Escherichia coli* K-12 can produce a variety of phenotypes. Interpretation of these phenotypes is complicated by the observation that the phenotypic expression of a *dnaB* mutation can change depending on the total genetic constitution of the cell carrying it (3, 4, 11). One common observation is that some *dnaB* mutants degrade their deoxyribonucleic acid (DNA) at nonpermissive temperatures (3, 14). This degradation seems to be dependent on exonuclease V since it can be blocked by mutations in the *recB recC* gene cluster (3). A second property of some *dnaB* mutants is their inability at any temperature to grow lambda phage with a normal *P* gene (*groP*) (5). This can sometimes be changed drastically by the presence of suppressors in the genetic background (11). Some *dnaB* mutants remain viable for several hours at high temperatures even though replication has stopped (14), whereas others quickly die (2, 4). This bacteriocidal effect seems to be dependent upon genetic factors distinct from those causing the inhibition of replication (4).

A mutation, called *bpo* (*dnaB* potentiator), has been identified in cells carrying the mutation *dnaB42*. *bpo* is genetically distinct from *dnaB* and potentiates the degradation of DNA that occurs at nonpermissive temperatures in cells carrying the *dnaB42* allele. Furthermore, it causes this *dnaB* mutation to have a lethal rather than simply a bacteriostatic effect at high temperature. The identification and characterization of *bpo* are described in this paper.

MATERIALS AND METHODS

Bacterial strains and phage stocks. The derivation and genotypes of bacterial strains used in this study are listed in Table 1. P1*clr* used throughout this study was a gift from J. E. Davies. Lambda phage used to assay for the GroP phenotype were a gift from I. Herskowitz.

Chemical reagents. Tryptone, vitamin-free Casamino Acids, yeast extract, and agar were obtained from Difco Laboratories, Inc. Amino acids, vitamins, nucleosides, and rifampin were purchased from Sigma Chemical Co. The maltose was "A grade" quality from Calbiochem. Radioactive thymine (21 Ci/mmol) and thymidine (5 Ci/mmol) were obtained from Amersham Radiochemical Centre.

General methods. General bacteriological media and methods, as well as procedures for P1 transduction, were described previously (10). The only deviation from the published method of P1 transduction was the use of a buffer containing 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.5), 0.01 M $MgCl_2$, and 0.005 M $CaCl_2$ for the absorption of the phage to the recipient cells. After absorption at 30°C, the infected cells were spun down and suspended for plating in M9 salts containing 0.004 M trisodium citrate. Plates were incubated at 30°C unless stated otherwise. Methods for preparation and counting of radioactive samples were described previously (16).

DNA synthesis determinations. Cultures (2 ml) were grown at 30°C in minimal glucose medium containing 0.2% vitamin-free Casamino Acids and 5 μg of thymidine per ml. Cultures were diluted repeatedly into fresh medium to assure log-phase growth of the cells for 24 h prior to labeling. Labeling was initiated by diluting the culture to a cell density of 5×10^6/ml in medium containing 8.5 μCi of [^{3}H]thymidine per ml. This culture was allowed to grow at 30°C for 2 h before sampling was begun. Thereafter, samples (50 μl) were removed, spotted on filter paper disks, and placed in cold 5% trichloroacetic acid. This sampling was continued until the cell density of the culture reached 2×10^8. The flask was shifted to a high-temperature water bath (40 to 43°C as indicated), and sampling was continued in duplicate.

Pulse labeling was performed in a similar fashion, except that cultures were grown in medium containing 10 μg of thymine per ml instead of thymidine. Cultures were grown at 30°C to a cell density of 10^8/ml in unlabeled medium. [^{3}H]thymidine was then added at a concentration of 10 μCi/ml. Samples (50 μl) were removed over a 15-min period of labeling at 30°C; then the cultures were shifted to the high temperature, and duplicate samples were taken at the times indicated.

