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Escherichia coli Mutants with Temperature-Sensitive Synthesis of DNA*

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Summary. Seven mutants of *E. coli* with temperature-sensitive synthesis of DNA have been isolated. Synthesis of RNA, protein and DNA precursors does not appear to be directly affected. The mutants can be divided into at least two groups on the basis of their pattern of DNA synthesis, their ability to support phage growth at 41° and their genetic mapping.

Mutants of the first group are heterogeneous in their pattern of DNA synthesis at 40°. Some mutants cease DNA synthesis abruptly upon transfer to 40° and any residual DNA synthesis is barely detectable. In others there is substantial residual synthesis at 40°. All these Group 1 mutants are alike, however, in that they support the growth of phage T4 but not Lambda at 41°. Two mutants with barely detectable residual DNA synthesis carry DNA mutations which have been mapped by P1 transduction and show about 72% linkage to the *malB* locus. It has not yet proved possible to map accurately the mutants showing substantial residual synthesis, and the possibility that these mutations are in a different gene(s) has not been excluded.

A single mutant has been placed in a second group. Like some Group 1 mutants it synthesizes substantial amounts of DNA at 40° before synthesis stops. However, unlike them it supports the growth of T4 *and* Lambda at 41°. The DNA mutation maps near the *leu* locus. Certain properties of this mutant are consistent with the idea that initiation of DNA synthesis is temperature-sensitive in this strain.

The analysis of bacteriophage mutants in which the synthesis of DNA is temperature-sensitive (TS)[1] has led to significant progress in our understanding of the mechanisms of DNA biosynthesis and its control. In particular, Waard, Paul and Lehman (1965) have shown that in T4, gene 43 is the structural gene for DNA polymerase, and Okazaki *et al.* (1967) have identified gene 30 as the structural gene of DNA ligase. In both cases the loss of biosynthetic ability *in vivo* has been confirmed *in vitro*. In bacteria in contrast, although mutations of both the DNA polymerase (De Lucia and Cairns, 1969) and DNA ligase (Pauling and Hamm, 1968) have been identified, neither appears to affect the rate of DNA synthesis *in vivo*. And although several papers have described the isolation and properties of *E. coli* mutants with TS synthesis of DNA (Bonhoeffer and Schaller, 1965), Kohiyama, Cousin, Ryter and Jacob, 1966), Fangman and Novick, 1968), Hirota, Ryter and Jacob, 1968), Kuempel, 1969), Inouye, 1969), little has been

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1 Abbreviations: BU, 5-bromouracil; βG_z, β-galactosidase; CAP; Chloramphenicol; IPTG, isopropyl-β-D-thiogalactoside; TR, temperature resistant; TS, temperature sensitive; UV, ultraviolet light (see also Table 1).

learned of the biochemical basis of these mutations, nor has much data on the genetic mapping of the mutations been presented.

This paper describes the isolation of some additional *E. coli* mutants with TS synthesis of DNA, and it reports some preliminary studies of their genetics and physiology with the ultimate goal of establishing the nature of the biochemical basis of the mutations.

Material and Methods

a) *Bacterial Strains.* The principal strains used in this study are all derivatives of *E. coli* K12. Their origin and genetic character are listed in Table 1.

Table 1[a]. *Description of Strains*

Strain	Source	Sex	Description
DG75[b]	B. Wolf	F⁻	*thy⁻leu⁻str*ʳ
AB1932[c]	D. Mount	F⁻	*metA28, arg (HBCE)⁻ lac⁻gal⁻xyl⁻tsx*
KG20	H. Bowman	F⁺	*ampA1, purA⁻, argH⁻, pro⁻ his⁻str*ʳ
JC12	A. J. Clark	Hfr	*met*B⁻*purC⁻lac⁻ gal⁻ xyl⁻ mtl⁻ ton tsx str*ˢ
P7201	B. Wolf	Hfr	*thr⁻ leu⁻ met⁻ thy⁻ str*ʳ
AB673 (J4)	P. Howard-Flanders	Hfr	*thr⁻ leu⁻ malB⁻ str*ˢ
AB257 (Cavalli)	A. J. Clark	Hfr	*met⁻ str*ˢ
AB259 (Hayes)	A. J. Clark	Hfr	*thi⁻ str*ˢ
KMBL82MPE1	M. Schwartz	F⁻	*malB⁻ uvrA⁻*

[a] The points of origin and direction of transfer of the Hfr strains used in this study are indicated in Fig. 1. Gene symbols are those used by Taylor and Trotter (1967) except for *dna* which stands for the genes involved in DNA synthesis, chromosome replication and the regulation of these.

[b] This is the parent strain of the present study. PC1, PC2... are mutants derived from this strain and carry allele numbers 301, 302 etc.

[c] The notation *arg (HBCE)* signifies that the arg mutation is one of these genes.

b) *Bacteriophage.* T4⁺ (obtained from G. Edlin) was grown and assayed on *E. coli* B/r/1 using standard phage methods. Strain AC169 (obtained from A. J. Clark) was UV induced to obtain Lambda⁺. The Lambda phage methods are described in Brooks and Clark (1967). PlvirA (hereafter referred to as P1) was obtained from B. Wolf. The procedures for assay and transduction were essentially those of Wolf, Newman, and Glaser (1968). Phage lysates were prepared on LCTG plates at 37°, or 30° if the donor bacteria were temperature-sensitive. In the latter case modified procedure of K. Eriksson-Grennberg (1968) was adopted.

c) *Media.* The minimal medium was that of Clark and Maaløe (1967). It was supplemented with thymine at 20 μg/ml, DL-leucine at 50 μg/ml and casamino acids at 2 mg/ml for growth of DG75 and its derivatives, or with 50 μg/ml DL-amino acids or 25 μg/ml L-amino acids as required for the growth of other strains. Thiamine at 10 μg/ml, adenine at 10 μg/ml and uracil at 40 μg/ml were added as required. Glucose or other carbon sources were used at 2 mg/ml.

