0021-9193/78/0133-0122$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 122-129

Vol. 133, No. 1
Printed in U.S.A.

# Chromosome Replication in *Myxococcus xanthus*

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Received for publication 23 August 1977

The rates of DNA synthesis during the cell-division cycle were measured in *Myxococcus xanthus* growing in three different media permitting a twofold variation in doubling time. In all three media, simple DNA cycles were observed. Synthesis of DNA occurred during 85% of the cell-division cycle, independent of generation time, from 5 to 11 h. Cells were observed to contain one bacterial nucleoid at birth that later divided synchronously midway through the cell cycle. Nucleoid segregation appeared to begin before chromosome replication was completed. The DNA content of exponential-phase bacteria was determined to be about $20 \pm 3 \times 10^{-9}$ μg per cell; newborn bacteria contained about $14 \pm 2 \times 10^{-9}$ μg of DNA per cell. Exponential-phase bacteria showed about a 50% increase in DNA in the presence of chloramphenicol (50 μg/ml). The number of randomly segregating chromosomes present in exponential-phase bacteria was determined by following the fate of prelabeled DNA during outgrowth in nonradioactive media. The results are consistent with a model in which cells are born with exactly one complete unreplicated chromosome. The molecular weight of such a chromosome is about $8.4 \pm 1.2 \times 10^9$.

*Myxococcus xanthus* is a gram-negative, rod-shaped bacterium which in nature commonly grows in soils on decaying organic material or by preying upon other microorganisms (8, 9, 26). The bacteria generally hunt by gliding over solid surfaces and by synthesizing extracellular enzymes and antibiotics (22, 24, 25). Under certain nutritionally limiting conditions or in the presence of certain chemical signals (4, 15), vegetative cells aggregate to form mounds and later sporulate. Mounds of mature myxospores are referred to as fruiting bodies. The complex life cycle of the myxobacteria makes them an attractive model system for the study of gene regulation and cell-cell interactions.

In this report, we expand our previous work on chromosome replication during the cell-division cycle (31) by studying DNA synthesis in cells growing vegetatively in a complex medium and in two defined media. It should be noted that all experiments were performed on exponential-phase cells without inducing cell synchrony. Synchronous patterns were reconstructed by sorting fixed bacteria on the basis of cell length, since we had previously found that cell length (but not cell width) increases as a function of cell age (29, 31). These experiments clarify the nature of the chromosome-replication cycle of *M. xanthus* and show a pattern of discontinuous replication cycles at different growth rates. This information, together with measurements of DNA per cell, allow us to estimate the size of the *M. xanthus* chromosome.

## MATERIALS AND METHODS

**Bacteria.** The bacterium used was *Myxococcus xanthus* strain DZ-1. DZ-1 is a nonmotile, nonfruiting, dispersed-growing strain which grows particularly well on the Witkin-Rosenberg defined medium (27). It was isolated as a streptomycin-resistant (1 mg/ml) clone of strain ER1 obtained from Eugene Rosenberg. Strain ER1 is a nonmotile descendent of strain FBmp described previously (31). Strain DZ-1 grows slightly faster than strain FBmp in defined (D16) medium and slightly slower in Casitone (CT) medium.

**Media and growth conditions.** CT medium contains 1% Casitone (Difco) and 0.1% $MgSO_4 \cdot 7H_2O$ in distilled water. The D16 medium contains 16 amino acids; it has been described previously as medium M1 minus tryptophan (27). The defined medium minus L-lysine and L-threonine (D14) has been used previously by Filer, Rosenberg, and Kindler (10). All cultures were grown at 30°C under conditions of vigorous gyratory shaking. *M. xanthus* strain DZ-1 was subcultured for several weeks to adapt the cells to the D16 and D14 media. The adaptation was lost if the cells were subsequently grown in rich medium. The generation times of the cells grown in D16 and D14 gradually improved until they were 6 and 11 h, respectively. Cells growing in CT medium showed generation times of about 4.5 to 5 h. This is the fastest growth rate we can obtain with this strain at 30°C.

