A Mutant in the Initiation of DNA Synthesis in 
Salmonella typhimurium

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(Accepted for publication 30 September 1970)

SUMMARY

A mutant of Salmonella typhimurium was isolated which showed temperature-sensitive synthesis of DNA. The mutant (I1G) increased in cell mass at 38° without concurrent DNA synthesis, resulting in loss of viability and the production of long filaments. DNA synthesis at 38° continues for approximately 40 min. at a gradually decreasing rate giving an increase of DNA in Casamino acid minimal medium of about 55% over that present at the time of the shift. This residual DNA synthesis is not reduced by increasing the temperature to 42°, but can be increased by enriching of the medium in which the organisms are grown before the shift. The effect of high temperature on DNA synthesis in I1G mimics the effect of chloramphenicol, which is known to allow the completion of rounds of replication of DNA without allowing new initiations. The lesion is essentially irreversible and is not corrected by increasing the osmotic pressure of the growth medium. Phage P22 can develop normally in I1G if added at the time of the shift or 30 min. later. Even if the phage is added 3½ h. after the shift substantial multiplication occurs. Cell division continues for several hours after the temperature shift, resulting in the production of DNA-less cells as reported in a separate publication.

INTRODUCTION

Although considerable advances have been made in our knowledge of DNA synthesis in the last few years, the processes involved in the control of DNA synthesis in bacteria are still poorly understood. The isolation of temperature-sensitive mutants of DNA synthesis in Escherichia coli and Bacillus subtilis has added to our knowledge of these processes. Several laboratories have isolated mutants of DNA synthesis in E. coli (Bonhoeffer, 1966; Fangman & Novick, 1968; Hirotta, Ryter & Jacob, 1968; Inouye, 1969; Kuempel, 1969) and B. subtilis (Gross, Karamata & Hempstead, 1968; Bazill & Retief, 1969) and these fall into two groups: class I mutants, in which DNA synthesis stops immediately after a shift from low to high temperature; class II, whose DNA synthesis continues for some time before it stops. The first class includes mutants affected in DNA synthesis per se, e.g. in an enzyme involved in DNA replication. The second class can be divided into two groups: first, those mutants that allow rounds of replication to be completed at the high temperature but cannot initiate new rounds of DNA synthesis (these are generally called initiation mutants); secondly, mutants that synthesize DNA after a temperature shift but which are not involved in initiation will appear in this group if the protein affected is only slowly inactivated at the high temperature.
The isolation and mapping of initiator mutants of bacteria should give valuable information about DNA synthesis and its control. The isolation of such mutants has been reported in *Escherichia coli* by Hirota *et al.* (1968) and Kuempel (1969) and in *Bacillus subtilis* by Gross *et al.* (1968).

We have isolated a temperature-sensitive strain of *Salmonella typhimurium* which appears to be defective in the initiation of DNA synthesis. It seems to be very similar to the strain CRT46 of Hirota *et al.* (1968) in that it continues cell division at the high temperature with the production of DNA-less cells. Details of cell division in our strain and the properties of the DNA-less cells are the subject of another publication.

**METHODS**

**Organisms.** The mutant 11G was isolated as described below from *Salmonella typhimurium* PG154. Strain PG154 has the following genetic constitution: F-, *metA22, tryB2, ilv-90, xyl-I, fla-66, malA1, strA210, tre-12, hi-b, h2-e, n, x* and was kindly supplied by Dr K. Sanderson (SA98 in Sanderson's collection). Strain 11GP22 is a derivative of the mutant 11G that has been made lysogenic for phage P22. Phage P22L4 is a virulent mutant of the temperate Salmonella phage P22 and was kindly supplied by Dr D. A. Smith.

