Relationship between nucleoside triphosphate pools and DNA replication in the cell cycle of *Escherichia coli*[1]

GEORGE G. KHACHATOURIANS[2] AND LYDIA HUZYK[3]
Department of Microbiology, University of British Columbia, Vancouver 8, British Columbia

Accepted January 23, 1974

KHACHATOURIANS, G. G., and L. HUZYK. 1974. Relationship between nucleoside triphosphate pools and DNA replication in the cell cycle of *Escherichia coli*. Can. J. Microbiol. **20**: 747–750.

The correlation between DNA replication and the nucleoside triphosphate pool fluctuation in the cell cycle of *Escherichia coli* B/r was examined. ^{32}P-labelled endogenous nucleoside triphosphates in normal synchronous cultures of *E. coli* B/r and those in which the chromosome replication cycle was inhibited by nalidixic acid, a specific inhibitor of DNA synthesis, were compared. No marked accumulation or depletion of nucleoside triphosphate pools was observed during the inhibition of DNA synthesis in the cell cycle. We suggest that changes in the pool levels during the cell cycle of *E. coli* occur independently of the DNA replication cycle.

KHACHATOURIANS, G. G., et L. HUZYK. 1974. Relationship between nucleoside triphosphate pools and DNA replication in the cell cycle of *Escherichia coli*. Can. J. Microbiol. **20**: 747–750.

Nous avons examiné la corrélation entre la replication de l'acide déoxyribonucléique et la fluctuation dans l'emmagasinage des nucléosides triphosphates dans le cycle cellulaire d'*Escherichia coli* B/r. Nous avons comparé les nucléosides triphosphates endogènes marquées au ^{32}P dans les cultures synchrones normales de *E. coli* B/r et dans celles où le cycle de replication des chromosomes est inhibé par l'acide nalidixique, un inhibiteur spécifique de la synthèse de l'ADN. Nous n'avons pas observé d'accumulation marquée ou d'enlèvement des nucléosides triphosphates durant l'inhibition de la synthèse de l'ADN au cours du cycle cellulaire. Nous suggérons que les changements dans les niveaux d'accumulation au cours du cycle cellulaire chez *E. coli* apparaissent indépendamment du cycle de la replication de l'ADN. [Traduit par le journal]

Introduction

In vivo deoxyribonucleic (DNA) and ribonucleic acid (RNA) synthesis requires the presence of precursor nucleoside triphosphate (NTP) pools.[4] Metabolic pathways leading to the formation of these precursors in *Escherichia coli* are now established (10). It has been shown that in synchronous cultures of *E. coli* B/r, a functional relationship between NTP pools and the DNA replication – cell division cycle exists (6). It was felt that changes in these pools could be evaluated in terms of the synchronous cell cycle when DNA replication was inhibited. We employed nalidixic acid (NAL), a specific inhibitor of DNA synthesis in bacteria (3), which apparently does not affect the dNTP pool levels of exponential cells of *E. coli* 15T$^-$ (9). It was hoped that NAL would not perturb the pools in synchronous cultures and would allow the monitoring of changes in NTP's to see if they are specifically associated with the cell or DNA replication cycle of *E. coli* B/r. We present results indicating that changes in the NTP levels during the cell cycle occur independently of DNA replication cycles.

Materials and Methods

General Experimental Plan

We used synchronous cultures of *E. coli* B/r with a 40-min generation time at 37°C. Newborn (zero age) cells in a synchronous culture contain half-replicated chromosomes (2, 5). Rounds of chromosome replication are completed at 20 min after the harvest of the cells. Cell division occurs at a cell age of 1.0, or about 20 min after the termination of a round of replication (2, 5).

NAL was used preferentially to block DNA synthesis (3) when administered to the synchronously growing cells. This inhibitor was attractive, since NAL-treated cells continue to grow and increase in cell mass identically as do untreated cultures.

Bacterial Growth and Cell Number Determination

The basal medium, low in orthophosphate, described previously (6), with 0.2% glucose (w/v) as a carbon source,

[1]Received November 20, 1973.
[2]Present address: Department of Microbiology, University of Massachusetts Medical School, 419 Belmont Street, Worcester, Mass. 01604.
[3]Present address: Fisheries Research Board, 6640 N.W. Marine Drive, Vancouver 8, British Columbia.
[4]Abbreviations: ATP, CTP, GTP, and UTP are the ribonucleoside triphosphates, and dATP, dCTP, dGTP, and dTTP are the deoxyribonucleoside triphosphates for the bases adenine (A), cytosine (C), guanine (G), uracil (U), and thymine (T).

was used. The strain used, *E. coli* B/r/1 (ATCC 12407), was grown and synchronized with the use of a 0.45-μm Millipore filter by the method of Helmstetter and Cummings (4) as described previously (2, 6). Cell counts and NAL treatment were carried out as described earlier (8). NAL was generously supplied by Dr. W. Goss of the Sterling–Winthrop Research Foundation (Rensselaer, N.Y.).

