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Envelope-Associated Nucleoid from *Caulobacter crescentus* Stalked and Swarmer Cells

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Envelope-associated nucleoids have been isolated from *Caulobacter crescentus* by using a modification of the procedure of T. Kornberg et al. (Proc. Natl. Acad. Sci. U.S.A. 71:3189-3193, 1974). The development of a Ludox density gradient procedure has permitted preparation of large quantities of synchronous cells. The sedimentation coefficients of the envelope-associated nucleoids of stalked and swarmer cells, prepared under conditions of equivalent cell lysis, were 3,000S and >6,000S, respectively. Small differences in the relative amounts of deoxyribonucleic acid, ribonucleic acid, and protein in stalked and swarmer cell envelope-associated nucleoids could not account for the large differences in sedimentation behavior. These characteristic sedimentation coefficients were retained in mixing experiments.

Cell division in *Caulobacter crescentus* is asymmetric and produces morphologically distinct progeny cells that have differing capacities for deoxyribonucleic acid (DNA) replication. The stalked cell is able to immediately reinitiate chromosome replication, whereas DNA replication in the swarmer cell is postponed until differentiation into a stalked cell is completed (4).

To investigate the properties of the replicating and nonreplicating *Caulobacter* chromosome, the envelope-associated nucleoid from stalked and swarmer cells was isolated and characterized. As reported in other bacterial systems (for review, see reference 16), the envelope-associated *Caulobacter* nucleoids are prepared by slight modification of procedures used for preparing such structures from *Escherichia coli* (10). Our studies demonstrate that the envelope-associated nucleoids isolated from stalked and swarmer cell types exhibit markedly different sedimentation behaviors on sucrose gradients. In this report we have explored, in *Caulobacter*, a number of parameters that have been shown to alter the sedimentation characteristics of the *E. coli* envelope-associated nucleoid. We present evidence that the differences in sedimentation behavior exhibited by stalked and swarmer cell chromosomes are intrinsic properties of the envelope-associated nucleoid from each of the *Caulobacter* cell types.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *C. crescentus* strain CB15 (ATCC 19089) was grown at 30°C to an absorbance at 660 nm (A_{660}) of 0.4 to 0.5 (10^9 colony-forming units per ml) in minimal glucose medium (HMG) (19). *E. coli* strain B^e, a gift from E. T. Young, was grown at 37°C to A_{550} = 0.5 (5×10^8 colony-forming units per ml) on M9 medium (7).

Isotopic labeling. DNA was labeled by incubating cultures at 30°C for approximately 1 h in the presence of [^{3}H]adenine (20 to 27 Ci/mmol; New England Nuclear) or [^{3}H]guanosine (10.5 Ci/mmol, Amersham/Searle). The relative incorporation of [^{3}H]adenine or [^{3}H]guanosine into DNA and ribonucleic acid (RNA) was determined by assay of trichloroacetic acid-insoluble radioactivity before and after alkaline hydrolysis (1 N NaOH, 60°C, 75 min) according to the method of Newton (13). With either [^{3}H]adenine or [^{3}H]guanosine, 6 to 10% of the total radioactivity incorporated into nucleic acid corresponded to DNA. Samples were filtered on Whatman GF/C filters and counted in Liquifluor (New England Nuclear), using a Packard Tri-Carb scintillation counter. In double-label experiments, the counting channels were chosen to minimize ^{14}C counts being recorded in the ^{3}H channel.

Cellular protein was labeled by incubating cultures at 30°C for 15 min in the presence of 1 μCi of a ^{14}C-labeled L-amino acid mixture (0.1 mCi/ml, New England Nuclear). The amount of ^{14}C-labeled amino acid incorporated into protein was determined by assay of trichloroacetic acid-insoluble radioactivity collected on Whatman GF/C filters.

