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Division Cycle of *Myxococcus xanthus*

II. Kinetics of Stable and Unstable Ribonucleic Acid Synthesis[1]

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The kinetics of stable and unstable ribonucleic acid (RNA) synthesis during the division cycle of *Myxococcus xanthus* growing in a defined medium was determined. Under these conditions, *M. xanthus* contains one chromosome which is replicated during 80% of the cell cycle. Stable RNA synthesis was measured by pulse-labeling an exponential-phase culture with radioactive uridine and then preparing the cells for quantitative autoradiography. By measuring the size of individual cells as well as the number of grains, the rate of stable RNA synthesis as a function of cell size was determined. Unstable RNA synthesis during the division cycle was determined by correlating the data for stable RNA synthesis with the relative amounts of stable and unstable RNA labeled during the short pulse. The data reported here demonstrate that: (i) cells synthesize both stable and unstable RNA throughout the division cycle; (ii) the rate of stable RNA synthesis increases in two discrete steps, corresponding to average ages of 0.15 and 0.75 generations; (iii) the rate of unstable RNA synthesis exhibits an initial rise, followed by a relatively constant rate of synthesis, and finally, a burst of unstable RNA synthesis prior to septum formation. The half-life of unstable RNA of *M. xanthus*, generation time of 390 min at 30 C, was 4 min. Comparison of the rates of stable and unstable RNA synthesis indicates noncoordinate RNA synthesis within the normal division cycle.

The relationship between chromosome replication, the rates of enzyme synthesis, and the bacterial division cycle has been the subject of several recent reviews (3, 10, 16). On the basis of studies of chromosome replication and enzyme synthesis in synchronous cultures, the bacterial cell cycle has emerged as a complicated prescribed sequence of discontinuous events. Recently, we reported quantitative evidence for a simple chromosome replication cycle in the bacterium *Myxococcus xanthus* growing exponentially in a defined medium (26). Each newborn sister cell inherits one bacterial chromosome with a molecular weight of 4.9×10^9 daltons (24); chromosome replication is initiated at approximately 0.12 generation and proceeds at a constant rate of 80% of the division cycle. The present investigation was undertaken to determine the kinetics of ribonucleic acid (RNA) synthesis during the division cycle and to relate these data to the defined deoxyribonucleic acid (DNA) cycle. To avoid possible artifacts of cell synchronization, all of the experiments reported here were performed on exponentially growing bacteria under conditions of balanced growth. In the following paper (25), the kinetics of both RNA and DNA synthesis are related to protein synthesis and cell growth.

[1] A preliminary report of this study was presented at the 70th Annual Meeting of the American Society for Microbiology, Boston, Mass., 26 April-1 May 1970.

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MATERIALS AND METHODS

Organism. The organism used was *M. xanthus* strain FBmp (26). Strain FBmp has an absolute requirement for glycine, isoleucine, leucine, and valine; growth is stimulated by alanine, asparagine, histidine, lysine, phenylalanine, proline, serine, and threonine (23).

Medium. The medium contained, per liter: 2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, 0.136 g of KH_2PO_4, 0.002 g of $FeCl_3 \cdot 6H_2O$, 0.002 g of $CaCl_2$, 1 g of alanine, 0.1 g of arginine, 0.5 g of asparagine, 0.1 g of cystine, 0.1 g of glycine, 0.1 g of histidine, 1 g of isoleucine, 2 g of leucine, 0.5 g of lysine, 0.5 g of methionine, 1 g of phenylalanine, 1 g of proline, 0.2 g of serine, 0.1 g of threonine, 0.4 g of tyrosine, 0.2 g of valine (all amino acids used were the L isomer and were obtained from Nutritional Biochemical Corp., Cleveland, Ohio), and 10 ml of 1 M tris(hydroxymethyl)aminomethane (Tris) buffer; final *p*H is 7.2.

Growth conditions. For each experiment, 50 ml of defined medium was inoculated with bacteria and aer-

ated by vigorous gyratory shaking for 48 hr at 30 C. At this time the cells were in exponential growth with a generation time of 6.5 hr. The medium was then supplemented with 10 μg of cytosine per ml, 5 μg of thymidine per ml (to minimize the incorporation of radioactive uridine into DNA), and 2 μg of uridine per ml 2 hr before the labeling period. All experiments were performed with cultures containing 1.2×10^8 cells/ml (optical density of 0.250 at 560 nm).

Labeling conditions for autoradiography experiments. An exponential-phase culture of *M. xanthus* was pulse-labeled for 2 or 40 min by incubating 1 ml of aerated culture with 500 μCi (22 Ci/mmole, International Chemical and Nuclear Corp., Irvine, Calif.) or 100 μCi (4 Ci/mmole, Schwarz BioResearch, Inc., Van Nuys, Calif.) of uridine-5-^3H, respectively. In some experiments, the cultures were then exposed to rifamycin SV (8 μg/ml, Calbiochem, Los Angeles, Calif.) and unlabeled uridine (500 μg/ml) for 20 min. The incubations were terminated by placing the cultures on ice and immediately harvesting by centrifugation at 6,000 × *g* for 7 min at 4 C. The pellets were washed with 0.01 M Tris buffer, *p*H 7.4, containing 0.001 M magnesium acetate and 0.5 mg of uridine per ml. The washed cells were then resuspended in the buffer (0.4 ml), spread on precleaned microscope slides, and air-dried. The bacteria were fixed with 2% formaldehyde and washed by dipping successively in four beakers containing cold 5% trichloroacetic acid and 0.01% uridine. The slides were finally rinsed with distilled water and air-dried.

Autoradiography. Ilford Nuclear Research emulsion L-4 (Ilford, Ltd., Ilford, Essex, England) was prepared and applied as described previously (26). After the slides were developed, the bacteria were examined under 1.875 magnification with an M20 microscope equipped with a screw micrometer eyepiece calibrated with a stage micrometer (Wild Heerbrugg, Ltd., Heerbrugg, Switzerland). The slides were examined by placing immersion oil directly on the slide without a cover slip. Cells were selected randomly for analysis of grain counts and length measurements. The bacteria were plainly visible and well dispersed. The standard deviation for length measurements was 0.13 μm. Those bacteria that contained a visible septum were scored as dividing cells and listed separately. The frequency distribution of cell size was similar to that obtained previously (26), indicating that the cells chosen for analysis were, indeed, chosen at random and that the grain distributions obtained represent the entire population. Background grain counts were less than 0.1 per cell. Control slides containing cells labeled for 20 min with radioactive uridine or valine showed that over 99% of the bacteria were metabolically active.

