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van Helvoort, J.M.L.M.; Woldringh, C.L.; Kool, J.

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NOTES

Chloramphenicol Causes Fusion of Separated Nucleoids in *Escherichia coli* K-12 Cells and Filaments

J. M. L. M. VAN HELVOORT,* J. KOOL, AND C. L. WOLDRINGH

Section of Molecular Cytology, Institute for Molecular Cell Biology, BioCentrum, University of Amsterdam, 1098 SM Amsterdam, The Netherlands

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Chloramphenicol is frequently used for better visualization of the *Escherichia coli* nucleoid. Here, we show that chloramphenicol causes not only rounding off of the nucleoid but also fusion of as many as four separated nucleoids. Nucleoid fusion occurred in fast-growing cells and in filaments obtained by *dicF* antisense RNA induction or in *ftsZ84*(Ts) and *pbpB*(Ts) mutants. Thus, treatment with chloramphenicol erroneously suggests that DNA segregation is inhibited.

Chloramphenicol is frequently used to enhance the visualization of nucleoids in *Escherichia coli* (1, 7, 13, 21). The nucleoid is pulled into an extended or lobular shape by the processes of cotranscriptional translation and translocation of envelope proteins (3, 9, 19). Because chloramphenicol causes disassembly of the translational complexes, the nucleoid rounds off (6, 20). Spherical nucleoids are visualized more easily by fluorescence microscopy because the DNA-binding fluorochrome DAPI (4′,6-diamidino-2-phenylindole dihydrochloride hydrate) is contracted in a single spot instead of being bound to an extended nucleoid (4).

Previously, we have shown that the use of chloramphenicol can give rise to misinterpretation of nucleoid partitioning in wild-type cells (16). After chloramphenicol treatment, condensed nucleoids are observed either at midcell or at the one-quarter and three-quarter positions in the cell. This has been interpreted (1, 5) to reflect a rapid movement of the nucleoids, because no intermediate positions were observed. However, this is an artifactual movement, which is caused by the contraction of unseparated nucleoids to the cell center and of separated nucleoids to their new centers. By contrast, nucleoids not treated with chloramphenicol move gradually along with cell elongation, as evident from the constant distance between the outer nucleoid border and the cell pole throughout the cell cycle.

In 1992 the chloramphenicol method was applied by Tétart et al. (13) in a study of the effect on nucleoid separation of lowering the FtsZ concentration. The observed delay in nucleoid separation during filamentation was ascribed to a decreased level of FtsZ. Much earlier, in 1961, Schaechter and Laing (10) showed by time-lapse microscopy that in rapidly growing cells chloramphenicol can induce a fusion of nucleoids within 10 min. This phenomenon was also observed by Steinberg (11). In view of these observations, we compared nucleoid partitioning in cells and filaments either fixed immediately with OsO_4 or fixed after pretreatment with chloramphenicol for a short period. We show that chloramphenicol causes fusion of separated nucleoids both in filaments obtained by DicF-RNA induction (13) and in filaments of cell division mutants *ftsZ* and *pbpB*.

Nucleoid partitioning in filaments of JS219/pJPB57 induced by *dicF* expression. First, we repeated the experiment described by Tétart et al. (13), in which filamentation is induced by means of inhibition of FtsZ synthesis with DicF-antisense RNA (14). JS219 (*lacI*$^{q+}$) containing the pJPB57 plasmid with *dicF* under the control of a *lac* promoter was grown for at least 20 doubling times at 37°C in L broth containing 0.5% NaCl, 0.4% glucose, and 100 μg of ampicillin per ml before induction with 2 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The mass doubling time (measured as optical density at 450 nm) was 22 min. At each time point, the following two samples were taken (Fig. 1; Tables 1 and 2): (i) 1 ml of cells was immediately fixed with OsO_4 (0.1% final concentration) and (ii) 10 ml of cells was incubated for 15 min with 200 μg of chloramphenicol per ml (Boehringer, Mannheim, Germany) at 37°C before fixation with 0.1% OsO_4. Fixed cells were immobilized on object slides coated with a dried layer of 2% agarose. All samples were analyzed for cell length, nucleoid number, and nucleoid length with the image cytometric program Object-Image (17).

