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Analysis of Cell Size and DNA Content in Exponentially Growing and Stationary-Phase Batch Cultures of *Escherichia coli*

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***Escherichia coli* strains were grown in batch cultures in different media, and cell size and DNA content were analyzed by flow cytometry. Steady-state growth required large dilutions and incubation for many generations at low cell concentrations. In rich media, both cell size and DNA content started to decrease at low cell concentrations, long before the cultures left the exponential growth phase. Stationary-phase cultures contained cells with several chromosomes, even after many days, and stationary-phase populations exclusively composed of cells with a single chromosome were never observed, regardless of growth medium. The cells usually contained only one nucleoid, as visualized by phase and fluorescence microscopy. The results have implications for the use of batch cultures to study steady-state and balanced growth and to determine mutation and recombination frequencies in stationary phase.**

When the growth and physiology of *Escherichia coli* cells are studied, batch cultures or continuous cultures may be used. Batch cultures are easy to set up, whereas continuous cultures, which include turbidostats and chemostats, require more complicated equipment and are therefore less commonly used.

When batch cultures are used, *E. coli* cells from an overnight culture are usually inoculated into Erlenmeyer flasks containing a complex or a defined medium. The flasks are incubated in shaking or rotary water baths at 37°C until the cell concentration reaches 10^7 to 10^8 cells per ml, whereafter the cells are studied. The state of a population in a batch culture may be divided into several phases (5). When stationary-phase cells are inoculated into fresh medium, there is an initial lag phase with little increase in cell mass. After some time, growth accelerates, a period of unrestricted growth begins, and the exponential phase starts. When cultures enter the stationary phase, growth declines and the metabolic activity is reduced.

The growth parameters of bacterial cultures may be described as either extensive or intensive. The extensive parameters include the amounts of the various components of the population, such as protein and DNA. Intensive parameters include the distributions of the extensive parameters, such as the distributions of cells with different protein or DNA contents. Examples of other intensive parameters are the distributions of cell size and cell age.

During unrestricted growth, all extensive properties of the culture increase by the same proportion over a given time interval, a condition known as balanced growth (6). If, for instance, the total protein content increases by the same factor over a given time interval, growth is balanced with respect to this parameter. It should be emphasized that balanced growth refers to the average behavior of the cells in a population and not to the behavior of individual cells. A cell goes through the different discontinuous processes of the cell cycle, such as chromosome replication, nucleoid partition, and cell division. Individual cells are therefore different, both in size and in metabolic activity, from one time point to another.

In steady state, the intensive parameters are time invariant (19). For instance, the distribution of cell sizes does not change over time. Steady state implies that the culture is exponentially growing and in balanced growth. In contrast, exponentially growing cultures need not display balanced growth, and cultures in balanced growth are not necessarily in a steady state. The latter statement is illustrated by the fact that cell division can be blocked without immediately interfering with growth; after the blockage, the biomass increases at the same rate whereas the cell size distribution changes over time. The terms steady state, balanced growth, and exponential growth have not been consistently used (for a discussion of this subject, see reference 8). Finally, it should be noted that these considerations have little relevance for the natural growth of *E. coli* populations. For example, in their natural environment (discussed in reference 20), the cells usually grow on surfaces, often under anaerobic conditions, and nutrient availability and other growth parameters change continuously and abruptly such that unrestricted growth seldom, if ever, occurs.

When unrestricted growth no longer can be maintained, the growth rate of the culture declines and the cells eventually enter the stationary phase. When this occurs depends on the medium used; generally, a culture grows exponentially up to a cell concentration of about 10^8/ml (15). During entry into stationary phase, the cells become smaller by repeated division. Also, initiation of chromosome replication stops, but elongation continues to termination; stationary-phase cells thus contain integer numbers of chromosomes. It is often assumed that a majority of the rounds of chromosome replication lead to cell division after some time in stationary phase; all or most stationary-phase cells should therefore eventually contain a single chromosome (reviewed in reference 17). It has, however, been reported that overnight cultures may contain cells with several chromosomes (2).

We have investigated various *E. coli* strains growing over a wide range of cell concentrations in batch cultures. The cells were grown in different media, and the cell size and DNA content were analyzed by flow cytometry. In rich medium, the cell size and DNA content started to decrease at cell concen-

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TABLE 1. *E. coli* strains used in this study

Strain	Genotype	Reference
PB103	*dadR trpE trpA tna*	7
EC1005	*metB1 nalA relA1 spoT1* λ^r F^-	11
MG1655	F^- λ^-	12
B/r A		13

trations that were considerably lower than that at the point at which the cells left the exponential growth phase. Also, the majority of stationary-phase cells grown in rich medium contained several chromosomes even after several days of incubation. These results have implications for the use of batch cultures to study balanced growth and steady state, as well as for studies of mutation and recombination frequencies in stationary phase.