Technique for synchronization of cells. Synchronous swarmer cells were isolated on Ludox density gradients by a modification of the technique of Pertoft et al. (15) and Shulman et al. (21). Ludox HS (DuPont) was a generous gift of Leland Hartwell. Swarmer cells have a higher buoyant density (ρ = 1.07 g/ml) than the remaining cell population (ρ = 1.01 g/ml); it therefore is possible to separate the swarmer cell type from heterogeneous cell cultures. Swarmer cells were isolated by centrifugation of 20

ml of heterogeneous cells at 43,000 × g for 15 min in a density gradient containing 23% (vol/vol) Ludox HS (pH 7.4) and 1.45% (wt/vol) dextran T-50 (Pharmacia). The swarmer cell band was concentrated by flotation on denser (41%, vol/vol) Ludox gradients at 43,000 × g for 15 min and then washed with culture medium. Residual stalked cells and Ludox remained in the supernatant after centrifugation at 5,900 × g for 9 min. Synchronous swarmer cells were obtained after a final centrifugation of the resuspended pellet at 2,500 × g for 5 min. The cells were then suspended in culture medium (30°C) and grown to the desired cell stage. These cells differentiated normally according to the temporal sequence outlined by Degnen and Newton (4) and remained synchronous throughout the cell cycle (see Results).

Lysis procedure for preparation of the envelope-associated nucleoid. The envelope-associated *Caulobacter* nucleoid was isolated by a modification of the procedure of Kornberg et al. (10) for the *E. coli* chromosome. Approximately 10^8 cells were pelleted by centrifugation at 12,000 × g for 10 min at 4°C. The cells were suspended in 0.25 ml of a solution containing 0.01 M tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (pH 8.2), 0.1 M NaCl, and 20% sucrose. A 0.05-ml amount of a freshly prepared solution of 4 mg of lysozyme (Sigma Chemical Co.) per ml in 0.12 M Tris-hydrochloride (pH 8.2)–0.05 M ethylenediamine tetraacetic acid (EDTA) was added, and the mixture was incubated at 0°C for 30 s. Lysis was achieved by incubating the mixture at 5°C for 3 to 4 min after adding 0.25 ml of a solution containing 1% Brij-58 (Atlas Chemicals), 0.4% sodium deoxycholic acid, 0.01 M disodium EDTA, and 0.01 M spermidine-3HCl (Sigma), pH 8.2; 0.5 ml of the lysis mixture was applied to a sucrose gradient at 4°C. Under these lysis conditions, 100% loss of cell viability occurred within 30 s after addition of detergent.

Preparation of cell envelopes. *Caulobacter* cells were labeled with ^{14}C-amino acids and [^{3}H]adenine and lysed as described above for preparation of the envelope-associated nucleoids. After a 10-fold dilution of the lysis mixture with 0.01 M Tris-hydrochloride (pH 6.8), 0.01 M $MgSO_4$, 50 μg of pancreatic deoxyribonuclease I (EC 3.1.4.5; Worthington Biochemicals Corp.), and 50 μg of pancreatic ribonuclease A (Sigma) were added, and the mixture was incubated at 37°C for 7.5 to 24 h. The cell envelopes were collected by centrifugation at 39,000 × g for 20 min and then washed twice in 0.01 M Tris-hydrochloride (pH 6.8)–0.01 M $MgSO_4$ by centrifugation at 39,000 × g for 15 min. Under these conditions less than 1% of the total acid-precipitable [^{3}H]adenine counts per minute in DNA and RNA remained associated with the envelope fractions. The envelopes were observed to retain their cellular shape when examined in the phase-contrast microscope. The sedimentation characteristics of the cell envelopes were assayed under centrifugation conditions as described below.

Sucrose density gradient centrifugation of envelope-associated nucleoids. Sucrose gradients were formed in 0.01 M Tris-hydrochloride (pH 8.2), 1 mM 2-mercaptoethanol, 1 mM EDTA, and 5 mM $MgCl_2$. For preparation of stalked cell envelope-associated nucleoids, 10 to 30% (wt/vol) sucrose gradients (5 ml) were centrifuged at 23,000 × g for 10 min in a Beckman L2-65B centrifuge, using an SW50.1 rotor. Because of their faster sedimentation rate, swarmer cell nucleoids were fractionated on denser (60 to 75%, wt/vol) sucrose gradients (11 ml) at 3,500 × g for 5 min in a Sorvall HB-4 rotor. All centrifugations were initiated between 4 and 6 min after lysis.

The recovery of rapidly sedimenting *Caulobacter* DNA complexes is largely dependent upon the rapidity with which they are separated from the remainder of the crude lysate. The *Caulobacter* nucleoid is very unstable in the lysis mixture before centrifugation, possibly because of the high levels of nucleolytic activity (Evinger and Agabian, unpublished data). Instability of the nucleoids in the lysis mixture has also been reported for *E. coli* (23). The envelope-associated nucleoids, however, remain stable for several hours after separation from the lysis mixture. As demonstrated for the *E. coli* nucleoid (24), the bulk of the *Caulobacter* chromosome material retains its characteristic sedimentation behavior after fractionation and recentrifugation.