DNA synthesis in thymine prototrophs was deter-

TABLE 1. *Derivation and genotype of bacterial strains used*

Strain	Genotype	Source and reference
AT2444	HfrH *thi metE*	E. Kort (1)
ATR2444	HfrH *thi metE rif*	Spontaneous *rif*^{r} isolate of strain AT2444
AW533	HfrG6 *thi his malB*	J. Adler (12)
P10-FM1	HfrP10 *thi dct thr leu*	R. Reader (8)
D_2TT	*thi thy str*	P. Kuempel (14)
$D_2$184	*thi thy str dnaB184*	P. Kuempel (14)
D_2TT228	*thi thy str malB*	P1 transduction of *malB* allele from strain AW533 into strain $D_2$184
Gr2	*thi argH metA str*	I. Herskowitz
Gr228	*thi argH str malB*	P1 transduction of *malB* allele from strain AW533 into strain Gr2
SA107	*thi metE malB rha lac str*	R. Buxton
Gr11	*thi argH metA str dnaB42 bpo*	I. Herskowitz
Gr11-M1	*thi argH str dnaB42 bpo*	Spontaneous *met*$^+$ isolate of strain Gr11
Gr11R	*thi argH metA str dnaB42 bpo rif*	Spontaneous *rif*^{r} isolate of strain Gr11
Gr11 (ATR2)	*thi str dnaB42*	P1 transduction of *arg*$^+$, *met*$^+$, and *bpo*$^+$ alleles from strain ATR2444 into strain Gr11
Gr11-P105	*thi str dnaB42*	*arg*$^+$ *met*$^+$ *bpo*$^+$ recombinant of a cross between strains P10-FM1 and Gr11
D_2TT42	*thi thy str dnaB42*	P1 transduction of *malB* region from strain Gr11 into strain D_2TT228
D_2TT42H	*thi thy str dnaB42 bpo*	P1 transduction of *malB* region from strain Gr11 into strain D_2TT228
JC1553/KLF10	*argG metB his leu mtl xyl malA gal lacYZ tonA recA supE str*/F'110	*E. coli* Genetic Stock Center (9)
JC1553/KLF12	*argG metB his leu mtl xyl malA gal lacYZ tonA recA supE str*/F'112	*E. coli* Genetic Stock Center (9)
NY100	*thi thy leu pyrB his lac recA val*^{r} *str*/F'118 *ampA*	J. Wechsler (15)
Gr11/KLF10	*thi argH metA str dnaB42 bpo*/F'110	*arg*$^+$ *met*$^+$ transconjugant of a cross between strains JC1553/KLF10 and Gr11
Gr11/KLF12	*thi argH metA str dnaB42 bpo*/F'112	*arg*$^+$ *met*$^+$ transconjugant of a cross between strains JC1553/KLF12 and Gr11
Gr11/KLF18	*thi argH metA str dnaB42 bpo*/F'118 *ampA*	*amp*^{r} transconjugant of a cross between strains NY100 and Gr11

mined by growing 10-ml cultures at 30°C in minimal glucose medium containing 25 μg (each) of arginine and methionine per ml. Cultures were grown to a cell density of 2×10^8/ml. Samples (1 ml) were placed at 30 or 43°C, 25 μCi of [^{3}H]thymidine was added, and samples were removed with time.

Cell viability at 30 and 41°C. Cultures (10 ml) were grown at 30°C in minimal glucose medium containing 0.2% Casamino Acids and 10 μg of thymine per ml. The cultures were sustained in log phase for at least 24 h by diluting them into fresh medium. To initiate the experiment, cultures with a cell density of approximately 10^8/ml were diluted either 10^4- or 2×10^5-fold into fresh 30°C medium and initial 0.1-ml samples were taken. The diluted cultures then were shaken at 30°C, and samples were taken during the next 90 min. The flasks were shifted to a 41°C water bath, and sampling continued in duplicate at the times indicated. Samples throughout the experiment were spread on minimal glucose plates supplemented with 0.2% Casamino Acids and 10 μg of thymine per ml. Plates were incubated at 30°C for 24 to 36 h before colonies were counted.

RESULTS

The *E. coli* K-12 strain Gr11 is one of a series of *groP* strains described by Georgopoulos and Herskowitz (5). It was constructed by transferring the *dnaB42* allele, first described by Hirota et al. (7), into strain PA505 MPE11 (12) by P1*kc* transduction. The strain will not grow at temperatures above 40°C since DNA synthesis stops immediately at these temperatures.