MATERIALS AND METHODS

Strains. The *E. coli* strains used are listed in Table 1.

Media and growth conditions. The cells were grown in Luria-Bertani (LB) medium containing 0.2% (wt/vol) glucose or in minimal (M9) medium containing either 0.2% glucose and 0.5% Casamino Acids, 0.2% glucose, or 0.2% sodium acetate (16). When LB medium or M9 medium containing glucose and Casamino Acids was used, the cultures were grown overnight, and the stationary-phase cells were diluted 10^5- to 10^7-fold in fresh medium in the morning. When M9 medium containing glucose or acetate was used, the cultures were diluted 10^7-fold in the evening and grown overnight, and samples were collected during the following days. Growth was measured as CFU on solid medium or as optical density at 550 nm (OD_{550}) on a Novaspec II spectrophotometer (LKB).

Flow cytometry. Samples for flow cytometry (0.05 to 2 ml) were, when LB medium was used, fixed in ethanol (70% final concentration) or, when M9 medium was used, centrifuged for 5 min and resuspended in 0.4 ml 10 mM Tris (pH 7.4), whereafter 99% ethanol was added to a final concentration of 70%. Prior to flow cytometry, the cells were centrifuged for 5 min and resuspended in 10 mM Tris (pH 7.4) containing 10 mM $MgCl_2$. After recentrifugation, the buffer was removed and the cells were resuspended in Tris-$MgCl_2$ buffer, whereafter 20 to 40 μl of the suspension was added to an equal volume of staining solution containing ethidium bromide (40 μg/ml) and mithramycin A (200 μg/ml) dissolved in the same buffer. The cells were kept dark at 4°C for about 2 h prior to flow cytometry. Calibration beads of uniform size and fluorescence (1.5-μm diameter; Bio-Rad) were included in each sample to monitor instrument performance. The samples were analyzed on an Argus 100 flow cytometer (Skatron). Small-angle forward light scatter was used as a measure of cell size, and DNA content was measured as fluorescence after staining with ethidium bromide and mithramycin A (see above). The DNA scale was calibrated by using cells with known DNA content. The software (ARGUS and AVN87, developed by Skatron and Torstein D. Schjerven, respectively) allowed further calculations, such as of average size and DNA content, as well as studies of the cell size distributions of subpopulations with different DNA contents to be performed (see Fig. 4). The theoretical and practical aspects of flow cytometry on microorganisms were recently reviewed by Skarstad et al. (21).

Microscopy. For microscopic studies, DNA staining was performed essentially as described in reference 1; DAPI (4′,6-diamidino-2-phenylindole; 0.5 μg ml^{-1}) was added to the cultures, which were further incubated for 30 min before the cells were transferred to microscope slides covered with thin 1% agar layers, containing the same growth medium. The cells were analyzed in a Nikon Optiphot-2 microscope, using simultaneous phase and fluorescence microscopy, and the images were digitalized by using a cooled charged-coupled device camera (Meridian) connected via an integrating device (Meridian model 440A) to a computerized image analysis system (software and hardware from Bergströms Instruments). The digitalized images were printed with a Sony UP-860/CE video printer.

RESULTS

Experimental outline. During flow cytometry studies of *E. coli* batch cultures, we observed that the cell size and DNA content distributions varied when the samples were collected at different ODs during the exponential growth phase. Similar observations were reported by Skarstad et al. (22). We therefore carefully investigated cell size and DNA content over a wide range of cell concentrations in batch cultures to determine whether cells in exponential growth phase also display steady-state growth (see the introduction). In all experiments, 1,000 ml Erlenmeyer flasks containing 100 ml of medium were used, and the cultures were incubated at 37°C in thermostatically regulated rotary water baths at 200 rpm. The flasks were inoculated from overnight cultures grown in the same medium. Growth was measured as OD_{550} and as CFU by spreading cells on agar plates containing growth medium. The cultures were usually diluted 10^7-fold to allow the cells to undergo at least eight generations before sampling.

After fixation and DNA staining, cell size and DNA content were measured by flow cytometry as light scatter and fluorescence, respectively (reviewed in reference 21). The DNA content scale was calibrated with cells of known DNA content, either stationary-phase cells or cells treated with rifampin, which results in replication runout and thus a DNA content corresponding to full chromosome equivalents. In contrast, light scatter is a complex parameter, and absolute cell size is not easily calculated from the light scatter signal. However, relative cell mass, which is the parameter considered here, can be considered to be roughly proportional to light scatter (3).

Cell size and DNA content in exponentially growing batch cultures. An overnight culture of strain MG1655 was diluted 10^7-fold into prewarmed LB medium containing 0.2% glucose, which yielded a doubling time of 19 min during exponential growth. Samples were withdrawn at regular time intervals and analyzed by flow cytometry (Fig. 1). Between 265 and 355 min after inoculation, the distributions and shapes of the flow profiles were nearly identical, indicating that the culture was in steady state, at least with respect to these parameters. After the 355-min time point (OD_{550} = 0.014), the average cell size and DNA content started to decrease. At 1,680 min, the cells were small and contained fully replicated chromosomes, showing that the culture had reached stationary phase.