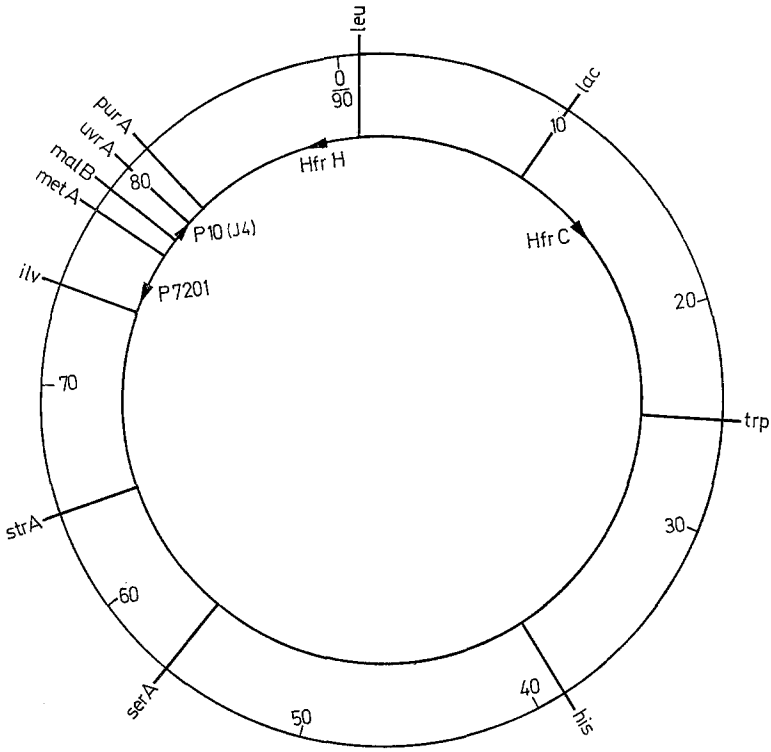

Fig. 1. The genetic map of *E. coli* K12 (adapted from Taylor and Trotter, 1967) showing the origin and direction of transfer of some of the Hfr strains used and the location of several markers mentioned in the text

Minimal agar plates were formulated from medium M9 (Wolf, Newman and Glaser, 1968) supplemented as above but without casamino acids.

LC broth (Luria, Adams and Ting, 1960) was used for growth of donor and recipient strains in all genetic experiments and elsewhere as noted in the text. It was supplemented with 20 μg/ml thymine for the growth of thymineless strains.

LCTG agar plates (Wolf, Newman and Glaser, 1968) were used for the preparation of P1 lysates.

Nutrient agar plates (23 g Difco nutrient agar/liter) were used for scoring temperature sensitivity during the initial isolation procedure and elsewhere as noted in the text. Nutrient yeast agar plates (23 g Difco nutrient agar + 5 g Difco yeast extract/liter) were used for scoring UV sensitivity.

d) *Mutagenesis.* Mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine was carried out according to the procedure of Adelberg, Mandel and Chen (1965).

e) *Isolation of Mutants.* The mutants were isolated by a modification of the technique described by Bonhoeffer and Schaller (1965). Their procedure is based on the fact that bacteria which have incorporated 5-bromouracil (BU) into their DNA are extraordinarily sensitive to inactivation by longwave ultraviolet (UV) light. Thus by shifting a mutagenized culture from 30° to 40° and simultaneously substituting BU for thymine one can obtain a culture in which wild type cells have incorporated BU while cells with TS synthesis of DNA have not. Subsequent irradiation with light at 313 nm preferentially inactivates normal cells and thereby enriches for the desired mutants which can be subsequently isolated by plating the cultures at 30° and picking TS colonies after replica plating to 40°.

As originally described, however, this technique appeared to suffer from several defects. Firstly, cells in which all energy dependent macromolecular synthesis is TS will fail to incorporate BU and hence be relatively UV resistant. Secondly, cells in which the cessation of DNA synthesis is only gradual at 40° will incorporate substantial amounts of BU and become UV sensitive. In an effort to circumvent both these difficulties the procedure was modified so that only after some time at 40° was the BU added and at the same time the cells were induced for β-galactosidase. After some further time at 40° the cells were irradiated and then grown on a medium with phenyl-β-D-galactoside as the principal carbon source. This compound is a substrate for β-galactosidase, but not an inducer (Monod, 1956). Hence cells which have not been preinduced for β-galactosidase should grow only slowly on a medium with phenyl-β-D-galactoside as the carbon source compared to preinduced cells. Under these conditions cells in which protein synthesis or all macromolecular synthesis is TS, and which were therefore not induced for β-galactosidase, should be discriminated against by subsequent growth on phenyl-β-D-galactoside. At the same time by adding BU only after the cells have been at 40° for some time, one helps to prevent incorporation of BU by cells with only a gradual cessation of DNA synthesis. The procedure adopted was, therefore, the following:

Mutagenized cultures were grown overnight for expression in minimal casamino acids medium at 30°. They were then diluted 1:100 into similar medium with glucose replaced by glycerol and grown until turbidity measurements indicated growth was exponential. They were then shifted to 40°. After one hour the cells were collected by filtration, washed in buffer and resuspended in a similar medium containing 20 μg/ml BU instead of thymine and supplemented with 10^{-3} M isopropyl-β-D-thiogalactoside. After 90 more minutes the cells were collected, washed and irradiated in buffer as described by Bonhoeffer and Schaller (1965). They were then diluted 1:9 into minimal casamino acids medium with phenyl-β-D-galactoside as the principal carbon source. After overnight growth at 30°, the culture was plated on nutrient agar at 30°. The colonies that appeared after three days were replicated to 30° and 40° to pick temperature-sensitive colonies. (More recent experiments have indicated that even uninduced cells are capable of substantial growth on phenyl-β-D-galactoside as principal carbon source when casamino acids are included in the medium. Hence the selection might better be carried out on phenyl-β-D-galactoside in the absence of casamino acids.)

f) *Determination of Cell Number.* Cell concentration was routinely determined by measurement of turbidity in a Klett photometer, or cell numbers by measurement with a Coulter counter, (modified with a Tennelec preamplifier, a Tennelec linear amplifier and a Nuclear Data pulse height analyzer). In our standard minimal-casamino acids medium the doubling time of DG75 was approximately 85 minutes at 30° and 45 minutes at 40°C.

g) *Radioactivity Measurements.* The incorporation of thymine-methyl-^{14}C (3mC/mmole) was usually used to measure DNA synthesis. In certain experiments thymine-methyl-^{3}H (6.7 C/mmole) was used. Incorporation of uracil-^{3}H (5.6 C/mmole) and proline-^{3}H (5 C/mmole) was used to measure RNA and protein synthesis respectively. All the above radiochemicals were obtained from the New England Nuclear Corp. and were usually added at a 1:100 dilution to growth media. The samples were processed by the batch method described in Byfield and Scherbaum (1966) except that following trichloracetic acid precipitation, the filters were washed two times with boiling water (Lark, Repko, and Hoffman, 1963). This method gave lower backgrounds than the use of the trichloracetic acid washing alone. The samples were placed in 5 ml of a toluene-based scintillation fluid prepared from Packard 25X concentrated scintillation mix, and counted in a Packard scintillation counter.

h) *Chromatography of Triphosphates.* The procedure of Randerath and Randerath (1967) was used for the chromatographic separation of the nucleoside triphosphates. The materials were the gift of G. Edlin.

i) *Other Chemicals.* Phenyl-β-D-galactoside was purchased from the Sigma Chemical Co. Isopropyl-β-D-thiogalactopyranoside and O-nitrophenyl-β-D-galactopyranoside were obtained from Mann Biochemical. Chloramphenicol was the gift of Parke-Davis and Co.

j) *β-galactosidase Assay.* The assay procedure for β-galactosidase was that of Pato and Glaser (1968).

k) *Genetic Techniques.* The standard mating techniques of Adelberg and Burns (1960) were used except that matings were usually carried out at 30° with parents grown at 30°. In a few cases the matings were done at 37° with parents grown at 30°C. The ratio of donor

to recipient cells was generally one to ten. Interrupted mating experiments were performed by mixing an aliquot of the mating mixture with an equal volume of 0.85% saline and treating with a vortex mixer for one minute before plating an appropriate dilution. Recombinants were scored after three days of incubation at 30°. Temperature-resistant recombinants were selected by first incubating zygotes at 30° for three hours and then incubating them at 40° for two days.

l) *UV Irradiation.* The source of UV was a 15 watt G.E. ultraviolet lamp producing a flux of approximately 35 ergs/mm² per second at the point of irradiation. Colonies to be tested for UV sensitivity were replicated onto a master plate which was in turn replicated onto two new plates, one of which was exposed to about 1500 ergs/mm². The plates were then incubated at 30° and scored after about 24 hours,

Results

Synthesis of DNA. Following the described procedure seven mutants were isolated in which the synthesis of DNA as measured by thymine incorporation is inhibited at 40°. The pattern of DNA synthesis in several of the mutants is illustrated for mutant PC-6 in Fig. 2. There is a barely detectable amount of synthesis which proceeds at a rate only about 2% of that of the parent strain DG75.

In mutant PC-2, however, there is a substantial increase in the amount of DNA synthesis at 40°, amounting to a net increase of about 33% of the DNA content at the time of the shift. Synthesis then apparently ceases altogether (Fig. 3A).

A similar behavior is seen when the cells are transferred to either 39° or 41° (Fig. 3B). Finally Fig. 3A also shows the amount of DNA synthesized at 30° when a high concentration of chloramphenicol is added to the culture to inhibit protein synthesis. As Lark (1966) has shown, starvation for a required amino acid or inhibition of protein synthesis by chloramphenicol allows DNA synthesis to continue along chromosomes already in the process of replication, but prevents further initiation of DNA synthesis. If the mutation in PC-2 similarly prevents the initiation of DNA synthesis, one would expect to find a similar increase in the DNA content of the culture after a shift to 40° and at 30° in the presence of CAP. The approximate agreement in the amount of residual DNA synthesis under these conditions (Fig. 3A) is therefore consistent with the idea that the mutation in PC-2 prevents the initation of DNA synthesis at 40°.

The final pattern of DNA synthesis observed is shown for mutant PC-7 in Fig. 4A.

