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Cosegregation of Cell Wall and DNA in *Bacillus subtilis*

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Cosegregation of cell wall and DNA of a lysis-negative mutant of *Bacillus subtilis* was examined by continuously labeling (i) cell wall, (ii) DNA, and (iii) both cell wall and DNA. After four to five generations of chase in liquid media it was found by light microscope autoradiography that the numbers of wall segregation units per cell are 29 and 9 in rich and minimal medium, respectively. Under the same conditions the numbers of segregation units of DNA were almost 50% lower: 15 and 5, respectively. Simultaneous labeling of cell wall and DNA (iii) provided figures almost identical to those obtained for cell wall alone (i), implying cosegregation of the two components. Statistical analysis ruled out their random distribution into daughter cells. Measurements of the positions of grain clusters at the end of the chase period along chains of cells, each derived from a single cell at the beginning of chase, show that cell wall units are localized according to a symmetrical pattern, whereas those of DNA are distributed in an asymmetrical but highly regular way. It appears that of two cell wall units of the same age one only has a strand of DNA attached to it. We present a simple diagrammatic model of cell wall organization and DNA-cell wall association which is compatible with our observations. Finally, we discuss previous experiments pertinent to cosegregation of cell wall and DNA obtained with cells grown on solid media as well as with germinating spores; an explanation for the independent segregation of cell wall and DNA observed in the latter case is advanced.

To explain coordinate DNA replication and cell division as well as the orderly segregation of chromosomes into daughter cells, Jacob et al. (14) advanced the "replicon" model which has as corollaries zonal insertion of new "surface" material and physical attachment of DNA to the cell envelope.

The hypothesis of zonal insertion, well documented in gram-positive cocci (3, 5, 12), has been studied for both membrane and cell wall synthesis of rod-shaped bacteria (17, 21). The majority of studies, but not all studies, favored diffuse intercalation of new material, in particular in the case of the cell wall of gram-positive rods (6). However, Schlaeppi et al. (24) have recently demonstrated zonal insertion by establishing that the cell wall of *Bacillus subtilis* consists of a limited number of large subunits which segregate during cell growth.

The first evidence for an association between DNA and the cell envelope came from electron microscopic observations (15). Several biochemical and genetic studies suggesting that both cell wall (8) and membrane (2) preparations contain specific regions of the *B. subtilis* chromosome provided further evidence of such an association. More recently Schlaeppi et al. (24) have recognized a certain analogy between mechanisms that regulate DNA and cell wall synthesis as a function of medium richness, suggesting a close association between the two components.

However, experiments aimed to establish cosegregation of cell wall and DNA by autoradiographic methods with gram-positive bacteria grown on solid media remain contradictory: Lark and collaborators (4, 9) observed a nonrandom segregation of the cell envelope and DNA and proposed a model involving a permanent association between the two components, whereas Ryter and Jacob (23) failed to observe such a cosegregation and interpreted their data in favor of a random segregation of chromosomes.

In the present study we report experiments on *B. subtilis* mutants with reduced cell wall turnover which allow long periods of chase and thus the comparison of the numbers and relative positions of segregation units of cell wall and DNA along chains of cells. They show a 100% cosegregation for DNA according to an apparently asymmetrical pattern.

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MATERIALS AND METHODS

Strains, media, and labeling conditions. Unless otherwise stated experiments were performed with strain FJ3, a *metC3 lyt-1*, mutant of *B. subtilis* deficient in autolytic enzyme activities (11). In the media described below this strain exhibits considerably reduced cell wall turnover and forms long chains of cells (24). Some control experiments were performed with strain Ni15 (*trpC2 thyA thyB xin-15 lyt-15* [18]).

Cells were grown in two media, a rich Casamino Acids-glucose medium (generation time of 20 min at 44°C) and a minimal salts-glucose medium (generation time of 40 min at 44°C) (24). When cells were labeled with [^{3}H]thymidine, both rich and minimal medium were supplemented with 1.5 mM of 2'-deoxyadenosine (dAdo) throughout the labeling and the chase period. The presence of dAdo slows the growth rate in minimal medium (generation time of 45 min), but apparently not in rich medium. Cultures were grown at 44°C with aeration and maintained in the exponential growth phase by dilution with fresh medium. The cell titer was kept below 5.10^7/ml in rich medium and 10^8/ml in minimal medium.

Labeling conditions. Each culture was divided into parts (i), (ii), and (iii) containing the following: (i) *N*-acetyl-D-[1-^{3}H]glucosamine ([^{3}H]GlcNAc) to label the cell wall, (ii) [*methyl*-^{3}H]thymidine ([^{3}H]dThd) and dAdo to label the DNA, and (iii) [^{3}H]GlcNAc and [^{3}H]dThd to label both cell wall and DNA.