To analyze cell size and DNA content in more detail (see Materials and Methods), the average size and DNA content were calculated at each time point from flow profiles of the kind shown in Fig. 1 and plotted against time together with the growth curve (Fig. 2A). Up to 260 min after the initial dilution, there was an increase in the size and DNA content of the cells, indicating that steady state had not been achieved. Thereafter, between 260 and 350 min, the values were nearly constant. At an OD of 0.02 (about 2×10^6 cells per ml), the cell size and DNA content started to decrease, and they continued to decrease gradually during the entire remainder of the growth curve even though the growth curve was exponential up to an OD of at least 0.2 (2×10^7 cells per ml). The experiment was performed three times, using the same growth conditions (see Experiment outline above), and the cell size and DNA content started to decrease between ODs of 0.02 to 0.04 in each experiment (data not shown).

Similar results were obtained in M9 medium containing 0.5% Casamino Acids and 0.2% glucose, in which the doubling time was about 30 min. An overnight culture was diluted 10^5-fold into prewarmed medium, whereafter samples for growth measurement and flow cytometry were withdrawn at different time points. During steady state, the cells were about twofold smaller than those grown in LB medium (Fig. 2B). Again, the cell size and DNA content started to decrease at an OD of about 0.02 (6×10^6 cells per ml), whereas the growth curve was exponential up to an OD of at least 0.1 (3×10^7 cells per ml). The experiment was performed twice, and the DNA content and cell size parameters decreased between ODs of 0.02 to 0.04 (not shown).

When M9 medium containing 0.2% glucose was used (Fig. 2C), the growth curve was exponential up to an OD_{550} of 0.6 to 0.8, which corresponded to a cell concentration of about 4 ×

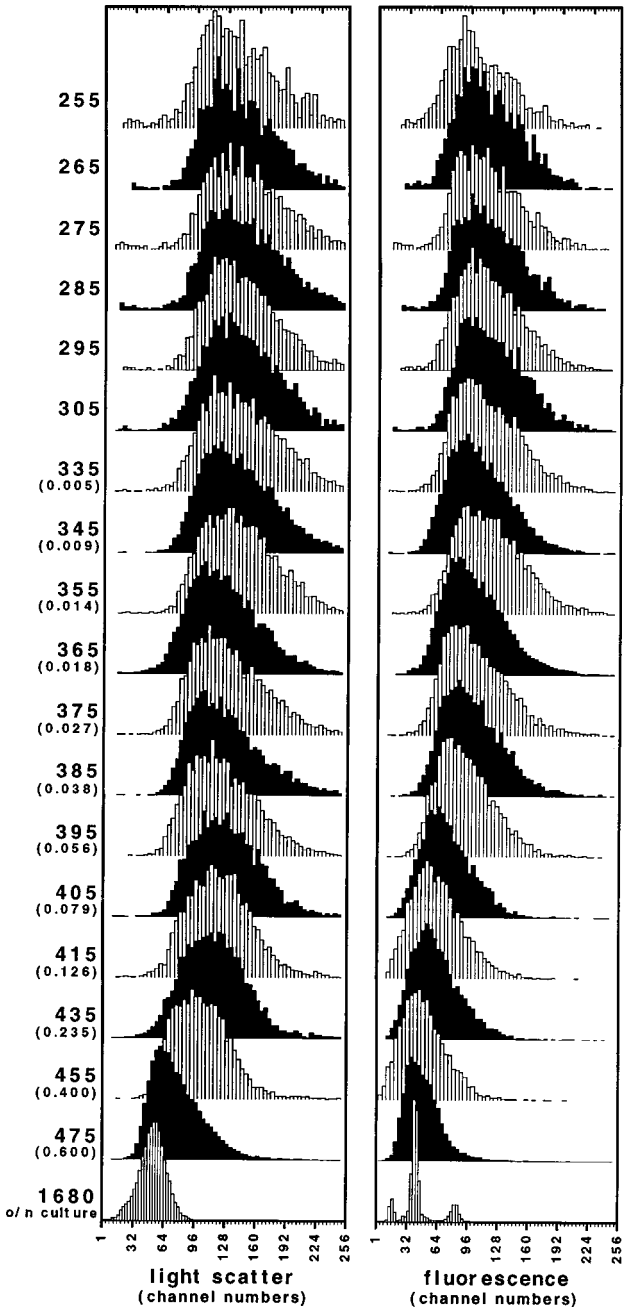

FIG. 1. Flow cytometry profiles of *E. coli* cells. At time zero, a stationary-phase culture of strain MG1655 was diluted 10^7-fold into fresh LB medium containing 0.2% glucose and grown at 37°C. The cells were collected at different time points during growth and analyzed by flow cytometry. The time points (minutes) and OD_{550} of the culture (in parentheses) are indicated to the left. The light scatter and fluorescence parameters represent the relative cell size and DNA content of the cells, respectively, in arbitrary units (instrument channel numbers). The *y* axis represents the number of cells.