Here it is evident that there is substantial DNA synthesis occurring at 40°, but synthesis fails to reach a clear plateau and appears to be substantially less than the amount of DNA synthesized in the presence of chloramphenicol. Furthermore, as shown in Fig. 4B (and in contrast to the case for PC-2) the amount of residual synthesis appears to be highly dependent on the temperature to which the cells are transferred.

Mutants PC-2 and PC-7 also differ in their patterns of DNA synthesis after a prior period of amino acid starvation. The pattern for PC-2 is illustrated in Fig. 5. Cells were grown at 30° and then starved for leucine, a required amino acid. DNA synthesis continued for a time, but synthesis levels off as expected after about two hours. One half of the culture was then shifted to 40° and left for a few minutes to allow for temperature equilibration. Then leucine was restored to

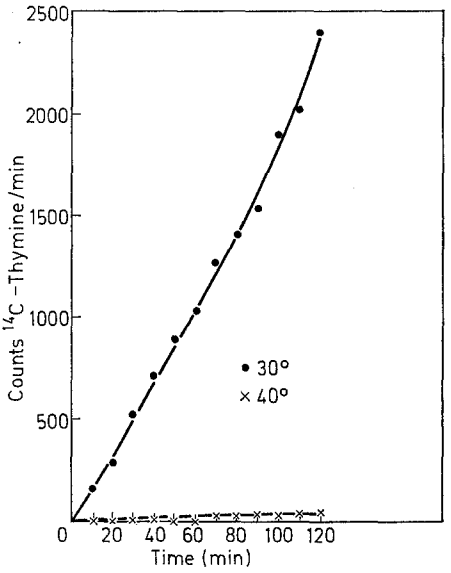

Fig. 2. DNA synthesis in PC-6. ^{14}C-thymine was added to an exponential culture of PC-6 at time 0 and one half of the culture was immediately shifted to 40°. The remainder of the culture was kept at 30°. The incorporation of ^{14}C-thymine into acid-precipitable counts was measured as described in Materials and Methods

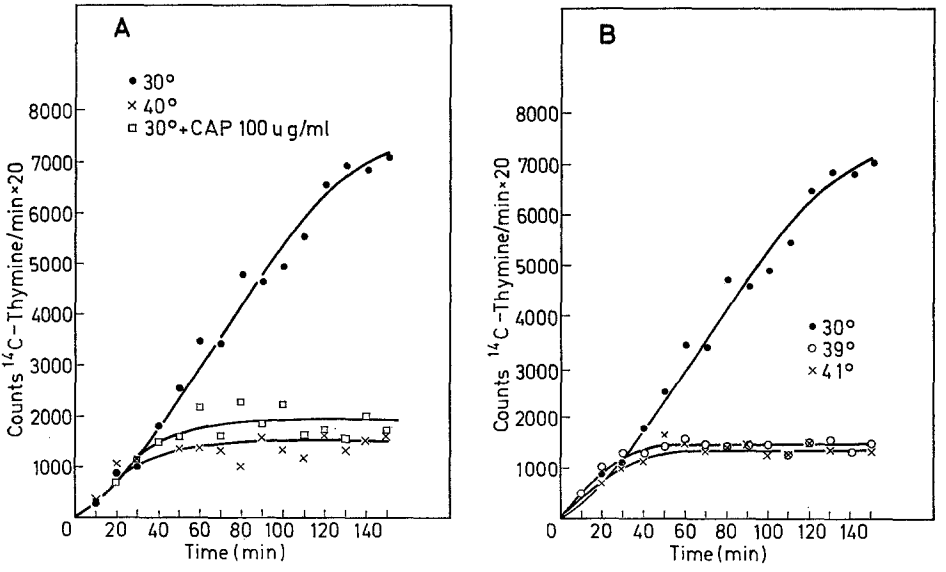

Fig. 3A. DNA synthesis in PC-2. ^{14}C-thymine was added to an exponential culture of PC-2 at time 0 and the culture was immediately divided in three. One third of the culture was immediately shifted to 40°. CAP (100 μg/ml) was added to another third kept at 30°, and the remaining third was left at 30° as a control. B. ^{14}C-thymine was added to an exponential culture of PC-2 at time 0 and the culture was immediately divided in three. One third was shifted to 41°, one third to 39° and the remaining third kept at 30° as a control

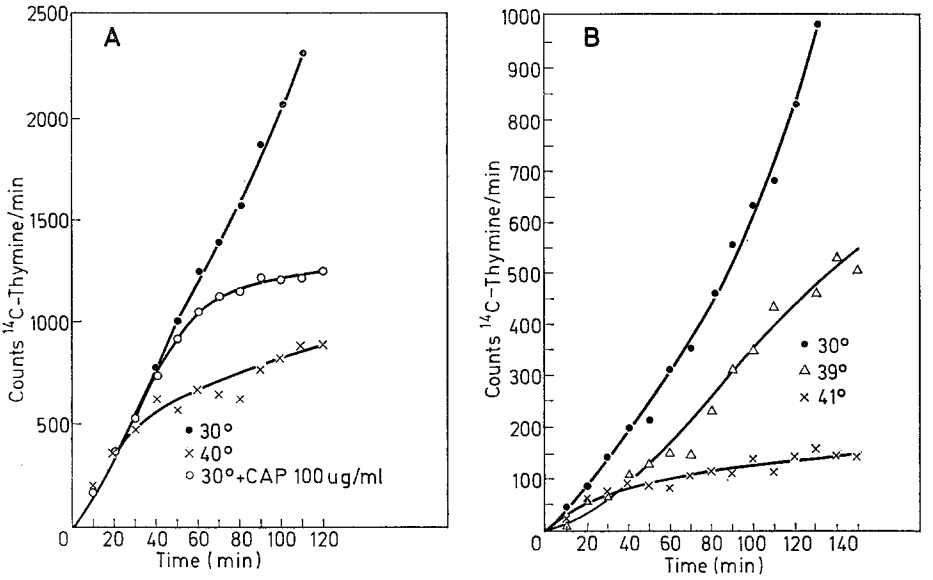

Fig. 4A and B. DNA synthesis in PC-7. The procedure was identical to that shown in Fig. 3 except that the mutant was PC-7