Kinetics of RNA degradation. An exponential-phase culture of *M. xanthus* was labeled for 2, 4, 10, 40, 80, or 390 min by incubating 10 ml of culture with uridine-5-^3H at a final concentration of 2.5×10^{-5} M. The culture was then divided into two parts. Rifamycin (8 μg/ml) and unlabeled uridine (500 μg/ml) were added to one part, and the other was maintained as the control. At timed intervals, duplicate samples (0.1 ml) were removed and spotted on sequentially numbered Whatman 3MM filter-paper pads. The filter-paper pads were immediately immersed in iced 5% trichloroacetic acid containing 0.01% uridine. After 2 hr at 0 C, the filter pads were washed as a group five times with 1 liter of 5% iced trichloroacetic acid, three times with 300 ml of 95% ethanol, and three times with 200 ml of anhydrous ether. The filter pads were then air-dried, and the radioactivity was measured in a liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.), with 10 ml of toluene containing 40 mg of 2,5-diphenyloxazole and 0.5 mg of *p*-bis-(2,5-phenyloxazolyl)-benzene per sample used as scintillation fluid.

Kinetics of protein synthesis after rifamycin addition. To 13 ml of exponentially growing culture of *M. xanthus* was added 0.13 ml of L-valine-2,3-^3H (2.3 Ci/mmole, 1 mCi/ml, Schwarz BioResearch, Inc.). After incubating for 4 min, the culture was divided into two parts. Rifamycin (8 μg/ml) was added to one part, and the other was maintained as the control. At timed intervals, quintuplicate samples (0.05 ml) were removed and assayed for acid-insoluble radioactivity, as described for the kinetics of RNA degradation.

Sedimentation analysis of RNA. An exponential-phase culture of *M. xanthus* (100 ml) was harvested by centrifugation at 6,000 × *g* for 10 min at 4 C. The pellet was then suspended in 6 ml of the supernatant medium. The concentrated culture (20×10^8 cells/ml) was placed in a tube and incubated at 30 C with vigorous bubbling. After 10 min, the culture was exposed to 1.5 mCi of uridine-5-^3H for 2 min. Half of the culture was harvested immediately and half was exposed to rifamycin (8 μg/ml) and unlabeled uridine (500 μg/ml) for 20 min before harvesting. Cultures were cooled rapidly and harvested by centrifugation at 6,000 × *g* for 10 min at 4 C. The pellets were then washed two times with 0.01 M Tris buffer, *p*H 7.4, containing 0.001 M magnesium acetate and 0.5 mg of uridine per ml. The pellets were stored at −20 C.

Radioactive cells were disrupted by resuspending the frozen pellets in 2 ml of Tris buffer, *p*H 7.4, containing 200 μg of lysozyme per ml, 10 μg of deoxyribonuclease I (Worthington Biochemical Corp., Freehold, N.J.) per ml, 0.001 M magnesium acetate, and 0.3% Macaloid (21). After repeated freezing and thawing, sodium dodecyl sulfate was added to a final concentration of 1%. RNA was extracted by vigorously shaking the suspension with an equal volume of redistilled buffered phenol, *p*H 8. The suspension was centrifuged at 5,000 × *g* for 10 min at 4 C, and the aqueous layer was removed. The aqueous fraction was made 0.1 M in NaCl, and RNA was precipitated by the addition of three volumes of 95% ethanol. The RNA precipitates were collected by centrifugation at 18,000 × *g* for 20 min at −5 C. The pellets were suspended in 1 ml 0.01 M Tris buffer, *p*H 7.4, containing 0.001 M magnesium acetate.

RNA (0.1 ml) was layered on 4.8 ml of 5 to 20% linear sucrose gradients and centrifuged in an SW39 rotor at 114,000 × *g* for 6 hr at 4 C. Fractions (0.059 ml) were collected from the bottom of the tubes directly onto Whatman 3MM filter-paper discs. The filter-paper discs were washed with iced 5% trichloroacetic acid and assayed for acid-insoluble radioactivity as described for the kinetics of RNA degradation.

RESULTS

Rate of stable RNA synthesis during the division cycle. The rate of uridine incorporation into cells

of different sizes was determined by pulse-labeling an exponential-phase culture of *M. xanthus* with ^{3}H-uridine for 40 min, removing acid-soluble radioactivity, and then preparing the cells for quantitative autoradiography. Each cell was scored both for length and number of grains. The population was divided into 10 arbitrary size groups, each of which contained an equal number of bacteria. Table 1 shows the distribution and mean number of silver grains for each size group; the mean number of silver grains is proportional to the rate of RNA synthesis.

Cells containing a visible septum presented a problem in the tabulation of the data. They would be scored as one large cell by standard Coulter Counter methods (6); however, physiologically, they might behave as individuals. Since these dividing cells are "older" than mature single cells and "younger" than small single cells, they were listed separately and placed in the first size groups.

Analysis of the distributions of grains observed in Table 1 is presented in Fig. 1. For a simple Poisson distribution, a straight line is obtained when the log $[P_{(n)} \cdot n!]$ is plotted against n, where n is the number of silver grains per cell and $P_{(n)}$ is the fraction of cells containing n number of grains. As presented in Fig. 1, the distribution of grains for most size groups closely fits a straight line. This indicates that almost all cells within each size group incorporated the same amount of radioactivity during the 40-min labeling period. Thus, the different slopes shown in Fig. 1 reflect the changing rates of RNA synthesis as a function of cell size. The means obtained from the slopes of the frequency distributions provide a check for the numerical average (Table 1).

The data replotted in Fig. 2 show the rate of stable RNA synthesis as a function of cell size. The abscissa contains the 10 size groups as an exponential function ($2^0 - 2^1$). This representation (25) corrects the size groups for the larger fraction of small cells relative to large cells present in an exponentially growing population (thereby approximating a linear time and age scale). The data in Fig. 2 show that (i) the rate of stable RNA synthesis during a 40-min pulse increases during approximately the first 35% of the division cycle, followed by (ii) a plateau period with almost no increase in rate and (iii) a period during which the rate of stable RNA synthesis again increases.