Preparation and Extraction of Nucleotide Pools

Labelling of the nucleotides was performed as given in detail in a previous paper (6). In summary, synchronized cells were labelled with $^{32}P_i$ (orthophosphate-free) at a final specific activity of 100 μCi of $^{32}P_i$ per micromole of phosphate. Subsequent incubation of the cells for 30 min at 37°C sufficiently equilibrates the pools (6). Acid-soluble pool material was extracted and prepared for thin-layer chromatography as described previously (6, 7).

Separation of Nucleotides

This was carried out by two-dimensional chromatography on poly(ethyleneimine)-impregnated cellulose (MN 300) layered plates prepared according to Randerath and Randerath (11). Extracted pools of 100 μl were spotted on a plate. The solvents for the stepwise elution were as follows. The first dimension consisted of two runs, the first in 1 N acetate : 1 M LiCl (1:1, v/v), and the second in 1 N acetate : 1.5 M LiCl (1:1, v/v). A wick made from Whatman No. 3 paper was used to allow the solvent to "run over" the plate for better separation. The second run in the first dimension took 5.5 h. After the second run, the plate was allowed to dry and was washed with methanol to remove the LiCl. The solvent for the second dimension was 3 M ammonium acetate and 4.3% borate, pH 7.0, and the run was continued for 4.5 h.

Localization and quantitation of nucleotides was accomplished by detecting the unlabelled nucleoside triphosphate markers on the chromatographic plates under a Mineralight lamp and by autoradiography (6). The areas showing radioactivity were clearly seen as exposed spots on X-ray film and matched with the UV-absorbing marker nucleotides. Quantitation of nucleotides was carried out by determining radioactivity cochromatographed with each marker nucleotide in a dioxane-BBOT scintillation fluid (Packard Instruments, Downers Grove, Ill.) in a Unilux scintillation counter (Nuclear-Chicago Corp.).

Chemicals

Orthophosphate-free $^{32}P_i$ was purchased from Tracerlab; the nucleotide references were obtained from Calbiochem Company (Los Angeles, Calif.); poly(ethyleneimine) was obtained from Chemirad Corporation of Canada; and cellulose MN 300 HR was from Canlab.

Results and Discussion

The synthesis of DNA from the nucleotides goes from ribonucleoside diphosphate to the deoxy form and is then converted to the deoxyribonucleoside triphosphate, which is utilized directly for DNA production. The ribonucleoside diphosphate may also be converted to the triphosphate, which is used in RNA synthesis. These reactions occur during the normal cell cycle and require the synthesis and utilization of pools of the ribo- and deoxyribo-nucleotides. The pools of nucleotides change during the cell cycle (6) but the reasons for these fluctuations have not been determined. We examined the role of DNA replication in determining the levels of the pools. If NTP concentrations in the cell are regulated by the DNA replication cycle, NTP's should accumulate when DNA synthesis is blocked. For this purpose, we used NAL as a specific inhibitor of DNA replication, although the precise mechanism of action of the drug is unknown (1).

Figure 1 illustrates the results presented earlier

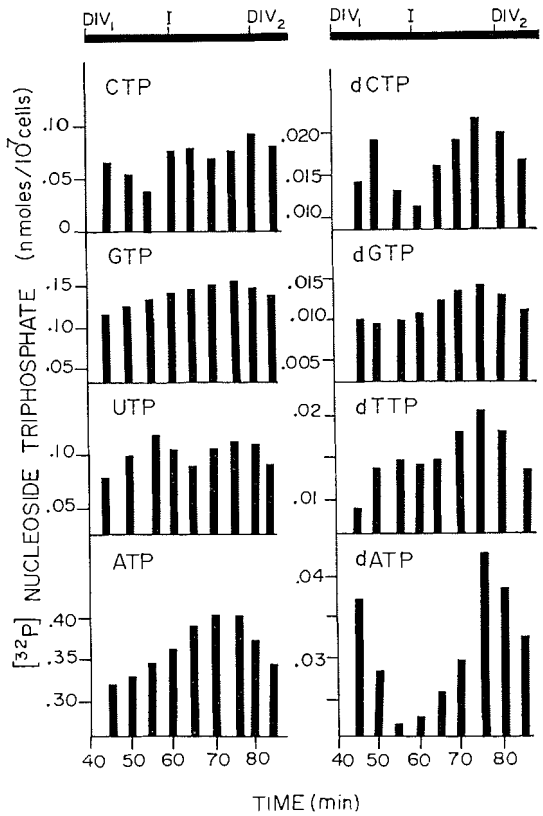

FIG. 1. Changes in the ribonucleoside triphosphate (left) and deoxyribonucleoside triphosphate (right) pools during the cell cycle of glucose-grown cells of *E. coli* B/r are shown. The ordinate represents nanomoles of ^{32}P-labelled pools for individual nucleotides as indicated in each quadrant; the abscissa indicates the cell age in minutes. In the upper part of each panel, the black bar indicates the timing of initiation (I) of DNA replication cycles and consecutive cell division (DIV) events.

