Cell Cycle Parameters of *Escherichia coli* K-12

RICHARD ALLMAN,†* TORSTEIN SCHJERVEN, AND ERIK BOYE

Department of Biophysics, Institute for Cancer Research, Montebello, 0310 Oslo 3, Norway

A computer simulation routine was used to calculate the DNA distributions of exponentially growing cultures of *Escherichia coli* K-12. Simulated distributions were compared with distributions obtained experimentally by flow cytometry. Durations of the DNA replication period (C) and the postreplication period (D) were found by minimizing the difference between theoretical and experimental DNA histograms. It was demonstrated that the K-12 strains AB1157 and CM735 had C and D periods that differed widely from each other and from those of the previously measured strain B/rA, while strain MC1000 was shown to have the same durations of the C and D periods as strain B/rA. The variation between K-12 strains may explain the divergence in the literature regarding their C and D periods. Strains W3110 and AB1157 *recA1* had DNA histograms that could not be adequately simulated by the classical Cooper-Helmstetter model, which is consistent with the asymmetrically located origin and terminus for W3110 and the asynchrony of initiation for AB1157 *recA1*.

Most cell cycle studies of bacteria, including *Escherichia coli*, have until recently been performed on synchronized or age-fractionated cultures (10) or indirectly after inhibiting initiation of DNA replication in exponentially growing cultures (8, 16, 24). However, even careful preparation of synchronous cultures introduces the risk that the normal replication and division pattern may be disturbed.

The development of a high-resolution flow cytometer utilizing arc lamp illumination and high-numerical-aperture microscope objectives (23) enables us to perform precise cell cycle analyses of unperturbed, exponentially growing cells. By using such instruments, the DNA content of individual *E. coli* cells can be measured with an accuracy of a few percent (21, 22). This has allowed the DNA replication cycle of *E. coli* B/r to be studied in detail at different growth rates (19), and precise determinations of the durations and variability of the cell cycle periods of *E. coli* B/r were obtained by using theoretical computer simulations of the experimentally produced histograms (20).

A large amount of data has been published on the DNA replication pattern of *E. coli* B/r (10, 12, 19, 20), and there is reasonable agreement about the lengths of the C and D periods, particularly for high growth rates. However, similar data for *E. coli* K-12 strains do not show the same agreement (reviewed in reference 12). In this study, we have determined the cell cycle periods of a number of different *E. coli* K-12 strains growing at different rates. Computer-simulated distributions of DNA content were compared with experimentally obtained histograms of cells in unperturbed, steady-state growth. The results show that K-12 strains as a group are not homogeneous with respect to their DNA replication patterns.

MATERIALS AND METHODS

Growth conditions. *E. coli* K-12 strains AB1157 [F⁻ *thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2* λ⁻ Rac⁻ *hisG4*(Oc) *rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*] (2), MC1000 [*araD139* (*ara leu*)*7697 lacX74*

galU galK strA] (7), CM735 (*metE46 trp-3 his-4 thi-1 galK2 lacY1 mtl-1 ara-9 tsx-3 ton-1 rps-8 supE44* λ⁻) (11), W3110 (F⁻ λ⁻ *thyA36 deoC2* IN1) (2), and AB1157 *recA1* (genotype as for AB1157 plus *recA1*) were grown in AB medium (9) containing 0.2% glucose and 0.2% Casamino Acids or 0.2% glycerol at 37°C. Batch culture doubling times were measured by monitoring the optical density of the culture at 450 nm. Samples were taken from exponentially growing cultures after at least 10 generations of steady-state growth, established by repeated dilution of cultures into flasks containing fresh, prewarmed growth medium.

Fixation and staining of samples for flow cytometry. Samples (1 ml) of cell suspensions were washed in 1 ml of ice-cold 10 mM Tris-HCl (pH 7.6) and fixed in ice-cold 77% ethanol. Samples were stored at 4°C for 24 h before staining. Fixed bacteria were washed twice in ice-cold 10 mM Tris-HCl (pH 7.6) and stained for 1 h at 4°C, using a combination of mithramycin (90 μg/ml) and ethidium bromide (25 μg/ml) in 10 mM Tris-HCl (pH 7.6) containing 10 mM MgCl₂.

Flow cytometry. Forward light scatter and DNA fluorescence were measured simultaneously, using an Argus flow cytometer (Skatron, Norway). DNA fluorescence was collected by using the Argus B1 filter block. System performance was monitored by using 1.75-μm fluorescent latex spheres.

