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Isolation of Membrane-Associated Folded Chromosomes from *Escherichia coli*: Effect of Protein Synthesis Inhibition

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The sedimentation properties of membrane-associated folded chromosomes prepared from *Escherichia coli* TAU-bar at 0 to 4 C were studied. Utilizing a modification of the procedure of Stonington and Pettijohn (1971), quantitative yields of membrane-associated folded chromosomes may be obtained. Folded chromosomes remained associated with the cell envelope during their replication and after completion of residual synthesis in the absence of required amino acids, as demonstrated by sedimentation velocities and the presence of high levels of cosedimenting protein. Membrane-associated folded chromosomes isolated from amino acid-starved cells sedimented more rapidly than membrane-associated folded chromosomes isolated from exponentially growing cells.

The genome of *Escherichia coli* can be isolated as a compact, rapidly sedimenting structure by detergent lysis of spheroplasts in the presence of 1.0 M NaCl (19, 21, 24, 25). The structure is apparently stabilized in part by ribonucleic acid (RNA)-deoxyribonucleic acid (DNA) interactions and functional RNA polymerase (8, 18, 21, 24). The chromosome possesses superhelical density typical of closed circular DNA molecules (24), but unlike other supercoiled molecules a single interruption in the continuity of a DNA strand is insufficient to destroy the superhelical properties of the molecule (18, 24). The chromosome is divided into many loops or folds, and each loop behaves as an independent superhelix unit, so that a nick in one loop only removes the superhelical turns of the DNA within that loop (7, 18, 24).

When the lysis procedure is performed at 22 C, folded chromosomes isolated from exponentially growing cells sediment through linear sucrose gradients as a broad peak at about 1,700*S* (19, 24). However, when the lysis is carried out at 0 to 4 C, the folded chromosomes are associated with a portion of the cell envelope (8, 19, 24). Such membrane-associated folded chromosomes sediment through sucrose gradients as a broad band with a peak at about 3,200*S* (19, 21, 25). Worcel and Burgi (25) recently reported results obtained with lysis of *E. coli* DG 75 cells at 10 C. At this temperature, a mixture of membrane-associated and membrane-free folded chromosomes was obtained. When folded chromosomes were isolated from amino acid-starved *E. coli* DG 75 cells at 10 C, membrane-associated folded chromosomes could no longer be isolated (25). Under their conditions all the recovered folded chromosomes were the more slowly sedimenting membrane-free form. These results were interpreted as evidence that chromosomes having completed rounds of replication are released from the cell envelope to become reassociated again after the resumption of DNA synthesis.

We report here studies of isolation, by a modification of the procedure of Stonington and Pettijohn (21), at 0 to 4 C of membrane-associated folded chromosomes from *E. coli* 15 TAU-bar during exponential growth and after amino acid starvation. Under these conditions, yields of membrane-associated folded chromosomes from *E. coli* 15 TAU-bar are nearly quantitative. Results obtained with this procedure indicate that folded chromosomes do not lose their membrane association after deprivation of required amino acids; to the contrary they become more rapidly sedimenting.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *E. coli* 15 TAU-bar (*thy⁻ura⁻arg⁻met⁻pro⁻try⁻*) (9) was used throughout these studies. Fresh overnight cultures were innoculated into prewarmed TG_0 medium (modified TPG medium [6] lacking sodium-pyruvate) containing 4 μg of thymine per ml, 20 μg of uracil per ml, and 20 μg each of L-arginine, L-methionine, L-proline, and L-tryptophan (minimal medium) per ml or with 20 μg of each naturally occurring L-amino acid (enriched medium) per ml. Labeling was performed with [^{14}C]thymine (0.5 to 1.0 μCi/ml, 47.5 mCi/mmol, New England Nuclear), [^{3}H]arginine (4.0 μCi/ml, 22 Ci/mmol, Amersham/Searle), or [^{3}H]leucine (1.25 to 10.0 μCi/ml, 6 Ci/mmol, Schwarz) and

[^{3}H]lysine (1.25 to 10.0 μCi/ml, 6.7 Ci/mmol, Amersham/Searle). Cultures were grown at 37 C with aeration. Transfer to medium lacking amino acids was accomplished by rapid filtration (15). When cultures were deprived of required amino acids 20 μg of L-methionine per ml was added to the medium. Chloramphenicol (Sigma) and tetracycline (Sigma) were freshly prepared before use.