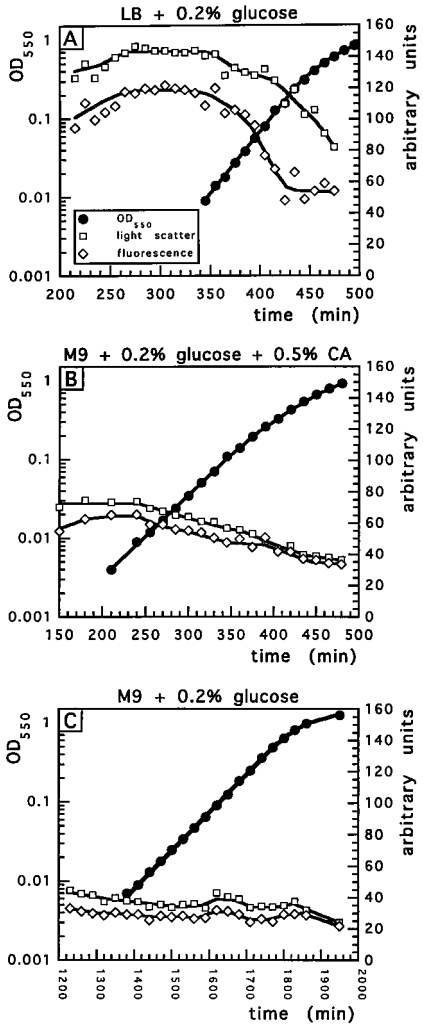

FIG. 2. Size and DNA content of *E. coli* cells in different media. A stationary-phase culture of strain MG1655 was diluted 10^7-fold (A and C) or 10^5-fold (B) into fresh medium and grown at 37°C. Samples were collected during growth, whereafter cell size (light scatter) and DNA content (fluorescence) were analyzed by flow cytometry (see Fig. 1). Growth (OD_{550}) and average light scatter and fluorescence values (i.e., the area under the curves in Fig. 1) were plotted against time. CA, Casamino Acids.

10^8/ml. The doubling time was about 63 min. The average cell size was about fourfold smaller at steady state than in LB medium. The changes in cell size and DNA content were less apparent in this medium, showing a small decrease up to an OD of 0.03, whereafter some fluctuations occurred. However, these variations, including the initial decrease, were small and within the error limits of the measurements, since these small fluctuations varied between individual experiments (±5 arbitrary units). The cell size and DNA content started to decrease significantly at an OD_{550} of 0.8, i.e., at the end of the exponential growth phase.

In conclusion, cell size and DNA content started to decrease at a very low cell concentration in the exponential growth phase when rich media were used, whereas the decrease was less pronounced in M9 medium with glucose.

Cell size and DNA content in batch cultures of other strains. To investigate the generality of the observations, we analyzed the cell size and DNA content of two other *E. coli* K-12 strains, PB103 and EC1005, as well as the commonly used strain B/r A (Table 1). The experiments were performed in LB medium containing 0.2% glucose as described above (Fig. 1 and 2), and the average cell size and DNA content were plotted against time (Fig. 3). The doubling time was about 20 min in all strains. The changes in the cell size and DNA content parameters during the exponential growth phase were similar to those of strain MG1655 (Fig. 2A), although there were minor differ-