Theoretical DNA distributions. The strategies used in the computer simulation have been previously described (20). Basically, the Cooper-Helmstetter model of DNA replication (10) predicts that if initiations of chromosome replication at multiple origins within a cell are synchronous, and if the rate of replication fork movement is constant, then the rate of DNA synthesis during the cell cycle can be described by a step function with two discontinuities, one at the time of initiation (step up) and the other one at termination (step down). The initiation and termination age and the rate of DNA synthesis as a function of cell age, $dG(a)/da$, have been derived by Cooper and Helmstetter (10). An integration of $dG(a)/da$ with respect to age yields the amount of DNA accumulated per cell, $G(a)$. The frequency of cells with a given DNA content, $n(G)$, in an exponential culture is found by combining $G(a)$ and the exponential age distribution as previously described (20). It may be concluded that in cells in which the simulated DNA distribution closely matches the

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* Corresponding author.

† Present address: Microbiology Group, School of Pure and Applied Biology, University of Wales College of Cardiff, P.O. Box 915, Cardiff CF1 3TL, Wales, United Kingdom.
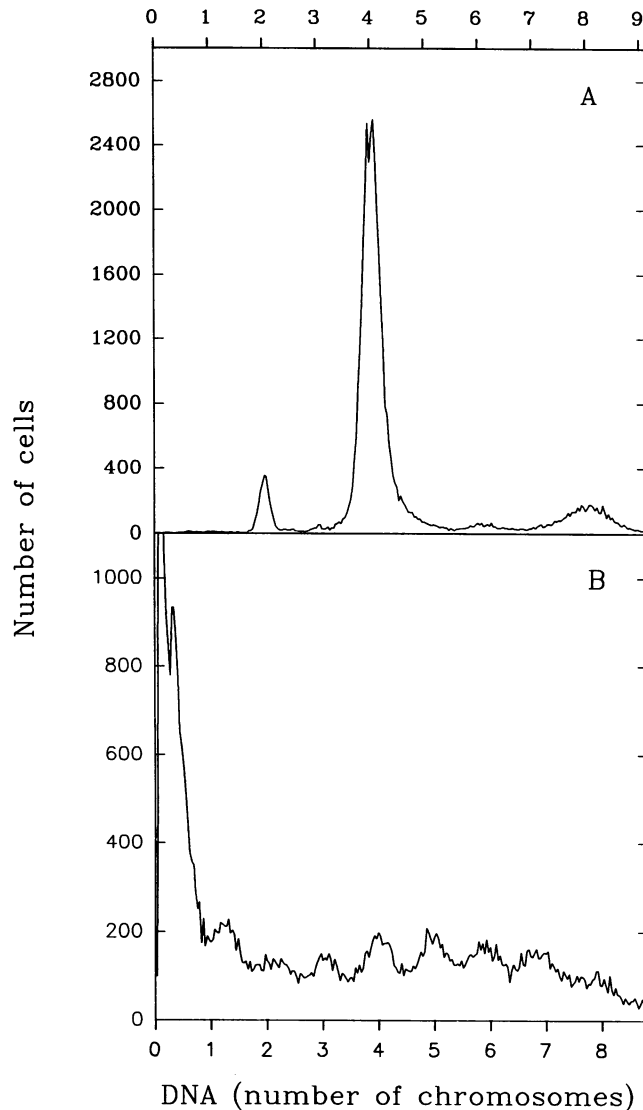
DNA (number of chromosomes)

FIG. 1. Experimentally obtained DNA histograms of exponentially growing cultures of Ab1157 (A) and AB1157 *recA1* (B) grown with doubling times of 28 and 39 min, respectively, and treated with rifampin (125 μg/ml for 90 min).

experimentally obtained values, the DNA replication cycle proceeds in accordance with the model.

Variability in individual generation times. The variability in individual generation times was taken into account, and two models for the variability in the postreplication (D) period were used, as previously described (20). Both models rely on a constant rate of movement of the replication fork and no variation in the DNA replication period from cell to cell. It was not possible to tell which of the models gave a better fit. This was also true of the present set of data. Thus, the data presented refer to only one of the biological variation models, namely, the one assuming an initial (deterministic) part of the postreplication period and thereafter a constant probability of division per unit time (4–6). This model assumes a negative mother-daughter correlation, in that the interinitiation time (time between successive initiations) remains constant.