Preparation of membrane-associated folded chromosomes. Cultures (10 ml) of *E. coli* 15 TAU-bar log phase cells (10^8 cells/ml) were quickly cooled to 0 C in a −80 C bath and harvested by centrifugation (8 min, 7,000 × *g*, 0 C), and the cell pellet was drained of any residual medium. Preparation and lysis of spheroplasts at 0 to 4 C was performed by a modification of the procedure of Stonington and Pettijohn (21). Pelleted cells were resuspended in 0.20 ml of a solution containing 0.10 M tris(hydroxymethyl)aminomethane (Tris)-chloride (pH 8.0), 0.01 M NaCl, 0.01 M NaN_3, and 20% (wt/vol) sucrose (density gradient grade, Mann) at 0 C. Incubation at 0 C was not allowed to exceed 4 min. A freshly prepared solution (0.050 ml) of 4 mg of egg white lysozyme (Worthington) per ml in 0.12 M Tris-chloride (pH 8.0), 0.05 M sodium-ethylenediaminetetraacetate was added; the solution was gently mixed and incubated at 0 C for 3 min. Lysis was accomplished by adding 0.25 ml of a solution containing 1% (wt/vol) Brij 58, 0.4% (wt/vol) sodium-deoxycholate, 0.01 M sodium-ethylenediaminetetraacetate and 2.0 M NaCl. After careful mixing, the lysate was incubated at 4 C for 20 to 30 min. Qualitatively identical results were obtained if the lysozyme treatment was shortened, or if the incubation time of the lysate at 4 C was reduced to as little as 2.0 min. The longer incubation periods seem to result in somewhat greater yields of membrane-associated folded chromosomes from *E. coli* 15 TAU-bar. Lysates were either layered directly onto a 5-ml linear 10 to 30% sucrose (density gradient grade, Mann) gradient, containing 0.01 M Tris-chloride (pH 8.0), 0.001 M sodium-ethylenediaminetetraacetate, 1.0 M NaCl, and 1 mM 2-mercaptoethanol, or were subjected to a 4,000 × *g* centrifugation (5 min, 0 C); the supernatant was subsequently layered onto a sucrose gradient. ^{32}P-labeled T4 bacteriophage were added as indicated as a sedimentation marker to some of the lysates or to 4,000 × *g* supernatants. Centrifugation was performed in a Spinco SW50.1 rotor at 16,000 rpm for either 10.0, 3.0, or 4.0 min at 2 C in an L3-50 ultracentrifuge.

Preparation of membrane-free folded chromosomes. Membrane-free folded chromosomes were prepared by the same method as were membrane-associated folded chromosomes, except that after the lysozyme was added the solution was incubated at 22 C. Lysis was performed at 22 C for 20 min, and the crude lysate was layered on sucrose gradients as described above. Centrifugation was performed in a Spinco SW50.1 rotor at 16,000 rpm for 25 min at 2 C.

Collection and analysis of sucrose gradients. Gradients were collected through a needle from the side of the centrifuge tube 5 mm from the bottom. Fractions were collected onto glass fiber filters (GF/C, Whatman) and dried. The fractions were washed in cold 5% trichloroacetic acid, rinsed with water, and then rinsed with 95% ethanol. After drying, the filters were placed in scintillation vials containing a toluene-based scintillation fluid. Radioactivity was determined in a liquid scintillation counter under conditions appropriate for discrimination of the isotopes.

RESULTS

Conditions which permit isolation of bacterial DNA in the form of compact, rapidly sedimenting membrane-associated folded chromosomes are not well understood. Lysis of cells in solutions of high ionic strength is one required condition, but once the cells have been lysed the chromosomes are stable in salt concentrations of 0.2 M and less (21; Simon and Ryder, unpublished data). The lysis procedure developed by Stonington and Pettijohn (21) has provided consistent and reproducible results in our laboratory; however, the total yields of chromosomes from exponentially growing cells were on the order of 30 to 50%. Because of the ambiguities in interpretation of results when a minority of the total DNA is isolated, attempts were made to increase total yields of chromosomes.

In an effort to improve recoveries of membrane-associated folded chromosomes, the effect of the 4,000 × *g* centrifugation in the Stonington and Pettijohn (21) procedure on recovery and sedimentation properties of the chromosomes was examined. Figure 1 illustrates the sedimentation profiles of membrane-associated folded chromosomes prepared at 0 to 4 C. The sedimentation profile obtained using the supernatant of the 4,000 × *g* centrifugation (Fig. 1a) was nearly identical to the sedimentation profile obtained when the crude lysate was directly layered onto a sucrose gradient (Fig. 1b).