Spectrophotometric assay of cell lysis. The specific activity of egg white lysozyme (EC 3.2.1.17; Sigma grade I) was 24,000 U/mg as determined by the assay of Shugar (20).

Lysis of *Caulobacter* CB15 stalked and swarmer cells and *E. coli* B^e cells was monitored spectrophotometrically as previously described (9). Upon addition of the lysis solution, the mixture was immediately transferred to a Gilford spectrophotometer. The A_{450} was monitored as a function of time; the initial A_{450} reading (0 s) was made exactly 5 s after the addition of the lysis solution to allow time for mixing and transfer to the cuvette.

Electron microscopy. Envelope-associated nucleoids were prepared for electron microscopy according to the cytochrome *c* monolayer spreading technique described by Delius and Worcel (5). Samples were examined in a Philips EM 201 electron microscope at 60 kV.

RESULTS

Isolation of synchronous swarmer cells. The Ludox density gradient technique provided large numbers of swarmer cells (6×10^{10} swarmer cells per liter of mid-exponential-phase culture) that were able to differentiate synchronously upon resuspension in HMG medium (Fig. 1). The rate of incorporation of [^{3}H]adenine into DNA during the cell cycle (Fig. 1A) established a pattern of DNA synthesis in Ludox-synchronized populations identical to that obtained by Degnen and Newton (4) for synchronous swarmer cells produced by selective elution from dividing cells attached to glass. Quantitation of cell number as a function of growth time in the experiment shown in Fig. 1B demonstrated that approximately 95% of the cells divided between 160 and 200 min. The level of synchrony obtained with this tech-

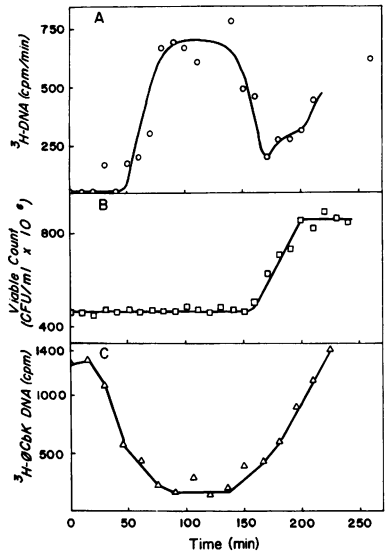

FIG. 1. *Assessment of swarmer cell synchrony obtained by the Ludox density gradient technique. (A) Pattern of discontinuous DNA synthesis was determined by measurement of the rate of DNA synthesis (counts/minute). 5 × 10⁸ to 5 × 10⁹ cells were incubated in the presence of [³H]adenine (0.4 μCi/ml) for 2 min. Cells were then incubated for 2 min in 1.0 ml of 7.5 mM adenine and processed as described previously (4). The [³H]adenine incorporated into DNA was measured as described in Materials and Methods. (B) Quantitation of cell number as a function of time of growth in HMG medium is expressed as the viable count (colony-forming units [CFU]/milliliter) at intervals throughout the cell cycle. (C) The φCbK differentiation assay (1) is quantitated as the counts/minute of ³H-labeled φCbK DNA (10⁴ cpm/2 × 10⁸ plaque-forming units) injected into cells at a multiplicity of infection of 0.1. φCbK specifically interacts with Caulobacter at the swarmer cell pole; as swarmer cells differentiate into stalked cells, surface phage receptors are lost. Differentiation of the stalked cell into the predivisional intermediate is accompanied by the reappearance of phage receptors and thus sensitivity to φCbK.*

nique was highly reproducible; similar measurements demonstrating that 93 to 97% of the cells divided during this 160- to 200-min interval were made in at least 10 independent experiments. Cellular differentiation may be assessed by assaying cell sensitivity to the swarmer-specific DNA bacteriophage φCbK (1); as shown in Fig. 1C, sensitivity of swarmer cells and predivisional cells to φCbK also indicated that differentiation proceeded according to the normal temporal sequence (4). Because the Ludox technique provided large quantities of swarmer cells that grew synchronously in culture, populations of cells at any stage of differentiation could be obtained for examination of the *Caulobacter* chromosomal structure during the cell cycle. Stalked cells used in the following studies were harvested after 75 min of synchronous growth in HMG medium.

