Mol Gen Genet (1981) 181:564–566

MGG

Short Communication

DNA Synthesis in *Escherichia coli* during a Nutritional Shift-Up*

Arieh Zaritsky** and Shmuel Zabrovitz***

Department of Biology, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva, Israel

Summary. DNA synthesis in a thymine-requiring *Escherichia coli* K12 strain was studied by exploiting deoxyguanosine, so simulating the behaviour of Thy$^+$ strains. DNA synthesis is inhibited during the first 25 min after a nutritional shift-up. The new DNA/mass is lower than that predicted by current models for initiation control.

The rate of DNA synthesis in a bacterial culture is determined solely by the frequency of initiation of new rounds of chromosome replication (Maaløe 1961; Helmstetter et al. 1968). This frequency, in turn, is equal to the frequency of total mass doublings (Donachie 1968; Pritchard et al. 1969). Therefore it is expected, that after transfer of a steady-state, exponentially growing culture to a new medium that supports faster growth (nutritional shift-up; Kjeldgaard et al. 1958), the rate of DNA synthesis will immediately accelerate, albeit slowly, to reach its new steady-state value asymptotically (Appendix and Fig. 1A). This prediction is based on extending the observations of constant replication velocity and mass at initiation to the transition period, which could well be false assumptions. In fact, published observations (Kjeldgaard et al. 1958; Cooper 1969; Bremer et al. 1977) are inconsistent with this prediction of the mass-controlled initiation model: it looks as if the rate of DNA synthesis maintains its preshift level for some 20 min, after which it accelerates quickly. More recent data (Fig. 8 in Brunschede et al. 1977) suggest that the actual rate is even slower than before during the first 25 min. The small number of measurements does not allow the fine analysis required. The main difficulties in obtaining detailed information are insufficient sensitivity of the chemical method of determining DNA (Cooper 1969) and the fact that the classical labeling technique with radioactive thymine or thymidine is not as straight forward as it is thought to be (O'Donovan 1978). Wild-type *Escherichia coli* cells do not incorporate efficiently externally-supplied thymine (Pritchard 1974), and the intracellular concentrations of its DNA-precursors are much lower in Thy$^-$-strains (Beacham et al. 1971), resulting in a change in the normal physiology of such cells (Zaritsky and Pritchard 1973; Zaritsky 1977).

A way of circumventing this difficulty has been devised (Zaritsky and Pritchard 1971, 1973) that exploits our knowledge of thymine metabolism and impaired mutants. Derivatives of Thy$^-$ strains that harbour a mutated *deo* gene can grow with low concentrations of thymine (Pritchard 1974; O'Donovan 1978) and are insensitive to deoxyguanosine. Supplementing the medium with this nucleoside restores the intracellular levels of thymine metabolites (Beacham et al. 1971) and simulates the behaviour of Thy$^+$ strains with even lower external concentrations (Zaritsky and Pritchard 1971; Zaritsky and Woldringh 1978). It is thus possible to refine the shift-up analysis by exploiting the advantages of thymine as a tracer.

A steady-state culture of *E. coli* K12 CR34 (*thr leu thy deo*; Lane and Denhardt 1974) growing in synthetic medium (Zaritsky and Woldringh 1978) supplemented with L-alanine and L-proline (400 μg/ml each) as carbon sources, L-threonine and L-leucine (50 μg/ml each), 0.03 μCi/l μg/ml ^{14}C-thymine and deoxyguanosine (100 μg/ml) (Sigma grade), was shifted-up by addition (with a dilution of 1:1.07) of prewarmed glucose (0.4%) and casein-hydrolysate (1%; Sigma Chemical Co., St. Louis, Mo., USA). Samples were withdrawn at the indicated times; absorbance (at 450 nm, Gilford microsample spectrophotometer) and ^{14}C-incorporation into TCA-insoluble material were measured (Fig. 1A).

The mass growth rate responds quickly to the nutritional shift-up with a slow asymptotic approach to the new steady-state rate. The expected curve for mass accumulation (Bremer and Dennis 1975; Grover et al. 1980) is almost superimposed and never exceeds that observed by more than 8%. This correlation justifies the calculation of expected mass increase as well as the choice of ribosomal efficiencies at the two growth rates (Appendix and legend to Fig. 1). It also means that the relative efficiencies in *E. coli* K12 do not markedly differ from those in *E. coli* B/r at corresponding doubling times.

DNA accumulation, on the other hand, stops almost completely during the first 25 min (Fig. 1A). The relative DNA concentration (Zaritsky and Pritchard 1973; Pritchard 1974) decreases faster, and approaches a lower steady-state level than expected (Appendix and Fig. 1B). These two features of the results differ significantly from currently accepted concepts and are related to DNA rather than to mass synthesis.