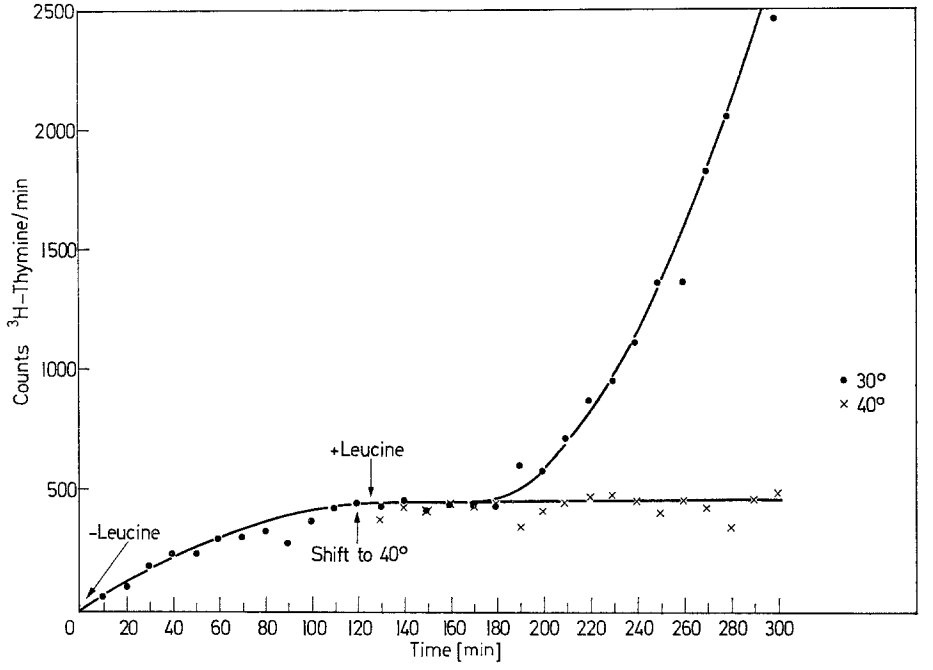

Fig. 5. A culture of PC-2 growing at 30° in our standard minimal medium but with casamino acids replaced by a mixture of 20 amino acids including leucine was collected by Millipore filtration, washed, and resuspended at time 0 in a similar medium containing ^{3}H-thymine and lacking leucine. After 120 minutes at 30° one half of the culture was shifted to 40°. After 5 additional minutes leucine was restored to both cultures. DNA synthesis was measured as described in Materials and Methods

Table 2. *Synthesis of β-galactosidase at 40° in mutant and parent strains*

Strain	Units βG_z per ml per 100 Klett units
PC-2	0.96
PC-6	0.76
PC-7	0.92
DG-75	1.97

An exponential culture grown in a glycerol minimal medium at 30°, was transferred to 40° and after five minutes induced with 10^{-3} M IPTG for 1 hour. Synthesis of βG_z was assayed as described in Materials and Methods.

both 30° and 40° cultures. At 30° DNA synthesis resumes after a lag period, but at 40° synthesis never resumes. When an identical experiment is performed using PC-7, however, synthesis resumes at both 30° *and* 40° (data not shown). This result is to be expected if the mutation in PC-2 (but not in PC-7) blocks the synthesis or functioning of an initiator protein.

The pattern of DNA synthesis at 40° in the remaining mutants was intermediate between the pattern seen in PC-6 and PC-7. That is, the amount of residual synthesis was usually more than the very low rate seen in PC-6, but less than the 20-30% increase seen in PC-7. (This increase can be calculated from the 85 minute doubling time of PC-7 at 30° and the uptake of ^{14}C-thymine into DNA shown in Fig. 4A).

Synthesis of RNA and Protein. The synthesis of RNA as measured by uracil incorporation and protein as measured by proline incorporation is normal for at least two hours at 40° (data not shown). Further evidence that RNA and protein synthesis remain relatively unaffected in these mutants at 40° is given by the results in Table 2.

As can be seen *de novo* synthesis of a functional protein can occur in the mutants although the amounts of enzyme obtained after one hour of induction at the restrictive temperature are less than that produced by the parent strain. The lower level of enzyme is understandable if one hypothesizes a loss in synthetic abilities at the restrictive temperature analogous to that which occurs in cultures undergoing thymineless death. Although cessation of DNA synthesis is the first event observed under conditions leading to thymineless death, the synthesis of enzymes is eventually affected possibly due to the loss of ability of cell's DNA to to serve as an efficient template for the production of m-RNA (McFall and Magasanik, 1965). Additionally, due to the lower amount of DNA synthesis which occurs in the present mutants at 40° in comparison to the parent DG75, the latter probably has more functioning *lacZ* genes at the end of one hour at 40°, leading to essentially a gene dosage explanation for the lower amounts of enzyme produced.