TABLE 1. Simulation of the DNA histogram for strain AB1157 growing with doubling time of 28 min

D (min)	Value of weighted deviation at given duration (min) of C[a]				
	53	54	55	56	57
21	2.93	2.73	2.55	2.43	2.37
22	2.59	2.44	2.37	2.32	2.33
23	2.38	2.33	<u>2.29</u>	2.35	2.49
24	2.30	2.32	2.38	2.58	2.82
25	2.37	2.49	2.64	2.91	3.18

[a] Values of deviation (*s*) between simulated and experimental histograms for different combinations of C and D. The lowest value for *s* (underlined) represents the optimal fit.

Instrumental and staining variability. Experimental DNA distributions were obtained by staining the cells and measuring the fluorescence intensity of each cell. It was assumed that for cells with exactly the same DNA content, the DNA histogram is represented by a normal distribution of constant relative width, i.e., constant coefficient of variation (CV). This methodological CV was estimated by measuring samples of cells containing integer numbers of chromosome equivalents and determining the CV of the peaks (3). Thus, theoretical DNA distributions were also convoluted with a normal distribution with a CV equal to the methodological CV.

Comparison of simulated and experimental DNA histograms. Theoretical histograms were fitted to experimental data by means of a computer algorithm which minimized the deviation between the respective histograms. The deviation is

$$s = \sqrt{\sum_{i=1}^{m} \frac{(\sqrt{y_i} - \sqrt{N_i})^2}{m-1}}$$

in which

$$\sum_{i=1}^{m} (\sqrt{y_i} - \sqrt{N_i})^2$$

is the sum of the squared differences between the square roots of the *m* individual points of the two data sets. The square roots of y_i and N_i values are used to avoid weighting factors which might lead to divergencies in the minimization procedure. The deviation, *s*, is a measure of goodness of fit.

RESULTS

One assumption in the simulation model used is that all chromosomal origins are initiated in synchrony. A high degree of synchrony was observed for strains AB1157, CM735, W3110, and MC1000 (Fig. 1A). In contrast, strain AB1157 *recA1* did not perform synchronous initiations (Fig. 1B), in accordance with earlier data (18). Simulated DNA histograms (DNA distributions) were compared with experimental histograms of strains AB1157, MC1000, and CM735 grown at two different growth rates. Parameters in the simulation were the time from initiation of DNA replication to termination of the same round of replication (the C period) and the time from termination to cell division (the D period). Values of the weighted deviation, *s*, between simulated and experimental data were obtained for a range of integer values of C and D and for both models of biological variation. When a minimum for *s* was found, C and D were determined more accurately, using combinations of integer and half-minute
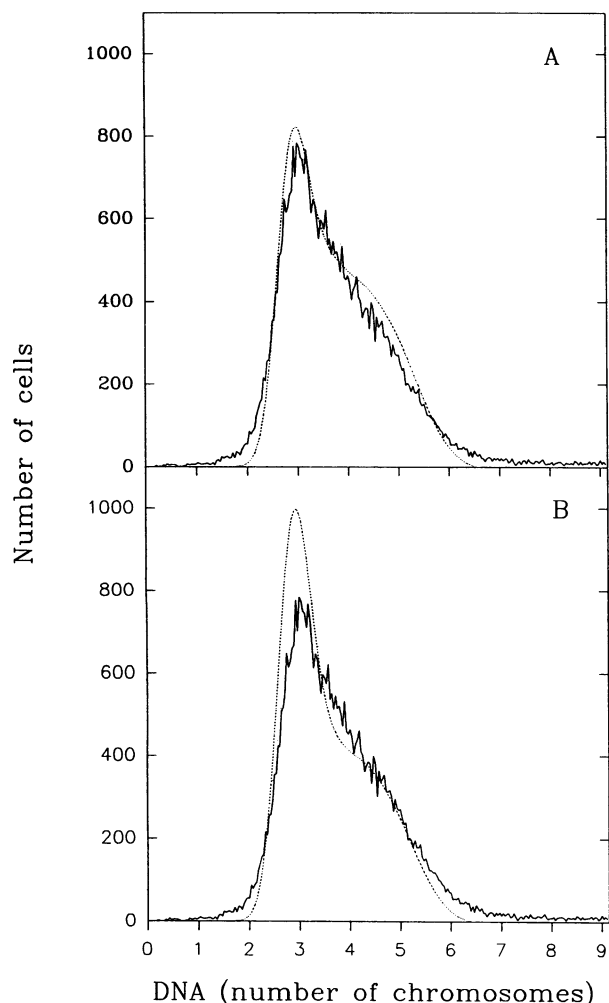
FIG. 2. Experimentally obtained histogram of an exponentially growing culture of strain AB1157 with a doubling time of 28 min (solid line) compared with a computer-simulated histogram (dashed line). (A) Optimal fit (C = 55 min, D = 23 min); (B) nonoptimal fit (C = 50 min, D = 24 min).
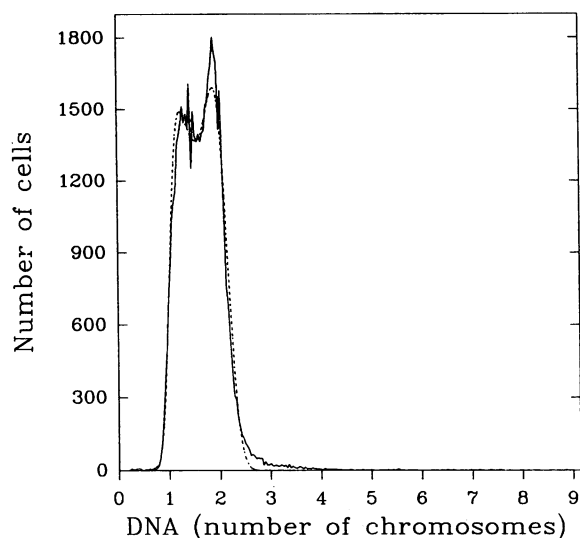

