DNA REPAIR REPLICATION IN TEMPERATURE-SENSITIVE DNA SYNTHESIS DEFICIENT BACTERIA

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Mutants of E.coli blocked in normal semiconservative DNA replication above 42°C have been isolated by Bonhoeffer and Schaller (1965) and by Rasmussen and Weywadt (1967). Bonhoeffer (1966) found that stimulated incorporation of P^{32} into DNA occurred at the restrictive temperature after ultraviolet (UV) irradiation of a CR34 temperature-sensitive mutant. In the present communication it is shown that this incorporation is of the nonconservative repair mode and that the amount of such repair at 42°C is as much as at the normal 36°C growth temperature. The t_3 TAU-bar mutant of Rasmussen and Weywadt (1967) was also found to undergo repair replication at the restrictive temperature. Our studies support the concept that different enzyme systems are involved in the normal semiconservative and the repair modes of DNA synthesis in E. coli.

Materials and Methods

E. coli strain CR34/43 Ts DNA $thy^-thr^-leu^-his^-B1^-$ obtained through the courtesy of Dr. Fredrich Bonhoeffer was cultured in a glucose-salts minimal medium (Maaløe and Hanawalt, 1961) and supplemented with 20μg/ml amino acids and 2 μg/ml thymine and vitamin B1. The TAU-bar mutant t_3 obtained through the courtesy of Dr. Knud Rasmussen was cultured in a similar medium with required supplements (Hanawalt, 1963).

Nonconservative and semiconservative replication at the high and low temperatures was determined by the method of Pettijohn and Hanawalt (1964).