**Autoradiography experiments of cells pulse-labeled with [$^3$H]thymidine.** Cultures were labeled by pipetting 2 ml of cells into 18- by 150-mm test tubes containing [*methyl*-$^3$H]thymidine (3.0 Ci/mmol, Schwarz BioResearch, Inc.) which had been lyophilized to dryness (CT medium, 400 μCi; D16 medium, 100 μCi; D14 medium, 100 μCi). The test tube cultures were incubated with shaking for 20 min at 30°C. The

incubation was terminated by adding 1 mg of thymidine and placing the cultures on ice. The bacteria were collected by centrifugation at 3,000 rpm for 10 min at 4°C. The pellets were then washed twice with 10 ml of cold 0.1% $MgSO_4$. The washed cells were suspended in 0.4 ml of 0.1% $MgSO_4$, spread on clean microscope slides, and air dried. The bacteria were fixed with 2% formaldehyde for 30 s and washed by dipping successively in four beakers containing 5% trichloroacetic acid and 0.5% sodium pyrophosphate. The slides were then rinsed with distilled water and air dried.

The slides were prepared for autoradiography as described previously (31). The developed slides were examined under a Zeiss microscope and photographed. Length measurements of cells were made from the calibrated photographs. Grain counts were made by locating the photographed fields under the microscope and focusing on the grains. Every intact bacterium photographed was analyzed.

**Nucleoid staining.** Cultures of *M. xanthus* DZ-1 were grown to $2 \times 10^8$ to $4 \times 10^8$ cells per ml in CT or D16 medium. The cells were then treated with chloramphenicol (50 μg/ml) for 20 min to allow the nucleoids to condense (16, 28). Then the bacteria were spread on microsope slides and allowed to air dry. The slides were placed in methanol for 2 min to fix the cells and then incubated for 30 min at 37°C in boiled (10 min) pancreatic ribonuclease (100 μg/ml). The slides were washed with water and then stained for 30 min in Giemsa solution. The stained bacteria were examined under the microscope and photographed. Cell length measurements were made from the calibrated photographs. Stained nucleoids were counted by locating the photographed fields under the microscope and focusing on the nucleoids.

**Diphenylamine measurements of DNA per cell.** Samples (10 ml) of cultures were removed for DNA analysis and cell counts. The cells were collected by centrifugation at 0°C and washed with 5 ml of 0.01 M phosphate buffer, pH 7.0. The cells were treated with 10% perchloric acid for 1 h at 0°C and then centrifuged at 10,000 rpm for 20 min. The DNA in the pellet was assayed as described by Burton (3). All assays were performed in quadruplicate, using both calf thymus DNA and deoxyadenosine as standards. Cell counts were performed using a Petroff-Hausser counting chamber. At least 1,000 cells were counted for each determination.

**Fluorometric measurements of DNA.** The amount of DNA per cell was also determined by the ethidium bromide fluorescence method (20). The procedure used was a modification of that first suggested by Klotz and Zimm (18). Cultures of *M. xanthus* were grown in CT medium. At about $2 \times 10^8$ cells per ml, 40 ml of culture was removed and centrifuged in the cold at 6,000 rpm for 10 min, then washed with cold 0.1 M boric acid buffer, pH 8.15. The pellet was resuspended in 5 ml of boric acid buffer, and triplicate 1-ml samples were lysed with 3.2 ml of standard lysis mix consisting of 12.0 ml of Pronase (4 mg/ml, B grade, Calbiochem), 2.0 ml of Brij 58 (10%, Pierce Chemicals), and 6.0 ml of lysozyme (2 mg/ml, A grade, Calbiochem). All solutions were in BA which contained 10 mM EDTA. The remainder of the cells were fixed in 2% formaldehyde and counted in a Petroff-Hausser chamber as described.

Lysates of *Escherichia coli* B/r, which are somewhat harder to obtain than those of *M. xanthus*, were prepared in the following manner. One milliliter of resuspended cells was treated with 0.6 ml of lysozyme (4 mg/ml) and 10% Brij for 60 min at 50°C. At this time, 0.5 ml of Pronase (4 mg/ml) was added, and the cells were incubated for an additional 30 min. Finally, 2.1 ml of standard lysis mix was added, and the mixture was left at 50°C for the remainder of the 3-h lysis period. Complete clearing was accomplished in 1.5 to 2 h.

Ribonuclease digestion was performed on all samples except for appropriate controls by adding 0.1 ml of boiled ribonuclease A (1 mg/ml, Worthington) to each tube and incubating for at least 2 h at 25°C. By this treatment, competing RNA was removed. Deoxyribonuclease controls were also done by adding 0.1 ml of deoxyribonuclease I (1 mg/ml, Worthington) in the presence of 0.1 M $CaCl_2$ and incubating for at least 4 h at 25°C. Ethidium bromide was then added to all samples at a final concentration of 10 mg/ml. Fluorescence was determined on a Hitachi-Perkin-Elmer fluorometer, using an excitation wavelength of 528 nm and an emission wavelength of 582 nm. The standard curve was made with salmon testes DNA (Worthington).