FIG. 3. Experimentally obtained histogram of an exponentially growing culture of strain AB1157 with a doubling time of 113 min (solid line) compared with a computer-simulated histogram (dashed line). Parameters of best fit: C = 77 min, D = 40 min.

values. Table 1 shows the values of the weighted deviation, s, for different values of C and D obtained for a culture of AB1157 growing with a generation time of 28 min. The best fit (the lowest s value) was obtained with C = 55 min and D = 23 min. These parameters gave a very nice fit between experimental and simulated histograms (Fig. 2A). Relatively small differences in parameter values caused significant misfit between theoretical and experimental histograms (Fig. 2B), illustrating that the error is small in the determination of parameter values.

Experimental and theoretical histograms obtained for cultures of AB1157 with a doubling time of 113 min are shown in Fig. 3. In this case, the parameter values were found to be C = 77 min and D = 40 min.

Durations of the C and D periods for AB1157, MC1000, and CM735, each grown at two different growth rates, are summarized in Table 2. The three K-12 strains had clearly different C and D periods. Two of the findings deserve special mention: first, the C period of strain AB1157 is significantly longer than for the two other strains at both high

and low growth rates; second, the D period of strain CM735 is exceptionally long at the higher growth rate.

It was not possible to obtain a good fit with strain W3110 or AB1157 recA1 (Fig. 4 and 5). This result is in accordance with the genetic characteristics of both of these strains: strain W3110 contains a large inversion including oriC (13, 14), which gives one replication fork a shorter distance to the terminus, terC, than the other. Given a constant rate of movement of the replication fork, one would not expect the two forks to reach terC simultaneously. As for strain AB1157 recA1, it is known that recA mutant strains are unable to coordinate initiation of DNA replication at multiple origin sites; initiations are not performed in synchrony (18). These deviations from the assumptions of our theoretical model may be sufficient to explain the lack of fit between theoretical and experimental histograms for the two strains.

DISCUSSION

The computer simulation routine used has been previously demonstrated to provide accurate measurements of cell cycle periods in E. coli B/rA. The results presented in this report demonstrate that the cell cycle periods of E. coli K-12 strains can be similarly determined. The data presented for the three wild-type strains, AB1157, MC1000, and CM735, clearly demonstrate that the different K-12 strains may differ