(6) on the patterns of the pools during the division cycle of synchronous cells, and represents the trends of pool accumulation and depletion. The NTP pool levels changed during the cell cycle in accordance with the division cycle, and shared some common features in their patterns. The point at which the dNTP pool levels were lowest occurred at the approximate time for initiation of a new round of replication, as can be seen for initiation of the second round at 60 min. Under the growth conditions used, the end of the round and initiation and start of a new round occurred very closely together at this point (5). The low levels could reflect depletion due to completion of the ongoing round of replication. CTP and possibly UTP (at 65 min) seemed to exhibit this characteristic. In both rNTP and dNTP pools, the highest levels occurred at the time of division, which is shown here as the second division at 80 min. UTP, however, had an equally high level at about 55 min of cell age. From these trends and from those presented in some detail previously (6), it seemed plausible that the changes in the pools reflect the cell growth but do not eliminate their dependence on the DNA cycle.

Figure 2 shows the division pattern of control and NAL-treated synchronous cells, the time during the division cycle at which samples for pool analysis were taken, and the comparative chromosome patterns for each of the cultures being examined. It can be seen that when NAL was added at the beginning of the first division, only one complete cycle of division was observed, after which cell numbers plateaud. This indicates that cell division does progress to completion in the presence of NAL, since the rounds of replication were completed before treatment. This is consistent with our earlier report (8).

The samples for analysis of pools during the cycle were taken at the times indicated in Fig. 2 for both treated and untreated cultures. These times were chosen for two reasons: first, they represent cells taken at intervals of one-third of the generation time from the time of the first division to that of the second; and second, if inhibition of the DNA replication cycle affected the accumulation and (or) utilization of the pools, a change from the normal pattern would be easily recognizable.

Results in Fig. 3 show the comparative levels of NTP's in samples taken at the times indicated

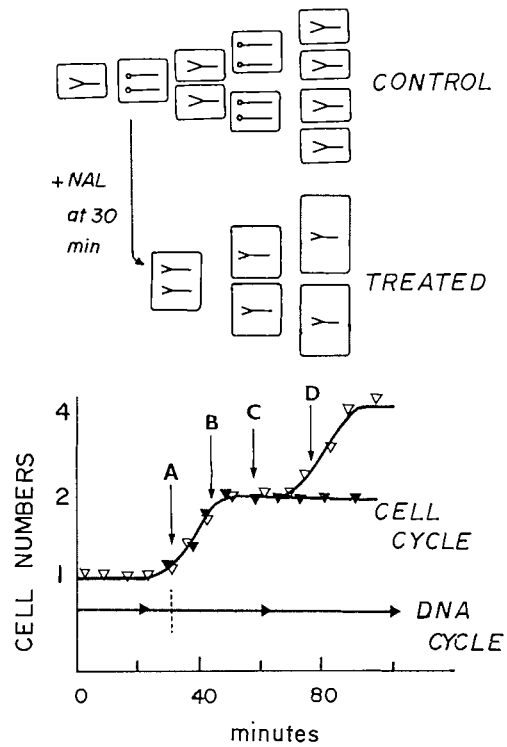

FIG. 2. Relationship between cell and DNA cycles in synchronous cultures of *E. coli* B/r. Theoretical patterns for cell, size cell division, and chromosome configuration are shown in the upper graph, and relate to the cell ages as fractions of the generation time during the cycle for control and NAL-treated cells. Chromosomes are represented as single lines for clarity. Increases in cell numbers measured by Coulter Counter for synchronous cultures grown in the presence of $^{32}P_i$ for control (▽) and NAL-inhibited (▼) cultures are shown in the bottom. The arrows at A, B, C, and D represent sampling times for the nucleotide pool analysis shown in Fig. 3. The timing of initiation of DNA synthesis (→) and NAL treatment (dotted line) are shown relative to the DNA replication cycle. The initial cell density was 3×10^7 cells per milliliter.

in Fig. 2. The levels of rNTP's were twofold to fourfold greater than the corresponding dNTP's in both treated and untreated cultures. Other than a slight increase in GTP and dGTP, and a slight decrease in dATP, dTTP, and UTP in NAL-treated cultures, pool levels were essentially identical with those of the control cultures.