The temporary inhibition of DNA synthesis observed after shifting the culture to a richer medium could result from

* Dedicated to the memory of Shmuel Zabrovitz, whose high spirits and good humor enabled him to complete the work while struggling with his lethal disease

** Present address (on leave): Department of Microbiology and Immunology, University of California, Berkeley, CA 94720, USA

*** Deceased

Offprint requests to: A. Zaritsky

0026-8925/81/0181/0564/$01.00

F is the difference between the number of chromosome origins ϕ and termini T; ϕ follows mass M accumulation directly and T becomes equal to ϕ exactly C min later (Donachie 1968; Pritchard et al. 1969; Bremer et al. 1977; K.V. Rasmussen, personal communication). Mass has been shown (Bremer and Dennis 1975; Grover et al. 1980) to accumulate with time t after a nutritional shift-up of *E. coli* B/r from medium supporting doubling time τ_1 to one of τ_2 according to

$$M(t)=M(0)\,[1+\nu_1(2^{t/\tau_2}-1)], \tag{2}$$

where $\nu_1=(\varepsilon_2\tau_2+\ln 2)/(\varepsilon_1\tau_1+\ln 2)$, and ε_j are constants proportional to ribosomal efficiency at τ_j. Since (Bremer and Churchward 1977; Bremer et al. 1977) $\phi(0)=N(0)\,2^{(C+D)/\tau_1}$, where D is the time between replication-termination and subsequent cell division (Helmstetter et al. 1968) and N is the total number of cells in the culture,

$$\phi(t)=\frac{M(t)}{M(0)}\,\phi(0)=N(0)\,2^{(C+D)/\tau_1}[1+\nu_1(2^{t/\tau_2}-1)], \tag{3}$$

$$\begin{aligned} T(t)=\phi(t-C)&=N(0)\,2^{(t+D)/\tau_1} && \text{for } t\leqslant C\\ &=N(0)\,2^{(C+D)/\tau_1}[1+\nu_1(2^{(t-C)/\tau_2}-1)] && \text{for } t\geqslant C \end{aligned} \tag{4}$$

and

$$\begin{aligned} F(t)&=N(0)\,2^{D/\tau_1}[2^{C/\tau_1}(\nu_1 2^{t/\tau_2}-\nu_1+1)-2^{t/\tau_1}] && \text{for } t\leqslant C\\ &=N(0)\,\nu_1 2^{(C+D)/\tau_1}(1-2^{-C/\tau_2})\,2^{t/\tau_2} && \text{for } t\geqslant C. \end{aligned} \tag{5}$$

The total amount of DNA in a shifted-up culture after t min is obtained by substituting Equation (5) in Equation (1) and integrating:

$$\begin{aligned} G(t)&=G(0)+\frac{N(0)\,G_0}{C}\,2^{(C+D)/\tau_1}(1-\nu_1)\,t\\ &\quad+\frac{N(0)\,G_0\nu_1\tau_2}{C(\ln 2)}\,2^{(C+D)/\tau_1}(2^{t/\tau_2}-1)\\ &\quad-\frac{N(0)\,G_0\tau_1}{C(\ln 2)}\,2^{D/\tau_1}(2^{t/\tau_1}-1) && \text{for } t\leqslant C\\ &=G(C)+\frac{N(0)\,G_0\nu_1\tau_2}{C(\ln 2)}\,2^{(C+D)/\tau_1}(1-2^{-C/\tau_2})\\ &\quad(2^{t/\tau_2}-2^{C/\tau_2}) && \text{for } t\geqslant C. \end{aligned} \tag{6}$$

Acknowledgement. We thank Norman B. Grover for help in deriving the Appendix and in the final styling of the manuscript.

References

Beacham IR, Beacham K, Zaritsky A, Pritchard RH (1971) Intracellular thymidine triphosphate concentrations in wild type and in thymine requiring mutants of *E. coli* 15 and K12. J Mol Biol 60:75–86

Beck C, Ingraham J, Maaløe O, Neuhard J (1973) Relationship between the concentration of nucleoside triphosphates and the rate of synthesis of RNA. J Mol Biol 78:117–121

Bremer H, Churchward G (1977) An examination of the Cooper-Helmstetter theory of DNA replication in bacteria and its underlying assumptions. J Theor Biol 69:645–646

Bremer H, Dennis PP (1975) Transition period following a nutritional shift-up in the bacterium *E. coli* B/r: stable RNA and protein synthesis. J. Theor Biol 52:365–382

Bremer H, Young R, Churchward G (1977) Initiation and termination of DNA replication in bacteria after a stepwise increase in the velocity of replication. J Bacteriol 130:92–99

