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Escherichia coli Mutants Temperature-Sensitive for DNA Synthesis

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Summary. A series of mutants of *E. coli* temperature-sensitive for DNA synthesis has been studied. The temperature-sensitive DNA mutations map in seven distinct genetic loci most of which have not been previously reported. Mutations in *dnaA* and in *dnaC* affect the initiation of DNA replication; those at the remaining loci affect chain elongation. A temperature-sensitive F'*lac* is shown to suppress a group A mutant with somewhat less efficiency than other F' factors previously reported by others. The gene products rendered temperature-sensitive by the mutations have not been identified for any of the loci.

This report attempts to analyze the complexity of normal DNA replication in *E. coli* by defining a number of genetic loci whose products are involved in replication. Previous work has defined three such loci (Hirota, Ryter and Jacob, 1968; Carl, 1970).

Of 501 temperature-sensitive mutants isolated without applying any selective pressure, fifteen were found to be specifically affected in DNA replication. These mutants will be designated Dna$^-$. We have mapped the mutations in fourteen of these, as well as in a number of strains isolated by other workers. The mutations occur at seven distinct locations on the *E. coli* chromosome.

Materials and Methods

Bacterial Strains. CR34: *thr leu* B_1 *thy* (low thymine requirer) *lac tonA strA.*

F'-containing strains were the gift of Dr. K. B. Low. AB1868 (AB312-type Hfr) and AB2271 (AB313-type Hfr) were the gift of Dr. E. A. Adelberg. The various Dna$^-$ mutants supplied by other workers were: BT series strains, Dr. F. Bonhoeffer; PC series strains, Dr. P. Carl; D247LT, Dr. P. Kuempel; MX74T2ts27, Dr. M. Inouye; and CRT46 and CRT83, Dr. Y. Hirota.

Bacteriophage. Plkc and Plvir were the gift of Dr. W. Brammar.

Media. Oxoid No. 2 Nutrient Broth (Oxo Ltd) supplemented with 20 μg/ml of thymine and M9 minimal medium supplemented with 20 μg/ml of thymine and the required amino acids, 0.35 μg/ml B_1 and 0.2% glucose were used as standard growth media. For preparation of P1 lysates and prior to transductions, strains were grown in P1 medium supplemented with 2.5×10^{-3} M $CaCl_2$ and 20 μg/ml thymine. P1 medium consists of 0.1 M tris-HCl pH 7.4, 1 liter; Bacto-tryptone (Difco Laboratories, Inc.), 10 g; yeast extract, 5 g; NaCl, 5 g; $MgSO_4$, 0.25 g; NH_4Cl_2, 1.0 g. Labelling medium was M9-glucose minimal medium supplemented with 0.05% Tryptone, 0.05% NaCl, 10 μg/ml thymine, 20 μg/ml of all amino acids except leucine, 0.35 μg/ml B_1, 100 μg/ml deoxyadenosine, 0.3 mC/ml ^{3}H-thymine (final specific activity 30 μC/μg), and 4 μC/ml ^{14}C-leucine (effective specific activity unknown because of tryptone supplement). (Thymine-methyl-^{3}H and ^{14}C-leucine were obtained from the Radiochemical Centre, Amersham.)

Mutant Selection. The 501 temperature-sensitive mutants in the "E series" were isolated from strain CR34 by Dr. Howard Goldfine using ethylmethane sulfonate mutagenesis at

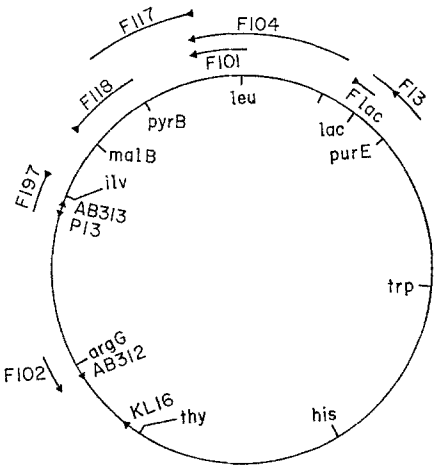

Fig. 1. *E. coli* K-12 chromosome map showing Hfr and F′ strains employed (adapted from Taylor and Trotter, 1967). Origin and direction of transfer of Hfr strains are denoted by arrows on the chromosomes map; the approximate extent of the chromosomal segments carried by the various F′ factors are indicated by their length and position. The origin and direction of transfer of the F′ factors are denoted by arrows. Genetic markers here and elsewhere in this paper conform to those of Taylor (1970). F′197 (Gross and Zusman, unpublished) is derived from HfrP72 (Glansdorff, 1967)