In rich medium the labeling conditions were as follows: (i) 75 μM [^{3}H]GlcNAc (specific activity, 0.65 Ci/mmol); (ii) 1.7 μM [^{3}H]dThd (specific activity, 58 Ci/mmol) with 1.5 mM dAdo; (iii) 70 μM [^{3}H]GlcNAc (specific activity, 0.36 Ci/mmol) and 1.7 μM [^{3}H]dThd (specific activity, 29 Ci/mmol) with 1.5 mM dAdo.

In minimal medium the conditions were as follows: (i) 250 μM [^{3}H]GlcNAc (specific activity, 0.23 Ci/mmol); (ii) 1.7 μM [^{3}H]dThd (specific activity, 58 Ci/mmol) with 1.5 mM dAdo; (iii) 250 μM [^{3}H]GlcNAc (specific activity, 0.23 Ci/mmol) and 1.7 μM [^{3}H]dThd (specific activity 58 Ci/mmol) with 1.5 mM dAdo. The final concentration of GlcNAc was always adjusted to 250 μM.

In the case of the thymine-requiring strain Ni15, cells were labeled in medium supplemented with 40 μg of cold thymine per ml and 150 μCi of [*methyl*-^{3}H]thymine (33 Ci/mmol) per ml.

At the end of the labeling period (four to five generations) cultures were harvested by filtration, washed, and appropriately diluted in prewarmed medium containing 100 μM (rich medium) or 250 μM (minimal medium) GlcNAc. After the chase period samples were filtered, treated for 1 min with cold trichloroacetic acid (5%, wt/vol), washed with distilled water, and processed for autoradiography. To eliminate the bulk of [^{3}H]GlcNAc incorporated into protein of cells grown in minimal medium they were treated with RNase-trypsin as previously described (24). In rich medium this treatment was omitted for the following reasons. First, the relatively small amount of [^{3}H]GlcNAc incorporated into protein (about 15%) was subtracted (by appropriate corrections) (24); second, the RNase-trypsin treatment results in a dispersion of the DNA along the cell which artificially increases the number of observed DNA segregation units. However, in minimal medium this RNase-trypsin treatment does not affect the number of segregation units per cell since the resolution of the method does not allow separation of two clusters within the same cell. Details of septum staining and preparation of autoradiographs by standard procedures are given elsewhere (20, 24). Although cross walls of RNase-trypsin-treated cells stained in red by crystal violet and fuchsine (24) were clearly distinguishable (despite a rather diffuse appearance) in each experiment, control samples were stained by standard procedures to ensure that the actually observed average cell length was the correct one.

Definition of a segregation unit. A segregation unit of either cell wall or DNA or cell wall and DNA was defined as a cluster of grains or a single grain, observed along a chain of labeled cells, which was separated from neighboring grain clusters (or single grains) by at least 1 μm (24). To avoid an underestimate of the number of cell wall segregation units, in [^{3}H]GlcNAc-labeled cells in experiments (i) and (iii), clusters centered on a septum or spreading on both sides of a septum were considered as two separate segregation units. In the case of [^{3}H]dThd-labeled cells (ii) such clusters were counted as single units (see below).

Determination of the average number of segregation units per cell. In each case corrections for grains due to emulsion background and for label incorporated into protein (in the case of [^{3}H]GlcNAc labeling) were performed as previously described (24). In each experiment the average number of segregation units per cell was calculated from the number of units per cell at the time of sampling corrected for the exponential increase in cell numbers during the corresponding chase period.

Statistical analysis of distributions of grain clusters. Average numbers of wall and DNA segregation units per cell were determined in separate experiments (Table 1). From figures thus obtained (15.1 DNA units per cell and 28.6 cell wall units per cell) a theoretical distribution of grain clusters (corresponding to either [^{3}H]dThd, [^{3}H]GlcNAc, or [^{3}H]dThd and [^{3}H]GlcNAc) which would have been obtained in the event of random segregation of labeled DNA or cell wall units was calculated as follows. Every cell was assumed to produce 32 cells (five generations of chase), and every unit (grain cluster) was assumed to be 1.5 μm long so that a chain of cells grown in rich medium (average cell length, 4.0 μm) could accomodate a unit in 85 positions (32 × 4 μm/1.5 μm). If the distribution of DNA labeled units and of wall labeled units are binomial and their segregation is independent, then for a doubly labeled chain the distribution of units (consisting either of DNA or cell wall or DNA and cell wall together) must be binomial. For a large number of possible locations (along the chain) such a distribution can be approximated by a normal one which in our particular case is characterized by a mean of 38.6 and a standard deviation of 4.6: N (38.6; 4.6) (25); pT = 0.18 and pN = 0.34 are the measured probabilities of obtaining a [^{3}H]dThd and a [^{3}H]GlcNAc cluster, respectively (see above and Table 1). The observed distribution obtained on 49 separate chains, was compared with the theoretical one as calculated above, by a χ^2 test with four classes (3 degrees of freedom).