**Isolation of mutant.** Strain 11G was one of 70 temperature-sensitive mutants isolated from *Salmonella typhimurium* PG154. Organisms grown in nutrient broth were mutagenized using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the method of Adelberg, Mandel & Chen (1965). After mutagenesis organisms were washed free of mutagen, allowed to grow at 25° for several hours in nutrient broth and then plated on nutrient agar at 25°. Colonies which failed to grow when replicated on nutrient agar at 38° were tested for filament formation at this temperature. Five of these mutants formed filaments and were tested for thymidine incorporation at 38°. Two mutants incorporated little thymidine; one of these was 11G.

**Media.** Oxoid nutrient broth no. 2 was used as a rich undefined medium and will be referred to as nutrient broth. Solidified with 2% Difco Bacto agar it will be referred to as *nutrient agar*. In one experiment Oxoid brain and heart infusion medium was used. The minimal (MM) was that of Davis & Mingioli (1950) with 0.2% glucose and 40 μg./ml. of the Dl-form of the required amino acids. It was enriched in some experiments with 0.1% Difco vitamin-free Casamino acids (Casamino MM). Difco Bacto agar (2%) was added to obtain solid media. Trypticase agar used as basal agar for phage assays consisted of 1% trypticase agar base, 1.4% Difco Bacto agar and 0.5% NaCl. The soft agar overlay consisted of 0.2% MgCl₂, 1% Oxoid no. 2 broth, 0.3% NaCl and 0.75% Difco Bacto agar.

**Growth of organisms.** In all experiments organisms were subcultured from a nutrient agar slope to a small volume of liquid medium, incubated overnight in a shaking water bath, and then diluted and grown for several hours keeping the extinction of the culture below about 0.2 before use. Extinction of the culture was measured in a Hilger photoelectric colorimeter against an uninoculated medium blank (filter 49 for minimal media and filter 55 for nutrient media).

**Measurement of cell viability.** Organisms growing in nutrient broth at the required temperature were diluted into phosphate buffer (0.05 M, pH 7.4) and appropriate dilutions were plated on nutrient agar. The plates were incubated at 25° for 40 h.
**Assay of phage.** In some experiments the multiplication of the virulent phage mutant P22 L4 in strains 11G and PG154 was studied. Organisms growing exponentially in nutrient broth were diluted in broth to 100 µg. dry wt/ml and about 10^6/ml. infective phage added. Incubation was continued and samples taken at zero and after various intervals were shakered with a few drops of chloroform, and the chloroform removed by bubbling air. Phage were estimated in sample dilutions using a soft agar technique with strain PG154 as indicator.

In other experiments induction of phage P22 in a lysogenic derivative of strain 11G was followed. In this case exponentially growing organisms were harvested, suspended in broth and irradiated for 90 sec. using a Philips TUV 6 W tube (854 ergs cm.²/sec.). Organisms were then diluted into nutrient broth and samples taken for phage counts (as above) at intervals during incubation.

**Synthesis of protein, RNA and DNA.** Protein synthesis was measured by the incorporation of [¹⁴C]phenylalanine. Organisms growing exponentially in MM were diluted to a cell density of 100 µg. dry wt/ml. in MM containing 0.05 % vitamin-free Casamino acids and [¹⁴C]phenylalanine (0.1 µCi/ml specific activity 12.6 mCi/mm) and incubated with shaking at the appropriate temperature. Samples (1 ml.) were removed at zero and at intervals into 1 ml. of ice cold 10 % trichloroacetic acid. After standing for at least 1 h. in the cold samples were filtered on Millipore filters (type HA) and the filters after washing with 4 x 2 ml. portions of ice cold 5 % trichloroacetic acid were dried and counted in 15 ml. of scintillation fluid (0.6 % butyl PBD in sulphur-free toluene–methanol, 3:1, v/v) using a Packard Tricarb liquid scintillation spectrometer.

Uracil incorporation was used as a measure of RNA synthesis. Organisms growing exponentially in MM were diluted to a cell density of 100 µg. dry wt/ml. in MM containing [³H]uracil (0.1 µCi/5 µg./ml.). Samples were taken at intervals into an equal volume of ice cold 10 % trichloroacetic acid and then treated as for phenylalanine incorporation.