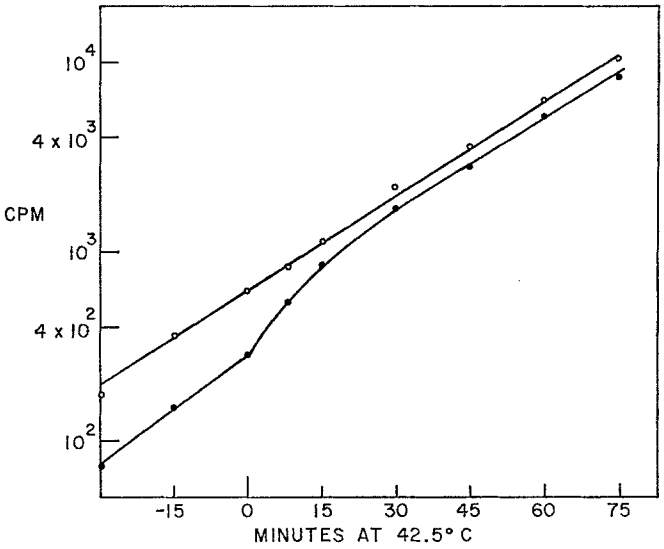

Fig. 2. Synthesis of DNA and protein by cells of strain CR34 before and after transfer from 30 to 42.5° C. An exponential phase culture was diluted into labelling medium and grown for 60 minutes at 30° C. The labelled culture was then shifted to 42.5° (0 time). Incorporation of label was measured as explained in Materials and Methods ○ ^{3}H-thymine, ● ^{14}C-leucine

30° C (Hechemy and Goldfine, 1971). They were screened for cells that were capable of growth at 30° C on nutrient media but incapable of growth at 42° C. Mutants were further screened for temperature-sensitive DNA synthesis by double-label experiments with ^{14}C-leucine and ^{3}H-thymine. The criterion for selection as a temperature-sensitive DNA deficient mutant was that DNA synthesis be reduced with no coincident perturbation of protein synthesis.

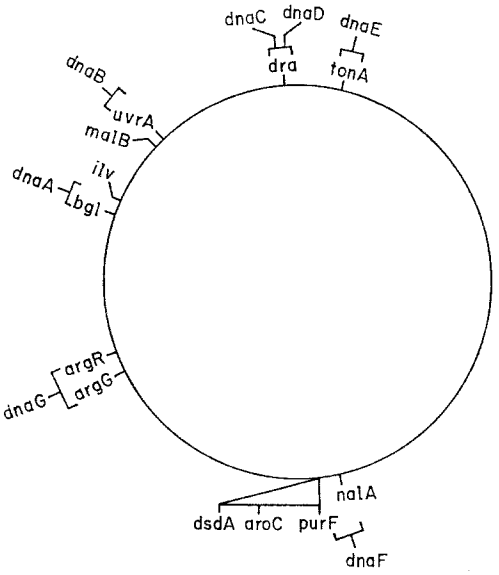

Fig. 3. Map locations of the *dna* mutations on the *E. coli* chromosome. Standard chromosome map after Taylor (1970)

Genetic Mapping. The positions of the various *dna* mutations were crudely determined by mating each mutant with various Hfr's and ascertaining which Hfr's transferred Dna$^+$ (temperature-resistance) with high frequency. The Hfr strains employed are shown in Fig. 1. By using a number of overlapping Hfr's many mutants were well localized by mating. Narrower limits were placed on the map position by a second series of matings employing a set of F′ strains as donors (Fig. 1). Final map positions were determined by co-transduction of the *dna* gene and a known genetic marker by P1 bacteriophage.