The resolution of this experiment is limited by the long pulse period (40 min, 10% of the doubling time) used to measure the uptake of radioactive uridine. To circumvent this problem, shorter pulse periods were employed. Although at one time it was assumed that short labeling periods measure the rate of synthesis of both stable and unstable RNA, it has recently been demonstrated that this is not the case experimentally. Nierlich has shown that exogenously supplied uracil enters the intracellular pools of RNA precursors in *Escherichia coli* only as nucleotides are removed from these pools by net synthesis of RNA (12, 14). Thus, short labeling periods are a measure of the rate of stable RNA synthesis. Accordingly, an exponential-phase culture of *M. xanthus* was pulse-labeled with ^{3}H-uridine for 2 min, washed with iced trichloroacetic acid, and prepared for quantitative autoradiography. The 1,500 bacteria scored in this experiment were divided into 20 equal size groups. The distribution of grains and the mean for each size group are

TABLE 1. *Autoradiographic data as a function of cell size for M. xanthus pulse-labeled for 40 min with ^{3}H-uridine*[a]

Size group[b]	Length (μm)	Grains per cell (*n*)												Mean	Mean$_p$[c]
		0	1	2	3	4	5	6	7	8	9	10	11		
1	1.80–2.56	17	25	37	28	11	2	0	0	0	0	0	0	1.98	2.14
2a	2.56–3.28	5	17	24	19	4	2	2	0	1	0	0	0	2.39	2.48
2b	2.00–2.52	3	7	17	7	7	3	1	1	0	0	0	0		
3	2.52–2.80	2	18	28	27	16	18	6	3	1	0	1	0	3.20	3.05
4	2.80–3.00	2	11	23	27	27	18	11	1	0	0	0	0	3.41	3.78
5	3.00–3.20	4	10	23	31	22	17	12	0	0	0	0	1	3.29	3.69
6	3.20–3.40	2	6	12	36	20	22	12	5	4	1	0	0	3.97	4.00
7	3.40–3.68	0	9	14	26	31	20	9	7	3	1	0	0	3.97	3.98
8	3.68–4.05	2	9	17	19	21	27	12	7	3	2	1	0	4.10	4.05
9	4.05–4.48	1	1	9	17	27	26	22	12	4	1	0	0	4.71	5.05
10	4.48–7.40	3	7	10	17	16	20	15	18	8	1	4	1	4.85	4.83
	Total	41	120	214	254	202	175	102	54	24	6	6	2	3.59	3.66

[a] *M. xanthus* was grown in defined medium, pulsed-labeled for 40 min (10% of doubling time), and prepared for autoradiography. Cells were selected randomly for analysis: grain counts and length measurements.

[b] The 1,200 bacteria scored in this experiment are divided into 10 arbitrary size groups, each of which contains 120 cells. A dividing cell containing visible septum is scored as two cells; these baby cells are presented first (size groups 1 to 2a); single cells are listed in size groups 2b to 10.

[c] Obtained from the slopes of the frequency distributions presented in Fig. 1, as described by Hanawalt et al. (5).

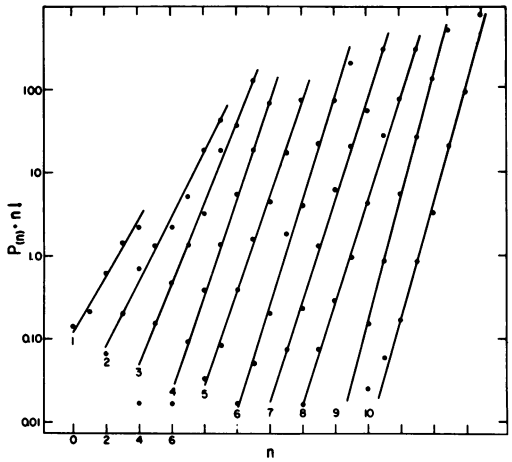

FIG. 1. *Distribution of rates of stable RNA synthesis within each size group. The distribution of silver grains for each of the 10 size groups shown in Table 1 is analyzed. The frequency function, log* $[P_{(n)} \cdot n!]$, *is plotted as the ordinate. The abscissa indicates the number of grains per cell for the first size group; the nine other curves are displaced successively to the right. For simple Poisson distributions, straight lines are obtained; since* $\ln P_{(n)} \cdot n! = n \ln \mathbf{n} - \mathbf{n}$, *the slopes yield* $\ln \mathbf{n}$, *where* $\mathbf{n}$ *is the mean number of grains per cell. The values obtained from this figure are listed in the last column of Table 1 and presented graphically in Fig. 2.*

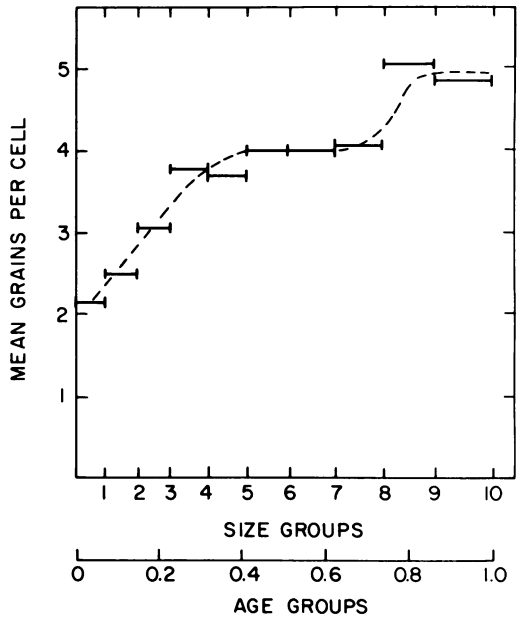

FIG. 2. *Rate of stable RNA synthesis as a function of cell size obtained from autoradiographic data of cells pulse-labeled with* ^{3}*H-uridine for 40 min. The mean number of grains per cell for each size group (Table 1) was obtained from the slopes of the frequency distributions presented in Fig. 1. The abscissa contains the 10 size groups as an exponential function* ($2^0 - 2^1$). *This representation corrects the size groups for the larger fraction of small cells relative to large cells present in an exponentially growing population (thereby approximating a linear time and age scale).*

presented in Table 2; the mean number of grains per cell as a function of cell size are presented graphically in Fig. 3.

The rates of RNA synthesis obtained from the 2-min pulse experiment (Fig. 3) are very similar to the data obtained from the 40-min pulse (Fig. 2). The observed 10% displacement in the division cycle is due to the different labeling periods, 0.005 and 0.10 generation. The close fit of the data obtained from short and long pulse periods further supports the contention that the 2-min pulse is also a measure of stable RNA synthesis. If the 2-min pulse measured both stable and unstable RNA synthesis, different results would be obtained because unstable RNA synthesis and stable RNA synthesis are noncoordinate during the division cycle (Fig. 8).