Since 11G does not require thymine for growth, DNA synthesis was measured by the incorporation of tritiated thymidine. In the strains used in this work as in *Escherichia coli* (Budman & Pardee, 1967) thymidine incorporation occurs at an appreciable rate for only a few minutes, falling off due to the induction of thymidine phosphorolase which breaks down the thymidine to thymine which cannot be utilized. DNA synthesis was therefore measured in the presence of uridine, which inhibits both the induction and the activity of thymidine phosphorolase. Exponentially growing organisms were diluted to 100 µg. dry wt/ml. in MM containing 1.5 mM uridine and [³H]thymidine (0.2 µCi/0.2 µg./ml.), and samples were taken (during incubation at the appropriate temperature with shaking) into an equal volume of ice cold 10 % trichloroacetic acid containing 40 µg./ml. unlabelled thymidine. Samples were then treated as previously except that filters were washed with ice cold 5 % trichloroacetic acid containing 40 µg./ml. unlabelled thymidine.

DNA synthesis in nutrient broth was performed in essentially the same way using nutrient broth plus 1.5 mM uridine and 0.2 µCi/ml. [³H]Thymidine (specific activity 5.0 Ci/mm).

**Chemicals.** Radioisotopes were obtained from the Radiochemical Centre, Amer- sham, Buckinghamshire. Methyl [³H]thymidine was supplied at 5.0 Ci/mm; L-4-[¹⁴C]phenylalanine at 12.6 mCi/mm and 2-[¹⁴C]uracil at 54.9 mCi/mm. Chloramphenicol was obtained from Parke, Davis and Co. All other chemicals were of at least analytical grade in purity.
Fig. 1. Growth and macromolecular synthesis in PG154 and II G.

(a) Growth of PG154 and II G at 38°. Organisms growing exponentially in nutrient broth at 25° were diluted to an extinction of 0.1 and the cultures transferred to 38° and the extinctions followed. □, PG154; ○, II G.

(b) Synthesis of protein in PG154 and II G. Organisms growing exponentially in MM at 25° were diluted into MM + 0.05% vitamin-free Casamino acids. [14C]Phenylalanine (0.1 μCi/ml; 12.6 mCi/mm) was added to each and the cultures transferred to 38° and samples taken at intervals for estimates of cold trichloroacetic acid insoluble radioactivity as described in Methods. □, PG154; ○, II G.

(c) Synthesis of RNA in PG154 and II G. Organisms growing exponentially in MM at 25° were diluted to approximately 100 μg. dry wt/ml into MM + [14C]uracil (0.1 μCi/5 μg./ml.). The cultures were shifted to 38° and samples taken as in (b). ○, PG154; □, II G.

(d) Synthesis of DNA in PG154 and II G. Organisms growing exponentially in Casamino acid MM were diluted into Casamino acid MM + 1.5 mM uridine. [3H]Thymidine (0.2 μCi/0.2 μg./ml.) was added and samples taken at intervals and treated as described in Methods. □, PG154 at 38°; ○, II G at 25°; △, II G at 38°.
RESULTS

Growth and macromolecular synthesis in strain \textit{IIG}

In nutrient broth, strain \textit{IIG} grew at approximately the wild-type rate at 25°C (data not shown). After a shift from 25 to 38°C cell mass as measured by extinction increased extensively and continued at a gradually decreasing rate for at least 5 h. (Fig. 1a). Viability, on the other hand, increased slightly for the first hour and then fell off exponentially with a half life of c. 20 min. Chloramphenicol (CAP) at 150 μg/ml to a large extent stops the loss of viability of \textit{IIG} (data not shown). When samples of the culture were examined under the phase contrast microscope it was seen that cell size increased little in the first hour at 38°C but then increased more rapidly to produce long filaments together with smaller cells.