The membrane-associated folded chromosomes are identified by the association of high levels of cosedimenting protein (19, 24) and by their sedimentation velocity (19, 21, 25). The relative sedimentation constant, determined by sedimentation velocity relative to ^{32}P-labeled T4 bacteriophage (1,025*S*; 5), was 3,600*S* for the peak fractions in Fig. 1a and b. These values are slightly higher than the value of 3,200*S* obtained by Stonington and Pettijohn (21) for *E. coli* D10 and by Dworsky and Schaechter (8) for *E. coli* D10 and RFS116. The more slowly sedimenting material which lacks appreciable levels of cosedimenting protein sedimented between 1,500 and 2,600*S* and probably represents folded chromosomes released from the cell envelope (see Fig. 2). A small proportion of membrane-free folded chromosomes were com-

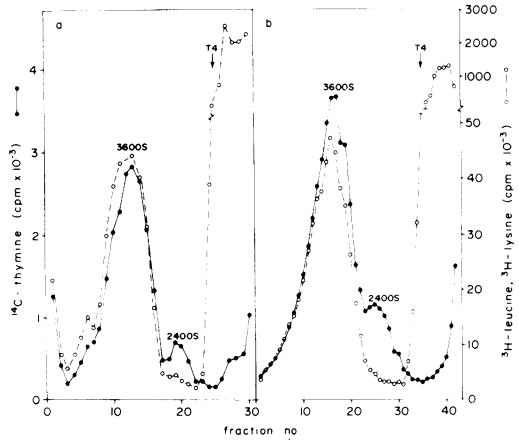

FIG. 1. *Effect of 4,000 × g centrifugation on sedimentation properties of membrane-associated folded chromosomes. (a) Cells were grown in enriched medium containing 1.0 μCi of [^{14}C]thymine per ml, 8 μCi of [^{3}H]leucine per ml, and 10 μCi of [^{3}H]lysine per ml. Membrane-associated folded chromosomes were prepared, and the lysate was subjected to a 4,000 × g centrifugation. The supernatant together with T4 phage marker was layered on a sucrose gradient. (b) Cells were grown in enriched medium containing 0.5 μCi of [^{14}C]thymine per ml, 5 μCi of [^{3}H]leucine per ml, and 5 μCi of [^{3}H]lysine per ml. The crude lysate together with T4 phage marker was layered on a sucrose gradient. Centrifugation of sucrose gradients was for 10 min at 16,000 rpm as described. The positions of the marker T4 phage are indicated by the arrows. Ninety percent of acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation in each case.*

monly observed in sucrose gradient displays of lysates prepared at 0 to 4 C; their relative proportion may be increased by prolonged incubation of the crude lysate at 4 C (Ryder, data not shown). The DNA which did not enter the gradient probably represents unfolded or otherwise disrupted chromosomes. Figure 1 illustrates data obtained from cells growing in medium containing a mixture of the naturally occurring L-amino acids; essentially identical results were obtained from cells grown in minimal medium (Ryder, data not shown).

Yields of membrane-associated folded chromosomes were higher when the 4,000 × *g* centrifugation was omitted in the lysis procedure (Table 1), although the membrane-associated folded chromosomes obtained were similar or identical in sedimentation behavior to those obtained when the 4,000 × *g* centrifugation was included.

Figure 2 illustrates the sedimentation profile obtained when exponentially growing cells were lysed at 22 C, and the crude lysate was displayed on a sucrose gradient. The DNA sedimented with a peak at 2,100*S*, and there was relatively little label from amino acids cosedimenting with the DNA (compare with Fig. 1 and Fig. 4).