Characterization of the *Caulobacter* nucleoids. The envelope-associated nucleoids were isolated in distinct forms from each of the two *C. crescentus* cell types by sucrose density gradient centrifugation as described in Materials and Methods. The sedimentation profile of the stalked cell envelope-associated nucleoid revealed a homogeneous peak of DNA with an average sedimentation coefficient of 3,000S (Fig. 2A). The swarmer cell envelope-associated nucleoid migrated much more rapidly than that of the stalked cells and with a somewhat narrower size distribution. From its mi-

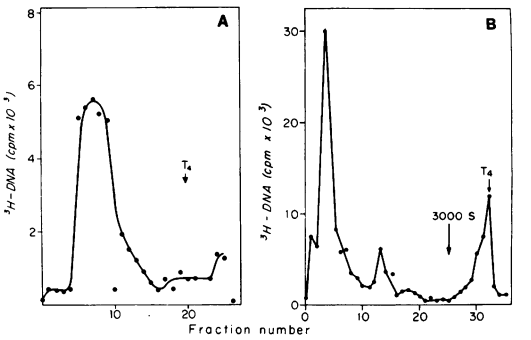

FIG. 2. *Sucrose gradient fractionation of Caulobacter envelope-associate nucleoids. (A) Stalked cell envelope-associated nucleoids were prepared as described in Materials and Methods, using stalked cells that had been incubated with 1 μCi of [³H]guanosine per ml for 60 min. After centrifugation of 0.5 ml of lysis mixture at 23,000 × g for 10 min on 10 to 30% sucrose gradients in an SW50.1 rotor, 0.2-ml fractions were collected from the bottom of the gradient. The direction of sedimentation is from right to left. The counts/minute of [³H]DNA in each fraction represents the trichloroacetic acid-insoluble radioactivity remaining after alkaline hydrolysis. 65% of the total [³H]DNA cpm applied to the gradient migrates with the envelope-associated nucleoid. (B) Swarmer cell envelope-associated nucleoids were prepared from cells that had been incubated for 60 min with 1 μCi of [³H]adenine per ml. A 0.55-ml amount of lysis mixture was layered on a preparative 60 to 75% sucrose gradient and subjected to centrifugation at 3,500 × g for 5 min in a Sorvall HB-4 rotor. The direction of sedimentation is from right to left. Fractions of 0.33 ml were collected from the bottom of the gradient. The counts/minute of [³H]DNA in each fraction represents the trichloroacetic acid-insoluble radioactivity remaining after alkaline hydrolysis. 52% of the total [³H]DNA counts/minute applied to the gradient migrates with the swarmer envelope-associated nucleoid.*

gration to the bottom of 10 to 30% sucrose gradients, a minimal value for the sedimentation coefficient of the swarmer cell envelope-associated nucleoid could be approximated by comparison with the sedimentation of a 1,000S T4 phage marker (3) for different lengths of time (9). The sedimentation coefficient of the swarmer cell envelope-associated nucleoid was estimated to be >6,000S (Fig. 2B). Because sedimentation values obtained for envelope-associated *Caulobacter* nucleoid forms were not corrected for rotor speed effects (8), we refer to the stalked and swarmer cell envelope-associated nucleoids as having sedimentation rates of approximately 3,000S and >6,000S, respectively. These values are used in a descriptive sense to identify the two nucleoid forms by their characteristic sedimentation behaviors.

The nucleoid complexes could be visualized in the electron microscope. The DNA was organized in structures of loops and whorls associated with fragments of the cell envelope (Fig. 3) and were similar in appearance to *E. coli* envelope-associated nucleoids (5, 6).

The sedimentation behavior of *Caulobacter* nucleoids could not be attributed to unlysed cells. Unlike *E. coli* (9), *Caulobacter* cells did not accumulate at the bottom of our sucrose gradients. The sedimentation profile of heterogeneous populations of stalked and swarmer cells extended from the top of 10 to 30% sucrose gradients to approximately 4,300S; whole cells remained near the top of 60 to 75% sucrose gradients.

The use of Ludox for cell synchronization did not appear to have residual effects on cell lysis or on chromosome sedimentation rates. Both swarmer cells and stalked cells lysed at the same rate as cells that were not in contact with the colloidal silica.