**Autoradiography of cells prelabeled with [$^3$H] thymidine and then grown in nonradioactive medium.** *M. xanthus* was grown for 3.5 generations (21 h) in D16 medium (2 ml) containing 10 μg of thymidine per ml and 200 μCi of [*methyl*-$^3$H]thymidine (3.0 Ci/mmol, Schwarz BioResearch, Inc.), which had been lyophilized to dryness. During this time, the cells grew from 6 to 70 Klett turbidity units. The cells were then chilled and centrifuged at 5,000 rpm for 10 min. The cells were washed with cold 0.1% $MgSO_4$ containing 200 μg of thymidine per ml, and then suspended in conditioned medium obtained by centrifuging out the cells from a parallel nonradioactive culture of similar turbidity. The medium was supplemented with 200 μg of thymidine per ml. The culture was grown at 30°C with shaking. The turbidity of the culture was monitored carefully so that samples could be taken at 1-generation time intervals. At 0, 1, and 2 generations, 0.2-ml samples were removed from the culture. The bacteria were spread on clean microscope slides and air dried. The slides were treated with trichloroacetic acid and prepared for autoradiography as described above. All slides were exposed and developed for an identical period of time (5 days). Control experiments indicated that this was important for comparing samples since the sensitivity of the gelatin emulsion was slightly higher initially and only became linear after several hours. The slides were stained with methylene blue and examined under the light microscope for grain counts. Only cells visibly dividing were scored in this experiment.

## RESULTS

**Rate of DNA synthesis during cell-division cycle in different growth media.** The rate of thymidine incorporation into individual

cells of *M. xanthus* growing in a defined medium D16 has previously been described (31). The results indicated the existence of a simple DNA cycle within the cell-division cycle, consisting of gap, synthesis, and second gap periods (21). Using the same technique of reconstructed synchrony, we have now measured the rates of DNA synthesis in cells growing in rich medium (CT) with generation times of about 5 h and in defined media (D16 and D14) with generation times of about 6 and 11 h, respectively (Fig. 1). The technique consists of exposing exponential-phase cultures of bacteria to relatively brief pulses of [$^3$H]thymidine, removing acid-soluble radioactivity, and then preparing the cells for autoradiography. The mean number of grains per cell in each size group was measured (Fig. 1). The data show that, in all three media, cells exhibit similar DNA synthesis cycles: (i) dividing cells synthesize little or no DNA; this is followed by (ii) a linear increase in grains per cell, (iii) a plateau region, and (iv) a period of decrease in grains per cell. Analysis of the distribution of silver grains within the size groups shows that all cells in the plateau regions of the cell cycles show a Poisson distribution of grains and are, therefore, synthesizing DNA at constant rates.

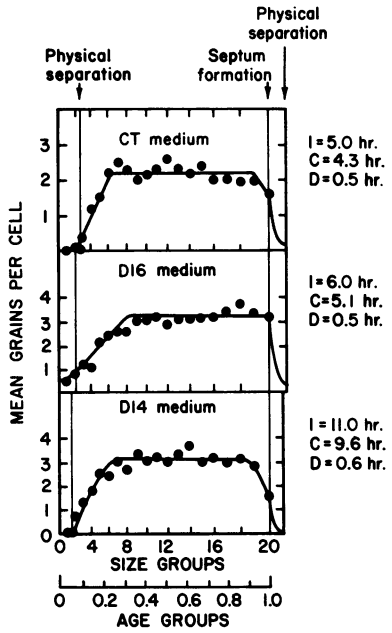



FIG. 1. *Rate of DNA synthesis during cell-division cycle. Exponential-phase cultures of M. xanthus were grown in CT or either D16 or D14 medium. The cells were pulse-labeled for 20 min with [$^3$H]thymidine and then prepared for autoradiography and cell length measurements as described in Materials and Methods. The mean number of silver grains for each size group is plotted as a function of the 20 size groups as an exponential function. This representation places the rate of DNA synthesis on a linear time and age scale. Dividing cells were scored as two small cells and were placed together in the first size groups, since they were assumed to be younger than the smallest nonseptated cells. Dividing cell data are also plotted after 1.0 generations to complete the cell cycle, since dividing cells can also be considered the oldest cells. The I, C and D periods are as described by Pierucci and Helmstetter (23). They were estimated directly from these data, assuming that the DNA cycle corresponds to the replication cycle of one chromosome.*