Identification of chains derived from one cell at the

beginning of chase. Slides processed for autoradiography reveal rather long chains of bacteria consisting generally of about 5 to 50 cells. However, a proportion of cells are organized in even longer chains which appear like clumps and are unsuitable for analysis. Among the longer isolated chains (16 to 32 cells) labeled with [^{3}H]GlcNAc in minimal medium, obtained after four to five generations of chase, those which exhibit dense clusters at both ends can be recognized easily (Fig. 1a). The presence of such clusters, corresponding to the poles of a single cell at the beginning of the chase (24), has been used as the criterion for identifying chains which have originated from one labeled cell ("unit chains"). The remaining isolated chains appear like fragments of broken unit chains or occasionally like a unit chain plus a chain fragment; the breaking is likely to have occurred during spreading onto slides. Nevertheless, analysis of cell fragments reveals on the average two dense clusters per equivalent of a unit chain, which is also supported by our previous observations (20, 24). The same criteria have been used to define unit chains of cells labeled in both cell wall and DNA. However, since longer chains (16 to 32 cells) obtained from [^{3}H]dThd-labeled cells exhibit generally a heavy cluster at one end only (and very rarely at both ends), the criterion of a unit chain defined above could not be applied. Therefore, we had to assume that a population of chains with one labeled end and of an average length corresponding to that of the unit chain was also derived from single cells. This was supported by the analysis of a representative population of chain fragments which revealed, on the average, one labeled end per equivalent of a unit chain.

RESULTS

Cosegregation of cell wall and DNA in media of different richness. Cell wall consisting of complete or partially completed polar sheath (24) and DNA consisting of complete or partially replicated strands of the chromosome may segregate independently of one another or cosegregate. To distinguish between these alternatives we compared the average number of such segregation units per cell obtained by light microscope autoradiography when either (i) cell wall or (ii) DNA or (iii) both cell wall and DNA were labeled. After an appropriate chase period the number of units (grain clusters) in experiment (iii) could either approach the sum of those obtained in experiments (i) and ((ii)—indicating independent segregation—or be equal to the highest of the figures obtained from experiments where only cell wall (i) or DNA (ii) is labeled—indicating cosegregation.

The results presented in Table 1 show that in both rich and minimal media, after four to five generations of chase, the number of segregation units obtained by simultaneously labeling cell wall and DNA (iii) was only marginally superior to that of cell wall segregation units (i).

To eliminate the possibility that a proportion of clusters obtained in the former case (iii) is due to a fortuitous overlapping of independently segregating components, we determined the number of segregation units per cell in about 50 chains chosen at random. A comparison of this distribution (Table 2) with the distribution predicted theoretically for independently segregating DNA and cell wall units (see above) completely rules out the latter possibility (probability of independence, <0.05%). The same conclusion has been reached for cells grown in minimal medium (data not presented). We believe that the small excess about 10% of the segregation units obtained with cells labeled in both DNA and cell wall (iii) over the figures obtained for GlcNAc units (i) is most likely due to the following reasons. First, an exchange between DNA sister strands results in DNA fragmentation (see below and reference 16) and thus a creation of labeled DNA segregation units which do not overlap any more with labeled cell

TABLE 1. Numbers of cell wall, DNA, and both cell wall and DNA segregation units per cell of *B. subtilis* FJ3 obtained by light microscope autoradiography from continuous-labeling and chase experiments

Medium	No. of generations of chase	Label[a]	Avg no. of grains per segregation unit	Mean no. of segregation units per cell	No. of cells examined
Rich	5.0	[^{3}H]GlcNAc	2.8	28.6	788
		[^{3}H]dThd	5.3	15.1	775
		[^{3}H]GlcNAc and [^{3}H]dThd	2.8	31.8	827
Minimal	4.5	[^{3}H]GlcNAc	3.1	8.6	1,637
		[^{3}H]dThd	4.4	5.0	1,137
		[^{3}H]GlcNAc and [^{3}H]dThd	4.2	9.5	1,146

[a] For labeling conditions see Materials and Methods.

TABLE 2. Statistical analysis of distributions of grain clusters[a]

Class	No. of segregation units per cell (DNA, cell wall, or DNA and cell wall)	Theoretical no. of chains[b]	No. of chains observed[c]
I	≤31.7	3.3[d]	26
II	31.8–36.3	11.8	9
III	36.4–40.9	18.8	9
IV	41.0–45.5	11.8	5
V	≥45.6	3.3[d]	

[a] Determined after five generations of chase in rich medium.