Synthesis of RNA and protein (as measured by the incorporation of [14C]uracil and [14C]phenylalanine) after a shift from 25 to 38°C proceeded fairly normally for the first 60 min. and then became approximately linear paralleling the extinction curve (Fig. 1b, c). DNA synthesis (as measured by the incorporation of [3H]thymidine) continued for about 40 min. and then stopped, resulting in an increase in DNA of about 55% over the amount present at the time of the shift in Casamino MM (Fig. 1d).

\textit{Phage multiplication in strain \textit{IIG} at 38°C}

The behaviour of the mutant at 38°C suggested that it was a DNA synthesis mutant of class II. The multiplication of phage \textit{P22} was studied to investigate the possibility that the lesion in \textit{IIG} affected the supply of DNA precursors and that the residual DNA synthesis at 38°C was due to the depletion of the remaining pool of DNA precursors. (\textit{P22} would presumably be dependent on host-produced precursors.) Furthermore, if \textit{IIG} is an initiator mutant the development of a phage with its own initiation apparatus should be unaffected.

Table 1. \textit{Multiplication of phage \textit{P22L4} in strain \textit{PG154} and strain \textit{IIG}}

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time of phage addition</th>
<th>Zero</th>
<th>40 min.</th>
<th>120 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{PG154}</td>
<td>At shift to 38°C</td>
<td>7 × 10^3</td>
<td>7.2 × 10^6</td>
<td>6.5 × 10^9</td>
</tr>
<tr>
<td>\textit{IIG}</td>
<td>At shift to 38°C</td>
<td>8.5 × 10^3</td>
<td>5.4 × 10^6</td>
<td>5.5 × 10^9</td>
</tr>
<tr>
<td>\textit{IIG}</td>
<td>30 min. after shift</td>
<td>2.1 × 10^4</td>
<td>1.5 × 10^6</td>
<td>2.5 × 10^9</td>
</tr>
<tr>
<td>\textit{IIG}</td>
<td>210 min. after shift</td>
<td>1.7 × 10^4</td>
<td>1.0 × 10^5</td>
<td>2.9 × 10^6</td>
</tr>
</tbody>
</table>

Phage development in \textit{IIG} was measured using a virulent mutant (L4) of phage \textit{P22}. Table 1 shows the results of experiments with the phage. The phage is able to develop normally whether it is added to \textit{IIG} at the time of the temperature shift or after 30 min. at the high temperature and even if added 3½ h. after the shift there is a 200-fold increase in phage. Similarly, when \textit{IIG} was made lysogenic for normal phage \textit{P22} and induced with u.v. the phage could still develop at 38°C, although the phage was not induced by growth at 38°C alone (data not shown).
Stability of DNA in strain IIG

The cessation of DNA synthesis could result from an uncontrolled breakdown of the DNA in IIG at the high temperature. This was ruled out by measuring the stability of the DNA in IIG at 38°. Cells labelled with [3H]thymidine at 25° were washed free of the label and transferred to 38° and samples taken over a period of 2½ h. The amount of label present in cold trichloroacetic acid insoluble material was constant for the first hour and then fell off slowly (Fig. 2). This loss of label was not found at 25° or at 38° in the presence of 150 μg./ml. of CAP. This very slow loss of label cannot explain the cessation of DNA synthesis in IIG and is probably similar to the slight breakdown of DNA found in several situations where DNA synthesis is inhibited under conditions allowing protein synthesis, representing the induction of nuclease activity (Cook, Deitz & Goss, 1966).