Labelling Experiments. Log phase cells grown in labelling medium minus the radioisotopes were diluted into 2 ml of labelling medium to a cell concentration of 5×10^6 cells/ml and incubated at 30° C with aeration. After one hour they were transferred to 42.5° C. Samples of 50 µl were removed at various times to Whatman 3MM filter paper discs and processed using a modification of a procedure of Bollum (1966). The dried filter discs were counted in a Packard Tri-carb scintillation counter using a toluene solution of PPO (2,5-diphenyloxazole) and POPOP (2,2-p-diphenylenebis (5-phenyl-oxazole) as the scintillator.

The behavior of the wild-type parent strain, CR34, in double-labelling experiments is shown in Fig. 2. The kinetics of protein synthesis and DNA synthesis are parallel at both temperatures with the exception that the rate of protein synthesis increases briefly following the shift to high temperature. This temporary increase in the rate of protein synthesis was always observed with CR34 and its derivatives. The 42.5° C temperature employed is apparently higher than optimal for this strain as, excepting the brief increase in rate of protein synthesis, the kinetics of macromolecule synthesis do not show the expected increase in rate at 42.5° C compared with 30° C but rather are identical at the two temperatures.

Results

Table 1 lists the thirty *dna* mutants we have utilized in this work. Their subdivision into genetic groups is indicated and their properties with respect to DNA synthesis at high temperature are summarized.

The map positions of the various *dna* loci are shown in Fig. 3. We will now summarize observations on mutants in each of the loci.

Table 1. *Mapped mutations conferring a Dna⁻ phenotype*

Mutant[a]	Cotransducible marker	Map position reference	Locus	DNA synthesis at 42°	Kinetics reference
E177, E508	ilv, bgl	A		residual synthesis, initiation-defective	A
CRT46, CRT83	ilv	A, B		residual synthesis, initiation-defective	E, F
D247LT (47)	ilv	A	*dnaA*	residual synthesis, initiation-defective	G
E517	ilv, bgl	A		residual synthesis	A
PC5	ilv	A		?	H

The dnaA Locus

The *dna* locus at approximately 73 minutes on the standard *E. coli* chromosome map has been designated *dnaA* (Hirota, Ryter, and Jacob, 1968). Mutations are considered to be at this locus if they are transferred early both by HfrP13 and by Hfr AB313 and are co-transducible with the *isoleucine-valine* or the *beta-glucosidase* loci (Figs. 1 and 3). All group A mutants tested are rendered Dna⁺ by the introduction of a newly isolated F′ factor, F′197 (Fig. 1).

Data for linkage between certain *dnaA* mutations and *ilv* are shown in Table 2. Co-transduction linkage with *bgl* is higher than with *ilv* but is somewhat difficult to score. Considerable evidence exists that *dnaA* mutants are defective in initiation of rounds of DNA replication (Hirota, Mordoh, and Jacob, 1970; Kohiyama, 1968; Kuempel, 1969; see Gross, 1971 for review). The results presented in Fig. 4 show that following a shift to high temperature, both mutants E177 and E508 synthesize an amount of DNA which is consistent with an ability to complete current rounds of DNA synthesis but an inability to initiate subsequent rounds. Also, incubation of E508 at the restrictive temperature in the absence of thymine for varying lengths of time followed by the addition of ^{3}H-thymine and continued incubation does not reduce the amount of DNA synthesized compared to that observed when thymine is present throughout the incubation (Monk and Gross, 1971).

Increase in Reversion by F-Prime Factors

We have observed that derivatives of strains E177 and E508 that harbor an F′ factor such as F′*lac* show an increased frequency of "reversion" to temperature-resistance compared with the parent F⁻ strain. During the course of this work, Nishimura *et al.* (1971) and Nandadasa and Pritchard (personal communication) reported similar observations with *dnaA* mutants CRT46 and CRT83. They presented evidence that reversion was associated with integration of the episome and termed this phenomenon integrative suppression.