When membrane-associated folded chromosomes were isolated from cells starved 60 min for required amino acids and the 4,000 × *g* centrifugation was included in the lysis procedure, total recovery of chromosomes was greatly decreased (Table 1). As reported by Worcel and Burgi (25), no membrane-associated folded chromosomes are isolated under these conditions (Fig. 3a). However, when the 4,000 × *g* centrifugation was omitted and the crude lysate was layered directly on the sucrose gradient, a different result was obtained (Fig. 3b). Under these conditions, no peak of rapidly sedimenting material was detected; neither membrane-free nor membrane-associated folded chromosomes were isolated. In both cases, the majority of the DNA was unresolved with respect to its

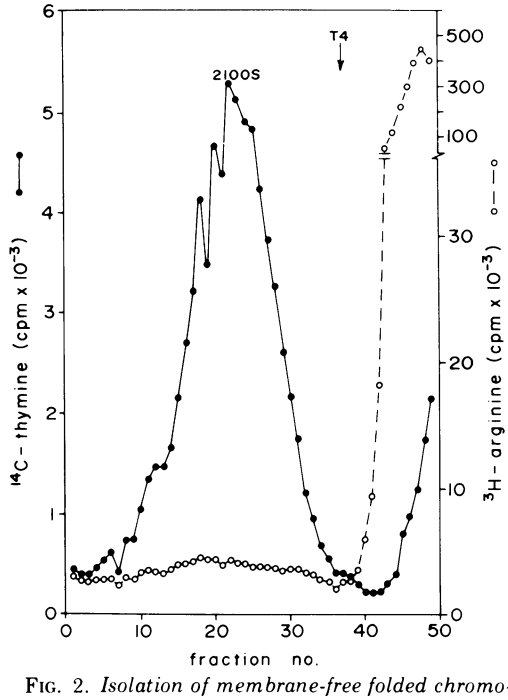

FIG. 2. *Isolation of membrane-free folded chromosomes. Cells were grown in minimal medium containing 0.5 μCi of [^{14}C]thymine per ml and 4 μCi of [^{3}H]arginine per ml. Membrane-free folded chromosomes were prepared at 22 C, and the crude lysate with T4 phage marker was layered on a sucrose gradient. Centrifugation was for 25 min at 16,000 rpm as described. The arrow indicates the position of the T4 phage marker. Seventy-one percent of the acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation.*

TABLE 1. *Recovery of labeled DNA*

Source of lysate	Fractionation of acid-insoluble [^{14}C]thymine counts in 4,000 × *g* centrifugation[a] (%)		Recovery of chromosomes from sucrose gradient centrifugation		
	Supernatant	Pellet	4,000 × *g* centrifugation	Time at 16,000 rpm (min)	Recovery of chromosomes[a] (%)
Exponentially growing cells	32	68	+	10	29
			−	10	96
			−	3	94
Amino acid-starved cells (60 min)	3	97	+	10	3
			−	10	11
			−	3	89

[a] Expressed as percentage of counts in the crude lysate.

sedimentation properties in the sucrose gradients. It is clear that if the 4,000 × *g* centrifugation is omitted, the DNA resolved on the sucrose gradient is not exclusively present as membrane-free material with sedimentation coefficient of about 1,300 to 1,500*S*.

Dworsky and Schaechter (8) reported that membrane-associated folded chromosomes isolated from cells treated with chloramphenicol or tetracycline are much more rapidly sedimenting than are chromosomes isolated from exponentially growing cells under similar conditions. If a similar result is obtained for folded chromosomes isolated from amino acid-starved cells, then such chromosomes might sediment with sufficient velocity to pellet either in the 4,000 × *g* centrifugation or in the sucrose gradient centrifugation. This is apparently the case, as is illustrated in Fig. 4. When membrane-associated folded chromosomes were prepared from amino acid-starved cells (Fig. 4b), they sedimented much more rapidly than did membrane-associated folded chromosomes from exponentially growing cells (Fig. 4a). In both cases, efficiency of the lysis of the cells was similar, since approximately 90% of acid-precipitable radioactivity from labeled amino acid remained at the top of the gradient. The rapidly sedimenting nature of membrane-associated folded chromosomes was observed over a 40-fold range of concentration of cells in the lysate. Thus, membrane-associated folded chromosomes isolated from amino acid-starved cells sedimented to the same position in sucrose gradients whether there were 2×10^9 cells or 5×10^7 cells in the crude lysate.

Similar results were obtained when the protein synthesis antibiotics chloramphenicol and tetracycline were incubated with exponentially growing cells for 60 min at concentrations sufficient to immediately inhibit growth (Fig. 5a and b).