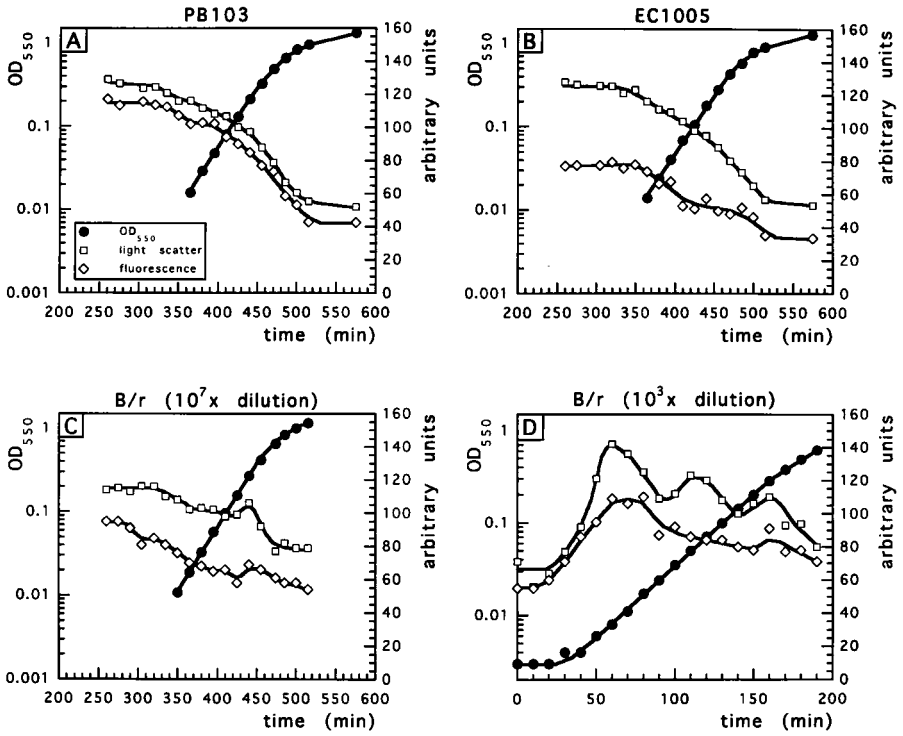

FIG. 3. Cell sizes and DNA contents of different *E. coli* strains. Stationary-phase cultures of different strains were diluted 10^7-fold (A to C) or 10^3-fold (D) into fresh LB medium containing 0.2% glucose and grown at 37°C. Growth (OD_{550}) and average light scatter and fluorescence values (arbitrary units) were plotted against time (see Fig. 2).

ences between the strains (Fig. 3A to C). The cell size and DNA content started to decrease at ODs of approximately 0.005 to 0.02, indicating that this decrease is a general phenomenon among *E. coli* strains. The growth experiments were performed twice with strains EC1005 and B/r A, and there were no significant differences between the experiments (data not shown).

Inoculum size. In the experiments described above, the initial dilutions of the cultures were high (10^5- to 10^7-fold). Commonly, considerably lower dilutions, in the range of 50- to 1,000-fold, are used in batch culture experiments. We therefore diluted an overnight culture of strain B/r A 10^3-fold into prewarmed LB medium containing 0.2% glucose. Cell size and DNA content were measured during the entire growth curve (Fig. 3D). After a lag phase of about 40 min, there was an exponential increase in OD. The cell size and DNA content increased and reached a peak at 60 to 70 min after the initial dilution. Thereafter, both parameters fluctuated significantly, and these variations differed between individual experiments (data not shown). Thus, even with this relatively high dilution, the cells never entered steady state despite the exponential increase in the OD.

Cell size and DNA content in stationary-phase cultures. We have often observed that a majority of the cells in a stationary-phase culture contain several chromosomes (Fig. 1). This needs to be emphasized, since it is often assumed that cells that enter stationary phase continue to grow and divide until all, or most, cells eventually end up with a single chromosome. To study this further, cultures were sampled for several days after entry into stationary phase and analyzed by flow cytometry. Strain MG1655 was diluted 10^7-fold into fresh LB medium containing 0.2% glucose, and in stationary phase, 28 h after the initial dilution, the cells were small and contained mainly two, four, or eight fully replicated chromosomes (Fig. 4). The culture had been growing exponentially for approximately 7 h (Fig. 2A), and the cells had thus been in stationary phase for more than 20 h. After one more day, a new peak corresponding to one chromosome appeared, and the cell size had decreased compared with the overnight culture. There were slight changes in the DNA distribution over the following days, but even after 6 days, a majority of the cells contained more than one chromosome.

The size distributions of the cells that contained different numbers of chromosomes were analyzed in more detail (Fig. 4C; see Materials and Methods). In the 28-h culture, there was a correlation between DNA content and cell size; the smallest cells contained two chromosomes, the medium-sized cells contained four, and the largest cells contained eight. However, in the 52- and the 76-h cultures, the cell size distributions of the cells that contained two or four chromosomes were similar, since the average size of the cells containing four chromosomes had decreased, while the average size of the cells containing two chromosomes had increased. This may indicate that the larger cells that contained four or eight chromosomes were capable of dividing whereas the smaller cells were not. At the 148-h time point, the cells containing two chromosomes were on the average larger than those containing four chromosomes.