A large set of F's have been shown to effect integrative suppression of *dnaA508* and *dnaA177*. Of the eleven F′ factors that we have tested only F′13 (Fig. 1)

Table 1 (Continued)

Mutant[a]	Cotransducible marker	Map position reference	Locus	DNA synthesis at 42°	Kinetics reference
E107, E125, E368	malB	A		immediate stop, DNA breakdown	A
E279	malB	A		immediate stop	A
E391	malB	A		residual synthesis	A
E173, E194	malB	A		residual synthesis	A
MX74T2ts27 (27)	malB	A	*dnaB*	immediate stop	I
FA21	malB	A		residual synthesis	J
FA22	malB	A		immediate stop	J
BT43	malB	A		immediate stop, DNA breakdown	K, L
HfrH165/59 (59)	malB	A		immediate stop	L
HfrH165/70 (70)	malB	A		immediate stop	K, L
PC1	dra	A		little residual synthesis	H
PC2	dra	A, C	*dnaC*	residual synthesis, initiation-defective	C
PC7	dra	A	*dnaD*	residual synthesis	C
E293	tonA	A		rate becomes arithmetic	A
E511	tonA	A	*dnaE*	immediate reduction in rate	A
E486	tonA	A		immediate stop	A
E101	nalA, purF, aroC	A	*dnaF*	immediate reduction in rate	A
PC3	—	A		residual synthesis	G
BT308	—	A, D	*dnaG*	immediate stop	L
BT399	—	A		immediate stop	L, M

[a] The allele number is generally the same as the strain designation minus the letter prefix. In those cases where the two are not identical, the allele number is given in parentheses following the strain number. In addition to the above, *dna* mutations 6, 8, 42, 43, 59, 313, 454, and 500 have been mapped at the *dnaB* locus (Ricard and Hirota, 1969; Carl, 1970). Reference notation: A = this paper; B = Hirota, Mordoh, and Jacob (1970); C = Carl (1970); D = Marinus and Adelberg (1970); E = Hirota, Ryter, and Jacob (1968); F = Kohiyama (1968); G = Kuempel (1969); H = P. Carl (personal communication); I = Inouye (1969); J = Fangman and Novick (1968); K = Bonhoeffer (1966); L = F. Bonhoeffer (personal communication); M = Wechsler and Gross (unpublished results).

lacked the ability to phenotypically suppress *dnaA508* and *dnaA177*. The presence of F′ *lac* in a strain containing *dnaA508* results in approximately a thirty to fifty-fold increase in reversion rate as determined by a test on solid medium which

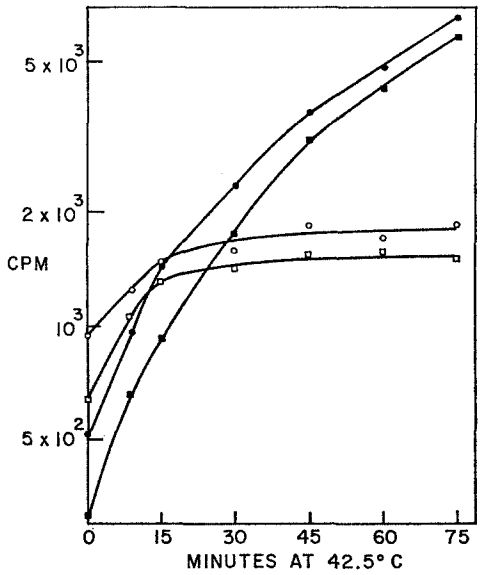

Fig. 4. Kinetics of DNA and protein synthesis of *dnaA* mutants. Cultures were pre-labelled for 60 minutes at 30° C and shifted to 42.5° C as in Fig. 2. Incorporation of label is shown for period at 42.5° C only. Mutant E177: ○ ^{3}H-thymine. ● ^{14}C-leucine; mutant E508: □ ^{3}H-thymine, ■ ^{14}C-leucine