Attempted reversal of temperature lesion in strain IIG

The lesion in DNA synthesis in IIG is essentially irreversible. After 90 min. at 38° in Casamino acid MM all DNA synthesis has stopped. If at this time the culture is shifted back to 25°, DNA synthesis only recommences slowly after 1½ h. and doubles in about 2½ h. This slow reversibility is stopped by the addition of 150 μg./ml. of CAP at the time of the shift to 25°. Increasing the osmotic pressure of the growth medium by the addition of 2 % NaCl to the Casamino acid MM does not reverse the lesion in DNA synthesis and allow increased synthesis of DNA at 38°.
DNA synthesis in strain IIG

Several experiments were performed to see if the behaviour of IIG at 38° was compatible with a mutation in the initiation of DNA synthesis. If the residual DNA synthesis after a shift to 38° is due to a mutation in some aspect of DNA synthesis per se which takes several minutes to be expressed due to the slow inactivation of some essential enzyme, then on increasing the temperature from 38 to 42° the residual synthesis should be drastically reduced. On the other hand, if this is a mutation in the initiation of DNA synthesis increasing the temperature should have no effect as rounds of replication will still be finished. Fig. 3 shows that the amount of DNA synthesized at 38 and 42° is exactly the same, a result compatible with IIG being an initiator mutant.

![Graph showing DNA synthesis over time](image)

Fig. 4. Effect of CAP on DNA synthesis in IIG. Organisms were grown in Casamino acid MM at 25° and diluted into Casamino acid MM + 1.5 mm uridine. [aH]Thymidine (0.2 μCi/0.2 μg./ml.) was added and the culture split into three portions. One was shaken at 25°, ○; one at 25° + 150 μg./ml. CAP, □; and the third at 38°, △; and samples taken and treated as in Methods.

As CAP is known to stop the initiation of further rounds of DNA replication without affecting rounds in progress (Maaløe & Hanawalt, 1961), the effect of CAP and the effect of high temperature on DNA synthesis in IIG should be essentially the same. Fig. 4 shows the result of an experiment where a culture of IIG growing in Casamino acid MM at 25° was split into three portions and DNA synthesis measured in one at 25°, in another at 38° and in the third at 25° plus 150 μg./ml. of CAP. The amount of DNA synthesized at 38° and the amount synthesized at 25° plus CAP are almost identical. In both cases the amount of DNA synthesized is between 50 and 55 %, the amount predicted if cells in this medium at this temperature have multiple replication forks per chromosome for a part of the replication cycle (Helmstetter & Cooper, 1968). Furthermore, if CAP and the high temperature have the same effect, i.e. allow the completion of rounds of DNA replication in the absence of new initiations, then ad-
dition of CAP at 38° should not further reduce the increment. Fig. 5 shows that CAP at 38° only reduces very slightly the amount of DNA synthesized.

Bacteria whose doubling time is less than the time for a round of chromosome replication have multiple replication forks in their DNA (Yoshikawa, O'Sullivan & Sueoka, 1964; Helmstetter & Cooper, 1968). Thus minimal grown cells of *Salmonella typhimurium* contain a single chromosome replicated by a single fork. As the doubling time permitted by the medium decreases to below about 40 min. at 37° multiple replication forks appear. It is easily seen that if rounds of replication are allowed to be completed in the absence of new initiations the amount of residual DNA synthesis will increase as the average number of replication forks increases. Fig. 6 shows the amounts of DNA synthesized by 11G after a shift from 25 to 38° in various media. Clearly the amount of DNA synthesized increases as the growth rate afforded by the medium increases as predicted for an initiator mutant.

Similarly, the amount of DNA synthesized at 38° in the presence of chloramphenicol increases as the richness of the medium increases.

*Cell division in 11G at 38°*

Observations of 11G growing on nutrient agar by phase contrast microscopy after a shift to 38° show that organisms divide once or twice in the first 90 min. These divisions may be normal ones resulting in the segregation of the newly finished chro-
mosomes. The daughter cells then elongate and after a period of elongation bud off cells at the ends of the short filaments. The details of this division process and the properties of the small cells will be described in a later publication.