In addition to qualitative assessments from electron micrographs that DNA and cell envelope material were present in both stalked and swarmer cell envelope-associated nucleoids (Fig. 3), the relative amounts of DNA, RNA, and protein were analyzed (Table 1). The proportions of these macromolecular components in *Caulobacter* nucleoids differed from those of intact *C. crescentus* cells and resembled those of *E. coli* envelope-associated nucleoids (16). As expected, the stalked cells, which were undergoing chromosome replication, contained a higher proportion of DNA (57 ± 8%) in their nucleoids than did the nonreplicating swarmer cells (46 ± 4%).

The role of the cell envelope in determining the sedimentation behavior of the *Caulobacter* envelope-associated nucleoids was assessed by extensively treating envelope-associated nucleoids with ribonuclease A and deoxyribonuclease I as described in Materials and Methods. A mean sedimentation rate of 1,975 ± 175S was determined from a total of eight separate experiments in which the envelopes of both heterogeneous populations and homogeneous stalked and swarmer cells were examined.

Rate of lysis of stalked and swarmer cells. The rates and extent of stalked and swarmer cell lysis (Fig. 4) were compared spectrophotometrically by measuring the change in A_{450} (ΔA_{450}) as described in Materials and Methods. The lysis procedure may be considered as two steps: the first involves the exposure of cells to the action of lysozyme, and the second involves exposure to detergent. Exposure of *C. crescentus* cells to lysozyme for intervals of 30 to 60 s did not alter subsequent lysis behavior. The rates of lysis of stalked and swarmer cells in the presence of detergent were similar when examined at either 5°C (Fig. 4A) or 10°C (Fig. 4B). Although the initial rate of lysis for either cell type was more rapid at 10°C than at 5°C, the extent of lysis appeared to be equivalent at both temperatures. The A_{450} in each case did not decrease substantially after 3 min. The ΔA_{450} was 0.4 for cells lysed for 5 min, the time when centrifugation of the lysis mixture was initiated. In comparison, when *E. coli* were lysed for 5 min under these conditions, the ΔA_{450} was 0.3 to 0.35.

Treatment with both lysozyme and detergent solutions was necessary for preparation of *Caulobacter* envelope-associated nucleoids. When either detergent or lysozyme was omitted from the lysis mixture, the ΔA_{450} was not as great for either *Caulobacter* cell type or for *E. coli* as when both components were present. Sedimentation of cell lysates prepared by detergent treatment alone resulted in a large amount of viscous material that did not migrate beyond the T4 phage marker. Similarly, cells did not appear to be fully lysed by treatment with lysozyme in the absence of detergent.

The primary effect of temperature during detergent treatment was reflected in the relative yield of nucleoids obtained from each cell type. Swarmer cell nucleoids could be prepared in good yield with lysis temperatures of 0 to 5°C. Lysis of swarmer cells at 10°C resulted in very poor yields of envelope-associated nucleoids and in increasing amounts of viscous, slowly sedimenting DNA. No distinct intermediate-size nucleoid forms were detectable; a 3,000S nucleoid was never obtained from swarmer cells under a wide variety of lysis conditions. On the other hand, the stalked cell