In the process of transferring the *dnaB42* mutation from strain Gr11 into a thymine-requiring genetic background, two classes of temperature-sensitive P1 transductants were observed. One class formed normal size colonies at 30°C but was totally inhibited at 41°C. The

second class formed colonies more slowly at 30°C; yet even at 41°C these transductants could form tiny colonies. The colonies never grew as large as those of the *dna*$^+$ parent, however. It thus seemed probable that strain Gr11 carried more than one mutation within the *dnaB* region of the chromosome that affected high-temperature growth of the strain and distinguished it from the recipient.

To substantiate the presence of a second mutation in strain Gr11, it was crossed with the Hfr strain P10, which has its origin of transfer within the *malB* gene. Such a cross replaces the genetic material on the *metA* side of *malB* with DNA from the Hfr strain while leaving the *dnaB* region of strain Gr11 unchanged. When methionine or arginine prototrophs of such a cross were selected, two classes of temperature-sensitive recombinants again were found. One class formed tiny colonies at 41°C, whereas the other showed no growth at all. This confirmed that strain Gr11 carried a second mutation and located it on the *metA* side of *malB*. This mutation seemed to potentiate the inhibitory effects of *dnaB42*. The mutation was named *bpo* (*dnaB* potentiator).

Mapping of *bpo*. (i) Hfr matings. Arginine prototrophs selected from three crosses between strain Gr11 (*argH pbo dnaB42*) and strain P10-FM1 (HfrP10 *agr*$^+$ *bpo*$^+$ *dna*$^+$) were screened for growth at 41°C. The percentage able to form tiny colonies ranged from 17 to 73. Among these partially temperature-resistant recombinants, 46 of 47 tested retained their GroP phenotype; thus, they still carried an unaltered *dnaB42* mutation.

(ii) F′ matings. When F′ factors complementary to the chromosomal region on the *dnaB* side of *malB* (KLF18) or both sides of *malB* (KLF12) were introduced into strain Gr11, the strains grew normally at 43°C. Strains merodiploid for the region on the *metA* side of *malB* (KLF11 or KLF10) did not form colonies when initially streaked on plates at 41 or 43°C. If colonies were allowed to form on plates at 30°C and then were transferred onto plates at 41°C, some growth, presumed to be due to *bpo*$^+$ homogenotes in the colonies, was seen.

(iii) P1 mapping. The position of the *bpo* mutation was determined relative to the markers *rif*, *metA*, and *argH*. Data showing the linkage among these loci are presented in Table 2. The cotransduction frequencies of *bpo* and *dnaB42* with these genes are given in Table 3. Data in this table substantiate the finding (5, 7) that *dnaB42* is located between *malB* and *uvrA* since it was 53% linked to *malB* (line 5) and 1.4% linked to *metA* (line 6), while its linkage with *rif* was less than 0.5% (line 3).

The *bpo* mutation is located on the *metA* side of *malB*. A linkage of 7% was found with *argH*. This was the only linkage that could be determined unambiguously since *argH* is unlinked to *dnaB42*. Linkages with *metA* and *rif* were calculated to be 90 and 45%, respectively. The *rif* locus has a very low cotransduction frequency with *dnaB42* (line 3). When temperature-resistant transductants of strain Gr11 are selected (line 2), if approximately half are *dnaB*$^+$ and half are *bpo*$^+$, the actual *bpo-rif* cotransduction frequency is twice that observed, or about 45%. When *met*$^+$ transductants are selected at 30°C (line 4), about 2% are expected to be *dnaB*$^+$ (line 6). Thus, the actual *bpo-metA* linkage is about 2% lower than that observed with *met*$^+$, temperature-resistant strains.

The relative location of *bpo* was determined by three- and four-factor crosses (Tables 4 and 5). The data indicate the gene order *argH rif metA bpo*.

The mapping experiments indicate that the *bpo* mutation is located near *metA* at a site substantially different from *dnaB*. It has no detectable effect on cell growth in the absence of the *dnaB42* mutation, and it seems to be dominant to the wild-type allele. It is possible that the *bpo* gene product is *cis*-acting. Unfortunately, this alternative could not be tested because of the absence of an F′ strain carrying the *bpo* allele. A summary of the mapping is shown in Fig. 1.