If the cells had continued to synthesize dNTP's while chromosome replication was blocked, there would have been a marked increase in the amounts of ^{32}P-labelled NTP pools. It is apparent that when DNA synthesis is inhibited during the cell cycle, the production of dNTP's is reduced or an equilibrium between synthesis

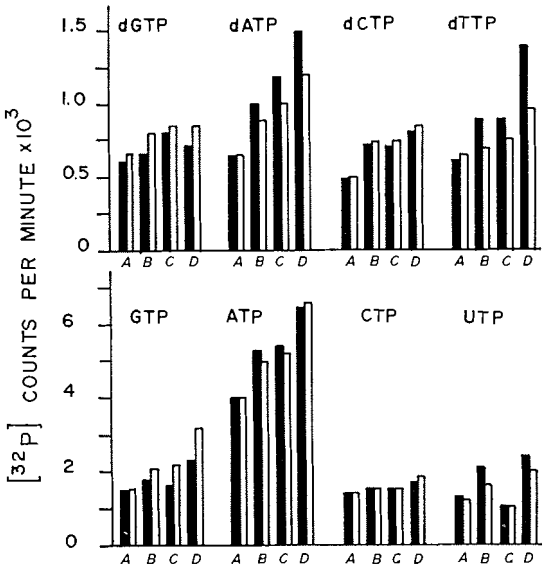

FIG. 3. Distribution of radioactivity in the acid-soluble nucleoside triphosphate pool materials. The amounts of $^{32}P_i$-labelled deoxyribo- or ribo-nucleoside triphosphates recovered from thin-layer chromatograms (adjusted to 3×10^7 cells per milliliter and normalized for backgrounds) for synchronous cultures of *E. coli* B/r (shaded bars) and treated with NAL (white bars) are shown. Ordinate: sampling time as described in the legend to Fig. 2.

and degradation of the pools is effective in maintaining the pools at their normal levels. This could be regulated via cell growth or other mechanisms in the cell cycle. The patterns of the pool levels (6; Fig. 3) in synchronous cultures of *E. coli* B/r therefore must be a complex process dependent on the regulation of NTP biosynthesis and turnover, and on cell growth. Measurement of the periodic enzyme synthesis and activities associated with these reactions should further help us visualize the behavior of these precursors.

Acknowledgment

This research was supported by the National Research Council of Canada (grant No. A4419 to Dr. D. J. Clark). We thank L. M. McGrath for help in preparation of the manuscript.

1. BOURGUIGNON, G. J., M. LEVITT, and R. STERNGLANZ. 1973. Studies on the mechanism of action of nalidixic acid. Antimicrob. Agents Chemother. **4**: 479–486.
2. CLARK, D. J. 1968. The regulation of DNA replication and cell division in *E. coli* B/r. Cold Spring Harbor Symp. Quant. Biol. **33**: 823–838.
3. GOSS, W. A., W. DEITZ, and T. M. COOK. 1964. Mechanism of action of nalidixic acid on *Escherichia coli*. J. Bacteriol. **88**: 1112–1118.
4. HELMSTETTER, C., and D. J. CUMMINGS. 1963. Bacterial synchronization by selection of cells at division. Proc. Natl. Acad. Sci. U.S.A. **50**: 767–774.
5. HELMSTETTER, C., S. COOPER, O. PIERUCCI, and E. REVELAS. 1968. On the bacterial life cycle. Cold Spring Harbor Symp. Quant. Biol. **33**: 809–822.
6. HUZYK, L., and D. J. CLARK. 1971. Nucleoside triphosphate pools in synchronous cultures of *Escherichia coli*. J. Bacteriol. **108**: 74–81.
7. IRR, J., and J. GALLANT. 1969. The control of RNA synthesis in *Escherichia coli*. II. Stringent control of energy metabolism. J. Biol. Chem. **244**: 2233–2239.
8. KHACHATOURIANS, G. G., and L. M. MCGRATH. 1973. Analysis of cell division in rapidly growing synchronous cultures of *Escherichia coli* recovering from an inhibition of deoxyribonucleic acid synthesis. Can. J. Microbiol. **19**: 1111–1114.
9. NEUHARD, J., and E. THOMASSEN. 1971. Turnover of the deoxyribonucleoside triphosphate in *Escherichia coli* 15T during thymine starvation. Eur. J. Biochem. **20**: 36–43.
10. O'DONOVAN, G., and J. NEUHARD. 1970. Pyrimidine metabolism in microorganisms. Bacteriol. Rev. **34**: 278–343.
11. RANDERATH, K., and E. RANDERATH. 1967. Thin-layer separation methods for nucleic acid derivatives. *In* Methods in enzymology. Vol. 12, Part A. *Edited by* S. P. Colowick and N. O. Kaplan. Academic Press Inc., New York. pp. 323–347.