[b] Theoretical distribution obtained by assuming independent segregation of DNA and cell wall units: N (38.6; 4.6) (see text).

[c] A total of 49 chains chosen at random were analyzed.

[d] For the χ^2 test, classes I and V were pooled.

wall segregation units; second, large units of both labeled wall and DNA can frequently produce grains in neighboring cells. Therefore, the observed almost complete overlap of grain clusters due to [^{3}H]dThd with those due to [^{3}H]GlcNAc shows that in both rich and minimal media cell wall and DNA cosegregate, suggesting that they are associated either directly or through a membrane component. Such an association, established at the time of synthesis of the two components, remains very stable and ensures orderly segregation of chromosomes into daughter cells.

This conclusion has been strengthened by experiments in which cells labeled in both components (iii) were chased in some instances for six or even eight generations. Results (not shown) showed that the largest clusters remained apparently unaltered and that the numbers of units per cell increased very slightly, probably due to DNA sister strand exchange. Indeed, similar experiments performed on cells labeled in the wall (i) or in DNA (ii) suggest that DNA only undergoes very limited fragmentation (around 5% per generation in minimal medium).

Since the growth conditions in which DNA was labeled (use of a *thy*$^+$ strain, dAdo, and [^{3}H]dThd) could have affected DNA synthesis, the number of segregation units was also determined with the thymine-requiring strain Ni15 (18). This control experiment, performed in rich medium where Ni15 has considerably reduced lytic activity (18), provided a figure of 14.4 DNA segregation units, which is very close to that obtained with *thy*$^+$ strains (Table 1).

Nature of excess cell wall segregation units. The data presented in Table 1 show that in both rich and minimal media the number of wall segregation units per cell is greater than that of DNA segregation units by a factor of 1.7 to 1.9. To decide whether this excess is due either to initiation of new cell wall insertion zones in advance of that of corrresponding rounds of DNA replication or to concomitant synthesis of four cell wall units and two strands of DNA, we have analyzed the relative positions of cell wall and DNA segregation units within a population of unit chains; a unit chain corresponds to a chain obtained from a single cell after four to five generations of chase (see above). Since in rich medium such chains were relatively rare and contained too many clusters to be readily analyzed (Table 1), we have confined our analysis to cells grown in minimal medium. As shown in Fig. 1a and as previously described (24) grain clusters of different size can be easily identified along chains of cells labeled with [^{3}H]GlcNAc. Two large clusters, designated A and B, comprising 51% of the total label are present at both ends of each unit chain. A large cluster designated C comprising about 21% of grains and spreading over two cells is located around the middle of each chain. In most chains two minor clusters designated D and E can be seen between A and C and C and B, respectively. As previously shown terminal clusters A and B should correspond to the oldest cell wall, i.e., to polar sheaths (pole plus part of the cylinder) (24) completed well before the beginning of the chase. C probably corresponds to completed cylindrical parts of two polar sheaths, and D and E probably correspond to segregation blocks only partly completed at the beginning of the chase. Thus we can assign an age to each cluster; it increases with increasing size. The specific locations of clusters A to E, obtained from about 30 chains, are given in Table 3. We would like to emphasize that grains of cluster C are distributed symmetrically over two neighboring cells forming very often a clearly visible "doublet." Grains corresponding to clusters D and E can also be seen on both sides of a septum. This type of segregation pattern supports symmetrical zonal insertion of new cell wall material, in agreement with the replicon model (14).

Similar analysis of the distribution of DNA segregation units along unit chains show a different picture which is clearly asymmetrical (Fig. 1b and Table 3). Two large clusters are located at positions A and C, and two smaller ones are located at positions D and E. It is striking that grains in clusters C, D, and E, due to labeled DNA, are mostly located over one cell (they are asymmetrical with respect to the nearest septum) and do not form doublets like clusters obtained with labeled GlcNAc. These observa-