The data presented in Fig. 1 show that at generation times of 5 to 11 h the chromosome replication time increased from 4.3 to about 9.6 h. However, the DNA synthesis period remained a constant fraction of the cell-division cycle, about 0.85. Similarly, the period between cell separation and the start of DNA synthesis increased from 0.4 to 0.9 h as the generation time increased from 5 to 11 h. In contrast, the period between the end of DNA synthesis and cell division was constant at all growth rates, about 0.5 h.

**Nucleoid replication during cell-division cycle.** Reconstructed synchrony can also be used to study bacterial nucleoid replication during the cell-division cycle. Exponential-phase cultures of *M. xanthus* were treated briefly with chloramphenicol to block protein synthesis and allow the nucleoids to condense (16). The bacteria were examined under the light microscope, and each cell was scored both for length and number of stained nucleoids. The data are grouped to show the number of nucleoids present in cells of different size groups or age groups (Fig. 2). In both CT and D16 media, almost all cells were born with one nucleoid; the number of nucleoids was observed to double about halfway through the cell cycle. However, since condensed dividing nucleoids are dumbbell shaped, they appear as two nucleoids prior to the completion of chromosome replication (5). Thus, the actual time of the completion of nucleoid division cannot be determined precisely by microscopic observations.

**Analysis of DNA per cell.** The amount of DNA present in exponential-phase cells of *M. xanthus* was determined chemically using the Burton diphenylamine procedure (Table 1). Measurements were made with cells grown in both the CT and D16 media. The amount of DNA per cell was $20 \pm 3 \times 10^{-9}$ $\mu$g in both media. The error in these measurements is estimated from the values obtained in several duplicate experiments. Since newborn cells have replicated about half their DNA midway in the cell-division cycle (Fig. 1), we can estimate the

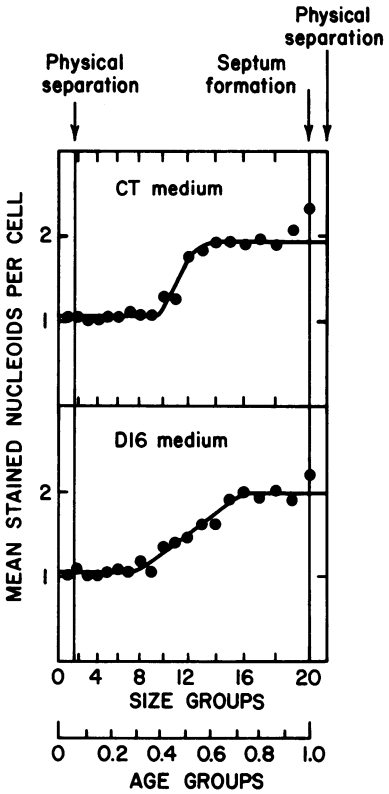



FIG. 2. *Nucleoids per cell during cell-division cycle. Exponential-phase cultures of M. xanthus were grown in CT medium or in D16 medium. Cells were spread on microscope slides and analyzed for nucleoid number and cell length. The bacteria scored in these experiments were divided into 20 approximately equal size groups on the basis of cell length.*

amount of DNA in these cells at about $14 \pm 2 \times 10^{-9}$ μg.

An alternate method for estimating the amount of DNA present in baby cells can be obtained by allowing chromosomes to complete their replication while blocking new chromosome initiation. This can often be achieved in bacteria by blocking protein synthesis with high levels of chloramphenicol (19). The results of such an experiment are summarized in Table 1. Exponential-phase cultures growing in CT or D16 medium were treated with chloramphenicol (50 μg/ml) and allowed to incubate at 30°C for 5.5 to 7 h, respectively. Under these conditions, the amount of DNA as well as cell number per milliliter increased. The amount of DNA per initial cell number (see Table 1), about $31 \times 10^{-9}$, should represent the amount of DNA present in cells with fully replicated chromosomes. Baby cells would then contain about half the amount of DNA, $15.5 \pm 2 \times 10^{-9}$ μg per cell.