Brunschede H, Dove TL, Bremer H (1977) Establishment of exponential growth after nutritional shift-up in *E. coli* B/r: accumulation of DNA, RNA and protein. J Bacteriol 129:1020–1033

Chandler M, Bird RE, Caro L (1975) The replication time of the *E. coli* K12 chromosome as a function of cell doubling time. J Mol Biol 94:127–132

Cooper S (1969) Cell division and DNA replication following a shift to a richer medium. J Mol Biol 43:1–11

Donachie WD (1968) Relationship between cell size and time of initiation of DNA replication. Nature (London) 219:1077–1079

Filpula D, Fuchs JA (1978) Regulation of ribonucleoside diphosphate reductase synthesis in *E. coli*: specific activity of the enzyme in relationship to perturbations of DNA replication. J Bacteriol 135:429–435

Grover NB, Zaritsky A, Woldringh CL, Rosenberger RF (1980) Dimensional rearrangement of rod-shaped bacteria following nutritional shift-up. I. Theory. J Theor Biol 86:421–439

Helmstetter CE, Cooper S, Pierucci O, Revelas E (1968) On the bacterial life sequence. Cold Spring Harbor Symp Quant Biol 33:809–822

Helmstetter CE, Pierucci O (1976) DNA synthesis during the division cycle of three substrains of *E. coli* B/r. J Mol Biol 102:477–486

Kjeldgaard NO, Maaløe O, Schaechter M (1958) The transition between different physiological states during balanced growth of *S. typhimurium*. J Gen Microbiol 19:607–616

Lane HED, Denhardt DT (1974) The *rep* mutation. III. Altered structure of the replicating *E. coli* chromosome. J Baceriol 120:805–814

Maaløe O (1961) The control of normal DNA replication in bacteria. Cold Spring Harbor Symp Quant Biol 26:45–52

O'Donovan GA (1978) Thymidine metabolism in bacteria (and "how, or how not, to label DNA"). In: Molineux I, Kohiyama M (eds) DNA synthesis: Present and future. Plenum Press, New York, pp 219–253

Pritchard RH (1974) On the growth and form of a bacterial cell. Philos Trans R Soc London Ser B 267:303–336

Pritchard RH (1978) Control of DNA replication in bacteria. In: Molineux I, Kohiyama M (eds) DNA synthesis: Present and future. Plenum Press, New York, pp 1–26

Pritchard RH, Barth PT, Collins J (1969) Control of DNA synthesis in bacteria. In: Meadow PM, Pirt SJ (eds) Microbial growth. XIX Symp. Soc. Gen. Microbiol. Cambridge University Press, Cambridge, pp 263–297

Zaritsky A (1977) Branching of fast-growing *E. coli* 15T$^-$ in low thymine concentrations. FEMS Microbiol Lett 2:65–69

Zaritsky A, Pritchard RH (1971) Replication time of the chromosome in thymineless mutants of *E. coli* 15 and K12. J Mol Biol 60:65–74

Zaritsky A, Pritchard RH (1973) Changes in cell size and shape associated with changes in the replication time of the chromosome of *E. coli*. J Bacteriol 114:824–837

Zaritsky A, Woldringh CL (1978) Chromosome replication rate and cell shape in *E. coli*: lack of coupling. J Bacteriol 135:581–587