When the same experiment was performed with cells grown in M9 medium containing glucose, 60 to 65% of the cells contained one chromosome and 35 to 40% contained two chromosomes after 44 h (Fig. 5). Note that the light scatter and fluorescence scales are the same as for those experiments in which the cells were grown in LB with glucose (Fig. 4), and the experiments are thus directly comparable. There was little change in cell size and DNA content over the following 2 days,

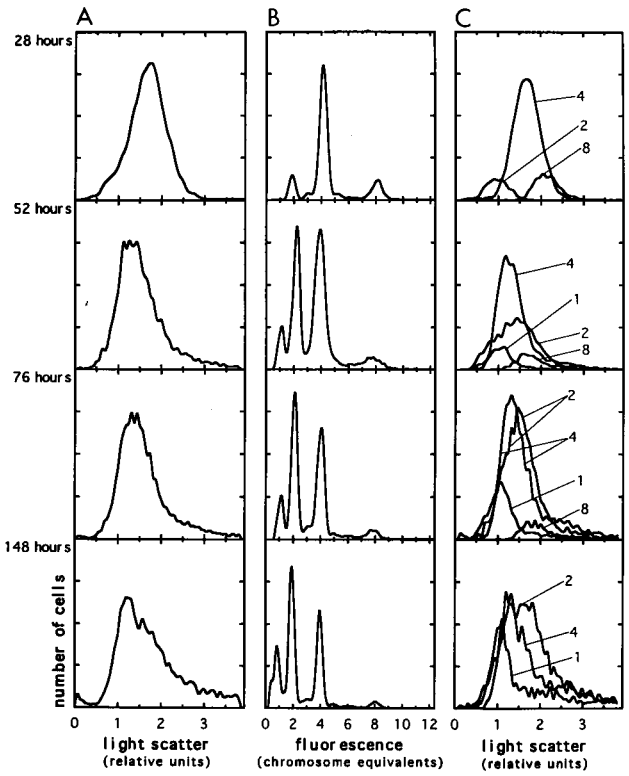

FIG. 4. Flow cytometry profiles of stationary-phase cultures. A stationary-phase culture of strain MG1655 was diluted 10^7-fold into fresh LB medium containing 0.2% glucose and grown at 37°C. Samples were collected after entry into stationary phase at the time points indicated to the left. (A and B) Light scatter and fluorescence of the entire population; (C) light scatter of subpopulations of cells containing different numbers of chromosomes (indicated by numbers). The light scatter and fluorescence parameters represent the relative cell size and the number of chromosome equivalents per cell, respectively.

in contrast to the changes observed between 28 and 52 h in rich medium (see above). The stationary-phase cells were about half the size of those that had been grown in LB with glucose (compare light scatter panels in Fig. 4 and 5). Cells grown at a slower growth rate, in M9 medium containing acetate (Fig. 6), were two- to threefold smaller than those in minimal glucose medium. A majority, about 80 to 90% of the population, contained one chromosome. Even after many days in stationary phase, 10 to 20% of the cells still contained two chromosomes.

In summary, after growth in rich medium, only a minority of the stationary-phase cells contained a single chromosome, even after several days of incubation. Even after growth in minimal medium, a substantial fraction of the populations contained cells with more than one chromosome.

Nucleoid distribution in stationary-phase cells. We wanted to know if the nucleoid distribution in stationary-phase cells was correlated to the number of chromosomes. Therefore, exponentially growing and stationary-phase cells (28 h after the initial dilution; Fig. 4) grown in LB containing glucose were stained with the DNA-specific dye DAPI (see Materials and Methods). A majority of the exponentially growing cells contained two separated nucleoids (Fig. 7), and the nucleoids were in turn often divided into two or more domains. In the stationary-phase cells, a majority of the cells contained only one nucleoid, and there was an apparent difference in the fluorescence intensity between different cells (Fig. 7). The flow cytometry data (see above) showed that most stationary-phase

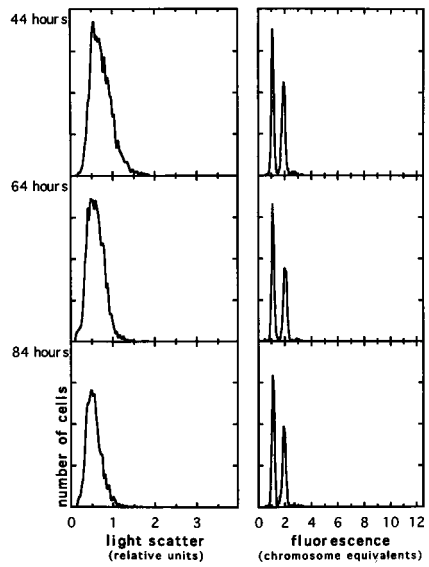

FIG. 5. Flow cytometry profiles of stationary-phase cultures. A stationary-phase culture of strain MG1655 was diluted 10^7-fold into fresh M9 medium containing 0.2% glucose and grown at 37°C. Samples were collected after entry into stationary phase at the time points indicated to the left. The light scatter and fluorescence parameters represent the relative cell size and the number of chromosome equivalents per cell, respectively.

cells contained four chromosome equivalents. Since the microscopic analysis revealed that few cells, if any, contained four nucleoids, most nucleoids contain two or more chromosomes in stationary-phase cells grown in rich medium.