In Fig. 1, we compare our results with the data described by Tétart et al. (see Fig. 3 in reference 13). In our experiment, the slopes of the curves for the increase in cell length (without and with chloramphenicol treatment) are much lower (Fig. 1A). This discrepancy is possibly due to the lower salt concentration, i.e., 0.5% versus 1% NaCl used by Tétart et al. (1a), which may have led to a less-efficient inhibition of *ftsZ* expression and to a continuation of cell divisions throughout the experiment.

In spite of the discrepancy described above, the results in Fig. 1A clearly show that in untreated cells, the average number of nucleoids per cell increased during four mass doublings from 2.0 to 4.5, without a lag and proportionally to a length increase from 4.6 to 10.3 μm (Tables 1 and 2). This is also indicated in Fig. 1B by the constancy of the partition index (Table 1), which decreased only after five mass doublings (see below), and in the images of Fig. 2C and 3A. Similar images of segregated nucleoids in *ftsZ* filaments have been reported previously (2, 18). We conclude that during the filamentation induced by inhibition of *ftsZ* expression, the process of parti-

* Corresponding author. Mailing address: Section of Molecular Cytology, Institute for Molecular Cell Biology, BioCentrum, University of Amsterdam, Kruislaan 316, 1098SM Amsterdam, The Netherlands. Phone: (31) 20-5256204. Fax: (31) 20-5256271. Electronic mail address: joop@mc.bio.uva.nl.

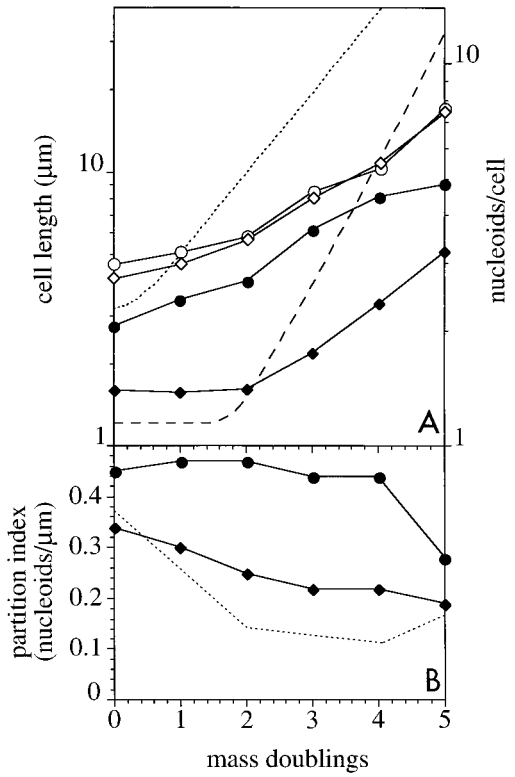

FIG. 1. Cell lengths, numbers of nucleoids per cell, and partition indices of *E. coli* JS219/pJPB57 cells during induction with IPTG plotted against numbers of mass doubling. Circles, untreated control cells; diamonds, cells treated with chloramphenicol. (A) Cell lengths (open symbols) and numbers of nucleoids per cell (closed symbols) compared with the results from the experiment described by Tétart et al. (see Fig. 3 in reference 13). Dotted line, cell length; dashed line, numbers of nucleoids per cell. Note the immediate increase in the number of nucleoids per cell in untreated cells and the decrease in the number of nucleoids per cell as a result of chloramphenicol-induced nucleoid fusion. (B) Partition indices plotted against numbers of mass doublings. Dotted line, partition index as described by Tétart et al. (13).

tioning continues normally and that FtsZ plays no role in nucleoid segregation. A similar conclusion was recently obtained for the function of FstZ in *Bacillus subtilis* (8).