Effect of *bpo* on DNA metabolism. It is clear that cells of genotype *dnaB42 bpo*$^+$ are quite different from the wild-type organisms. Their growth at 43°C is blocked completely and even at 40°C is restricted severely. The double mutant *dnaB42 bpo*, on the other hand, is completely prevented from growth even at 40°C. One possible explanation of this phenotype is that *dnaB42* is "leaky" enough at 40°C to allow some growth. The *bpo* mutation then potentiates the effects of *dnaB42* to slow replication,

TABLE 2. *Linkage of argH, rif, metA, and malB by P1 transduction*

Strain transduced (relevant genotype)	Donor strain (relevant genotype)	Selected phenotype (no.)	Screened phenotype (no.)	Linkage (%)
(1) Gr11 (*argH rif*^{s})	ATR2444 (*arg*$^+$ *rif*^{r})	Arg$^+$ (484)	Rif^r (343)	*argH-rif* (71)
(2) Gr11 (*argH metA*)	ATR2444 (*arg*$^+$ *metA*$^+$)	Arg$^+$ (484)	Met$^+$ (92)	*argH-metA* (19)
(3) Gr11 (*rif*^{s} *metA*)	ATR2444 (*rif*^{r} *metA*$^+$)	Met$^+$ (447)	Rif^r (236)	*rif-metA* (53)

TABLE 3. *Mapping of bpo and dnaB42: P1 transduction*

Strain transduced (relevant genotype)	Donor strain (relevant genotype)	Selected phenotype (no.)	Screened phenotype (no.)	Linkage (%)
(1) Gr11 (*argH bpo*)	ATR2444 (*arg*$^+$ *bpo*$^+$)	Arg$^+$ (692)	tr(40°)[a] (60)	*argH-bpo* (7)
(2) Gr11 (*rif*^{s} *bpo dnaB42*)	ATR2444 (*rif*^{r} *bpo*$^+$ *dnaB*$^+$)	tr(40°)[a] (128)	Rif^r (30)	*rif-bpo* plus *rif-dnaB42* (23)
(3) Gr11 (*rif*^{s} *dnaB42*)	ATR2444 (*rif dnaB*$^+$)	tr(43°)[a] (325)	Rif^r (1)	*rif-dnaB42* (0.3)
(4) Gr11 (*metA bpo dnaB42*)	ATR2444 (*metA*$^+$ *bpo*$^+$ *dnaB*$^+$)	Met$^+$ (303)	tr(40°)[a] (257)	*metA-bpo* plus *metA-dnaB42* (85)
Gr11 (*metA bpo dnaB42*)	ATR2444 (*metA*$^+$ *bpo*$^+$ *dnaB*$^+$)	Met$^+$ (132)	tr(40°)[a] (128)	*metA-bpo* plus *metA-dnaB42* (97)
(5) Gr228 (*malB dnaB*$^+$)	Gr11-M1 (*malB*$^+$ *dnaB42*)	Mal$^+$ (39)	ts(43°)[a] (21)	*malB-dnaB42* (54)
D_2TT228 (*malB dnaB*$^+$)	Gr11 (*malB*$^+$ *dnaB42*)	Mal$^+$ (121)	ts(43°)[a] (57)	*malB-dnaB42* (47)
Gr228 (*malB dnaB*$^+$)	Gr11-M1 (*malB*$^+$ *dnaB42*)	Mal$^+$ (60)	GroP (34)	*malB-dnaB42* (57)
Gr11-M1 (*malB*$^+$ *dnaB42*)	AW533 (*malB dnaB*$^+$)	tr(43°)[a] (34)	Mal (18)	*malB-dnaB42* (53)
(6) Gr2 (*metA dnaB*$^+$)	D_2TT42 (*met*$^+$ *dnaB42*)	Met$^+$ (363)	ts(43°) (5)	*metA-dnaB42* (1.4)

[a] Form colonies at the indicated temperatures.

TABLE 4. *Linkage of bpo, metA, and rif by P1 transduction*

Strain transduced (relevant genotype)	Donor strain (relevant genotype)	Selected phenotype (no.)	Screened phenotype (no.)	Linkage (%)
Gr11 (*rif*^{s} *metA bpo dnaB42*)	ATR2444 (*rif*^{r} *met*$^+$ *bpo*$^+$)	Met$^+$ Rif^r (198)	tr(40°)[a] (157)	(*metA-rif*)-*bpo* (80)
		Met$^+$ tr(40°)[a] (257)	Rif^r (157)	(*metA-bpo*)-*rif* (61)

[a] Form colonies at the indicated temperatures.