TABLE 2. Cell cycle periods of wild-type K-12 strains

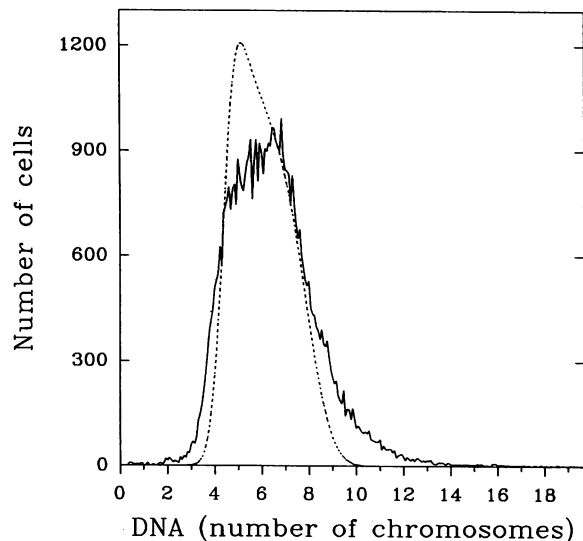| Bacterial strain	Value (min) for:		
	Doubling time	C period	D period
AB1157	28	55	23
AB1157	113	77	40
MC1000	21	41	27
MC1000	68	46	21
CM735	29	44	40
CM735	111	64	43

FIG. 4. Experimentally obtained histogram of an exponentially growing culture of strain W3110 with a doubling time of 26 min (solid line) compared with a computer-simulated histogram (dashed line). Parameters of best fit: C = 76 min, D = 26 min.

widely in their C and D periods. These K-12 strains therefore cannot be considered a homogeneous group in terms of DNA replication control, which may explain the divergence in the values previously reported for C and D in K-12 strains (12). Thus, data for DNA replication kinetics obtained for one K-12 strain are not necessarily valid for another.

Inspection of the experimental and theoretical histograms for strains AB1157, MC1000, and CM735 shows that they deviate from one another at the lower and upper boundaries and also in a region in the middle (for example, the deviation in the shoulder in Fig. 2A). In the latter region are cells that have just initiated DNA replication. A similar deviation has
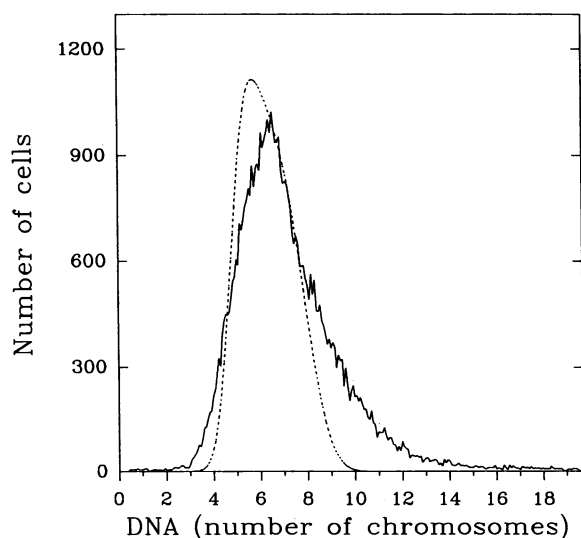

FIG. 5. Experimentally obtained histogram of an exponentially growing culture of strain AB1157 *recA1* culture with a doubling time of 39 min (solid line) compared with a computer-simulated histogram (dashed line). Parameters of best fit: C = 105 min, D = 50 min.

been reported earlier for B/rA (20), and it was suggested that the rate of fork movement is not constant shortly after initiation. Recently, different pieces of evidence indicate that there may be mechanisms to slow down the rate of fork movement in the vicinity of *oriC* (1, 15). The variability in C is probably not caused by different chromosomal sizes, since they differ by less than 10% according to the flow cytometric analyses. Thus, a difference in the rate of fork movement is the most likely reason for the variation in C-period duration. For strains W3110 and AB1157 *recA1*, good fits between experimental and theoretical histograms were impossible to obtain, which is explained by the aberrant DNA replication in these strains. The lack of fit serves as a validation of the method used: violation of the assumption in the theoretical model precludes a meaningful simulation unless the model is changed.

We can conclude that strains AB1157, MC1000, and CM735 but not W3110 or AB1157 *recA1* replicate their DNA as described in the model of Cooper and Helmstetter (10), with synchronous initiation at all origins within a cell and constant rates of fork movement. This has also been shown directly by measuring the number of chromosomes after treatment with rifampin. It is also readily apparent that *E. coli* K-12-type organisms show heterogeneity with regard to their cell cycle periods. This finding emphasizes the need to measure the actual C and D periods for each strain used rather than accepting published values for other strains.