When amino acids were resupplied to an amino acid-starved culture, the sedimentation properties of membrane-associated folded chromosomes were altered; they then resembled the sedimentation properties of chromosomes isolated from exponentially growing cells (Fig. 6). After 90 min of amino acid starvation, the bulk of the chromosomes isolated at 0 to 4 C did not appear in the gradient profile and presumably had been deposited at the bottom of the centrifuge tube (Fig. 6a). After restoration of required amino acids for 45 min, the sedimentation velocities of the isolated chromosomes reverted to the range of velocities observed for chromosomes prepared from exponentially growing cells (Fig. 6b). (DNA synthesis was resumed by 30 min after restoration of amino acids to amino acid-starved cells [data not shown].) The presence of relatively large amounts of cosedimenting label in protein with the DNA in Fig. 6b confirms that these chromosomes possess the characteristics of membrane-associated folded chromosomes.

DISCUSSION

We have observed that omission of the 4,000 × *g* centrifugation step in the procedure for isolation of bacterial chromosomes in a compact, highly folded state developed by Stonington and Pettijohn (21) allows quantitative yields of membrane-associated folded chromosomes. Up to 96% of total label incorporated in a uniform labeling with thymine (Table 1) or from a short pulse labeling with thymidine (Ryder, data not shown) may be recovered as rapidly sedimenting material. These high levels of recovery routinely observed under our conditions suggest that the structure isolated does not represent a unique subfraction of the total population of chromosomes which is somehow in a state favorable for isolation.

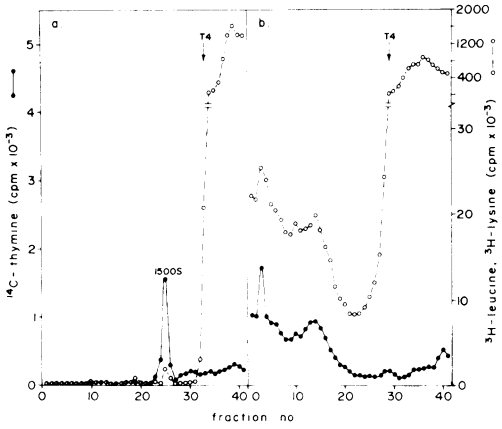

FIG. 3. *Effect of 4,000 × g centrifugation on sedimentation properties of chromosomes from amino acid-starved cells. (a) Cells were grown in enriched medium containing 0.5 μCi of [^{14}C]thymine per ml, 2.5 μCi of [^{3}H]leucine per ml, and 2.5 μCi of [^{3}H]lysine per ml to 10^{8} cells/ml and transferred to medium lacking amino acids, except for methionine (1). After 60-min amino acid starvation, the culture was lysed at 0 to 4 C. The lysate was subjected to a 4,000 × g centrifugation. T4 phage marker was added to the supernatant which was layered on a sucrose gradient. Ninety percent of the acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation. (b) Cells were grown in enriched medium containing 0.5 μCi of [^{14}C]thymine per ml and 2.5 μCi of [^{3}H]lysine per ml. After transfer to medium lacking amino acids except methionine, the culture was incubated for 60 min and subsequently lysed at 0 to 4 C. The crude lysate along with T4 phage marker was layered on a sucrose gradient. Eleven percent of the acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation. Both gradients were centrifuged for 10 min at 16,000 rpm as described. The positions of T4 phage marker are indicated by the arrows.*

When lysates of cells starved for required amino acids for periods of time sufficient to allow the completion of the residual DNA synthesis, which occurs in amino acid-starved cells (60 or more min in *E. coli* 15 TAU-bar under our growth conditions, data not shown), are subjected to a 4,000 × *g* centrifugation and the supernatant is displayed on sucrose gradients run for more than 4 min at 16,000 rpm, no membrane-associated folded chromosomes are obtained (Fig. 3). With the inclusion of the 4,000 × *g* centrifugation step, all the recovered chromosomes lacked the levels of cosedimenting protein indicative of membrane association and sedimented at the position of membrane-free folded chromosomes, approximately 1,500*S* (Fig. 3a). Under these same conditions of centrifugation, crude lysates displayed on sucrose gradients revealed no unique peak of rapidly sedimenting material (Fig. 3b). If the time of centrifugation is greater than about 4 min, the recovery of chromosomes is very low, whether or not the 4,000 × *g* centrifugation is included in the lysis procedure (Table 1). Thus, either the chromosomes are not recovered from the 4,000 × *g* centrifugation (Table 1, Fig. 3a), or they are not recovered from the sucrose gradient under these conditions (Table 1, Fig. 3b and 6a).