Because of the large errors observed in the diphenylamine measurements of DNA, the amount of DNA per cell was also determined by the ethidium bromide fluorescence method of LePecq and Paoletti (20). Table 1 shows that exponential-phase cells growing in the CT medium contain $20 \pm 1 \times 10^{-9}$ μg of DNA per cell, a value which agrees with our other determinations. DNA per cell was also measured in exponential-phase *E. coli* B/r grown in glucose minimal medium as a control. The value obtained, $9.03 \times 10^{-9}$ μg per cell, is similar to the literature value, $9.1 \times 10^{-9}$ μg per cell (5).

TABLE 1. *Chemical analysis of DNA per cell in exponential-phase cells and in chloramphenicol-treated cells*[a]

| Medium | DNA (μg) per: | | | |
|---|---|---|---|---|
| | Untreated | | Chloramphenicol treated | |
| | Cell | Baby cell[b] | Cell[c] | Baby cell[d] |
| CT | $20.2 \times 10^{-9}$<br>$20.5 \times 10^{-9}$[e] | $13.5 \times 10^{-9}$ | $32.5 \times 10^{-9}$ | $16.2 \times 10^{-9}$ |
| D16 | $21.6 \times 10^{9}$ | $14.4 \times 10^{-9}$ | $30.2 \times 10^{-9}$ | $15.1 \times 10^{-9}$ |

[a] *M. xanthus* strain DZ-1 was grown in CT medium (generation time, 5 h) or D16 medium (generation time, 6 h). At time zero, chloramphenicol (50 μg/ml) was added to the cultures. Samples (10 ml) were immediately removed for DNA analysis, using the Burton diphenylamine procedure (see Materials and Methods), and for cell counts. The remainder of the cultures was allowed to incubate for an additional 5.5 h (CT medium) or 7 h (D16 medium) to allow for chromosome completion. Samples were also removed at the end of the incubation period for DNA analysis and cell counts.

[b] The values are calculated from the data presented in this Table and Fig. 1. Figure 1 shows that the average cell has replicated about 50% of its DNA.

[c] The values are calculated by using the cell number at time zero, since we are interested here in the amount of DNA present in cells with fully replicated chromosomes. The cell number was observed to increase 29.8% (CT medium) and 28.4% (D16 medium) in the presence of chloramphenicol.

[d] The values are one-half those found in cells incubated in chloramphenicol. They are based on the assumptions that chromosomes are completed in the presence of chloramphenicol, but not initiated, and that dichotomous multifork replication does not normally occur in *M. xanthus*.

[e] This value was obtained using the ethidium bromide fluorescence technique (see Materials and Methods). As a control, this technique showed *E. coli* B/r grown in glucose minimal medium to have $9.0 \times 10^{-9}$ μg/cell.

**Determinations of number of segregating chromosomes per cell.** The number of segregating chromosomes present in exponential-phase bacteria can be determined by following the fate of prelabeled DNA from parent to daughter cells. These experiments require scoring the radioactivity in individual cells. A synchrony procedure was devised for these experiments based on the hypothesis that dividing cells, sampled from an exponential-phase culture at generation time intervals, can be considered a synchronous subpopulation. Accordingly, an

exponential-phase culture was labeled for 4 generations with [$^3$H]thymidine and then suspended in a nonradioactive conditioned medium. Samples were removed after 0, 1, and 2 generations of growth in the nonradioactive medium, and the cells were prepared for autoradiography. Only the silver grains above dividing sister cells were scored in each sample. The predicted pattern for random segregation of semiconservatively replicated DNA is illustrated in Table 2 for cells with one or two chromosomes. It should be noted that this kind of experiment could not distinguish between one chromosome and multiple chromosomes with a linked pattern of segregation, such as described for F′ and the *E. coli* chromosome (6). The experimental results are presented in Table 3. The mean number of silver grains per sister cell declined during outgrowth from 4.90 to 1.21. The more interesting information is the mean number of silver grains in individual sister cells. To obtain this information the distributions of silver grains for sister cells were analyzed (Fig. 3). Figure 3a and b show Poisson distributions for all sister cells sampled at 0 and 1 generations. Therefore, sister cells A and B in each matched pair of dividing cells have the same means (Table 3). Figure 3c does not show a Poisson distribution, indicating nonequivalence between sisters. Accordingly, each pair of sisters was considered separately as sister A, the more radioactive sister, and sister B, the less radioactive sister. The new distributions were analyzed separately in Fig. 3d. Now a Poisson distribution is obtained for the more heavily labeled sister A. Sister B has only a small amount of radioactivity; it does not show a Poisson distribution, however, indicating heterogeneity in this class. Some of this heterogeneity can be accounted for by the occasional (about 5%) birth of a cell with two nucleoids (see Fig. 2); some may be due to genetic recombination. In addition, it is possible that these cells may contain some plasmid DNA. Plasmids have been reported in a different strain of *M.*