TABLE 5. *Linkage of bpo, metA, rif, and argH by P1 transduction*

Selected phenotypes[a] (no.)	Unselected phenotypes (no.)	Linkage (%)
(1) Arg$^+$ Rif^r (343)	Met$^+$ (79)	(*argH-rif*)-*metA* (23)
(2) Arg$^+$ Rif^r (343)	tr(40°)[b] (29)	(*argH-rif*)-*bpo* (8)
(3) Arg$^+$ Rif^r (343)	Met$^+$ tr(40°)[b] (5)	(*argH-rif*)-*metA-bpo* (1.5)
(4) Arg$^+$ Met$^+$ (92)	Rif^r (79)	(*argH-metA*)-*rif* (86)
(5) Arg$^+$ Met$^+$ (92)	tr(40°)[b] (6)	(*argH-metA*)-*bpo* (7)
(6) Arg$^+$ Met$^+$ (92)	Rif^r tr(40°)[b] (5)	(*argH-metA*)-*bpo-rif* (6)
(7) Arg$^+$ tr(40°)[b] (45)	Rif^r (29)	(*argH-bpo*)-*rif* (64)
(8) Arg$^+$ tr(40°)[b] (45)	Met$^+$ (6)	(*argH-bpo*)-*metA* (13)
(9) Arg$^+$ tr(40°)[b] (45)	Rif^r Met$^+$ (5)	(*argH-bpo*)-*metA-rif* (11)

[a] Transductants of strain Gr11 were selected at 30°C after infection with phage P1 grown on strain ATR2444.

[b] Formed colonies at the indicated temperatures.

enough so that the double mutant can not grow at all.

To test this possibility, DNA synthesis rates at various temperatures were determined in strains D_2TT (*dna*$^+$ *bpo*$^+$), D_2TT42 (*dnaB42 bpo*$^+$), and D_2TT42H (*dnaB42 bpo*). Figure 2A shows results obtained from cultures grown at 30°C and shifted to 40°C. The rate of thymine incorporation at 30°C into all three cultures was similar (sometimes D_2TT42 incorporation was

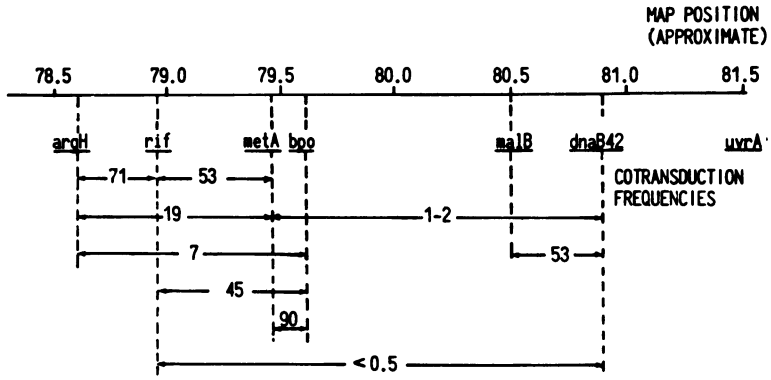

FIG. 1. *Location of bpo, as determined by P1 transduction, indicated relative to positions assigned previously for genes argH, rif, metA, and malB (13).*