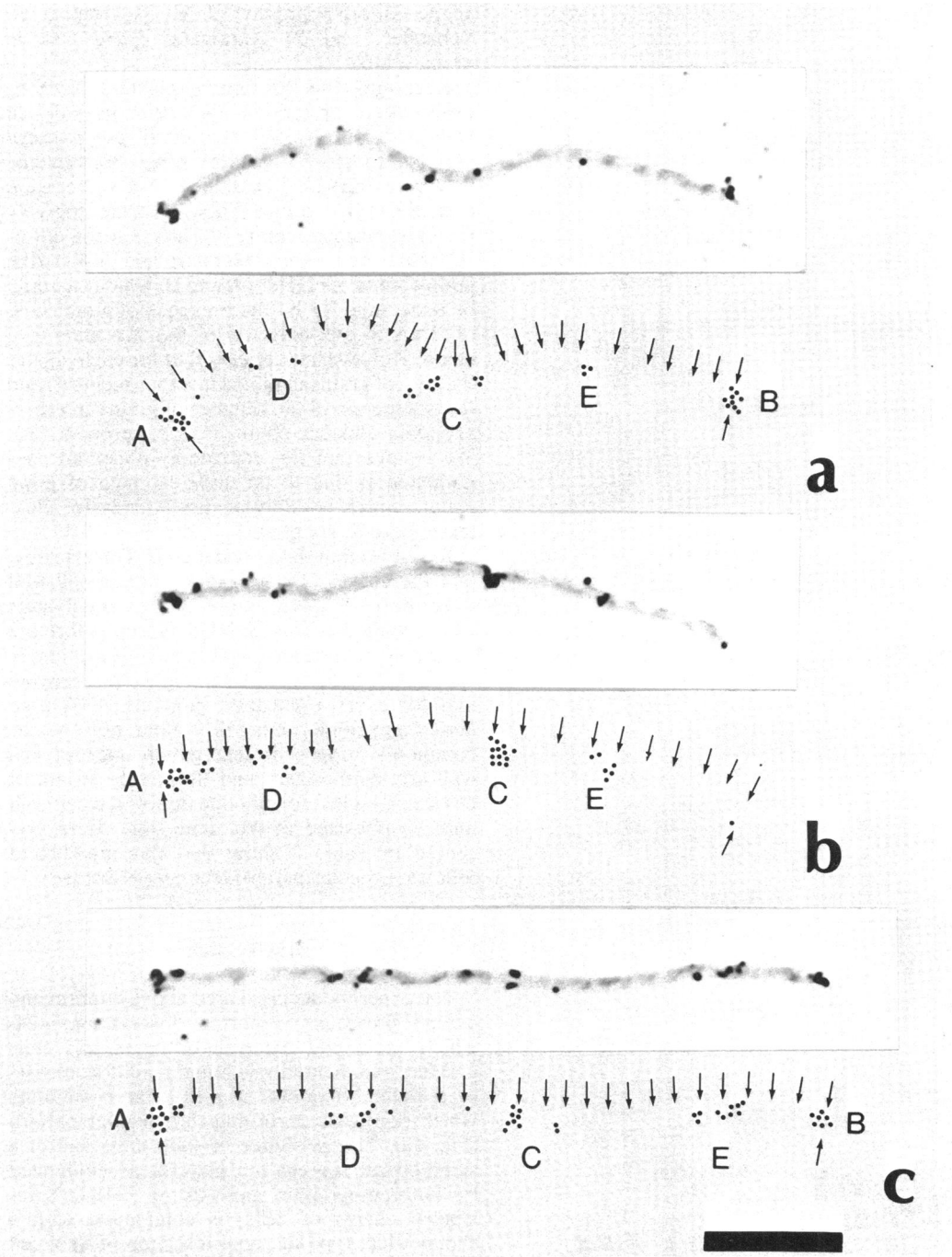

FIG. 1. Cells of *B. subtilis* strain FJ3 (*lyt-1*, *metC3*) growing exponentially in minimal medium at 44°C were labeled for four generations with [^{3}H]GlcNAc (a), [^{3}H]dThd (b), or [^{3}H]GlcNAc and [^{3}H]dThd (c) of appropriate specific activities. After 4.5 generations of chase in cold medium, cells were harvested, treated with trichloroacetic acid, washed, treated with RNase and trypsin to remove [^{3}H]GlcNAc incorporated into protein, and finally processed for autoradiography. Septa stained by crystal violet and fuchsin appeared red. Their position, as revealed by visual inspection, is indicated by arrows. Grain clusters designated by A, B, C, D, and E define corresponding positions in Table 3 and Fig. 3. Bar, 10 μm.

TABLE 3. Positions of main clusters observed in chains derived each from a single cell after 4.5 generations of chase in minimal medium

Label	No. of chains examined	Avg no. of cells per chain	Average no. of grains in cluster					Mean position of cluster in chain[a]					
								C[b]		D[b]		E[b]	
			A[b]	B[b]	C[b]	D[b]	E[b]	Observed	Theoretical[c]	Observed	Theoretical[c]	Observed	Theoretical[c]
[^{3}H]GlcNAc	34	21.7	9.2	7.4	6.9	4.9	4.2	0.51 ± 0.04[d]	0.50	0.25 ± 0.05[d]	0.25	0.76 ± 0.04[d]	0.75
[^{3}H]dThd	55	21.7	10.2		9.0	4.0	4.3	0.57 ± 0.07	0.54	0.32 ± 0.07	0.29	0.80 ± 0.06	0.79
[^{3}H]GlcNAc and [^{3}H]dThd	25	25.4	14.7	6.7	12.5	6.3	6.7	0.55 ± 0.08	0.52	0.32 ± 0.08	0.27	0.78 ± 0.07	0.77