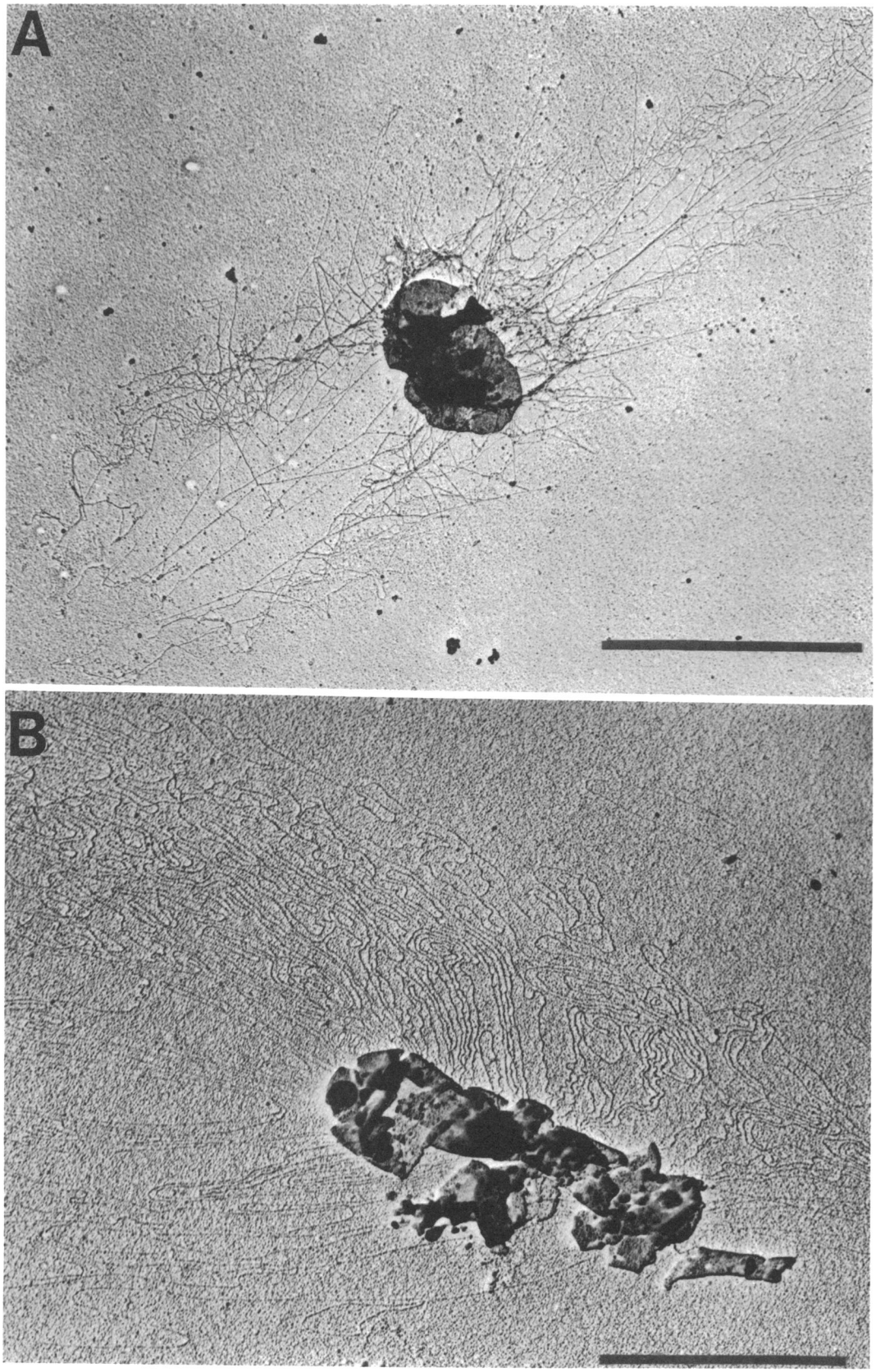
A
B

TABLE 1. *Comparison of envelope-associated nucleoids (EANs) and intact cells from Caulobacter stalked and swarmer cells and from E. coli*

EANs or cells studied	Determination					
	DNA content (wt fraction)[a]	RNA content (wt fraction)[a]	Protein content (wt fraction)[a]	Approx sedimentation rate	Sedimentation rate of cell envelopes[b]	Fraction of total cellular protein bound[c]
Stalked cell EANs	0.57 ± 0.08	0.15 ± 0.01	0.29 ± 0.02	3,000S		0.10
Swarmer cell EANs	0.46 ± 0.04	0.26 ± 0.05	0.28 ± 0.03	>6,000S		0.04-0.10
E. coli EANs[d]	0.40	0.15-0.35	0.40	3,200S		0.2
Stalked cells	0.12 ± 0.01	0.20 ± 0.07	0.68 ± 0.12	1,300S-4,300S[e]	1,975 ± 175S	1.0
Swarmer cells	0.08 ± 0.01	0.29 ± 0.05	0.62 ± 0.11	1,300S-4,300S[e]	1,975 ± 175S	1.0

[a] The amount of DNA was determined by the diphenylamine assay (2), that of RNA was determined by the orcinol assay (14), and that of protein was determined by the Folin phenol assay (11). Errors are expressed as standard deviations from the mean of three estimations.

[b] Sedimentation range of ribonuclease- and deoxyribonuclease-treated cell envelopes prepared as described in Materials and Methods.

[c] Fraction of ^{14}C-labeled amino acids incorporated into trichloroacetic acid-insoluble material in 30 min as described in Materials and Methods.

[d] Data based on weight fraction of total *E. coli* nucleoid dry weight and sedimentation rates as reported by Pettijohn (16).