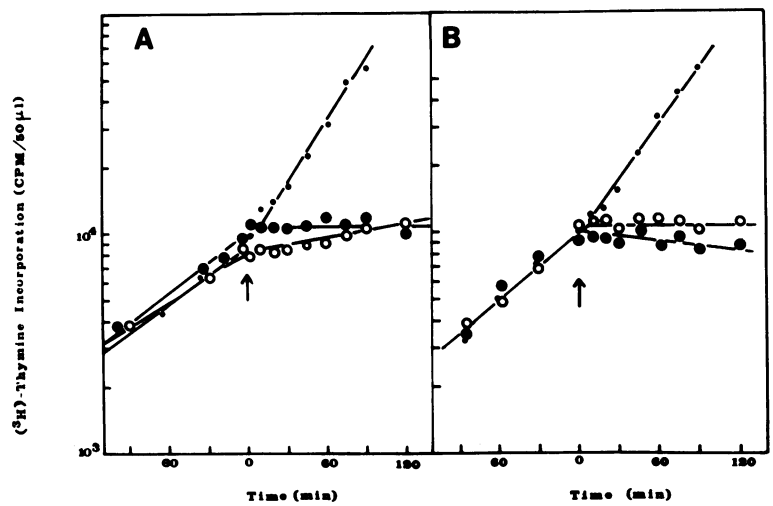

FIG. 2. *DNA synthesis at permissive and nonpermissive temperatures for strains D_2TT (dna^+ bpo^+) (•), D_2TT42 (dnaB42 bpo^+) (○), and D_2TT42H (dnaB42 bpo) (●). Cultures were grown in the presence of [^{3}H]thymine for many generations prior to shifting them to 40°C (A) or 43°C (B). The time of sampling is indicated either before or after the shift (time zero, marked by arrow).*

as much as 10% slower). At 40°C, however, strain D_2TT42H immediately stopped incorporating label, whereas D_2TT42 continued incorporating at a rate about 20 to 25% that observed at 30°C. Cells without the *dnaB* mutation more than doubled their rate of incorporation after a similar temperature shift. When the nonpermissive temperature was 41°C instead of 40°C, D_2TT42H again stopped making DNA, whereas D_2TT42 was still able to incorporate thymine at 10 to 15% its 30°C rate (data not shown).

Figure 2B illustrates the data obtained when the nonpermissive temperature was raised to 43°C. At this temperature, D_2TT42 was no longer able to make DNA, but the radioactivity incorporated prior to the shift was stable for at least 2 h. The DNA of strain D_2TT42H, on the other hand, was unstable at 43°C, and acid-precipitable counts were lost throughout the high-temperature incubation.

The degradation observed in Fig. 2B represents overall chromosomal instability since the cultures had been labeled for many generations. When cultures were pulse labeled with thymidine and then shifted to a nonpermissive temperature, the counts incorporated should have been predominately in nascent DNA near the replication forks. Figure 3 shows that both D_2TT42H and D_2TT42 degrade some of this nascent DNA. Here again, though, the DNA of D_2TT42H is more unstable during the first hour at 43°C than its bpo^+ counterpart. This result suggests that degradation in these strains is similar to that reported previously for other *dnaB* alleles (14). Similar results were obtained with strains Gr11 and Gr11 (ATR2).

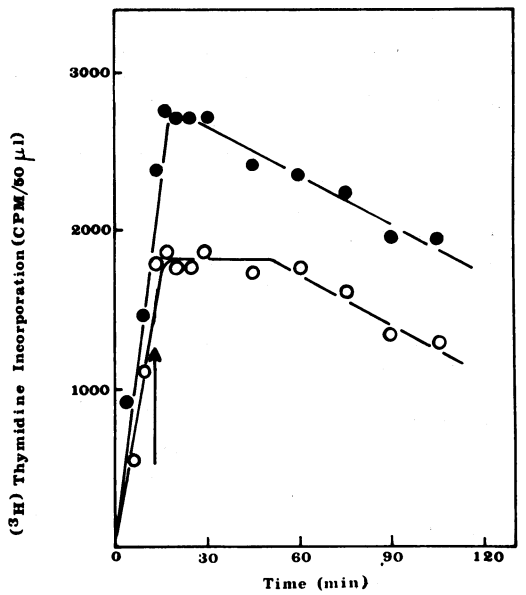

FIG. 3. *[3H]thymidine incorporation into cultures of strains D2TT42 (dnaB42 bpo+) (○) and D2TT42H (dnaB42 bpo) (●). Cultures were labeled at 30°C for 15 min prior to shifting them to 43°C (marked by the arrow).*

DNA synthesis in merodiploids. Genetic studies described previously indicated that the *bpo* gene product was either *cis*-acting or dominant to the *bpo*+ allele. When DNA synthesis rates of various merodiploids of strain Gr11 were compared (Table 6), this conclusion was substantiated. Recombinants that were *bpo*+ *dnaB42* (12 were tested but only results for strain Gr11-P105 are shown) gave a two- to threefold greater rate of synthesis at nonpermissive temperatures than did strain Gr11. Cultures heteroallelic for *bpo* did not show this increased rate. When a *dnaB*+ allele was present on the episome, synthesis returned to the rate expected for wild-type organisms. These results are consistent with the genetic indications that *bpo* is dominant or *cis*-acting whereas *dnaB42* is recessive.