Table 2. *Co-Transduction Linkage of dnaA Alleles*

(A) Donor	(B) Recipient	(C) Selected marker (No. tested)	(D) Unselected marker (No.)	(E) % Linkage [(D)/(C) × 100%]
ilv⁻dna⁺	*ilv⁺dna-508*	*dna⁺* (100)	*ilv⁻* (4)	4
ilv⁺dna-508	*ilv⁻dna⁺*	*ilv⁺* (100)	*dna⁻* (11)	11
ilv⁻dna⁺	*ilv⁺dna-177*	*dna⁺* (100)	*ilv⁻* (13)	13
ilv⁺dna-177	*ilv⁻dna⁺*	*ilv⁺* (100)	*dna⁻* (17)	17
ilv⁻dna⁺	*ilv⁺dna-517*	*dna⁺* (100)	*ilv⁻* (4)	4
ilv⁺dna⁺	*ilv⁻dna-46*	*ilv⁺* (100)	*dna⁺* (7)	7
ilv⁺dna⁺	*ilv⁻dna-46*	*dna⁺* (100)	*ilv⁺* (8)	8

directly measures mutational events. Suprisingly, the presence of the temperature-sensitive F′ factor, F′ts62*lac*, which is itself spontaneously cured at 42° C (Jacob, Brenner and Cuzin, 1963) also results in an increased reversion frequency though at a rate approximately one-tenth of that observed with the wild-type F′*lac*. This result suggests that integrative suppression may involve a more complex mechanism than was originally believed.

We did not note integrative suppression in the initial F′ mapping experiments with *dnaA517*, and it may, therefore, be different from the other *dnaA* mutations.

The dnaB Locus

The largest class of *E. coli dna* mutants carry mutations which are co-transducible with the *malB* locus. This class has been designated *dnaB* (Hirota, Ryter, and Jacob, 1968). The eleven mutants which we have mapped at this locus are rendered Dna$^+$ by F'118 (Fig. 1). Siccardi *et al.* (1971) and Carl (1970) have mapped several *dnaB* mutations very close to *uvrA*.

Co-transduction linkages of a number of *dnaB* alleles with *malB* are listed in Table 3.

In studying recombination between various mutants by P1 transduction, we noted that infection of certain *dnaB* mutants with P1 grown on a strain carrying the same *dnaB* mutation resulted in a significant number of temperature-resistant "transductants". This phenomenon is probably explained by the recent observation that certain P1 mutants can suppress all *dnaB* mutants tested (A. Jaffe-Brachet, D. Schwartz, and M. Yarmolinsky, personal communication). This P1 suppression may account for some of the variation in co-transduction frequencies in the data of Table 3.

Table 3. *Co-Transduction Linkage of dnaB Alleles*

(A) Donor	(B) Recipient	(C) Selected marker (No. tested)	(D) Unselected marker (No.)	(E) % Linkage [(D)/(C) × 100%]
malB101 dna$^+$	*malB*$^+$dna-107	*dna*$^+$ (99)	*malB*$^-$ (6)	6
malB101 dna$^+$	*malB*$^+$dna-125	*dna*$^+$ (99)	*malB*$^-$ (79)	79
malB$^-$ *dna*$^+$	*malB*$^+$*dna-125*	*dna*$^+$ (98)	*malB*$^-$ (73)	73
malB101 dna$^+$	*malB*$^+$*dna-173*	*dna*$^+$ (100)	*malB*$^-$ (7)	7
malB$^-$ *dna*$^+$	*malB*$^+$*dna-173*	*dna*$^+$ (94)	*malB*$^-$ (11)	12
malB$^+$ *dna-173*	*malB*$^-$*dna*$^+$	*malB*$^+$ (100)	*dna*$^-$ (33)	33
malB101 dna$^+$	*malB*$^+$*dna-194*	*dna*$^+$ (90)	*malB*$^-$(5)	5
malB$^-$ *dna*$^+$	*malB*$^+$*dna-279*	*dna*$^+$ (100)	*malB*$^-$ (5)	5
malB101 dna$^+$	*malB*$^+$*dna-368*	*dna*$^+$ (76)	*malB*$^-$ (6)	8
malB$^-$ *dna*$^+$	*malB*$^+$*dna-391*	*dna*$^+$ (100)	*malB*$^-$ (8)	8
malB$^+$ *dna-391*	*malB*$^-$*dna*$^+$	*malB*$^+$ (100)	*dna*$^-$ (35)	35
malB101 dna$^+$	*malB*$^+$*dna-21*	*dna*$^+$ (100)	*malB*$^-$ (11)	11
malB$^-$ *dna*$^+$	*malB*$^+$*dna-21*	*dna*$^+$ (96)	*malB*$^-$ (25)	25
malB101 dna$^+$	*malB*$^+$*dna-22*	*dna*$^+$ (92)	*malB*$^-$ (45)	49
malB$^-$ *dna*$^+$	*malB*$^+$*dna-22*	*dna*$^+$ (100)	*malB*$^-$ (8)	8

The *malB101* allele is a *malB* deletion (strain supplied by M. Schwartz). The allele denoted as *malB*$^-$ is due to the insertion of F to form Hfr P10. We have also co-transduced *dna* mutations 27, 43, 59, and 70 with *malB* (data not shown).