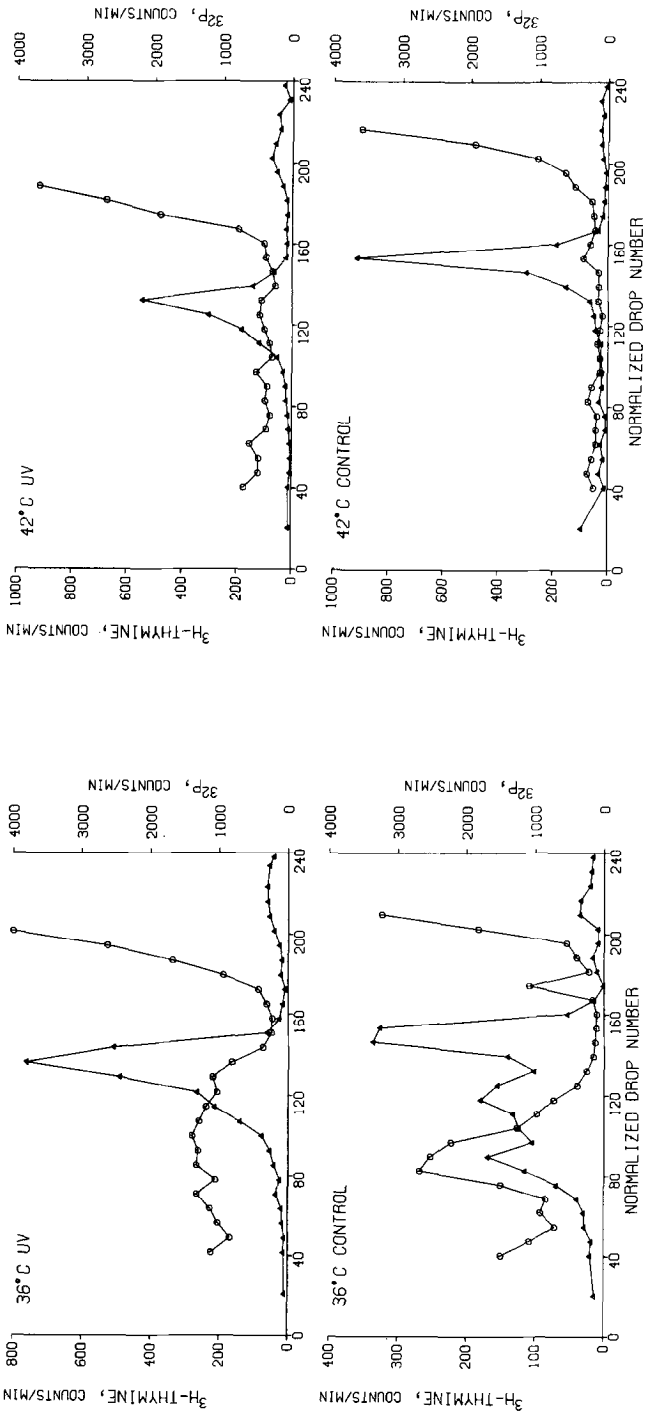

Fig. 1. Density distribution of H^3 and P^{32} counts in a CsCl gradient after prelabeling for several generations in H^3-thymine at 36°C and subsequent growth at the temperature shown on the graph in $P^{32}O_4$, 5-BU medium containing no thymine. The graphs marked "UV" were irradiated with UV after prelabeling as described in the text. Radioactivity was assayed as described in the text. Δ , H^3-thymine prelabel; O , $P^{32}O_4$.

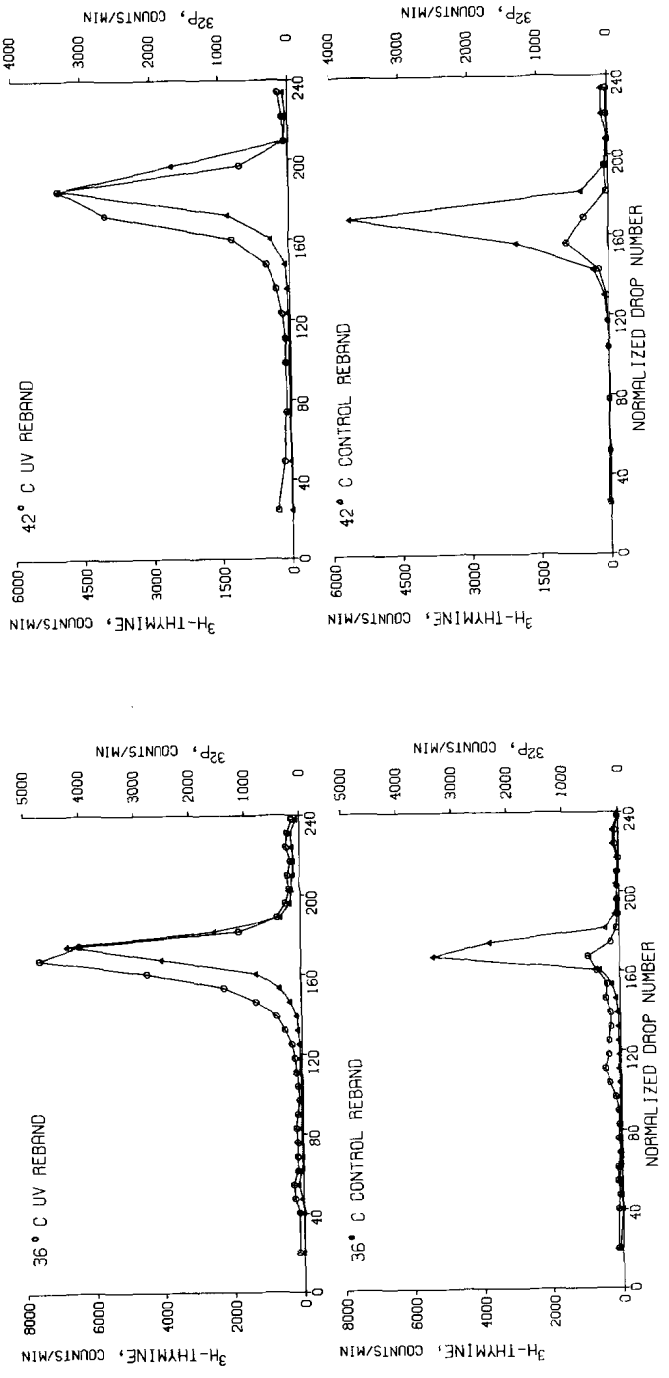

Fig. 2. Rebanding of the normal density DNA fractions from Fig. 1.
Δ , H^3-thymine prelabel; O , $P^{32}O_4$.