[a] The position was determined by the ratio of the number of cells located between cluster A and any given cluster to the total number of cells forming that particular chain.
[b] Clusters A and B are located at ends of chains; C is in the middle, D is between A and C, and E is between C and B (Fig. 1).
[c] Deduced from the model (see discussion).
[d] Standard deviation.

tions have been confirmed by electron microscope autoradiography (J.-M. Schlaeppi, O. Schaeffer, and D. Karamata, manuscript in preparation).

Regarding the position of clusters A to E, observations on unit chains labeled in both cell wall and DNA provide a distribution nearly identical to that obtained by superimposing distributions of the cell wall and DNA segregation units described above (Table 3). Quantitatively the most striking feature of Table 3 is the difference between experiments (i) and (iii) in the grain counts of clusters A and B, which can only be accounted for by the presence of a molecule of DNA in one only (A) of the two ends of a chain. However, it appears that for each cluster the sum of grains obtained in experiments (i) and (ii) is superior to the number of grains in corresponding clusters obtained in experiment (iii). We believe that this repeatedly observed phenomenon is due to an underestimate of grain counts which possibly occurs already for clusters of five to six grains.

It follows that data presented in Table 3 show that the excess of segregation units of cell wall over those of DNA is not due to small units whose synthesis was initiated shortly before the beginning of the chase and in advance of corresponding DNA units. On the contrary, it appears that for every replication point of DNA (two new segregation units) four (and not two as commonly supposed) segregation units of cell wall are synthesized, and that every strand of DNA is attached to only one out of two cell wall units synthesized at the same time. Data presented in Table 3 show that this attachment follows a regular pattern (see model below).

DISCUSSION

The experiments presented above confirm and extend previously reported observations (24) which show that the wall of *B. subtilis* cells growing exponentially in liquid medium consists of a limited number of rather large subunits which segregate according to a regular pattern (Fig. 1a). The existence of such units and of a symmetrical segregation pattern, as evidenced by autoradiographic analysis of [^{3}H]GlcNAc-labeled chains of cells, is compatible with a symmetrical type of zonal insertion of new cell wall material as suggested by Jacob et al. (14). The existence, in minimal medium, of at least four large segregation units per cell (apparently one-half cell long) is in full agreement with our observation of long delays required to visualize segregation of labeled cell wall subunits by light microscope autoradiography (24). Indeed, such segregation delays—which last about two gener-

ations in minimal medium (24)—would correspond to a lapse of time required for large blocks, most likely organized in layers, to slide one with respect to the other and, only after they have reached the outside cell surface, to become separated from each other by the extrusion of new wall onto the outer surface. A layer-type organization comprising an inside and an outside wall layer could also account for the observations of Pooley (19) and Archibald (1), which show a difference in age between the inner and the outer wall surfaces. We have shown that DNA cosegregates with cell wall according to a regular but asymmetrical pattern, strongly implying an association between the two structures which would ensure orderly segregation of chromosomes into daughter cells. It appears that from two symmetrically arranged cell wall units synthesized at any time only one will have a molecule of DNA attached to it. This attachment is permanent. Figure 2 provides a simple model which fully accounts for our observations and predicts distributions of major cell wall and DNA segregation units (after appropriate periods of chase) identical to those obtained in our experiments (Fig. 3 and Table 3). Although obviously oversimplifying the situation, the model has some specific implications: it requires, for example, a layer-like organization of cell wall, a structure of cell wall subunits which should allow their reorganization while maintaining constant wall thickness, a specific structural association between cell wall and DNA, and timing of septum synthesis relative to that of the corresponding cylindrical part of the cell wall. These predictions are currently being investigated by high-resolution electron microscope autoradiography and will be presented with a more elaborate model. Our picture can easily account for the surprisingly large number of wall segregation units (Table 1). Indeed, cells grown in minimal medium are 1.9 μm long, are mostly mononucleate, and have one DNA replication point (J.-M. Schlaeppi, unpublished observation). Therefore they should consist on the average of at least four DNA and eight cell wall segregation units. In rich medium cells are 4.0 μm long, are mostly binucleate, have three replication points of DNA per chromosome, and should consist of 16 DNA and 32 cell wall segregation units. Both estimates are in good agreement with our results (Table 1).