Fusion of nucleoids caused by chloramphenicol treatment. Treatment of the cells with chloramphenicol for 15 min as performed by Tétart et al. (13) caused in the control cells (0 mass doublings in Table 1 and Fig. 1) a decrease from 2.0 to 1.4 in the average number of nucleoids per cell and a decrease from 0.45 to 0.34 in the partition index. As evident from the DAPI-stained cells and filaments in Fig. 2, this decrease is caused by a fusion of previously separated nucleoids.

In the chloramphenicol-treated filaments, the partition index continued to decrease, especially during the first two mass doublings (Fig. 1B; Table 1). This decrease has been interpreted by Tétart et al. (13) as an indication of a transient inhibition of nucleoid separation. However, although in the control cells (zero mass doublings), on average, 2 nucleoids could fuse into 1, as many as 4 nucleoids could fuse in individual filaments (Fig. 2). As a result, the average number of nucleoids per cell remained constant (Fig. 1A), and the partition index decreased (Fig. 1B; Table 1). These observations have led Tétart et al. to the erroneous interpretation that nucleoid segregation is transiently inhibited during early filamentation (13).

Residual division and constriction initiation during chloramphenicol treatment. In Table 2, it can be seen that, compared with the control culture (zero mass doublings), the 15-min chloramphenicol treatment caused a decrease in average cell length from 4.6 to 4.1 μm, which can be ascribed to residual division. Although residual division should give a decrease in the percentage of constricting cells, we observed an increase from 29 to 32% (Table 2). This implies that new constrictions are initiated during chloramphenicol treatment, concomitant with a 20% increase in optical density (see also reference 16). Since 85% of the cells in the untreated population had 2 or more nucleoids, many were apparently still able to initiate a constriction.

The concomitant initiation of constrictions and fusion of nucleoids may cause the appearance of a constriction at the site of the nucleoid. The present observations suggest that such constrictions may have initiated before fusion, between previously separated nucleoids, in accordance with the nucleoid occlusion model (18).

Nucleoid partitioning in long filaments. After five mass doublings, the partition index of nontreated cells decreases from 0.44 to 0.28 nucleoids per μm (Table 1), indicating that in these long filaments partitioning is retarded. However, Fig. 2C shows that the nucleoids are still evenly distributed throughout the entire filament, as opposed to cells treated with chloramphenicol (Fig. 2D). In many filaments, some of the central nucleoids do not seem to have separated, resulting in an increase in average nucleoid length (Table 1). We suggest that in such individual filaments, unbalanced growth has occurred and

TABLE 1. Values for *dicF*-induced *E. coli* JS219/pJPB57 cells untreated or treated with chloramphenicol[a]

Mass doublings[b]	Value for untreated cells			Value for chloramphenicol-treated cells[c]		
	No. of nucleoids per cell	Partition index[d]	Nucleoid length (μm)	No. of nucleoids per cell	Partition index[d]	Nucleoid length (μm)
0	2.0	0.45	1.3	1.4	0.34	1.1
1	2.4	0.47	1.3	1.4	0.30	1.3
2	2.7	0.47	1.4	1.4	0.25	1.5
3	3.7	0.44	1.5	1.9	0.22	1.7
4	4.5	0.44	1.4	2.4	0.22	1.8
5	4.8	0.28	1.9	3.1	0.19	2.4

[a] The cells were induced with 2 mM IPTG at the start of the experiment.
[b] Mass doublings were measured as doublings in optical density at 450 nm.
[c] The cells were treated with 200 μg of chloramphenicol per ml for 15 min (13).
[d] Partition index, number of nucleoids per μm of cell length.

TABLE 2. Cell lengths and percentages of constrictions of *dicF*-induced *E. coli* JS219/pJPB57 cells untreated or treated with chloramphenicol[a]

Mass doublings[b]	Untreated cells			Chloramphenicol-treated cells[c]		
	No. of cells scored	Cell length (μm)	Constricted cells (%)	No. of cells scored	Cell length (μm)	Constricted cells (%)
0	162	4.6	29	300	4.1	32
1	163	5.1	28	300	4.6	20
2	120	5.8	14	279	5.7	6
3	132	8.4	11	231	8.0	7
4	116	10.3	5	153	10.8	3
5	96	17.0	2	135	16.5	3

[a] Cells were induced with 2mM IPTG at the start of the experiment.
[b] Mass doublings were measured as doublings in optical density at 450 nm.
[c] Cells were treated with 200 μg of chloramphenicol for 15 min (13).