![Graph showing the effect of growth medium on DNA synthesis in strain 11G.](image)

**Fig. 6.** Effect of growth medium on DNA synthesis in 11G. Organisms growing in various growth media at 25° were diluted into the same media + 1.5 mM uridine. [PH]Thymidine was added and DNA synthesis measured at 38° as described in Methods. Brain and heart infusion (mean generation time at 25° = 50 min.), □; Casamino MM (mean generation time at 25° = 105 min.), △; MM (mean generation time at 25° = 260 min.), ○.

**DISCUSSION**

The continuing increase in extinction, RNA and protein synthesis with concomitant loss of viability and formation of filaments is typical of growth in the absence of DNA synthesis, and is shown by all DNA synthesis mutants and in other treatments that stop DNA synthesis without affecting RNA or protein synthesis, e.g. thymine starvation of a thymine-requiring strain or treatment with nalidixic acid. The viable count of 11G at 38° increases for about an hour and this is probably due to normal cell division continuing for one or two divisions in this mutant after a shift from low to high temperature. Following this increase viable count falls exponentially.

All the physiological experiments performed on 11G are suggestive of or compatible with a mutation in the initiation of DNA synthesis. The development of phage P22 L4 in 11G at 38° can occur because the phage can control the initiation of its own DNA synthesis as implied by the replicon theory of Jacob, Brenner & Cuzin (1963). The development of phage also suggests that the supply of DNA precursors is not affected at 38° since phage P22 is unlikely to code for enzymes involved in the supply of deoxynucleotide triphosphates. Since phage P1 (though not phage λ) can multiply in a DNA synthesis mutant of *Escherichia coli* which is not an initiator mutant (165/70 studied by Lanka & Schuster, 1970), the experiments with phage P22 cannot, however, be considered to differentiate conclusively between an alteration in initiation and one in DNA synthesis per se.
A direct effect on an enzyme involved in DNA synthesis per se but only slowly inactivated is made unlikely by the fact that increasing the temperature from 38 to 42° (which should increase the rate of inactivation of such an enzyme) did not decrease the amount of DNA synthesized after a temperature shift. Increased breakdown of DNA is also ruled out as a cause of the lesion.

The effect of CAP on DNA synthesis after a shift is exactly as predicted. The amount of DNA synthesized in Casamino acids MM is about 50 to 55 % at 25° plus CAP or at 38°, and this is in good agreement with the amount expected if cells in this medium contain two replication forks for a part of their replication cycle. This agreement is not fortuitous (as it was in the mutant of Fangman & Novick, 1968) since the increment in DNA synthesis in 11G increases as the growth rate afforded by the medium increases. In brain–heart infusion the amount of DNA synthesized after a temperature shift is about that expected of a very rich medium. Similarly, in MM alone the amount of DNA synthesized by 11G after a shift is about 30 %; this is to be expected, as growth of 11G in this medium is very slow at 25° (mean generation time of about 260 min.) and there would probably be a gap between rounds of replication. This would mean that some organisms would not be synthesizing DNA at the time of the temperature shift, and this would decrease the amount of DNA synthesized in the absence of new initiations to below the 38 % expected for organisms where DNA synthesis is continuous with a single replication fork (Maaloe & Hanawalt, 1961).