ACKNOWLEDGMENTS

REFERENCES

1. Atlung, T., A. Løbner-Olesen, and F. G. Hansen. 1985. Overproduction of dnaA protein stimulates initiation of chromosome and minichromosome replication in *Escherichia coli*. Mol. Gen. Genet. 206:51–59.
2. Bachmann, B. J. 1987. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p. 1190–1219. *In* F. C. Neidhardt, J. L. Ingraham, B. Magasanik, K. B. Low, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
3. Boye, E., H. B. Steen, and K. Skarstad. 1983. Flow cytometry of bacteria: a promising tool in experimental and clinical microbiology. J. Gen. Microbiol. 129:973–980.
4. Bremer, H. 1982. Variation of generation times in *Escherichia coli* populations: its cause and implications. J. Gen. Microbiol. 128:2865–2876.
5. Bremer, H., and L. Chuang. 1981. The cell cycle in *Escherichia coli* B/r. J. Theor. Biol. 88:47–81.
6. Bremer, H., and L. Chuang. 1981. Cell division after inhibition of chromosome replication in *Escherichia coli*. J. Theor. Biol. 93:909–926.
7. Casabadan, H. J., and S. N. Cohen. 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. 138:179–207.
8. Churchward, G., and H. Bremer. 1977. Determination of deoxyribonucleic acid replication time in exponentially growing *Escherichia coli* B/r. J. Bacteriol. 130:1206–1213.
9. Clark, D. J., and O. Maaløe. 1967. DNA replication and the division cycle in *Escherichia coli*. J. Mol. Biol. 23:99–112.
10. Cooper, S., and C. E. Helmstetter. 1968. Chromosome replication and the division cycle of Escherichia coli B/r. J. Mol. Biol. 31:519–540.
11. Hansen, E. B., T. Atlung, F. G. Hansen, O. Skovgaard, and K. von Meyenburg. 1984. Fine structure, genetic map, and complementation analysis of mutations in the DnaA gene of *Escherichia coli*. Mol. Gen. Genet. 196:387–396.

12. **Helmstetter, C. E.** 1987. Timing of synthetic activities in the cell cycle, p. 1594–1605. *In* F. C. Neidhardt, J. L. Ingraham, B. Magasanik, K. B. Low, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.

13. **Hill, C. W., and B. W. Harmish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:7062–7069.

14. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the *E. coli* chromosome: applications of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.

15. **Løbner-Olesen, A., K. Skarstad, F. G. Hansen, K. von Meyenburg, and E. Boye.** 1989. The dnaA protein determines the initiation mass of *Escherichia coli* K12. Cell **57**:881–889.

16. **Pritchard, R. H., and A. Zaritsky.** 1970. Effect of thymine concentration on the replication velocity of DNA in a thymineless mutant of *E. coli*. Nature (London) **226**:126–131.

17. **Skarstad, K., E. Boye, and H. B. Steen.** 1986. Timing of initiation of chromosome replication in individual *Escherichia coli* cells.

EMBO J. **5**:1711–1717.

18. **Skarstad, K., and E. Boye.** 1988. Perturbed chromosomal replication in *recA* mutants of *Escherichia coli*. J. Bacteriol. **170**:2549–2554.

19. **Skarstad, K., H. B. Steen, and E. Boye.** 1983. Cell cycle parameters of slowly growing *Escherichia coli* B/r studied by flow cytometry. J. Bacteriol. **154**:656–662.

20. **Skarstad, K., H. B. Steen, and E. Boye.** 1985. *Escherichia coli* DNA distributions measured by flow cytometry and compared with theoretical computer simulations. J. Bacteriol. **163**:661–668.

21. **Steen, H. B., and E. Boye.** 1980. Bacterial growth studied by flow cytometry. Cytometry **1**:32–36.

22. **Steen, H. B., and E. Boye.** 1981. *Escherichia coli* growth studied by dual-parameter flow cytophotometry. J. Bacteriol. **145**:1091–1094.

23. **Steen, H. B., and T. Lindmo.** 1979. Flow cytometry: a high resolution instrument for everyone. Science **204**:403–404.

24. **Zaritsky, A., and R. H. Pritchard.** 1971. Replication time of the chromosome in thymineless mutants of *Escherichia coli*. J. Mol. Biol. **60**:65–74.