Many *dnaB* mutants show virtually immediate cessation of DNA synthesis at 42° C (Figs. 5A and B). Others exhibit considerable residual synthesis (Fig. 5C). The DNA breakdown which immediately or closely follows a shift to high temperature in three E series *dnaB* mutants (Fig. 5A) has been reported to involve the *recB, recC* nuclease (Buttin and Wright, 1968). In our hands, the time of appearance and degree of breakdown following the temperature shift has not been

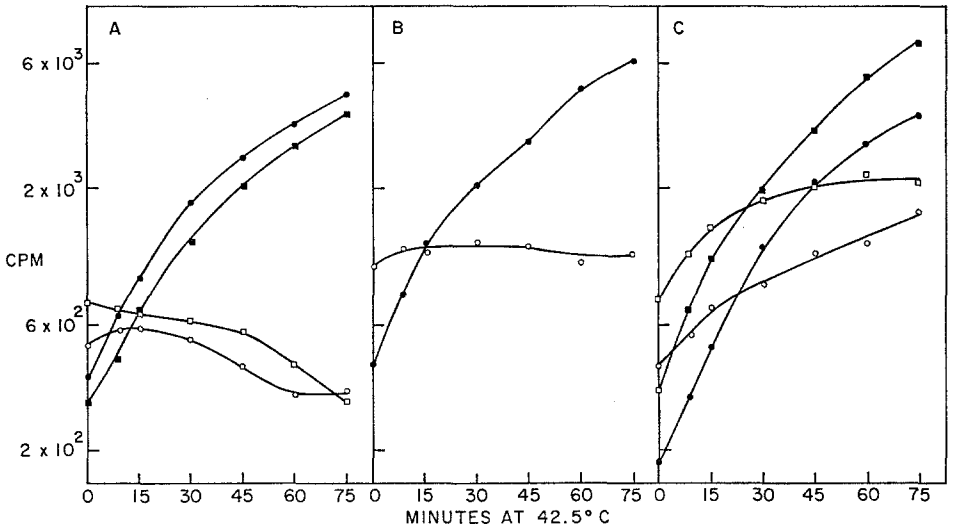

Fig. 5A–C. Kinetics of DNA and protein synthesis of *dnaB* mutants. Procedure as in Fig. 4. A Mutant E107: ○ ^{3}H-thymine, ● ^{14}C-leucine; mutant E125: □ ^{3}H-thymine, ■ ^{14}C-leucine. B Mutant E279: ○ ^{3}H-thymine, ● ^{14}C-leucine. C Mutant E173: ○ ^{3}H-thymine, ● ^{14}C-leucine; mutant E391: □ ^{3}H-thymine, ■ ^{14}C-leucine