Cell viability at nonpermissive temperatures. When cell viability of strains Gr11 and Gr11(ATR2) or D2TT42 and D2TT42H were compared, the effect of replacing the *bpo* mutation with its wild-type allele was striking. Figure 4 shows the effect on D2TT42 and D2TT42H of a temperature shift from 30 to 41°C. The viability of the D2TT42H (*bpo dnaB42*) culture remained constant for about 30 min but then fell exponentially. In contrast, D2TT42 (*bpo*+ *dnaB42*) retained its viability throughout the course of the experiment. If the temperature was raised to 43°C instead of 41°C, the exponential death of the D2TT42H culture began without the 30-min lag. Again the D2TT42 culture was stable for the 2 h tested. Identical results were obtained with strains Gr11 and Gr11(ATR2).

Identity of the *bpo* and *metH* cistrons.

TABLE 6. *[3H]thymidine incorporation at 43°C*

Strain	Genotype	Rate of [3H]thymidine incorporation at 43°C (cpm/min per 2 × 10⁷ bacteria)[a]
Gr11	*bpo dnaB42*	50
Gr11-P105	*bpo*+ *dnaB42*	130
Gr11/KLF10	*bpo dnaB42/bpo*+	40
Gr11/KLF18	*bpo dna B42/dnaB*+	1,380
Gr11/KLF12	*bpo dnaB42/bpo*+ *dnaB*+	1,400

[a] Parallel cultures were labeled at 30°C. Sample from all such cultures incorporated 450 to 550 cpm/min. The observed 43°C rate of incorporation was normalized to a 30°C rate of 500 cpm/min.

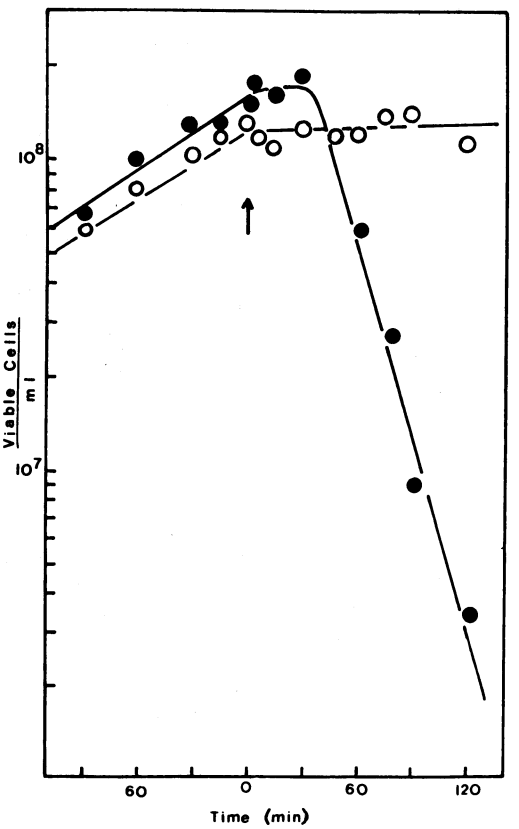

FIG. 4. *Viability of strains D2TT42 (dnaB42 bpo+) (○) and D2TT42H (dnaB42 bpo) (●). The cultures were grown at 30°C and then shifted to 41°C (time zero, marked by arrow). Times before and after the shift when samples were removed and streaked on plates are indicated. Plates were incubated at 30°C.*

Ahmed (1) has shown that the *metH* gene lies on the *malB* side of *metA* and probably adjacent to it. Because of the high cotransduction frequency between *bpo* and *metA*, it seemed possible that *bpo* and *metH* could be the same gene. The *metH* cistron codes for 5-methyltetrahydrofolate-homocysteine methyl transferase, which catalyzes an *S*-adenosylmethionine (SAM)-vitamin B_{12}-N^5-methyltetrahydrofolate-dependent methylation of homocysteine to methionine (1). The product of the *metE* gene can also carry out this methylation (1). Thus, the methionine requirement of a *metH* strain can only be detected in the absence of an active *metE* gene product.