[e] Sedimentation range of heterogeneous unlysed cells.

envelope-associated nucleoid could be obtained in good yield at lysis temperatures between 5 and 10°C. Below 5°C a greater proportion of the cells remained near the top of the gradient and appeared to be unlysed.

Mixing experiments. Stalked cell and swarmer cell nucleoids were labeled with [^{3}H]adenine and [^{14}C]adenine, respectively. When both cell types were lysed together before centrifugation, the bulk of the nucleoid material migrated to its characteristic position (Table 2): stalked cell DNA was found in the upper (3,000S) band, swarmer cell DNA was found in the lower (>6,000S) band, and a small fraction (<10%) of the DNA migrated more slowly than the 1,000S T4 marker band. A small amount (16%) of the stalked cell material was present in the swarmer cell nucleoid peak; about 7% of the swarmer material remained in the upper band. The characteristic DNA distribution obtained for each cell type remained essentially unchanged when centrifuged on sucrose gradients individually or in combination with the lysis mixture of the other cell type. No DNA peaks were observed at intermediate sedimentation rates. If the two cell types were lysed separately and then layered on the sucrose gradients, the material first applied to the gradient represented the minor chromosome contaminant present in the other nucleoid band. These results are consistent with those of Snustad et al. (22) for *E. coli* chromosomes obtained from T4-infected cells.

Cell number. The sedimentation behavior of stalked and swarmer cell envelope-associated nucleoids was examined over a wide range of cell concentrations. Meyer et al. (12) reported anomalous sedimentation behavior when gradients contained too much lysate material (greater than 10^9 cells per 5-ml gradient) and suggested that a nonspecific entangling and aggregation of the DNA in these cases produced artificially high sedimentation rates. The sedimentation coefficients of *C. crescentus* stalked and swarmer cell envelope-associated nucleoids were examined over a 1,000-fold range of cell concentrations. When 2×10^7 to 2×10^9 cells per lysate on 5-ml gradients or as many as 10^{10} cells per lysate on 10-ml preparative gradients were examined, the sedimentation coefficient in nine separate preparations of 75-min stalked cell envelope-associated nucleoids varied from 2,750S to 3,300S, with a mean coefficient of 3,150 ± 120S. The swarmer cell envelope-associated nucleoid sedimented with a characteristic peak of >6,000S; in this case, small differences in sedimentation coefficients determined from 60 to 75% sucrose gradients are not meaningful. These results suggest that within these ranges of cell concentrations, the sedimentation coefficients for envelope-associated nucleoids from either cell type are not appreciably altered.

FIG. 3. *Electron micrographs of Caulobacter envelope-associated nucleoids. The nucleoids were prepared for electron microscopy as described in Materials and Methods. (A) Swarmer cell envelope-associated nucleoids isolated from the >6,000S peak of chromosome material (see Fig. 2B). (B) Stalked cell envelope-associated nucleoids from the 3,000S peak of chromosome material (see Fig. 2A). Bar represents 1 μm.*

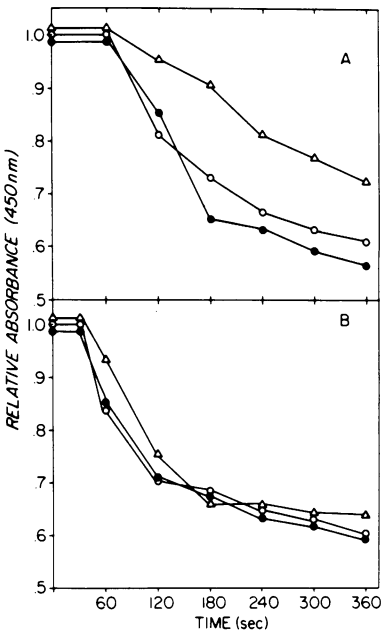

FIG. 4. *Spectrophotometric assay of cell lysis. Caulobacter stalked and swarmer cells and E. coli B^e cells were treated with lysozyme at 0°C for 30 s before addition of detergent solution at either 5°C (A) or 10°C (B). Lysis was monitored as described in Materials and Methods. The relative absorbance at 450 nm is an expression of the amount of remaining A_{450}-absorbing material relative to that at 0 s. Symbols: (○) Stalked cells; (●) swarmer cells; (△) E. coli B^e cells.*

DISCUSSION

In this study, the sedimentation properties of envelope-associated nucleoids from *C. crescentus* stalked and swarmer cells were analyzed under a variety of conditions affecting the sedimentation properties of the *E. coli* envelope-associated nucleoid. The nucleoids of *Caulobacter* are also sensitive to temperature, to the duration of lysis conditions, and to the extent of lysozyme treatment. However, unlike the situation in *E. coli* (9), if lysis is performed in such a way as to effect an equivalent rate and extent of lysis in stalked and swarmer cells, two distinct forms of the envelope-associated nucleoid may be isolated: a 3,000S chromosome complex is obtained from stalked cells, and a >6,000S chromosome complex is obtained from swarmer cells.