The rate of stable RNA synthesis (i) increases during approximately the first 25% of the division cycle, (ii) remains constant from 0.25 to 0.65 generation, and (iii) increases again during the last third of the division cycle. These data present a more detailed analysis of the kinetics of stable RNA synthesis than are presented in Fig. 2.

Half-life of unstable RNA of M. xanthus. Although the rate of uridine incorporation is proportional to only the rate of stable RNA synthesis, the nucleotide enters both the stable and unstable fractions (12, 14). The fraction of stable RNA labeled during the 2-min pulse period was determined by labeling a culture with ^{3}H-uridine for 2 min and then exposing the bacteria to rifamycin (8 μg/ml) and unlabeled uridine (500 μg/ml). Rifamycin has been shown to bind to RNA polymerase of *M. xanthus* (Okano, Bacon, and Rosenberg, *unpublished data*), thus preventing the enzyme from initiating RNA polymerization, as has been described for other bacteria (19, 22). After a 15-sec initial lag, radioactive uridine was incorporated into acid-insoluble material at a linear rate (Fig. 4). After rifamycin addition at 2 min, the amount of acid-insoluble radioactivity decreased for 20 min, reaching a limit level of about 50%. The half-life of the unstable RNA (3.5 to 4.0 min) was calculated from the semilogarithmic plot of the per cent acid-insoluble radioactivity remaining as a function of the time after rifamycin addition (insert, Fig. 4).

When the length of the radioactive uridine pulse was increased from 2 min to 4, 10, 40, or 80 min, the percent loss of insoluble radioactivity after rifamycin addition was consistently too high compared to the predicted value based on a 4-min

TABLE 2. *Autoradiographic data as a function of cell size for M. xanthus pulse-labeled for 2 min with ^{3}H-uridine*

Size group[a]	Length (μm)	Grains per cell (*n*)														Mean
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	1.76–2.40	24	15	24	9	2	1	0	0	0	0	0	0	0	0	0	1.37
2	2.40–2.68	12	22	21	10	6	2	2	0	0	0	0	0	0	0	0	1.87
3	2.68–3.08	4	17	22	20	4	4	3	0	1	0	0	0	0	0	0	2.44
4a	3.08–4.32	3	3	4	4	3	4	2	2	2	0	0	0	0	0	0	2.98
4b	1.84–2.60	7	8	7	12	8	3	1	1	0	1	0	0	0	0	0	
5	2.60–2.84	1	9	17	19	9	13	5	1	0	1	0	0	0	0	0	3.30
6	2.84–2.96	2	9	8	13	22	12	3	5	0	0	1	0	0	0	0	3.67
7	2.96–3.12	5	3	9	17	15	11	3	8	3	0	1	0	0	0	0	3.94
8	3.12–3.20	6	7	12	12	19	10	5	3	1	0	0	0	0	0	0	3.36
9	3.20–3.28	3	4	11	14	15	13	5	2	4	3	0	1	0	0	0	4.09
10	3.28–3.40	4	3	12	13	17	12	4	6	3	1	0	0	0	0	0	3.91
11	3.40–3.52	3	7	7	18	19	8	7	4	1	0	0	1	0	0	0	3.73
12	3.52–3.64	4	3	10	15	12	15	7	6	2	1	0	0	0	0	0	4.00
13	3.64–3.78	3	4	14	12	16	11	5	4	5	1	0	0	0	0	0	3.92
14	3.78–3.88	4	4	13	15	14	9	3	8	3	1	0	0	1	0	0	3.94
15	3.88–4.00	2	4	13	12	12	10	7	11	1	1	2	0	0	0	0	4.27
16	4.00–4.17	1	6	7	14	21	11	5	5	1	2	1	1	0	0	0	4.18
17	4.17–4.32	1	3	10	9	14	7	11	8	7	2	1	2	0	0	0	4.92
18	4.36–4.57	1	3	12	14	11	6	11	5	3	0	3	2	2	1	1	4.95
19	4.57–4.88	1	2	7	8	15	12	9	10	8	1	0	1	0	1	0	5.07
20	4.88–7.50	3	3	7	7	9	14	9	7	5	6	1	2	1	1	0	5.31
	Total	94	139	247	267	263	188	107	96	50	21	10	10	4	3	1	3.76

[a] The 1,500 bacteria scored in this experiment are divided into 20 arbitrary size groups, each of which contains 75 cells. Dividing cells are scored as described in Table 1. Baby cells are presented in size groups 1 to 4a; single cells are listed in size groups 4b to 20.

half-life (Table 3). In fact, direct measurement of the half-lives of unstable RNA as a function of pulse length indicated multiple unstable RNA species with average half-lives of 4 and 10 min.

To determine whether both unstable RNA species code for protein, changes in the rate of protein synthesis after rifamycin addition were measured. A culture of *M. xanthus* was labeled with radioactive valine for 4 min and then exposed to rifamycin and unlabeled uridine. Two minutes after rifamycin addition, the rate of radioactive valine incorporation decreased (Fig. 5). The half-life of messenger RNA as measured by this decrease in the rate of protein synthesis is 4.2 min. This indicates that the unstable RNA with a half-life of 10 min does not serve as template for protein synthesis.

Sedimentation analysis of the RNA present after rifamycin treatment of bacteria. An exponentially growing culture was pulse-labeled with radioactive uridine for 2 min and then exposed to rifamycin and unlabeled uridine for 20 min (five half-lives). Sucrose gradient centrifugation of the RNA extracted from these bacteria (Fig. 6) indicated distinct peaks of 23*S*, 16*S*, and 4*S* RNA; approximately 7% of the stable RNA was 4*S* RNA. In contrast, RNA extracted from bacteria harvested immediately after a 2-min pulse yielded a broad sedimentation profile.

These data (Fig. 4–6) establish the usefulness of rifamycin to measure the fraction of stable RNA labeled in a short pulse. These studies were then extended to each of the 20 size groups.

Fraction of stable RNA labeled in a 2-min pulse. An exponential-phase culture of *M. xanthus* was pulse-labeled with ^{3}H-uridine for 2 min and then exposed to rifamycin and unlabeled uridine for 20 min. Acid-soluble radioactivity was removed and the cells were prepared for quantitative autoradiography. Table 4 contains both the distribution of grains and the mean for each size group; the mean number of grains per cell as a function of cell size is presented in Fig. 7. The abscissa contains the 20 size groups as an exponential function as described previously for Fig. 2. The data in Fig. 7 show: (i) that the amount of stable RNA labeled in a short pulse increases linearly during the first 25% of the division cycle, and (ii) that this is followed by a plateau region with a relatively small increase in rate.