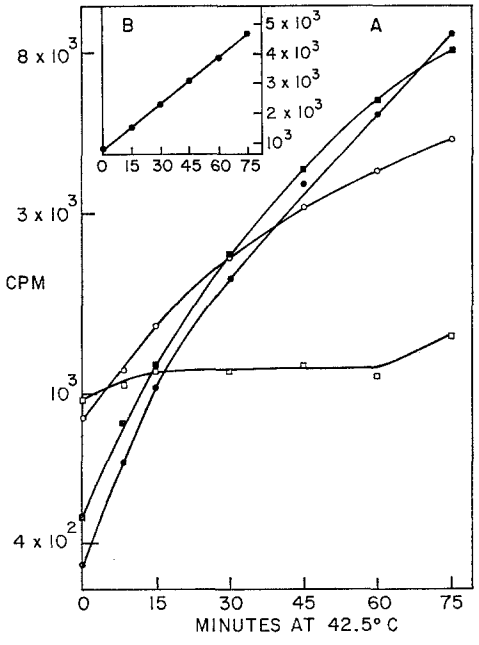

Fig. 6A and B. Kinetics of DNA and protein synthesis of *dnaE* mutants. Procedure as in Fig. 4. A Mutant E293: ○ ^{3}H-thymine, ● ^{14}C-leucine; mutant E486: □ ^{3}H-thymine, ■ ^{14}C-leucine. B Incorporation of ^{3}H-thymine at 42.5° C of mutant E293 plotted arithmetically

reproducible from experiment to experiment. One mutant, E279, in which DNA synthesis appears to halt immediately at high temperature, shows no detectable breakdown of DNA (Fig. 5B).

We are currently performing complementation experiments to determine whether the *dnaB* locus contains one or more cistrons.

The dnaC and dnaD Loci

The *dnaC* and *dnaD* loci are defined by mutations *dna-1, dna-2* and *dna-7* all of which are co-transducible with *dra*. One of these, *dna-2* has been reported to be initiation-defective; and *dna-1* is subject to integrative suppression (J. Zeuthen, personal communication). Linkages to *dra* for *dna-1* and *dna-2* are 20–30% whereas *dna-7* is approximately 50% co-transducible with *dra*. All three of these mutations are covered by F'101 and F'104 (Fig. 1).

Besides the linkage differences and the fact that both PC1 (*dna-1*) and PC2 (*dna-2*) have some characteristics of initiation-defective mutants, there is an important physiological difference among these mutants; PC7 (*dna-7*) does not support the growth of λ bacteriophage at 40° C but PC2 (*dna-2*) does (Carl, 1970). PC1 (*dna-1*) has not been fully tested for λ growth. These mutations have, therefore, been assigned to two groups. The *dnaC* locus contains *dna-1* and *dna-2*, and *dnaD* contains *dna-7*. Complementation studies with these mutations are currently in progress.

The dnaE Locus

Three mutations which are co-transducible with *tonA* define the *dnaE* locus. The 54–69% linkage observed places the mutations close to *tonA*. High linkage to *tonA* for a general mutator gene, *ast*, has also been reported (Zamenhof, 1966); no mutator activity, however, has been observed in the *dnaE* mutants at 30° C or at temperatures between 30 and 42° C (R. Hall, personal communication). The *dnaE* locus is covered by F'104 but not by F'101 (Fig. 1).

All three *dnaE* mutants are immediately affected by transfer from 30 to 42.5° C. Mutant E486 halts synthesis immediately at high temperature (Fig. 6A). Mutant E293 changes abruptly from exponential to arithmetic synthesis with the shift from 30 to 42.5° C (Figs. 6A and B). It is not clear whether the reproducible arithmetic linearity of the synthesis is a coincidence or whether it reflects some significant property of the mutation.

The dnaF Locus

Mutation *dna-101* is the only lesion so far obtained in the *dnaF* locus. Dna^+ is transferred early by Hfr KL16 (Fig. 1) and *dnaF101* has been found to be loosely linked in P1 transduction to *aroC* and *purF* (Table 4). No co-transduction could be obtained with *dsdA* (Fig. 3). Mutation *dnaF101* has recently been found to be approximately 80% co-transducible with *nalA* (M. Monk, personal communication).

Strain E101 displays an immediate reduction in the rate of DNA synthesis in response to the temperature shift (Fig. 7).

The dnaG Locus

The *dna* lesions in strains PC3, BT308, and BT399 do not map at any of the above loci. Dna^+ recombinants occur with high frequency in matings with Hfr