Cell wall and DNA cosegregation and a nearly constant ratio of cell wall to DNA segregation units in media of different richness suggest a coordinate regulation of initiation of DNA and cell wall subunit synthesis. In particular, it follows that the rate of cell wall extension—like that of DNA synthesis (28)—as a function of media richness is determined by the frequency of initiation of new cell wall insertion sites and thus possibly by the density of wall insertion zones per unit cell length. Identical conclusions have been reached by determining the number of cell wall segregation blocks per unit cell length in three media of different richness (24).

The model presented above (Fig. 2), obtained from experiments on cells growing exponentially in liquid media, is in apparent conflict with conclusions on DNA and envelope segregation derived from data on gram-positive bacteria grown on solid media (4, 9, 23). When [^{3}H]thymine-labeled cells of a *B. subtilis* chain-forming mutant are chased for several generations on solid medium (where single cells result in long chains), clusters corresponding to DNA segregation units are clearly not distributed at random; although the majority of chains have label in at least one end cell, some chains are labeled at both ends and others are labeled at neither end (9). These observations were explained by the following model. Each complete chromosome is attached to the cell envelope by only one strand, the older one. The newly synthesized strand becoming permanently attached during the subsequent round of replication to either the other old cell envelope unit (resulting in chains labeled in both ends) or to a newly initiated unit (chains with only one labeled end). This model cannot explain our observations, i.e., DNA strands are not found attached to both of the oldest envelope units. We believe that the discrepancies between our results and those of Eberle and Lark (9) are most likely due to DNA sister strand exchange (DNA fragmentation). The latter occurs much more frequently on solid media (27) than in the liquid media used in our work and could have generated chains labeled at both ends as well as chains with no labeled poles. Further support for Lark's model has been sought by Chai and Lark (4) in another experimental system. Cells of *Lactobacillus acidophilus* labeled by a pulse of [^{3}H]thymine and immediately coated with fluorescent cell wall antibody and chased on solid medium show no cosegregation of DNA and cell wall. However, if pulse-labeled cells are chased for over one generation before coating with fluorescent antibody and spreading on solid medium, over 50% of wall units cosegregate with DNA. These observations are in good agreement with both Lark's and our models. Indeed, according to the latter only the outside cell wall layer to which old DNA strands are attached can be labeled by fluorescent antibody. Since DNA labeled at the time of pulse is attached to the inside wall layer (Fig. 2) which cannot be labeled by fluorescent antibody, no cosegregation should be observed unless a period of about one generation elapses between labeling of DNA and that of cell wall.

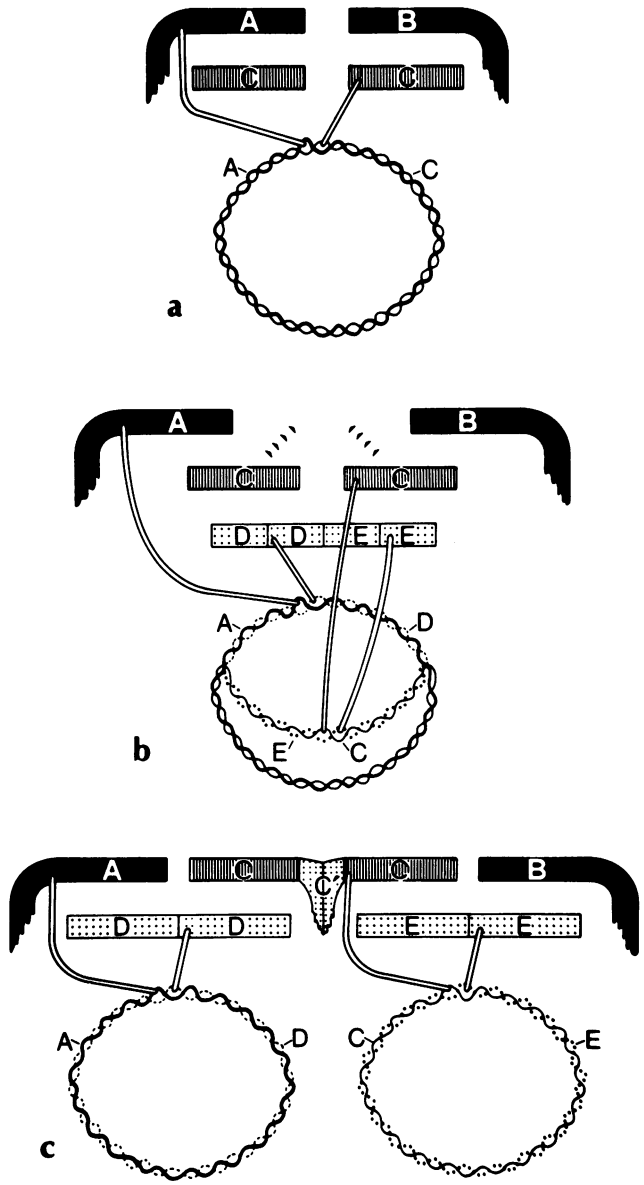

FIG. 2. Model of cell wall organization and DNA-cell wall association which, upon sufficient chase periods, would provide segregation patterns of cell wall and DNA compatible with our observations. a, b, and c correspond to three different stages in the cell cycle. Segregation units A, B, C, D, and E seen in Fig. 1 should correspond to subunits designated by the same letters. C′ designates the new septum.