Differing rates of sedimentation have been described for envelope-associated nucleoids isolated from a single bacterial species. In particular, rapidly sedimenting forms (3,700S) have been described for *E. coli* grown on carbon sources other than glucose (3,100S) (9) and for *E. coli* undergoing amino acid starvation (5,000 to 6,000S) (9, 18). We do not believe that the differences in sedimentation coefficients of nucleoids prepared from *C. crescentus* can be attributed to either of these nutritional factors. Both stalked and swarmer cells exist concurrently in heterogeneous cultures grown on the same medium, and both cell types are in balanced growth, synthesizing proteins and RNA throughout the entire cell cycle. The differential capacity for DNA replication in stalked and swarmer cells is a developmental feature of the *Caulobacter* cell cycle and as such provides a powerful system for analyzing differences in bacterial chromosome structure.

Our results agree with the finding that the nonreplicating *E. coli* nucleoid remains membrane associated (9, 18). In the isolation of the envelope-associated nucleoid structure, the rate of lysis appears to be very important. Slight increases in the rate of lysis, for example between 5 to 10°C in swarmer cells, result in decreased yields of envelope-associated nucleoids and a proportional increase in the amount of viscous DNA at the top of the gradient, suggesting that a fragile balance exists between the disruption of the cell envelope and the maintenance of envelope associations that

TABLE 2. *Sedimentation of mixed lysates of stalked and swarmer cells*

Lysis	Distribution of total [^{3}H]- or [^{14}C]DNA in sucrose gradients[a]		
	>6,000S	3,000S	≤1,000S
Expt I			
Individual cell lysis			
Stalked cell [^{3}H]DNA	0.06 ± 0.02	0.78 ± 0.15	0.12 ± 0.04
Swarmer cell [^{14}C]DNA	0.84 ± 0.07	0.05 ± 0.03	0.11 ± 0.08
Expt II			
Combined stalked and swarmer cell lysis			
Stalked cell [^{3}H]DNA	0.16 ± 0.09	0.78 ± 0.04	0.06 ± 0.05
Swarmer cell [^{14}C]DNA	0.88 ± 0	0.06 ± 0.05	0.07 ± 0.06

[a] Values are expressed as the fraction of total [^{3}H]- or [^{14}C]DNA recovered from the regions of the gradients as designated. [^{3}H]- and [^{14}C]DNA refer to counts per minute [^{3}H]- or [^{14}C]adenine incorporated into DNA and assayed as described in Materials and Methods. Errors are expressed as the standard deviations from the mean of three determinations in experiment I and two determinations in experiment II.

may stabilize the nucleoid structure. It is also very difficult to prepare significant proportions of the swarmer cell nucleoid in an envelope-free state (data not shown), further indicating that these cell envelope associations may be of greater relative importance in maintaining the nonreplicating versus the replicating DNA structures.

In comparison with stalked cell nucleoids, the swarmer cell envelope-associated nucleoid has less DNA mass, more RNA, and an equivalent percentage of the total cellular protein. These differences in the composition of the envelope-associated nucleoid roughly correspond to those of intact cells. The relatively small increase in the RNA and protein content and decrease in DNA mass is unlikely to account for the difference in the swarmer cell envelope-associated nucleoid sedimentation rate. Furthermore, the fact that both stalked and swarmer cell envelopes demonstrate identical sedimentation characteristics on sucrose gradients suggests the presence of other interactions that could alter the sedimentation properties of the nucleoid. For example, compaction might result from DNA-protein interactions involving either the cell membrane or another class of specific proteins. The possibility that nucleolytic or proteolytic activity may constitute additional factors in determining the nucleoid structure is likewise recognized and is currently being assessed. Preliminary studies do indicate that both quantitative and qualitative differences exist in the proteins isolated from each of the *Caulobacter* envelope-associated nucleoids.

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