Rate of unstable RNA synthesis during the division cycle. The rate of unstable RNA synthesis as a function of cell age is obtained by correlation of the data from Fig. 3, 4, and 7. The rate of stable RNA synthesis during the division cycle (Fig. 3) minus the amount of stable RNA labeled in a 2-min pulse (Fig. 7) yields the fraction of unstable RNA labeled during a 2-min pulse. [This corrects the data in Table 4 for the fraction (50%) of stable RNA labeled in a 2-min pulse (Fig. 4); it

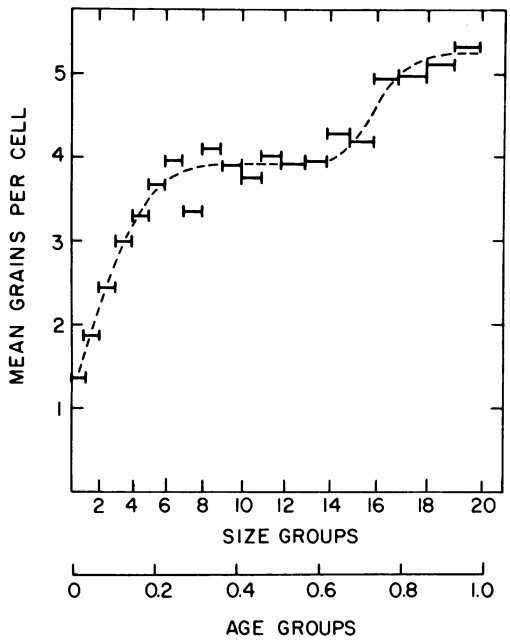

FIG. 3. *Rate of stable RNA synthesis as a function of cell size obtained from autoradiographic data of cells pulse-labeled with ^{3}H-uridine for 2 min. The 1,500 bacteria scored in this experiment are divided into 20 arbitrary size groups, each of which contains an equal number of bacteria. The mean number of silver grains for each size group (Table 2) is plotted as a function of cell size groups. The abscissa contains the 20 size groups as an exponential function as described in Fig. 2.*

TABLE 3. *Decay of unstable RNA in the presence of rifamycin*[a]

Length of ^{3}H-urjdine pulse (min)	Loss of acid-insoluble radioactivity after rifamycin addition		Half-life of unstable RNA[d] (min)
	Observed[b] (%)	Predicted[c] (%)	
2	50	50	3.5
4	51	47	4
10	46	40	4
40	35	17	4 + 10
80	29	8	10
360	5	1.9	

[a] Cells were grown in the presence of ^{3}H-uridine for 2, 4, 10, 40, 80, or 360 min. After the labeling period, rifamycin (8 μg/ml) and unlabeled uridine (500 μg/ml) were added. At timed intervals, duplicate samples (0.1 ml) were removed and assayed for acid-insoluble radioactivity.

[b] After rifamycin addition, the amount of acid-insoluble radioactivity decreased until a limit level was reacted (e.g., see Fig. 4). The per cent loss of radioactivity after rifamycin addition was calculated from this limit level.

[c] These values were obtained from the observation that, during a 2-min pulse, the label was distributed equally between stable and unstable species and making the assumption that the half-life of the unstable RNA was 4 min.

[d] The half-life of unstable RNA was calculated from the per cent acid-insoluble radioactivity remaining at time intervals after rifamycin addition (e.g., see insert of Fig. 4).

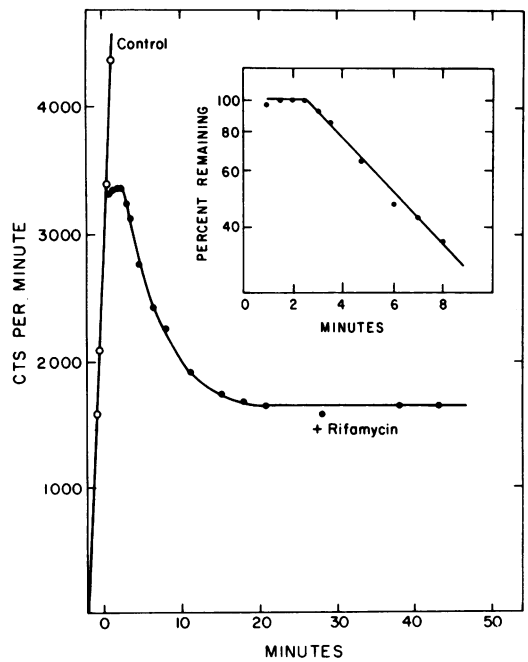

FIG. 4. *Kinetics of RNA degradation in the presence of rifamycin. A culture (9 ml) was pulse-labeled with ^{3}H-uridine (100 μCi/ml) for 2 min. The culture was then (defined as time zero) divided into two parts. To one part, rifamycin (8 μg/ml) and unlabeled uridine (500 μg/ml) were added (●); the other part was maintained as the control (○). At timed intervals, duplicate samples (0.1 ml) were removed and assayed for acid-insoluble radioactivity. The insert is a semilog plot of the per cent acid-insoluble radioactivity remaining as a function of the time after rifamycin addition.*

relates the two autoradiography experiments independent of such factors as exposure time, lag of rifamycin action, and changes in specific activity. More explicitly, before subtracting, the mean of each size group in Table 4 was multiplied by half the mean of all cells in Table 2, divided by the total mean of all cells in Table 4, or 0.50 × (3.76/2.93).] In this way the ratio of unstable to stable RNA synthesized in a 2-min pulse was determined for each size group (Fig. 8). The data clearly demonstrate that the relative amounts of stable and unstable RNA synthesis vary through the cell cycle, i.e., noncoordinate RNA synthesis.