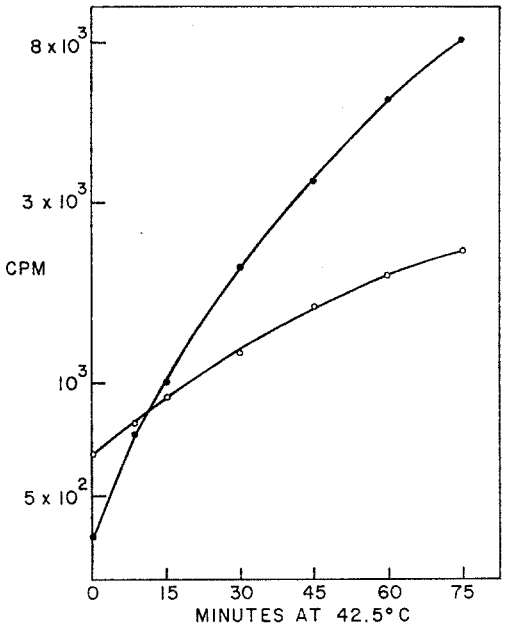

Fig. 7. Kinetics of DNA and protein synthesis of *dnaF101* mutant. Procedure as in Fig. 4. ○ ^{3}H-thymine, ● ^{14}C-leucine

Table 4. *Co-Transduction Linkage of dnaF101*

A) Donor	(B) Recipient	(C) Selected marker (No. tested)	(D) Unselected marker (No.)	(E) % Linkage [(D)/(C) × 100%]
aroC^{+}*dnaF101*	*aroC*$^{-}$*dnaF*$^{+}$	*aroC*$^{+}$ (500)	*dnaF101* (1)	0.2
purF^{+}*dnaF101*	*purF*$^{-}$*dnaF*$^{+}$	*purF*$^{+}$ (500)	*dnaF101* (8)	1.6
dsdA^{+}*dnaF101*	*dsdA*$^{-}$*dnaF*$^{+}$	*dsdA*$^{+}$ (138)	*dnaF101* (0)	<0.7
aroC^{+}*purF*$^{-}$	*aroC*$^{-}$*purF*$^{+}$	*aroC*$^{+}$ (100)	*purF*$^{-}$ (28)	28
purF^{+}*aroC*$^{-}$	*purF*$^{-}$*aroC*$^{+}$	*purF*$^{+}$ (97)	*aroC*$^{-}$ (24)	24

AB313, with Hfr AB312 and with F'102, but not with Hfr P13 (Fig. 1). Attempts to show co-transduction of *dna-3* with the *argG*, *metC*, *strA* or *spc* loci have been unsuccessful. Mutations *dna-3*, *dna-308*, and *dna-399* are tentatively placed together in the *dnaG* locus.

Discussion

Genetics

Seven widely scattered loci have been defined as playing a role in cellular DNA replication in *E. coli*. Nine genetic loci resulting in Dna^{-} phenotypes have previously been reported in *B. subtilis* (Karamata and Gross, 1970). The distribution of mutations within the seven *E. coli* loci is clearly not random as the *dnaD*

and *dnaF* loci are defined by one mutation each while *dnaA* and *dnaB* are defined by six and twenty-one respectively. Since there are groups with very few representatives, it is likely that there are *dna* loci for which no mutants have been isolated. The number of cistrons within the defined groups has not yet been determined.

Correlation of Gene and Function

None of the E series mutants are defective in DNA polymerase I (Wechsler, unpublished), and none of the *dna* loci maps near *polA*, the structural gene for DNA polymerase I (DeLucia and Cairns, 1969; Gross and Gross, 1969; Kelley and Whitfield, 1971). Similarly, examination of all E series mutants for altered polynucleotide ligase activity has given negative results (M. Gellert, personal communication). The E series mutants have been screened by a rapid technique designed to detect defective ribonucleotide reductase with negative results (O. Karlström, personal communication). Mutants at one of the *dna* loci in *B. subtilis* have been found to be defective for ribonucleotide reductase (D. Karamata and G. Bazill, personal communication). Mutants in *dnaA* and *dnaB* have been shown to be temperature-sensitive for DNA synthesis after toluene treatment in a system developed by Moses and Richardson (Moses and Richardson, 1970a; Mordoh, Hirota, and Jacob, 1970; Kohiyama and Kolber, 1970). Mutants in *dnaE* and *dnaG* have also been shown to be defective in this system (Gross and Peacey, unpublished). It is probable, therefore, that these loci are directly involved in DNA synthesis rather than in precursor synthesis. No alteration in DNA polymerase II was noted in two *dnaB* mutants examined (Moses and Richardson, 1970b).

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