Deoxyribonuleotide Pools. The cessation of DNA synthesis in the mutants could conceivably be due to a temperature-sensitive deoxyribonucleotide kinase or some other enzyme involved in converting the ribonucleotides to deoxyribonucleotide triphosphates. That this is not the case was demonstrated by comparing the relative amounts of labelled material in the deoxyribonucleotide pools in the mutant and parent strains one hour after a shift to 40°. The results are expressed in Table 3.

Table 3. *Distribution of ^{32}P-labelled nucleotides in percent of total cold 10% TCA soluble nucleotides recovered*

Strain	ATP	CTP	GTP	UTP	dATP	dCTP	dGTP	dTTP
PC-2	29.0	15.2	18.7	10.1	5.5	3.8	2.2	4.9
PC-6	34.6	16.1	15.3	16.7	9.3	2.2	2.8	3.2
PC-7	28.6	14.6	9.6	27.5	10.7	3.1	1.9	3.8
DG-75	48.9	10.2	16.7	15.7	1.2	3.0	1.4	2.8

Cells growing at 30° in the low phosphate medium of Edlin and Maaløe (1965) were transferred to 40° and after five minutes $^{32}PO_4$ was added. After 1 hour the cells were collected and the distribution of ^{32}P-labelled nucleotides in the cold 10% TCA soluble fraction was measured as noted in Materials and Methods.

Except for the anomalously high level of dATP in the mutants the pools are qualitatively comparable in the mutants and DG75. This increase in the dATP is also seen in cells undergoing thymineless death (Munch-Peterson and Neuhard, 1964) and was also noted by Fangman and Novick (1968) in their mutants with TS synthesis of DNA.

Phage Growth. In order to characterize the mutants further, they were tested for the ability to support the growth of bacteriophage T4 and Lambda at 30° and 41°. The results are shown in Table 4.

Table 4. *The Effect of the TS mutations on the yield of bacteriophage*

Strain	T4 burst size		Lambda burst size	
	30°	41°	30°	41°
DG-75	27	40	168	26
PC-2	14	22	48	8
PC-6	37	55	38	6×10^{-3}
PC-7	14	20	51	7×10^{-3}

Cultures grown in broth at 30° were collected by centrifugation, resuspended in absorption medium (broth + 0.002 M KCN for T4 or 0.01 M $MgSO_4$ for Lambda) and infected with phage at a multiplicity of about 3. The cultures were incubated at 30° for 30 minutes, collected and washed by centrifugation and resuspended in absorption medium. They were then diluted 10^4 into broth prewarmed to 30° or 41°. After 150 minutes $CHCl_3$ was added and the bursts were assayed by the pour plate method using indicator bacteria described in Materials and Methods. Plates were incubated at 37°.

As can be seen, all the mutants support the growth of T4 at both 30° and 41°. In addition all support the growth of Lambda at 30°. However, only PC-2 gives rise to normal bursts of Lambda at 41°. This important result suggests that mutant PC-2 should be clearly distinguished from PC-7 in spite of the fact that both show some residual DNA synthesis. There may, of course, be a mutation other than the *dna* mutation which is preventing Lambda multiplication in PC-7. Temperature-resistant revertants of PC-6 have been isolated, however, and these have completely

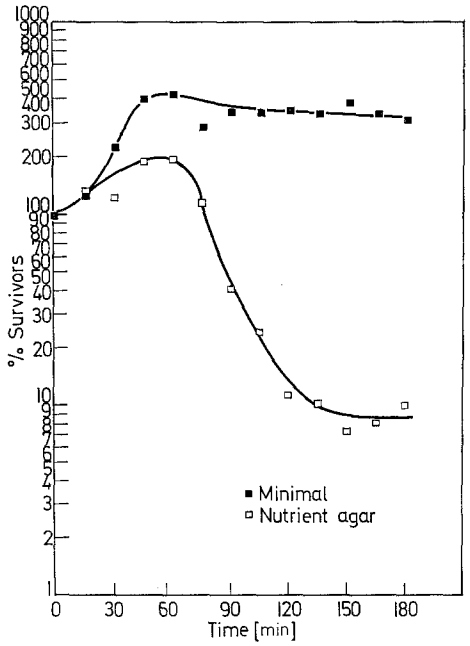

Fig. 6. An exponential culture of PC-6 growing in LC broth at 30° was shifted at time 0 to 40°. Samples were taken at the times indicated, diluted in saline and plated on either minimal or nutrient agar plates

regained ability to support Lambda growth at 41°. This argues that the cessation of DNA synthesis at 41° and the inability to support the growth of Lambda at this temperature are due to a single mutation in PC-6 (M. Golomb, personal communication.) PC-7 remains to be tested. On the basis of their inability or their ability to support Lambda phage multiplication at the restrictive temperature we distinguish Group 1 and Group 2 mutants respectively. The only Group 2 mutant found among those described here is PC-2.

Viability. All the mutants lose viability when transferred from 30° to a restrictive temperature, and in general the higher the temperature of incubation and the faster the rate of cellular mass increase, the faster is the loss of viability. Cell death is accompanied by the formation of filamentous forms. The loss of viability of mutant PC-6 growing in a rich broth medium is illustrated in Fig. 6. As can be seen the apparent loss of viability is much less when the cells are plated on a minimal rather than a complex medium. The more rapid kinetics of cell death seen after cells are plated on complex media may be a general result of the failure of cells in which DNA synthesis has been inhibited by any of several methods to restore a normal DNA/mass ratio (Maaløe and Kjeldgaard, 1966) or failure to repair lesions to their DNA under conditions of rapid growth. Similar phenomenon have been observed when cells irradiated with UV (Alper and Gillies, 1958) are plated on a minimal rather than complex medium. This phenomenon (known as minimal medium recovery) has been extensively studied in certain *rec*$^-$ and *uvr*$^-$ mutants of *E. coli* by Ganesan and Smith (1968) and is considered more fully in the discussion.