Communicated by G. O'Donovan

Received May 1 / August 25, 1980

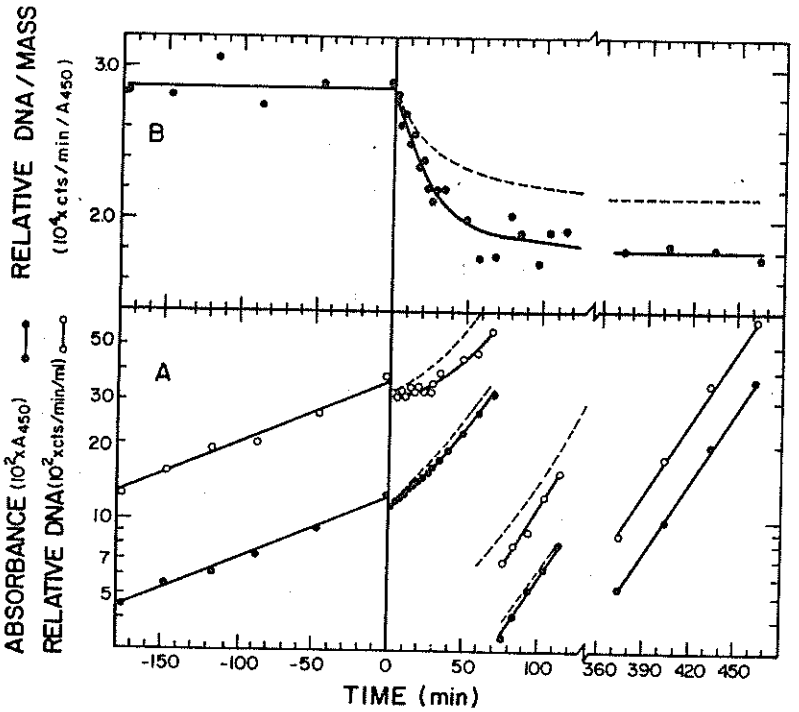

Fig. 1 A Total relative mass (●) and DNA (○) and B relative DNA/mass (●) in a culture of *E. coli* K12 CR34 before and after shifting up from $\tau_1 = 120$ min to $\tau_2 = 32$ min at $t = 0$. Dashed lines are the predicted curves (see Appendix), with $\varepsilon_1 = 0.0665$ and $\varepsilon_2 = 0.1455$ (obtained by linear regression of data extracted from Bremer and Dennis (1975) and see Grover et al. (1980)

reduction in the intracellular concentrations of the immediate DNA-precursors, the four deoxyribonucleoside-triphosphates. Their precursors (the four ribonucleoside-diphosphates) are likely to become exhausted by the dramatic increase in the rate of RNA synthesis (Kjeldgaard et al. 1958; Brunschede et al. 1977). A marked reduction in the pool levels of the four ribonucleoside-triphosphates has indeed been found (Beck et al. 1973) to occur during the early phase of a shift of another *E. coli* K12 strain from glucose-minimal to an amino acid-enriched medium. The levels of the four deoxyribonucleoside-triphosphates behave similarly (J. Neuhard, personal communication). Furthermore, it has recently been reported (Filpula and Fuchs 1978) that the enzyme responsible for conversion of the ribo-nucleotides to the deoxyribo-nucleotides is transiently overproduced before adjusting to the new steady-state conditions in the shifted-up cultures of *E. coli* strain CR34, used here. The maximum rate of increase in its specific activity occurs at about the same time (25 min) as the recovery of DNA synthesis (Fig. 1A) and of precursor pool sizes (J. Neuhard, personal communication).

The DNA concentration achieved (0.627 of its preshift value) is 16.3% lower than expected (0.749) from the two models propsed (Donachie 1968; Pritchard et al. 1969) for initiation-control. Both predict that cell mass per chromosome origin at the time of replication-initiation, M_i, should not change and that DNA/mass should increase with the doubling time, τ, according (Zaritsky and Pritchard 1971; 1973) to $(1-2^{-C/\tau})/M_i C(\ln 2/\tau)$, where C is the replication time (Helmstetter et al. 1968). The observed reduction in DNA/mass is consistent with an *increase* in C from 40 to 60 min during shift-up, whereas C has been reported to remain constant or rather to *decrease* with increasing growth rate (Helmstetter et al. 1968; Chandler et al. 1975; Helmstetter and Pierucci 1976). Therefore M_i must increase by *at least* 16.3% during the transition described. This value might still be an underestimate, since the predicted mass-growth is somewhat higher than that observed (Fig. 1A), causing the predicted DNA/mass (Fig. 1B) to be somewhat lower than its real value.

Similar deviations from the concept of a constant M_i have recently been observed (N. Grossman C.L. Woldringh and E.Z. Ron; H. Bremer and L. Chuang, both personal communication). Grossman et al. demonstrated that amino acid starvation of an auxotrophic *E. coli* resulted in a reduction in M_i. This is consistent with the data of Bremer and with those presented here and may well be complementary, because a block in protein synthesis may be looked on as an extreme case of a nutritional shift-down. Thus, the direction of change of M_i with growth rate is identical in both perturbations. As has recently been recognised (Pritchard 1978), the small number of chromosome origins per cell introduces a stochastic element into their response to effector(s) of initiation. A more thorough study, one which describes how M_i changes with τ over the entire range, would be necessary to construct a more appropriate model for initiation control.

Both of the unusual features of DNA synthesis observed after a shift-up are expected to affect the rate of cell division during the same period and beyond, because these two basic processes are tightly coupled (Helmstetter et al. 1968). Small deviations from rate-maintenance were indeed observed when a glucose-grown culture of *E. coli* B/r was shifted-up by addition of casamino acids (Helmstetter et al. 1968; Cooper 1969).

Appendix

DNA Accumulation During Shift-Up

The rate of total DNA synthesis in a culture is equal to the product of the number of pairs of replication forks, F, × the rate of replication per fork-pair G_0/C, where G_0 is the amount of DNA in an unreplicating chromosome (Helmstetter et al. 1968). The total amount of DNA $G(t)$ at time t is therefore

$$G(t) = G(0) + \int_0^t \frac{G_0}{C} F(t)\, dt. \qquad (1)$$