Another set of experiments with [^{3}H]thymine-labeled *B. subtilis* chased for several generations on solid medium has provided results compatible with random segregation of chromosomes (22). Analysis of whole populations of chains (and not of selected individual chains) revealed that during the second hour of chase the proportion of labeled cell ends decreased by a factor of two per generation. However, in these experiments cells were no more in the exponential growth phase at the beginning of the chase; they were labeled and grown in a rich medium (A. Ryter, personnel communication) where they form long chains which split and yield short chains or individual cells (required for spreading on solid medium) only in the stationary phase. We believe that the physiological conditions of the cells at the beginning of the chase, as in the case of spore germination, could have influenced the DNA segregation pattern (see below).

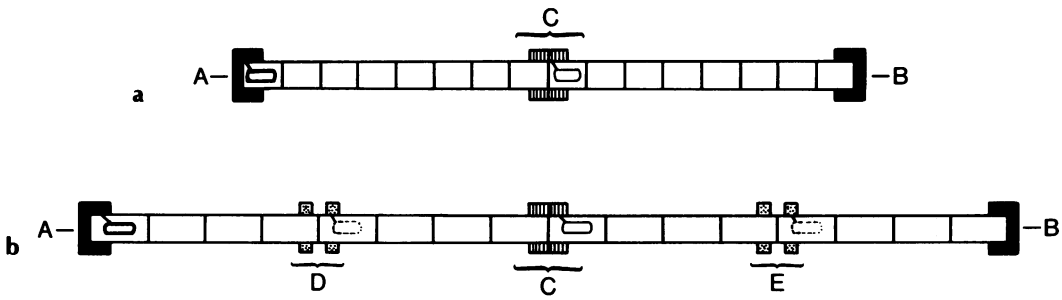

FIG. 3. Positions of segregation units of cell wall and DNA obtained after four generations of chase according to the model represented in Fig. 2. At the beginning of chase (0 generation) we have chosen a cell which has just divided (a) and a cell halfway through its division cycle (b).

In conclusion, our model (Fig. 2) could be reconciled with all data obtained on cells growing exponentially on solid media. Furthermore, observations on DNA segregation, obtained with strain Ni15 growing exponentially on solid minimal medium (J.-M. Schlaeppi, data not shown), show an asymmetrical nonrandom distribution as predicted; after approximately three to four generations of chase the majority of chains derived from single cells exhibit one heavily labeled pole, the other pole being devoid of label.

Data obtained on germinating spores of *B. subtilis* (21, 26) are not in agreement with observations of a regular pattern of cell wall and chromosomal cosegregation in exponentially growing cells. Ryter and Jacob (22) have shown that after the first two divisions after germination, DNA segregation units were distributed randomly between new and old poles. However, we believe that, due to the absence of a normal cell envelope, spore DNA is loose and becomes permanently attached only after several rounds of replication to either of the old or the newly synthesized wall subunits segregating afterward according to the pattern established here for exponentially growing cells. Dissociation of DNA from the cell envelope or some randomness during germination seems to be paralleled by a similar phenomenon during the last segregation events before sporulation, as evidenced by observations of Hitchins (13) on *Bacillus megaterium*, and could also account for the observed randomness of DNA segregation in chains originating from stationary-phase cells of *B. subtilis* (23).

For reasons already given our model has been derived from experiments carried out on a lysis-negative mutant. In a previous report (24) we could not find any difference between *lyt*$^+$ and *lyt* strains with respect to cell wall segregation. Our experiments (Schlaeppi, unpublished observations) aimed to study DNA segregation in *B. subtilis* 168 *thy trp*, a *lyt*$^+$ strain (10, 18), reveal that chains formed from single cells growing exponentially in minimal medium split between the first and the second hour after spreading on nutrient agar; each initial cell gives rise on the average to two to four chains which slide one next to the other. Such situations have also been observed in *Escherichia coli* (7). However, despite chain breaking, DNA segregation follows an asymmetrical pattern comparable to that obtained with *lyt* strains. Therefore we believe that our observations, suggesting that the cell wall plays a role of a primitive mitotic apparatus in *B. subtilis*, are not restricted to *lyt* mutants.

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