Genetic Mapping. Crosses between various TR (i.e. *dna*$^+$) Hfr strains viz. JC 12, Hfr H, P7201 and the TS (i.e. *dna*$^-$) mutant PC-6 were performed to locate roughly the *dna*-306 mutation carried by this mutant. Times-of-entry for the *dna*$^+$ allele were measured by selection of TR recombinants using Ade$^-$, Str, and Thr$^-$ respectively as contraselective properties. Because PC-6 lost viability at 37° the matings were run at 30° C. This meant that the times-of-entry could not be directly translated into the units used by Taylor and Trotter in the construction of the standard *E. coli* map since these units are minutes of transfer time at 37° C. Nonetheless the *dna-306* mutation was roughly located between 88 min (i.e. 0 Hfr H) and 75 min (i.e. 0 Hfr P7201) on the *E. coli* map by these conjugational crosses. Transductional crosses were used to pinpoint *dna-306*. TR (i.e. *dna*$^+$) strains carrying three different mutations in the region implicated were used as recipients and PC-6 was used as the donor. Prototrophic transductants were scored for their inheritance of *dna-306* by testing their growth at 40° C. From the data in Table 5 it can be seen that *dna-306* is co-transducible with *malB*$^+$ at a higher frequency than with *metA*$^+$ and not at all with par A$^+$. This implies that *dna-306* lies close to *malB* and the fact that the linkage *dna-306—metA* is less than the 20% *malB—metA* linkage reported by Schwartz (1967) implies *dna-306* is on the *uvrA* side of *malB*. This is further supported by the following data. One of the *malB*$^-$ *dna*$^+$ strains used, KMBL82MPEl carried an *uvrA* mutation so that linkage between *dna-306* and *uvrA*$^-$ could be examined. Of the 242 mal$^+$ transductants, 173 had inherited *dna-306* and 178 had inherited *uvrA*$^+$ (i.e. were UV^r). 166 of the Mal$^+$ transductants had inherited both *dna-306* and *uvrA*$^+$. The linkage between *uvrA* and the *dna* locus is greater thus than 90% indicating that both genes are on the same side of *malB*. Furthermore the data are consistent with the order *malB*, *uvrA*, *dna* although they do not conclusively prove it. The difference in co-transduction frequencies of *malB*$^+$ and *dna-306* obtained with J4 and KMBL82MPEl as recipients may be attributable to marker or strain effects. Several TS transductants in these crosses were tested at random and all were found to show TS synthesis of DNA. A second Group 1 mutant PC-8 which like PC-6 shows very little residual DNA synthesis at 40° shows a similar high linkage to the *malB* gene. The symbol *dnaB* has been provisionally assigned to the TS locus in PC-6 and PC-8 in agreement with the notation adopted for the apparently identical locus by Hirota, Ryter and Jacob (1968). Complementation tests are required to confirm this provisional assignment.

Since PC-2 appeared to be a different type of mutant on the basis of its ability to support the growth of Lambda and its pattern of residual DNA synthesis, it was of interest to see if its *dna* mutation mapped near the two *dnaB* mutations. An experiment with Hfr Cavalli (HfrC) showed that the TR allele entered PC-2 slightly before *leu* in an interrupted mating experiment. This result was confirmed in the cross shown in Fig. 7. Again TR enters closely linked to *leu*. Thus it is clear that the mutation in DNA synthesis of strain PC-2 is in a different gene from that of strain PC-6. Attempts to demonstrate co-transduction of the TS gene with several markers near *leu* were unsuccessful.

I have been unable to map the mutant loci carried by strain PC-7 and several other Group 1 mutants which synthesize substantial amounts of DNA at the restrictive temperature. These strains are not as sensitive to temperature as PC-6 or PC-2, and recombinants are difficult to score accurately as there is only a slow

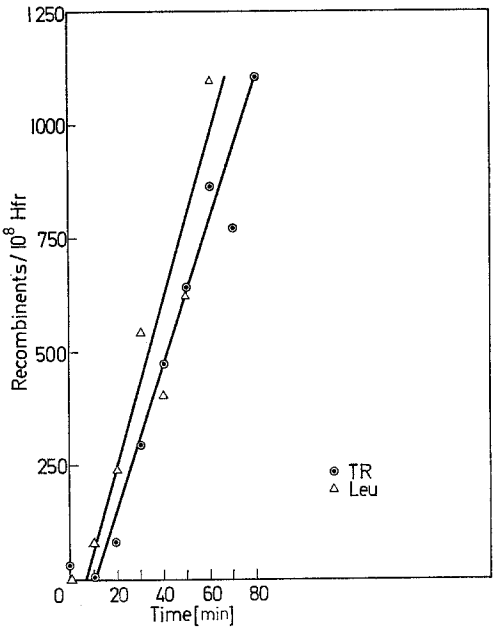

Fig. 7. Entry times of *leu* and TR in an interrupted mating experiment between Hfr H and PC-2. The parent strains were grown at 30° and shifted at time 0 to 37°. Streptomycin was used to contraselect against Hfr H

Table 5. *Co-transduction by phage P 1 of a gene conferring TS DNA synthesis with several prototrophic markers*

Recipient strain (and relevent genotype)	Selected phenotype[a]	dna$^+$ donor		dna$^-$ donor	
		No. of Trans-ductants tested	No. of Trans-ductants which were TS	No. of Trans-ductants tested	No. of selected Transduc-tants which were TS
KG20 (*purA*$^-$)	Ade$^+$	—	—	200	0
AB1932 (*metA*$^-$)	Met$^+$	400	0	462	10
J4 (*malB*$^-$)	Mal$^+$	52	0	28	6
KMBL82MPE1 (*malB*$^-$)	Mal$^+$	22	0	242	173

[a] Selection was performed at 30°C.

loss of viability at 40° with the result that a "lawn" of phenotypically TR colonies appears on selective plates.