In contrast, when crude lysates of cultures starved for required amino acids are sedimented through sucrose gradients for 3.0 min at 16,000 rpm, the recovery of chromosomes is greatly increased (Fig. 4b, Table 1). Under these conditions, 80 to 90% of the total acid-insoluble label from thymine is recovered from the sucrose gradient and is recovered in membrane-associated folded chromosomes.

Consequently, membrane-associated folded chromosomes isolated from cells which have completed DNA synthesis are much more rapidly sedimenting than membrane-associated folded chromosomes obtained from exponentially growing cells. Very rapidly sedimenting membrane-associated folded chromosomes are also obtained from cells treated with chloramphenicol or tetracycline for sufficient periods of time (8; Fig. 5). We estimate that these chromo-

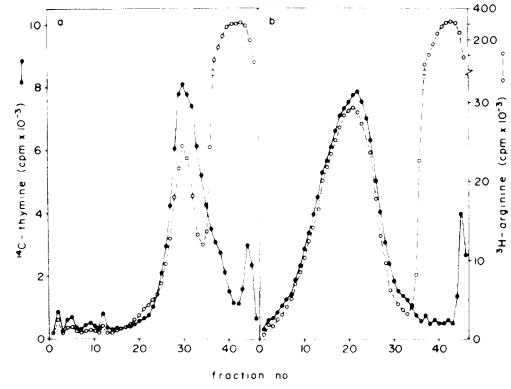

FIG. 4. *Comparison of sedimentation properties of membrane-associated folded chromosomes from exponential and amino acid-starved cells. Cells were grown in minimal medium containing 0.5 μCi of [^{14}C]thymine per ml and 2.0 μCi of [^{3}H]arginine per ml. Membrane-associated folded chromosomes were prepared, and the crude lysates were centrifuged through identical sucrose gradients for 3.0 min at 16,000 rpm in the same rotor. (a) Exponentially growing cells. Ninety-four percent of the acid-soluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation. (b) Cells were transferred to non-radioactive medium lacking required amino acids except methionine and incubated for 60 min. Eighty-nine percent of the acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation.*

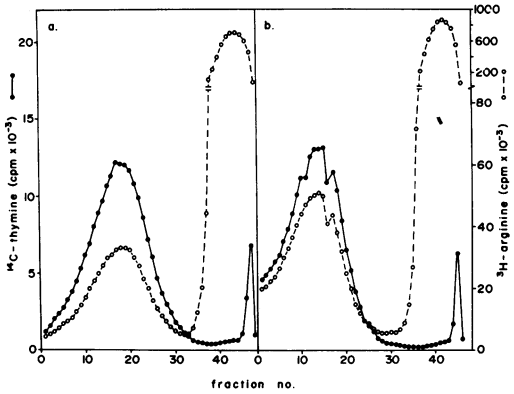

FIG. 5. *Effects of protein synthesis inhibitors on sedimentation properties of membrane-associated folded chromosomes. Cells were grown in minimal medium containing 1.0 μCi of [^{14}C]thymine per ml and 4 μCi of [^{3}H]arginine per ml to 10^{8} cells/ml. Antibiotics were added and the cultures were incubated for an additional 60 min. Membrane-associated folded chromosomes were prepared and the crude lysates were centrifuged through identical sucrose gradients for 3.0 min at 16,000 rpm in the same rotor. (a) Chloramphenicol (200 μg/ml) was added to the growing culture. Eighty-five percent of the acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation. (b) Tetracycline (1 μg/ml) was added to the growing culture. Seventy-eight percent of the acid-soluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation.*

somes have relative sedimentation coefficients of 5,000 to 6,000*S*.

The rapidly sedimenting character of membrane-associated folded chromosomes from cells deprived of required amino acids is reversed once amino acids are restored (Fig. 6b). The change in sedimentation properties of membrane-associated folded chromosomes observed after amino acids are restored to a starved culture requires de novo protein synthesis; if 200 μg of chloramphenicol per ml is added to a culture immediately before amino acids are restored, no decrease in sedimentation velocities of membrane-associated folded chromosomes is observed (Ryder, data not shown).

Worcel and Burgi (25) recently reported that membrane-associated folded chromosomes cannot be isolated from amino acid-starved *E. coli* DG 75 cells which have completed residual DNA synthesis; all of the DNA then appears as folded chromosomes lacking associated membrane. They interpreted these results to indicate that attachment of folded chromosomes to a site associated with the cell envelope might be a required step in the regulation of the process of initiation of DNA replication, as suggested in the replicon model (11) for the regulation of rounds of bacterial DNA replication.