A 100 ml culture of CR34/43 Ts DNA was prelabeled with H^3-thymine at a specific activity of 0.17 μc/μg for several generations of balanced exponential growth. It was then filtered, washed, and resuspended to a titer of 4×10^8/ml in 120 ml of the tris buffer without phosphate or nutrients (Maaløe and Hanawalt, 1961). The resuspended culture was halved and the first 60 ml portion was kept as a control and the second 60 ml portion was irradiated for $2\frac{1}{2}$ minutes in a 50 cm Petri dish 1 m from a 15 watt Westinghouse Sterilamp. This dose gave a survival level of 2.5% as determined by colony counts. The control and UV portions were then each halved into 30 ml fractions and the temperatures adjusted to give a 36°C control, a 36°C UV, a 42°C control, and a 42°C UV culture. Twenty milliliters of a concentrated amino acid, B1, glucose, 5-bromouracil, and $P^{32}O_4$ solution of the proper temperature was then added to each culture to yield the normal concentrations of all nutrients except that 10 μg/ml of 5-BU replaced the thymine. A specific activity of 1.53μc/μg of $P^{32}O_4$ was used. The cultures were grown with aeration for 45 minutes at their respective temperatures, harvested by filtration, washed, and resuspended in 2 ml of NET buffer (0.1 M NaCl, 0.01 M EDTA, 0.01 M tris, pH 8). The cells were lysed by treatment with 200γ/ml lysozyme for 1 hour followed by 200γ/ml pronase and 0.1% sarkosyl detergent at 60°C for 2 hours and finally vortex mixing for 2 minutes. The density distribution of the DNA was then analyzed by cesium chloride density gradient centrifugation as previously described (Pettijohn and Hanawalt, 1964).

Drop fractions of each cesium chloride gradient were collected and every seventh drop was then treated with 2 M KOH for 2 hours at 37°C to hydrolyze the RNA. The DNA in the fraction was then precipitated with 5% trichloroacetic acid (TCA) and collected on a Millipore HA filter. The H^3 and P^{32} on the filter was assayed in a TriCarb liquid scintillation counter using a toluene, PPO, POPOP scintillation fluid. The unassayed fractions from the normal density region of the gradient were then rebanded in a

second cesium chloride gradient. KOH resistant TCA precipitable counts were then determined on ten drop fractions of the rebanded gradient to ascertain the P^{32}/H^3 ratio in the normal density region of the gradient.

The fraction of the chromosome nonconservatively replicated f_{nc} is computed from the P^{32}/H^3 ratio $(P/H)_{hy}$ in the reband of the hybrid density peak of the 36°C control and the P^{32}/H^3 ratio in the normal density peak $(P/H)_{nc}$. The assumption is made that the ratio of P^{32} to H^3 in the hybrid DNA represents a 1:1 ratio of parental and newly replicated daughter P^{32} labeled DNA. From this assumption the following equation for the fraction of nonconservative replication can be derived:

$$f_{nc} = \frac{(P/H)_{nc}}{(P/H)_{nc} + (P/H)_{hy}}$$

The values of f_{nc} for the CR34/43 Ts DNA and the TAU-bar mutant are given in Table 1 for both 36°C and 42°C and with and without UV irradiation. Within the accuracy of the experiment the extent of nonconservative replication at 42°C is the same as at 36°C for both the UV treated cells and the controls.

PERCENT OF NONCONSERVATIVE REPLICATION

Strain	36°C		42°C	
	Control	UV	Control	UV
CR34/43	0.27%	2.3%	0.29%	1.8%
TAU-bar t_3	0.17%	2.1%	0.18%	1.9%

Table 1. The fraction of the chromosome which is replicated nonconservatively with and without UV irradiation and above and below the restrictive temperature is shown for the E.coli strains CR/34 and TAU-bar t_3.

These data demonstrate that the ability to perform repair in these mutants is retained even though the ability to perform normal semiconservative synthesis is inhibited at the restrictive temperature. It is clear that the genetic defect which is blocking normal synthesis in these mutants is at a later step in the DNA synthesis pathway than the

kinase steps, since the nucleoside triphosphates would be needed for repair synthesis also. Furthermore, the results are suggestive that the normal and the repair DNA replication enzyme systems are not identical.

Acknowledgements

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