When P1 phage were grown on strain Gr11R (*bpo rif*^{r}) and used to transduce AT2444 (*metE*) to rifampin resistance, 20% of the transductants grew very poorly on plates supplemented with homocysteine and vitamin B_{12} but grew well on methionine. This phenotype is expected for a *metE* cell with a partially inactive *metH* gene product. The presence of this abnormal *metH* activity suggests that *bpo* and *metH* are indeed the same gene. Further verification of this conclusion was sought by using the P1 stock to transduce strain SA107 (*metE malB*) to *mal*$^+$ and looking for transductants that grew poorly on homocysteine plus vitamin B_{12}. In this case none was found. At the same time, however, less than 1% cotransduction of *metA* with *malB* was found in this strain (5% was expected). No reason for this low linkage is known, but as a result a firm conclusion about the presence of a *metH* mutation in strain Gr11R could not be drawn from this experiment. The unambiguous demonstration of the identity of the *bpo* and *metH* cistrons awaits further analysis.

DISCUSSION

A mutation, named *bpo*, that potentiates the effects of the *dnaB42* mutation in *E. coli* strain Gr11 has been identified. P1 transduction and conjugation with HfrP10 have located it between *metA* and *malB* on the *E. coli* chromosome. Merodiploids complementary to various parts of the region around *malB* indicate that *bpo* is dominant to its wild-type allele but has no detectable effect on cell growth or DNA synthesis in the presence of a wild-type *dnaB* gene product.

The presence of *bpo* and *dnaB42* in a cell is lethal at temperatures greater than 40°C. When the *bpo*$^+$ allele is present, *dnaB42* is simply bacteriostatic for several hours at these temperatures. The chromosome of the *bpo dnaB42* cell is also more unstable at temperatures where replication is inhibited than its *bpo*$^+$ *dnaB42* counterpart. Experiments not reported here have indicated that other processes involving DNA-metabolizing functions, such as recombination and repair, seem to be unaffected by variations at the *bpo* locus.

Several *dnaB* mutations have been reported that are lethal at high temperature (2, 4). In one case, isolates were selected that remained thermosensitive but did not die at nonpermissive temperatures (4). It thus seems likely that mutations like *bpo* can exert an effect on several different *dnaB* alleles.

An interesting feature of the *bpo*$^+$ *dnaB42* isolates is their poor growth at 30°C relative to that of the double mutant. These cells form very small colonies on plates and take hours to come out of lag when inoculated into liquid medium. Once growing, however, they have a doubling time very similar to that of their *bpo dnaB42* or *dna*$^+$ relatives. The long lag time is unaffected by the nutritional supplements present in the growth medium. Likewise, neither the killing nor the nucleolytic effects of the *bpo* allele are affected by the type of growth medium used. Since *bpo* is located in the *dnaB* region and since the double mutant grows much better at 30°C, one possibility for the genesis of *bpo*-type mutations is that they arise during the same mutagenizations that produce *dnaB* mutations and are selected preferentially to the slower-growing *bpo*$^+$ *dnaB* cells.

The lethal effects of some *dnaB* mutants can be overcome by the addition of chloramphenicol to the culture at the time it is placed at high temperature (4). This suggests that the cells die because of an imbalance in their cellular processes caused by continued growth in the absence of DNA synthesis. The *bpo* mutation may mimic this chloramphenicol effect. The retardation of growth seen at 30°C in *bpo*$^+$ cells may be enough to prevent cell death when DNA replication stops at higher temperatures.

The indication of an altered *metH* gene in strain Gr11 raises the possibility that this gene product is involved in a cellular role other than biosynthesis of methionine. Alternatively, since a known cofactor of the *metH* product is SAM, which is also involved in the methylation of DNA (6), an altered *metH* gene could sequester or hydrolyze SAM. The lower intracellular level of SAM that results could cause the DNA to be undermethylated. This, in turn, could lead to increased chromosomal breakdown and cell death and would be dominant to the wild-type *metH* gene product. Further studies are planned to test these possibilities.

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