Finally, the kinetics of unstable RNA synthesis

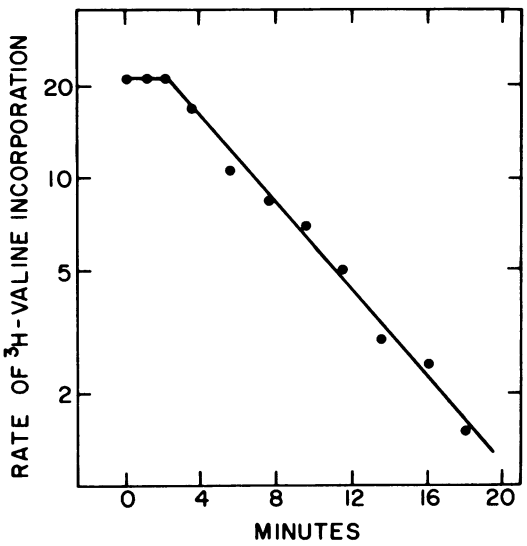

FIG. 5. *Change in rate of protein synthesis after rifamycin addition. A culture (13 ml) was labeled with ³H-valine (10 μCi/ml) for 4 min. The culture was then divided into two parts. To one part, rifamycin (8 μg/ml) was added; the other part was maintained as the control. At timed intervals, quintuplicate samples (0.05 ml) were removed and assayed for acid-insoluble radioactivity. The average rate of ³H-valine incorporation into acid-insoluble material is plotted as a function of the time after rifamycin addition. This semilog plot is corrected for the 5% residual valine incorporation that persists at a constant level in the presence of rifamycin.*

during the division cycle is obtained (Fig. 9) as follows: unstable RNA = stable RNA × (fraction unstable RNA/fraction stable RNA). These data indicate that unstable RNA: (i) increases during the first 25% of the division cycle, followed by (ii) a plateau region and (iii) a large burst of RNA synthesis during the last quarter of the division cycle. Comparison of the rates of stable and unstable RNA during the division cycle indicate the noncoordinate pattern of synthesis.

DISCUSSION

Stable RNA synthesis and chromosome replication. Evidence for the existence of a DNA cycle in *M. xanthus* growing in the defined medium was presented previously (24, 26). The position of the DNA cycle within the division cycle is summarized in Fig. 9: chromosome replication extends from 0.12 to 0.90 generation, septum formation occurs at 1.0, and physical separation of sister cells takes place 0.10 generation later. Although DNA synthesis occurs during 80% of the cycle, cells synthesize both stable and unstable RNA throughout the division cycle (Fig. 9). The rate of stable RNA synthesis increases in two discrete steps, corresponding to average ages of 0.15 and 0.75 generation. Correlation of these cell ages with the DNA cycle would "map" these events at 0.05 and 0.80, respectively, on the *M.*

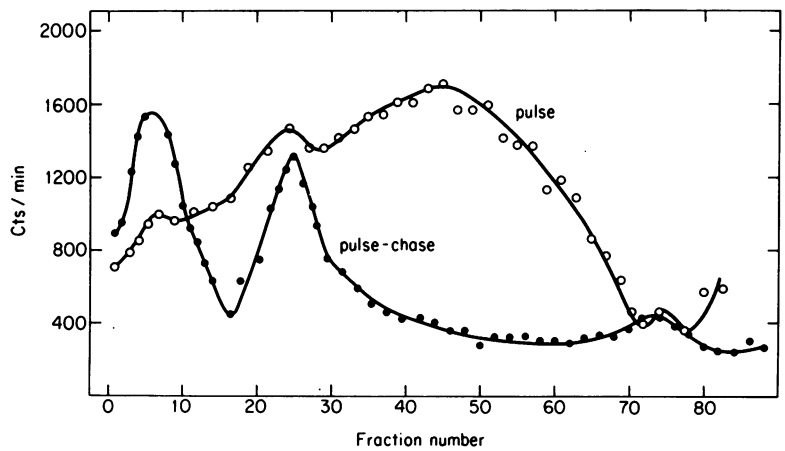

FIG. 6. *Sedimentation analysis of RNA present after rifamycin treatment of bacteria. An exponentially growing culture of M. xanthus was pulse-labeled with ³H-uridine (250 μCi/ml) for 2 min. Half of the culture was harvested immediately (pulse, ○), and half was exposed to rifamycin (8 μg/ml) and unlabeled uridine (500 μg/ml) for 20 min before harvesting (pulse-chase, ●). RNA was extracted, layered on sucrose gradients, and centrifuged for 6 hr at 114,000 × g as described in Materials and Methods. Fractions were collected from the bottom of the tube and analyzed for acid-insoluble radioactivity.*

TABLE 4. *Autoradiographic data as a function of cell size for M. xanthus pulse-labeled for 2 min with ^{3}H-uridine followed by rifamycin chase for 20 min*

Size group[a]	Length (μm)	Grains per cell (*n*)											Mean
		0	1	2	3	4	5	6	7	8	9	10	
1	1.76-2.52	23	34	11	6	1	0	0	0	0	0	0	1.04
2	2.52-2.88	19	25	14	12	5	0	0	0	0	0	0	1.45
3a	2.92-3.60	10	13	7	5	1	2	0	0	0	0	0	1.48
3b	1.80-2.48	11	12	6	4	1	3	0	0	0	0	0	
4	2.48-2.76	8	14	17	15	11	6	2	2	0	0	0	2.57
5	2.76-2.92	8	13	16	18	12	3	2	1	2	0	0	2.63
6	2.92-3.00	5	12	20	15	9	9	3	2	0	0	0	2.80
7	3.00-3.12	4	6	15	18	7	14	7	2	2	0	0	3.47
8	3.12-3.24	8	10	11	17	15	9	3	1	1	0	0	2.95
9	3.24-3.32	3	6	18	19	15	2	10	1	1	0	0	3.25
10	3.32-3.40	1	7	16	18	10	13	5	4	0	0	1	3.55
11	3.40-3.52	4	10	9	19	14	10	7	2	0	0	0	3.29
12	3.52-3.64	3	8	18	12	9	17	4	1	2	1	0	3.43
13	3.64-3.78	5	12	17	16	8	7	3	5	2	0	0	3.07
14	3.78-3.88	3	8	19	13	15	9	7	1	0	0	0	3.19
15	3.88-3.96	2	10	16	15	13	6	6	4	3	0	0	3.43
16	3.96-4.12	2	13	18	20	3	8	7	1	2	1	0	3.38
17	4.12-4.28	4	11	19	13	8	8	6	4	1	0	1	3.23
18	4.28-4.60	7	7	18	14	7	7	9	5	1	0	0	3.27
19	4.60-5.00	2	12	15	8	14	14	3	4	2	0	1	3.52
20	5.00-7.30	5	8	11	14	12	12	6	2	4	1	0	3.61
	Total	137	251	311	291	190	159	90	42	23	3	3	2.93

[a] The 1,500 bacteria scored in this experiment were analyzed as described in Table 2. Baby cells are presented in size groups 1 to 3a.