Although we were also unable to obtain membrane-associated folded chromosomes from amino acid-starved cells when the 4,000 × *g* centrifugation was included in the lysis procedure, it is apparent that, at least for *E. coli* 15 TAU-bar, this result was due to the extremely rapidly sedimenting nature of membrane-associated folded chromosomes isolated from amino acid-starved cells. Clearly, under the conditions of lysis described here, release of the chromosome from the cell envelope does not appear to occur as a result of amino acid starvation in *E. coli* 15 TAU-bar. However, because of the differences in temperature of lysis and in the *E. coli* strains used, the results of this study and that of Worcel and Burgi (25) may not be directly comparable.

Inhibition of protein synthesis by deprivation of required amino acids (2, 4, 14, 15, 23) or by antibiotics (13) blocks initiation of new rounds

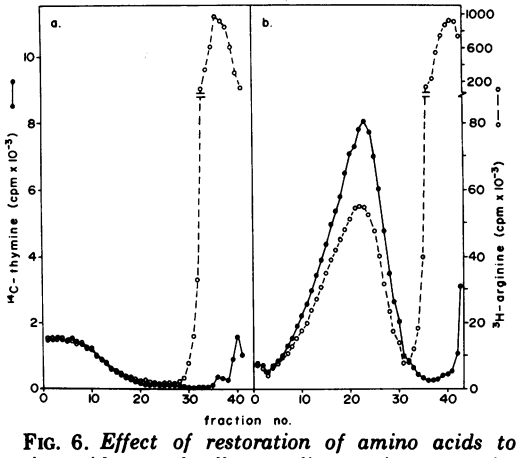

FIG. 6. *Effect of restoration of amino acids to amino acid-starved cells on sedimentation properties of membrane-associated folded chromosomes. Cells were grown in minimal medium containing 1.0 μCi of [^{14}C]thymine per ml and 4 μCi of [^{3}H]arginine per ml to 10^{8} cells/ml, transferred to medium lacking [^{14}C]thymine and required amino acids except methionine, and incubated for 90 min. A portion was removed, and the cells were harvested and stored at 0 C until membrane-associated folded chromosomes were prepared. Non-radioactive required amino acids were then added and the culture was incubated for 45 min. Membrane-associated folded chromosomes were prepared, and the crude lysates were centrifuged through identical sucrose gradients in the same rotor for 4.0 min at 16,000 rpm as described. (a) Amino acid starvation (90 min). Nineteen percent of the acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation. (b) Amino acid starvation (90 min), 45-min amino acid restoration. One hundred and four percent of the acid-insoluble ^{14}C-labeled radioactivity applied to the gradient was recovered after centrifugation.*

of chromosome replication, but allows residual replication of duplicating chromosomes to a point up to or close to the end (2, 4, 14, 15). Recent evidence (16) suggests that, in the absence of protein synthesis, DNA replication does not proceed to the very end of the chromosome. A terminal segment representing about 0.5% of the chromosome remains unreplicated. This result would suggest that the chromosomes of cells which had been starved for required amino acids should have a mass approximately twice that of initiating chromosomes.

Sedimentation velocities depend both upon molecular weight and conformational properties of the sedimenting particle. The relative contributions each of these factors makes to the sedimentation properties of membrane-associated folded chromosomes has not been determined. Therefore, the explanation for the increased sedimentation velocities of membrane-associated folded chromosomes isolated from amino acid-starved cells could be due to increases in molecular weight, alterations in hydrodynamic properties, or both. In electron microscope studies, conformational changes in the structure of bacterial nuclear regions have been observed after protein synthesis inhibition (17, 26).

It is tempting to suggest that changes in sedimentation properties of membrane-associated folded chromosomes isolated from amino acid-starved cells and the subsequent sedimentation changes observed after resupplying amino acids to a starved culture are correlated with the events in the DNA replication cycle. However, the observed changes may also be correlated with the activity of the protein synthesizing machinery of the cell. Studies utilizing *E. coli* mutants thermosensitive in functions involved in the process of initiation of DNA synthesis (3, 10, 12, 20, 22) should resolve whether the sedimentation properties of membrane-associated folded chromosomes obtained from amino acid-starved cells are due to events in the DNA replication cycle or are directly related to protein synthesis inhibition.

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