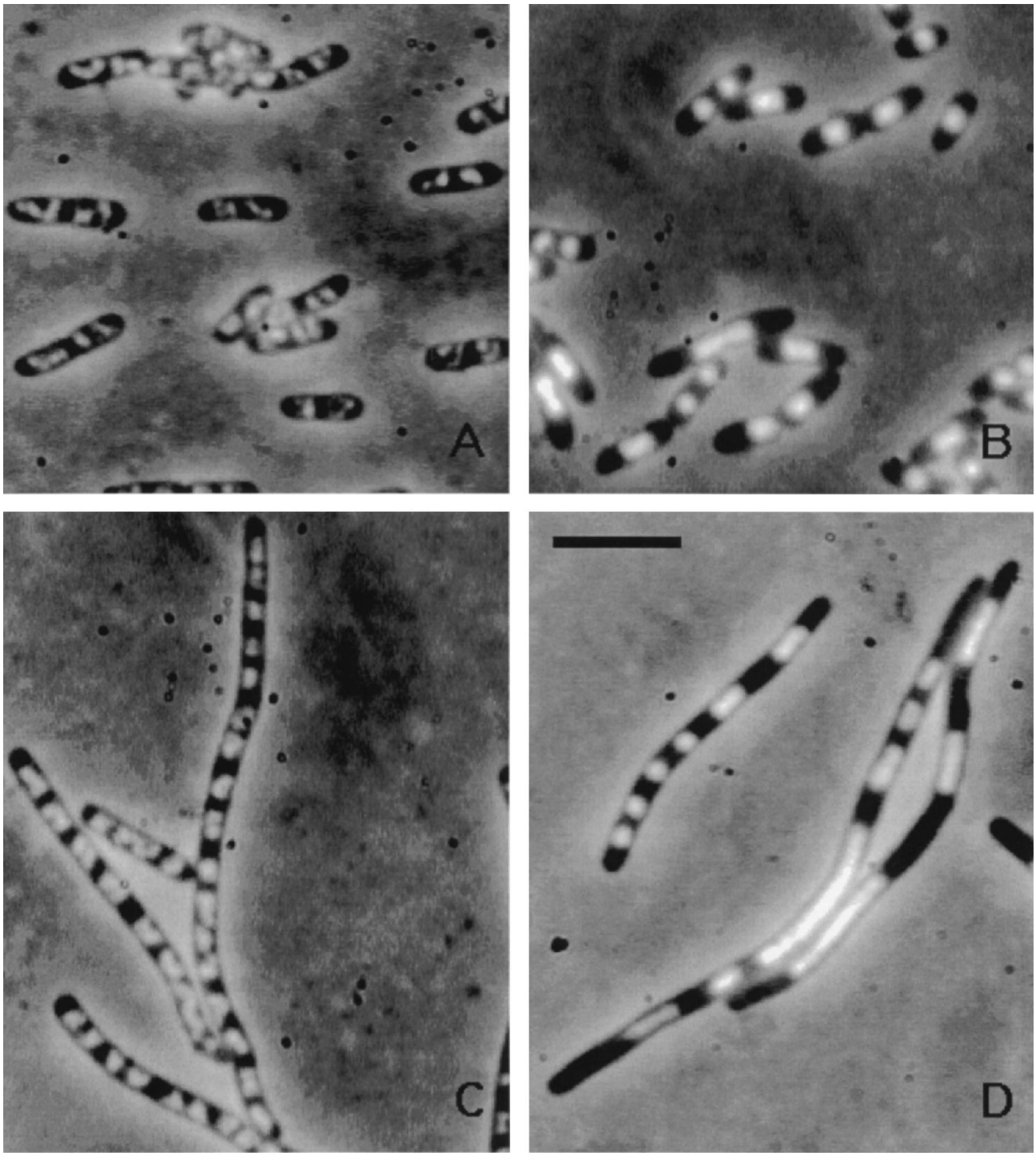

FIG. 2. DAPI-stained cells of *E. coli* JS219/pJPB57 before (A and B) and after (C and D) induction of *dicF* antisense RNA with 2 mM IPTG for five mass doublings. (A) Uninduced, untreated control cells (many cells contain four separated nucleoids). (B) cells treated for 15 min with 200 μg of chloramphenicol per ml; (cells contain one or two rounded nucleoids); (C) untreated filaments, showing well-segregated nucleoids; (D) filaments treated for 15 min with 200 μg of chloramphenicol per ml, showing fused nucleoids.

that a decreased rate of protein synthesis has caused a slowing down of both growth and nucleoid partitioning (see also reference 16).

Chloramphenicol causes nucleoid fusion in *ftsZ84*(Ts) and *pbpB*(Ts) filaments. The effect of chloramphenicol on the number and shape of the nucleoid was also tested in *ftsZ84* (strain LMC509) and *pbpB2158* (strain LMC510) cell division mutants. The strains have been described previously (12). Cells of *ftsZ84* were grown in low salt glucose minimal medium (114 mosM) to prevent suppression of cell division at the restrictive temperature (12). Cells of *pbpB* were grown in normal glucose minimal medium (300 mosM). Both strains were first grown at