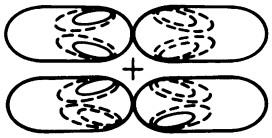
TABLE 3. *Autoradiographic data on segregation of DNA after cell growth and division*[a]

| Generations in nonradioactive medium | No. of sister cells scored | Mean grains/sister cell | Mean grains/sister cell[b] | |
|---|---|---|---|---|
| | | | Cell A | Cell B |
| 0 | 204 | 4.90 | 4.90 | 4.90 |
| 1 | 410 | 2.47 | 2.47 | 2.47 |
| 2 | 832 | 1.21 | 2.22 | 0.20 |

[a] *M. xanthus* was grown in the D16 medium supplemented with [$^3$H]thymidine for 3.5 generations. The cells were then washed free of the radioactivity and suspended in nonradioactive, conditioned medium as described in Materials and Methods. At the times indicated, samples were removed and prepared for autoradiography. Only dividing cells were scored for grain counts since, at one-generation time intervals, dividing cells represent a synchronous subpopulation. Background grain counts were less than 0.05 grains/cell and were not subtracted from the data.

[b] The basis for discriminating between the two sister cells is presented in Fig. 3 and is described in the text.

TABLE 2. *Predicted segregation patterns for prelabeled chromosomes*

| Generations in unlabeled medium | MODEL 1: 1 Chromosome Per Cell | | | | MODEL 2: 2 Chromosomes Per Cell | | | |
|---|---|---|---|---|---|---|---|---|
| | Chromosomes per dividing cell | | Relative radioactivity | | Chromosomes per dividing cell | | Relative radioactivity | |
| | Cell A | Cell B | Cell A | Cell B | Cell A | Cell B | Cell A | Cell B |
| 0 |  | | 4.90 | 4.90 |  | | 4.90 | 4.90 |
| 1 |  | | 2.45 | 2.45 |  | | 2.45 | 2.45 |
| 2 |  | | 2.45 | 0 |  | | 2.45 + 1.23 | 0 + 1.23 |

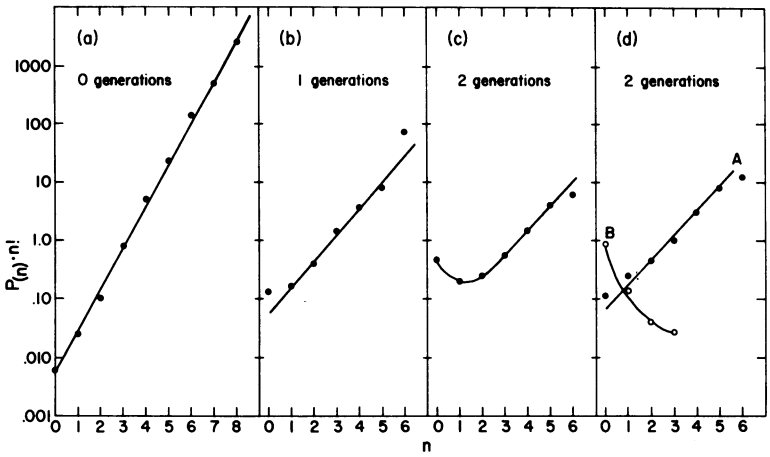



FIG. 3. *Distribution of radioactive DNA in dividing cells following growth and division in nonradioactive medium. The distribution of silver grains in the dividing cells presented in Table 3 was analyzed as suggested by Hanawalt et al. (12). The frequency function, log [$P_{(n)} \cdot n!$], is plotted as the ordinate, where n is the number of silver grains per cell and $P_{(n)}$ is the fraction of cells containing n number of grains. The abscissa is n, the number of grains per cell. A straight line indicates a Poisson distribution, since ln $P_{(n)} \cdot n! = n \ln n - n$. (a, b, and c) Distribution of silver grains observed after 0, 1, and 2 generations. The means obtained from the slopes of (a) and (b) are presented in Table 3. Since (c) does not show a Poisson distribution, the data were replotted in (d) to show the distribution obtained if a dividing cell is considered as one heavily labeled sister (A) and one lightly labeled sister (B).*

*xanthus* (2). The results obtained in this experiment agree very closely with model 1 (Table 2), and are inconsistent with a two-chromosome model in which there is random segregation of DNA. These data to not rule out the possibility, however, that cells contain two chromosomes and that daughter cells always receive both Watson strands or both Crick strands during segregation.