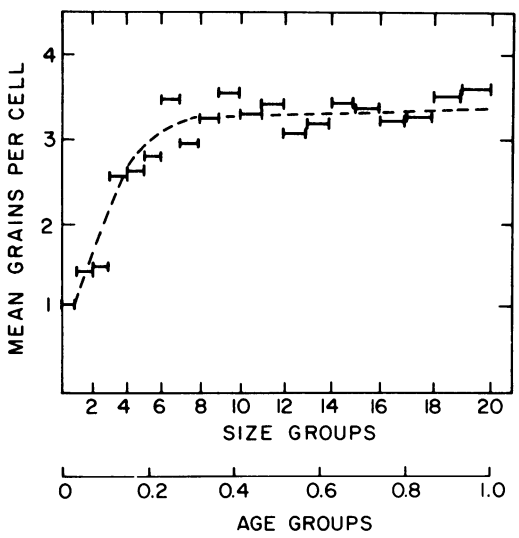

FIG. 7. *Amount of stable RNA labeled in a 2-min pulse. An exponentially growing culture of M. xanthus was pulse-labeled with ^{3}H-uridine (250 μCi/ml) for 2 min and then exposed to rifamycin (8 μg/ml) and unlabeled uridine (500 μg/ml) for 20 min. The cells were then washed and prepared for autoradiography. The 1,500 bacteria scored in this experiment were analyzed (Table 4) and are presented as described in Fig. 3.*

xanthus chromosome. These data are consistent with the hypothesis that the rate of stable RNA synthesis is template-controlled and dependent on gene dosage. This hypothesis cannot be tested in *M. xanthus*, however, until techniques for genetic mapping are developed.

RNA-DNA hybridization experiments with *E. coli* (2) and density transfer experiments with *Bacillus subtilis* (1, 20) indicate two clusters of genes complementary to stable RNA. In *B. subtilis*, 60 to 80% of these genes are located in a single cluster near the origin of the genetic map;

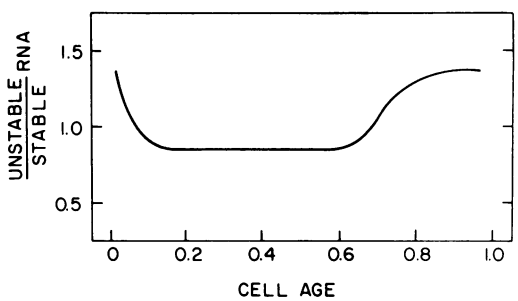

FIG. 8. *Noncoordinate RNA synthesis during the division cycle. The relative rate of unstable/stable RNA synthesis is plotted as a function of cell age. Data from Fig. 3, 4, and 7 are correlated.*

the remaining 20 to 40% of these genes is located in the terminal portion of the chromosome. The significance of two clusters of stable RNA genes on the bacterial chromosome is not understood at this time.

Direct measurements of the rate of stable RNA synthesis during the division cycle have been reported for *E. coli* (9, 17) and in *Salmonella typhimurium* (4). Since the DNA replication cycles of *E. coli* and *S. typhimurium* are complex and may include multiple growing points (7, 8), it is difficult to compare the data with those for *M. xanthus*.

Half-life of unstable RNA of M. xanthus. The kinetics of unstable RNA degradation in the presence of rifamycin (Fig. 4, Table 3) indicates two species of unstable RNA in *M. xanthus*. The major species of unstable RNA synthesized in a short labeling period has a half-life of about 4 min (Fig. 4). After long labeling periods with radioactive uridine, the other unstable RNA species predominates (half-life approximately 10 min). The function of this species is unknown; since the half-life of messenger RNA measured by the change in rate of protein synthesis after rifamycin addition is 4.2 min, it apparently does not code for protein synthesis. This unstable RNA fraction may represent a small quantity of precursor ribosomal RNA (15) that is not incorporated into mature ribosomes.

The half-life of the messenger RNA of *M. xanthus* growing with a generation time of 390 min at 30 C is of the same magnitude as that of *E. coli* (18) growing with a doubling time of 66 min at 30 C. Thus, bacteria with vastly different generation times can have messenger RNA species with similar half-lives.

Unstable RNA synthesis during the cell division cycle. To our knowledge, the rate of unstable RNA synthesis has never previously been measured during the bacterial division cycle. Previous studies have not considered the fact that even a short pulse of labeled precursor measures the rate of stable RNA synthesis (12). Our approach has been to measure the rate of stable RNA synthesis (2-min pulse) and then to determine the relative amount of stable and unstable RNA synthesized during the 2-min pulse. Correlating these data for each size group yields the kinetics of unstable RNA synthesis during the cell cycle.

The rate of unstable RNA synthesis during the division cycle exhibits three distinct phases: an initial rise, followed by a relatively constant rate of synthesis, and finally, a burst of unstable RNA synthesis prior to septum formation. Although the plateau period observed in Fig. 9 appears to occupy only 45% of the cell cycle and 56% of the

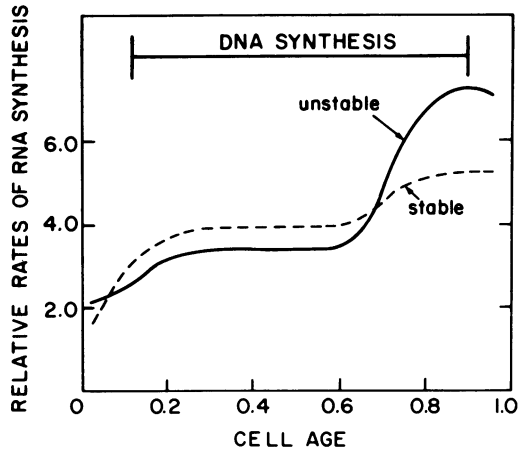

FIG. 9. *Relative rate of stable and unstable RNA synthesis as a function of cell age. Correlation of the data from Fig. 3, 4, and 7 yield the relative rates of stable (dashed line) and unstable (solid line) RNA synthesis as a function of cell age. The DNA cycle is presented to relate RNA synthesis to chromosome replication (26).*

DNA replication time, it probably represents the dominant pattern of unstable RNA synthesis during the cell cycle. Since the autoradiography data relate rates of synthesis to cell size, and since there is a definite distribution of size as a function of age (26), the transition periods are artificially broadened. Thus, the length of the plateau period represents a minimum value. The fact that the rate of unstable RNA synthesis remains relatively constant at a time when most of the DNA is replicated argues against any model for enzyme synthesis that is both continuous and gene-dosage-dependent. The rate of unstable RNA synthesis during this period is almost proportional to the constant rate of DNA synthesis (26) but certainly not proportional to total DNA.