Discussion

The seven mutants isolated in the present study can be grouped into at least two classes on the basis of the experiments reported here. The first group of mutants

will support the growth of T4 but not Lambda at 40°. It is typified by PC-6 which appears to be mutant at a locus designated *dnaB* closely linked to *malB*. Several additional mutants of similar phenotype, including the mutant originally described by Bonhoeffer and Schaller (1965), have been found to carry mutations which map in this region (Hirota, Ryter and Jacob, 1968). These additional mutants are phenotypically heterogenous in that some cease DNA synthesis abruptly upon transfer to the restrictive temperature while others stop more slowly. Whether this phenotypic heterogeneity is a reflection of the existence of several *dna* genes lying close together, or whether it is a reflection of the effects of differently mutant products of a single gene is not yet entirely clear. The available evidence from F′ complementation tests favors the former possibility (Hirota, Ryter and Jacob, 1968). The Group 1 mutants found in the present work are similarly phenotypically heterogeneous, but I have not yet succeeded in showing that the mutants such as PC-7 with substantial residual synthesis do in fact carry mutations which map at a site near *malB*.

The nature of the biochemical lesion in these Group 1 mutants is unknown. However, recently Ricard and Hirota (1969) have reported that some, but not all, mutants mapping in the *malB* region are no longer thermo-sensitive if 2% NaCl is added to the growth medium. PC-6 is also "salt-rescueable" (M. Golomb, personal communication) and this supports its similarity to the mutants of the *dnaB* locus described by these authors. It is tempting to speculate that the osmotic sensitivity of these mutants is due to the fact that the DNA replicating machinery is membrane bound as has often been suggested (Hirota, Ryter and Jacob, 1968).

The marked minimal medium recovery in PC-6 (Fig. 6) suggests that normally the events leading to lethality in this strain are distinct from the blockage of DNA replication itself, in that the lethal event can be phenotypically uncoupled from the cessation of DNA synthesis merely by appropriate subsequent treatment of the cells after exposure to high temperature. Fangman and Novick (1968) were able to genotypically uncouple lethality from the cessation of DNA synthesis by isolating from one of their mutants "death resistant" mutants which although they retained the original TS mutation in DNA synthesis no longer lost viability. These results show that cessation of DNA synthesis is by no means in itself lethal to the cell, but that lethality must reflect some coupling of DNA synthesis to other cell processes, e.g. cell division.

PC-2 clearly represents a second class of mutants distinguished from the above mutants by the fact that Lambda replication can occur at the restrictive temperature and that the mutation appears to map near *leu* rather than near *malB*. In most respects this mutant resembles mutants T46 and T83 described by Hirota, Ryter and Jacob (1968) and Kohiyama (1968) i.e. the amount of residual synthesis does not appear to depend strongly on temperature (Fig. 3B), and is approximately equal in magnitude to the amount of synthesis observed when DNA initiation is blocked by CAP (Fig. 3A). Furthermore, a period of prior amino acid starvation at 30° prevents residual synthesis from occuring at 40°. (Fig. 5). All these properties are consistent with the notion that the TS mutation in PC-2 affects the initiation of DNA synthesis though this is by no means firmly established. However mutants T46 and T83 map near the *ilv* locus while, as we have seen, the mutant locus of PC-2 appears to be close to *leu*. Although we have not ruled out the

presence of a suppressor of *dna-302* lying near *leu* in the Hfr strain used for the preliminary mapping, it is possible that *dna-302* is a mutation in a gene hitherto unreported. Recently Kuempel (1969) reported a mutant in which DNA initiation appeared to be TS. However, as no mapping data were presented it is impossible to decide if his strain is mutant at the locus near *ilv* or the one near *leu* or at some third site. Lark and his co-workers have presented evidence (reviewed in Lark, 1966) that there may be at least two proteins whose functioning is involved in the initiation of DNA synthesis. Thus the TS mutation mapping near *ilv* may represent the gene coding for one such protein and the site near *leu* may represent the second protein. It is interesting that Gross, Karamata and Hempsted (1968) have also found two genes in *B. ubtilis* which appear to govern DNA initiation. It is, of course, entirely possible that more than two genes are involved in the initiation of DNA replication.

Fangman and Novick (1968) have reported some TS DNA mutants which unlike any of the mutants discussed here carry mutations which appear to map near the *strA* gene. T4 can grow in these mutants at the restrictive temperature but Lambda can not, i.e. they appear phenotypically rather like *dnaB* mutants. Fangman and Novick's mutants also appear to be two types: those with a very rapid shut off of DNA synthesis and those in which synthesis continues for a period before stopping. In both types of mutants the exact kinetics of DNA synthesis are very temperature dependent. It has not yet been clearly established if more than one locus is involved, but preliminary mapping data suggest at least two sites. None of these mutants have the properties expected of initiator mutants, however. It is possible that some of the mutants I have as yet been unable to map could be at these loci.

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