The amount of DNA synthesized plus CAP at 25° and to a greater extent the amount at 38° plus CAP is generally slightly lower than in 11G at 38° in the absence of CAP. This would occur if new rounds of replication could be initiated in a few organisms after the temperature shift. Lark & Lark (1964) have dissected the initiation of DNA synthesis in Escherichia coli into two steps on the basis of different sensitivities to CAP. One step (the CAP-resistant step) is resistant to low levels of CAP but sensitive to high levels, and the other step (the CAP-sensitive step) is sensitive to low levels of CAP. Lark & Renger (1969) have separated these two steps temporally and find that the CAP-resistant step occurs 15 min. before the initiation event, and the CAP-sensitive step occurs 30 min. before the initiation event. Ward & Glaser (1969) have confirmed these results using more physiological conditions (and using E. coli B/r and not E. coli 15T-) and find that the CAP-resistant step occurs at the time of initiation of DNA synthesis and the CAP-sensitive step occurs about 21 min. before initiation. If a mutant of initiation was affected in this early CAP-sensitive step then the amount of DNA synthesized by 11G plus high levels of CAP (e.g. 150 μg./ml. as used in the experiments with 11G) should be very much less than the amount synthesized by the mutant at the high temperature, as new initiations could occur for about 20 min. in this kind of mutant. The amount of DNA synthesized by 11G at 38° is only slightly more than in the presence of CAP, and so it would appear that 11G is not affected in an early stage of initiation.

Several mutants of DNA synthesis can be corrected by increasing the osmotic pressure of the growth medium (Ricard & Hirota, 1969), and this may reflect the involvement of a membrane protein in the DNA lesion. The absence of any effect of increasing the osmotic pressure on the amount of DNA synthesized after a shift argues against the involvement of the membrane in the lesion in 11G.

The lesion in 11G is completely irreversible when a culture is held at 38° for 90 min. and then transferred back to 25° in the presence of high levels of CAP, and only very
A mutant of DNA synthesis

A mutant of DNA synthesis =37 slightly reversible in the absence of CAP. This at first suggested that the protein altered by the temperature shift cannot be reactivated by lowering the temperature, but since synthesis of new initiator at 25° still cannot allow initiation, irreversibility is probably due to the formation of a faulty initiation complex or to the accumulation of irreparable damage to the DNA after 90 min. at the high temperature. Like most processes that stop DNA synthesis but allow RNA and protein synthesis, some degradation of the DNA occurs (Cook et al. 1966). Although this is only a few per cent after 90 min. this may be too much to be repaired in most cells and may also contribute to the loss of viability. In some experiments on DNA synthesis this slight breakdown of the DNA was noticed as a loss of counts after about 60 min. at 38°, but as the maximal increment in DNA synthesis in 11G at 38° is reached by this time this loss of label probably has no effect on the estimate of the amount of DNA synthesized after the shift.

The critical test for an initiation mutant of DNA synthesis is to show that the DNA synthesized at the high temperature corresponds to the completion of rounds of chromosome replication. The double labelling method of Lark (Lark, Repko & Hoffman, 1963) makes it possible to analyse this DNA. By synchronizing the cells by amino acid starvation for a required amino acid, the origin and terminus regions of the chromosome can be labelled with 3H and 14C labels during growth at the low temperature. On shifting to 38° in the presence of 5-bromouracil, samples are removed and the DNA analysed by isopycnic centrifugation in caesium chloride. Initiation mutants should show a preferential replication of the terminus label into hybrid density material. Attempts to reinitiate replication in presumed initiator mutants and show the preferential replication of the origin region have failed due either to the lesion being irreversible, e.g. 11G, or to the accumulation of a large pool of thymidine triphosphate at 38° making pulse labelling with labelled thymine impossible (Kuempel, 1969). The first kind of double labelling experiments are being done with the mutant 11G.

The DNA synthesis mutants of Escherichia coli studied by Hirota et al. (1968) map in two regions on the E. coli genome. One group that maps very close to the ilv loci contains mutants that appear to be initiation mutants. The second group, mapping between malB and pyrB, contains mutants that stop DNA synthesis immediately after a shift from low to high temperature and some that continue DNA synthesis for a short while, but in which the residual DNA synthesis is dependent on the external conditions. Strain 11G is very similar to the first group of mutants, but preliminary mapping of the DNA lesion suggests that it maps close to and to the right of metA on the Salmonella typhimurium map as it is generally represented. More precise mapping and dominance studies are in progress.

One of us (B. G. S.) wishes to acknowledge the receipt of a Science Research Council Studentship.

REFERENCES