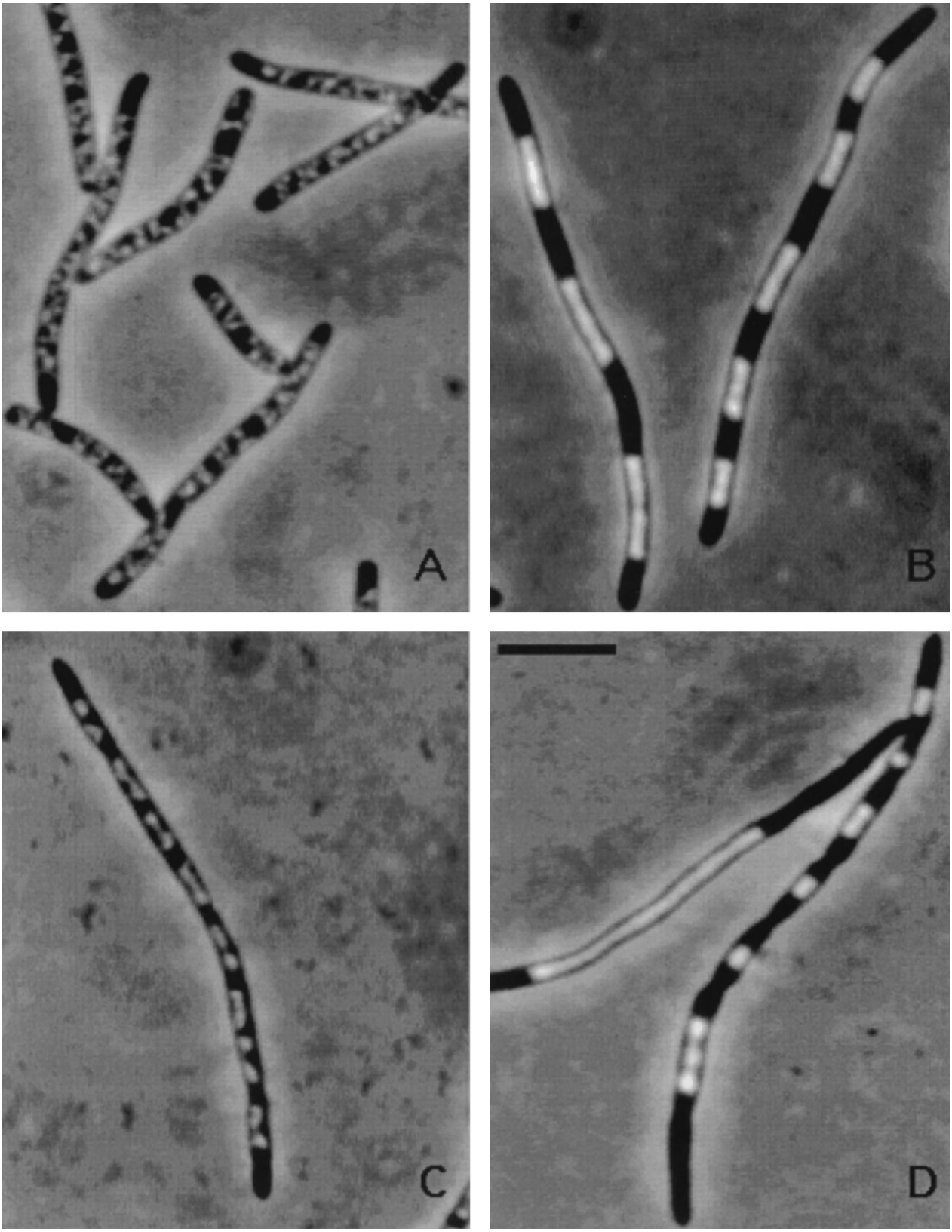

FIG. 3. DAPI-stained filaments of *ftsZ84*(Ts) (A and B) and *pbpB2158*(Ts) (C and D), grown for three mass doublings at 42°C. (A and C) Filaments not treated with chloramphenicol. Note the different shapes of the nucleoids caused by growth in low and high salt concentrations, respectively. (B and D) Filaments treated with 300 μg of chloramphenicol per ml for 60 and 80 min, respectively. Note that in the *pbpB* filaments, fusing nucleoids must have passed through the persisting constriction sites.

permissive temperature (30°C) for several doubling times before shifting to the restrictive temperature (42°C). After three mass doublings at 42°C, samples were either fixed directly with 0.1% OsO_4 or first treated with chloramphenicol (300 μg/ml) at 42°C for 60 min (*ftsZ84*) or 80 min (*pbpB*).

Figure 3A shows filaments of *ftsZ* with rather extended nucleoids due to growth at a low salt concentration (19). Figure 3C shows *pbpB* filaments grown in 300 mosM medium, with more confined nucleoids and persisting constrictions (see reference 12). After chloramphenicol treatment, the filaments of both strains showed fused nucleoids (Fig. 3B and D), sometimes reminiscent of the "fused, spherical nucleoids arranged linearly" as described by Zusman et al. (20). The process of nucleoid fusion is similar for chloramphenicol concentrations between 5 and 500 μg/ml (results not shown), as was already demonstrated by Steinberg (11). We conclude that chloramphenicol-induced nucleoid fusion is not restricted to the JS219 strain and its *dicF* filaments but represents a general phenomenon in *E. coli* K-12 cells.

Conclusion. As previously suggested by various authors (3, 9, 19), the nucleoid is shaped in an extended or even lobular form through numerous interactions with the cytoplasm. Abolishment of these interactions causes the rounding off of the nucleoid. We now show that, in addition, already separated nucleoids can fuse together into single nucleoids. Possibly, this fusion is related to the same exclusion mechanism that causes the phase separation between nucleoid and cytoplasm in living cells (15).

The rapid fusion with chloramphenicol (within 15 min) renders this antibiotic unsuitable for visualizing DNA in studies of nucleoid partitioning (1, 13, 21). In such studies, care should be taken to fix cells or filaments directly in their growth medium and to omit unbalanced growth conditions or procedures that affect protein synthesis and thereby enhance the visibility of nucleoids. Presently, we are investigating whether the rate and extent of the fusion change with growth and energy conditions, and whether fusion varies in different strains. Preliminary observations have shown that the fusion process is reversible and that nucleoids can resegregate upon growth recovery.

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