## DISCUSSION

The data presented clarify the pattern of chromosome replication during the cell-division cycle of *M. xanthus*. Chromosome replication does not take place throughout the cell-division cycle but rather is restricted to a specific interval within the cycle. The length of the DNA-replication period increases at slower growth rates but remains a constant fraction (0.85) of the cell cycle. Throughout the synthesis periods, the rates of replication remain constant as reported previously (31).

The amount of DNA present in exponential-phase bacteria was found to be constant, within experimental error, in all growth media used. The amount of DNA, $20 \pm 3 \times 10^{-9}$ μg/cell, is actually not very different from the value obtained for *E. coli* growing in rich broth (5). However, it is now known that *E. coli*, under these rapid growth conditions, contains multiple copies of its chromosome, each of which is replicating with multiple pairs of replication forks (7). Our data indicate that *M. xanthus* growing in the media we used probably does not reinitiate chromosome replication prior to completion of a round of replication, since we only observe about a 50% increase in DNA following chloramphenicol addition (Table 1). These results agree with those of Kimchi and Rosenberg (17), who found a 40% increase in thymidine incorporation after chloramphenicol addition.

The pattern of DNA segregation (Table 3) indicates that all or almost all of the DNA in *M. xanthus* segregates as a single unit. It is not possible to determine from these experiments, however, if this single segregation unit represents one or several genetically linked chromosomes. This information can be obtained by physical studies of the size of the DNA. Viscoelastic experiments (18), performed in collaboration with Brian Bowen in Bruno Zimm's laboratory, indicate that the molecular weight of *M. xanthus* DNA is very large, about $8.2 \pm 1.5 \times 10^9$ (unpublished data). This value is large enough to account for all the DNA present in newborn cells. If the amount of DNA reported in Table 3, $14 \pm 2 \times 10^{-9}$ μg/newborn cell, were organized into a single chromosome, it would have a molecular weight of $8.4 \pm 1.2 \times 10^9$. This value is between three and four times the size of the chromosome of *E. coli*. Further, the reassociation kinetics (1) of *M. xanthus* DNA have

recently been studied by Fred Schachat and Johnathan Hodgkin of Stanford University (personal communication). Analysis of their data indicates a genome complexity about 3.5 times greater than that of *E. coli*. Thus, the simplest interpretation of the data available is that cells of *M. xanthus* contain a single, very large, nonreplicating chromosome at birth (division) and that the chromosome replicates once during the cell-division cycle.

It should be noted that the value for the chromosome molecular weight reported here is larger than in our earlier report (30). That study was based on autoradiographic experiments following outgrowth of glycerol-induced spores. We think that the experiments reported here, using exponential-phase cells, are easier to interpret and subject to fewer errors.

In conclusion, the experiments reported here show the chromosome replication cycle in *M. xanthus* to consist of three well-defined segments. This DNA cycle is similar to that of *E. coli* growing at slow growth rates (11) since synthesis is discontinuous. However, unlike many strains of *E. coli*, two gap periods are observed. Recently, Helmstetter and Pierucci (13) have found that some strains of *E. coli* B/r, but not others, show two gap periods at slow growth rates. The simplicity of the DNA cycle in *M. xanthus* may be important in nature for rapid segregation of cellular DNA during starvation and the initiation of fruiting-body formation.

The chromosome of *M. xanthus* appears to be very large. At least some of this complexity might be accounted for by the developmental cycle, which involves cellular aggregation and fruiting-body formation. *M. xanthus* is not noted for its nutritional diversity, however (14). The function and selective advantage for this large amount of DNA remains a mystery.

## ACKNOWLEDGMENTS

This research was supported by Public Health Service grants GM 20509-04 from the National Institute of General Medical Sciences and BMS75-09231 and PCM75-09231 AO1 from the National Science Foundation.

The authors would like to thank Dale Kaiser and Colin Manoil for reading the manuscript.

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