DISCUSSION

We have used flow cytometry to analyze cell size and DNA content of *E. coli* cells grown in batch cultures. In rich medium,

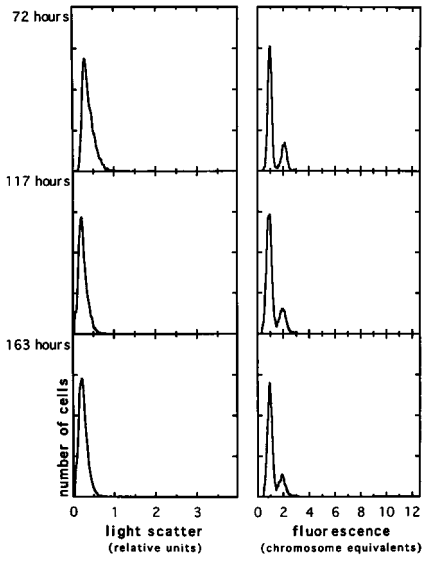

FIG. 6. Flow cytometry profiles of stationary-phase cultures. A stationary-phase culture of strain MG1655 was diluted 10^7-fold into fresh M9 medium containing 0.2% sodium acetate and grown at 37°C. Samples were collected after entry into stationary phase at the time points indicated to the left. The light scatter and fluorescence parameters represent the relative cell size and the number of chromosome equivalents per cell, respectively.

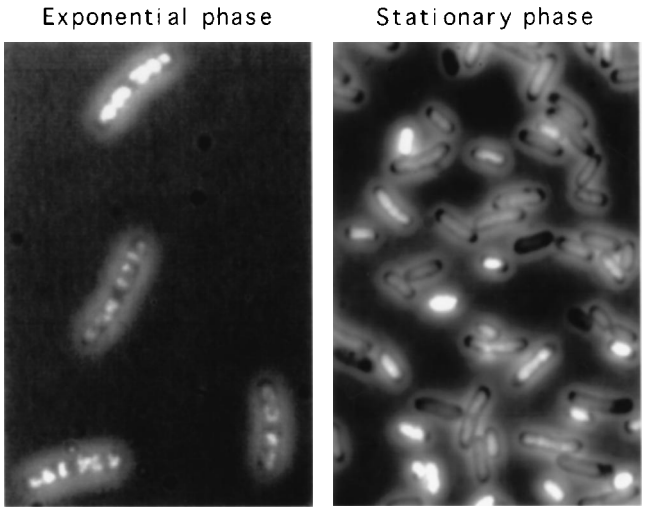

FIG. 7. Size and nucleoid distributions of exponentially growing and stationary-phase *E. coli* cells. Exponentially growing cells and cells from an overnight culture (28 h after the initial dilution; see Fig. 4) of MG1655 grown in LB containing 0.2% glucose were stained with the DNA-specific dye DAPI (see Materials and Methods). The nucleoids were visualized by combined phase and fluorescence microscopy.

the cell size and DNA content started to decrease at a concentration that was about 5 to 10 times lower than that at the transition point between the exponential phase and the stationary phase. Thus, steady state was not maintained throughout the exponential growth phase.

The transition between the exponential and stationary phases is relatively distinct in defined minimal media, whereas the growth curve of cultures grown in rich media shows a more gradual transition between the two phases (18). In agreement with this finding, there was a better correspondence between the end of steady state and the end of the exponential growth phase in minimal medium. Rich media contain a mixture of nutrients which are depleted at different times during the growth of a batch culture, thus resulting in changes in the metabolic activity of the cells, and this is probably one of the reasons why steady state was not maintained. Change in the growth medium, for example, a change in pH, is another factor that affects growth and cell division. Acidic waste products are produced during growth in rich medium, which might affect the pH at lower cell concentrations than in minimal medium (18). However, we found no evidence of a major pH change at the time point when the cell size and DNA content started to decrease (not shown). (The pH was not monitored at later time points.) Furthermore, even in M9 medium containing Casamino Acids and glucose, which is a buffered medium, the cell size and DNA content started to decrease early in the exponential phase. The amino acids are present in different concentrations in this medium, and amino acids in low concentrations might thus be consumed earlier during growth (see discussion of LB medium above) and thereby cause an unbalanced situation in the culture. We have not tested whether the cell size and DNA content decrease early during exponential growth in minimal media in which amino acids are present in equal, unlimiting, amounts.

If the rates of increase of total DNA and cell mass are the same, a culture is in balanced growth with respect to these parameters (see the introduction). These rates of increase were not directly measured in the experiments; only the distributions of cell size and DNA content were measured. Thus, even when the cultures were not in steady-state growth, they may still have displayed balanced growth. For example, if the rate of increase in cell mass and DNA content was unchanged while the interdivision time gradually became shorter, the cell size and DNA content should decrease in parallel. This also implies that the DNA-to-mass ratio should not change. By dividing the average cell mass by the average DNA content (obtained from the data shown in Fig. 2 and 3), this ratio was found to vary during the exponential growth phase (not shown). Thus, the cultures were neither in steady state nor in balanced growth.