During the last quarter of the division cycle, the rate of unstable RNA synthesis increases sharply. This burst of unstable RNA synthesis prior to division occurs at a time when the rate of stable RNA synthesis increases slightly and DNA synthesis is terminated. The data demonstrate noncoordinate RNA synthesis during the normal division cycle. Noncoordinate RNA synthesis has been reported previously only during non-steady-state conditions (13, 11). The function of the unstable RNA made before division and the mechanism for its control are not understood. However, the proximity of the burst in synthesis to the end of the cell cycle suggests that the information transcribed at this time may be related to the ill-defined series of events culminating in cell division.

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LITERATURE CITED

1. Colli, W., and M. Oishi. 1969. Ribosomal RNA genes in bacteria: evidence for the nature of the physical linkage between 16 *S* and 23 *S* RNA genes in *Bacillus subtilis*. Proc. Nat. Acad. Sci. U.S.A. **64**:642-649.
2. Cutler, R. G., and J. E. Evans. 1967. Relative transcription activity of different segments of the genome throughout the cell division cycle of *Escherichia coli*. The mapping of ribosomal and transfer RNA and the determination of the direction of replication. J. Mol. Biol. **26**:91-105.
3. Donachie, W. D., and M. Masters. 1969. Temporal control of gene expression in bacteria, p. 37-76. *In* G. M. Padilla, G. L. Whitson, and I. L. Cameron (ed.), The cell cycle. Academic Press Inc., New York.
4. Ecker, R. E., and G. Kokaisl. 1969. Synthesis of protein, ribonucleic acid, and ribosomes by individual bacterial cells in balanced growth. J. Bacteriol. **98**:1219-1226.
5. Hanawalt, P. C., O. Maaløe, D. J. Cummings, and M. Schaechter. 1961. The normal DNA replication cycle. II. J. Mol. Biol. **3**:156-165.
6. Harvey, R. J., A. G. Marr, and P. R. Painter. 1967. Kinetics of growth of individual cells of *Escherichia coli* and *Azotobacter agilis*. J. Bacteriol. **93**:605-617.
7. Helmstetter, C. E. 1969. Regulation of chromosome replication and cell division in *Escherichia coli*, p. 15-35. *In* G. M. Padilla, G. L. Whitson, and I. L. Cameron (ed.), The cell cycle. Academic Press Inc., New York.
8. Lark, C. 1966. Regulation of deoxyribonucleic acid synthesis in *Escherichia coli:* dependence on growth rates. Biochim. Biophys. Acta **119**:517-525.
9. Manor, H., and R. Haselkorn. 1967. Size fractionation of exponentially growing *Escherichia coli*. Nature (London) **214**:983-986.
10. Mitchison, J. M. 1969. Enzyme synthesis in synchronous cultures. Science **165**:657-663.
11. Morris, D. W., and N. O. Kjeldgaard. 1968. Evidence for the non-coordinate regulation of ribonucleic acid synthesis in stringent strains of *Escherichia coli*. J. Mol. Biol. **31**:145-148.
12. Nierlich, D. P. 1967. Radioisotope uptake as a measure of synthesis of messenger RNA. Science **158**:1186-1188.
13. Nierlich, D. P. 1968. Amino acid control over RNA synthesis: a re-evaluation. Proc. Nat. Acad. Sci. U.S.A. **60**: 1345-1352.
14. Nierlich, D. P., and W. Vielmetter. 1968. Kinetic studies on the relationship of ribonucleotide precursor pools and ribonucleic acid synthesis. J. Mol. Biol. **32**:135-147.
15. Pace, B., R. L. Peterson, and N. R. Pace. 1970. Formation of all stable RNA species in *Escherichia coli* by posttranscriptional modification. Proc. Nat. Acad. Sci. U.S.A. **65**:1097-1104.
16. Pierucci, O., and C. Helmstetter. 1969. Chromosome replication, protein synthesis and cell division in *Escherichia coli*. Fed. Proc. **28**:1755-1760.
17. Rudner, R., E. Rejman, and E. Chargaff. 1965. Genetic implications of periodic pulsations of the rate of synthesis and the composition of rapidly labeled bacterial RNA. Proc. Nat. Acad. Sci. U.S.A. **54**:904-911.
18. Salser, W., J. Janin, and C. Levinthal. 1968. Measurement of the unstable RNA in exponentially growing cultures of *Bacillus subtilis* and *Escherichia coli*. J. Mol. Biol. **31**: 237-266.
19. Sippel, A., and G. Hartmann. 1968. Mode of action of rifamycin on the RNA polymerase reaction. Biochim. Biophys. Acta **157**:218-219.
20. Smith, I., D. Dubnau, P. Morell, and J. Marmur. 1968. Chromosomal location of DNA base sequences complementary to transfer RNA and to 5*S*, 16*S* and 23*S* ribosomal RNA in *Bacillus subtilis*. J. Mol. Biol. **33**:123-140.
21. Stanley, W. M., and R. M. Bock. 1965. Isolation and physical properties of the ribosomal ribonucleic acid of *Escherichia coli*. Biochemistry **4**:1302-1311.
22. Wehrli, W., J. Nuesch, F. Knusel, and M. Staehelin. 1968. Action of rifamycins on RNA polymerase. Biochim. Biophys. Acta **157**:215-217.
23. Witkin, S., and E. Rosenberg. 1970. Induction of morphogenesis by methionine starvation in *Myxococcus xanthus:* polyamine control. J. Bacteriol. **103**:641-649.
24. Zusman, D., and E. Rosenberg. 1968. Deoxyribonucleic acid synthesis during microcyst germination in *Myxococcus xanthus*. J. Bacteriol. **96**:981-986.
25. Zusman, D., P. Gottlieb, and E. Rosenberg. 1971. Division cycle of *Myxococcus xanthus*. III. Kinetics of cell growth and protein synthesis. J. Bacteriol. **105**:811-819.
26. Zusman, D., and E. Rosenberg. 1970. DNA cycle of *Myxococcus xanthus*. J. Mol. Biol. **49**:609-612.