Since the cell size and DNA content started to decrease without a decrease in growth rate, measured as OD, the rate of increase in cell number must have become faster than the increase in mass. There was, however, no apparent difference between the increase in cell mass and cell number as measured by OD and viable counts (not shown), respectively. The expected difference is, however, within the error limits of the methods used and may therefore have escaped detection.

Commonly, stationary-phase cultures are diluted 50- to 1,000-fold into fresh media to achieve exponential growth. When we analyzed cultures that were diluted 1,000-fold in rich medium, large fluctuations in cell size and DNA content were observed after dilution, even though exponential growth was maintained (Fig. 3). Thus, large dilutions, more than 10^5-fold, are required to allow batch cultures in rich medium to enter steady state and probably also balanced growth. Repetitive dilution in the exponential growth phase is commonly used to maintain exponential growth. However, we have observed that in rich media, dilutions performed above an OD of 0.05 result in fluctuations in cell size and DNA content (not shown), similar to the results obtained when stationary-phase cultures were diluted (Fig. 3D). When minimal medium containing glucose was used, no or very small changes were observed when repeated dilutions were performed (not shown). Therefore, if steady state must be maintained in rich medium, repeated dilutions have to be performed below an OD of 0.05. Thus, batch cultures containing rich medium may not be the first choice, particularly since steady state occurs only at cell concentrations which are at or below the detection limit for OD measurements. Also, at such low cell concentrations, large sample volumes are required to collect enough cells for analysis.

The majority of the cells in stationary-phase cultures grown in rich medium contained two, four, or eight chromosomes; i.e., every round of chromosome replication did not lead to cell division. The average cell size and DNA content decreased during the first days in stationary phase. In minimal medium, the cells contained mainly one or two chromosomes and no major changes occurred over time. The amount of DNA thus depended on the growth rate of the cells as they entered stationary phase.

Upon entry into stationary phase, growth declines but cell division continues for some time, resulting in a reduction in cell size (15, 17). It is possible that cells have to grow until a minimal distance between the nucleoids is reached, to allow the cell division machinery to act. Rapidly growing cells contain many replication forks (4), and there will be several chromosomes in the cells after termination. Since cellular growth decreased upon entry into stationary phase, the cells may not have been able to grow until sufficient distance between the nucleoids was reached to allow cell division. In support of this view, no separated nucleoids were detected by phase and fluorescence microscopy in stationary-phase cells. Also, when subpopulations were analyzed, the average size of the cells that contained four or eight chromosomes decreased in stationary

phase. Another explanation for the lack of division in cells with several chromosomes is that replication termination and/or decatenation may not have been properly finished. Finally, the decrease in chromosome numbers could be explained by degradation of entire chromosomes. However, this should have resulted in cells in which the number of chromosomes was different from 2^n (21), and this was not observed. Also, if degradation of chromosomes occurred, the degradation must have been specific for the large cells, and it therefore appears more likely to us that the large cells in these populations continued to divide for some time in stationary phase, thereby reducing the average cell size. It should be added that the number of chromosomes in stationary phase was strain dependent. For example, cells of strain B/r A grown in rich medium had mainly one, two, or four chromosomes in an overnight culture (not shown). However, we found no strains in which all the cells had only one chromosome in stationary phase.

Recently, directed (also known as adaptive or Cairnsian) mutations have been debated in the scientific literature. These mutations arise among nongrowing cells, and it has been speculated that they specifically occur in genes which are advantageous during the particular growth conditions (9). It is not known which mechanism that could generate such mutations, but some adaptive mutations require the RecA protein. Duplication of DNA regions occurs at a frequency of 10^{-3} to 10^{-4}, and a model for how adaptive mutations arise that includes gene amplification and recombination between duplicated regions on the same single chromosome has been proposed (10). Our results indicate that the cells often have several chromosomes located within a single nucleoid, and therefore the contribution of recombination to generate directed mutations may be larger than previously assumed. Furthermore, the presence of multiple chromosomes allows for an increased general mutation rate, since generation of a deleterious mutant allele is not lethal if a wild-type counterpart is still present on another chromosome. Finally, multiple chromosomes may allow general recombination events to occur at higher frequencies than has previously been assumed for cells in stationary phase.

To summarize, cell populations in batch cultures show complex growth patterns, particularly in rich media (14). Exponential increase in cell mass does not reveal whether a batch culture is in steady state or whether it is in balanced growth. If steady state is required, parameters such as cell size and DNA content should be monitored during the growth of the culture. It should be pointed out that this study considers only two intensive properties of the population, the distributions of cell size and DNA content; other parameters may change even earlier during the exponential phase. Finally, the assumption that all cells in stationary phase contain only one chromosome is not valid.

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