JOURNAL OF BACTERIOLOGY, Jan. 1986, p. 66–71
0021-9193/86//010066-06$02.00/0

Vol. 165, No. 1

# SOS-Independent Coupling between DNA Replication and Cell Division in *Escherichia coli*

ALINE JAFFÉ,* RICHARD D'ARI, AND VICTOR NORRIS

*Institut Jacques Monod, Centre National de la Recherche Scientifique, Université Paris 7, 75251 Paris Cedex 05, France*

Received 22 July 1985/Accepted 22 October 1985

**Inhibition of DNA synthesis in *Escherichia coli* mutants in which the SOS-dependent division inhibitors SfiA and SfiC were unable to operate led to a partial arrest of cell division. This SOS-independent mechanism coupling DNA replication and cell division was characterized with respect to residual division, particle number, and DNA content. Whether DNA replication was blocked in the initiation or the elongation step, numerous normal-sized anucleate cells were produced (not minicells or filaments). Their production was used to evaluate the efficiency of this coupling mechanism, which seems to involve the cell division protein FtsZ (SulB), also known to be the target of the division inhibitors SfiA and SfiC. In the absence of DNA synthesis, the efficiency of coupling was modulated by the cyclic-AMP–cyclic-AMP receptor protein complex, which was required for anucleate cell production.**

Cultures of *Escherichia coli* bacteria grown in steady-state conditions contain very few anucleate cells (18; this work), reflecting the tight coupling between DNA replication and cell division. Similarly, when DNA synthesis is blocked, cell division also comes to a halt, leading to filamentous growth with little formation of anucleate cells (8, 18).

One coupling mechanism has been well characterized and is known to be involved in the SOS response (19, 21). Treatments which interfere with the integrity of the *E. coli* DNA or with its synthesis induce the expression of different genes negatively controlled by the LexA repressor. This expression and its consequences constitute the SOS response (28). After the induction of the SOS response, an inhibition of cell division occurs, resulting in the formation of filaments. This filamentation is related to the expression of the *sfiA* (or *sulA*) gene, which codes for an inhibitor of septum formation. It has been shown that the SfiB protein is the target for the inhibitory action of the SfiA protein (26a); the *sfiB* (or *sulB*) gene corresponds to *ftsZ*, the product of which is involved in cell division (26, 29). In the case of *sfiB* mutants, this protein is altered but still functional. The SfiC system is another SOS-dependent inhibitory mechanism found in certain strains; it is also suppressed by *sfiB* mutations (9).

Induction of the SOS response in *recA* (Tif) strains at 42°C does not cause division inhibition in *sfiA sfiC* or *sfiB* mutants (9, 13). SfiA and SfiC are thus the only SOS-dependent division inhibitors. However, these mutants still form filaments under conditions of DNA arrest resulting from UV irradiation, nalidixic acid treatment, or thymine starvation (5, 20). These observations demonstrate the existence of an SOS-independent pathway which coordinates DNA replication and cell division.

We have previously shown that during thymine starvation, anucleate cells were produced if the SfiA and SfiC division inhibitors were absent; when the SfiA protein was induced, the accumulation of anucleate cells was totally abolished (24). The SfiA and SfiC systems were shown not to regulate cell division and chromosome segregation when DNA replication was not perturbed (22).

* Corresponding author.

To identify the mechanisms involved in the coupling between DNA replication and cell division, we investigated the residual cell division and formation of anucleate cells under different conditions of blocked DNA synthesis using *sfiA sfiC* or *sfiB* mutants. In the experiments reported here, the efficiency of the SOS-independent coupling mechansim was measured by counting the number of anucleate cells produced by different mutants. To block the initiation of DNA synthesis, *dnaA*(Ts) and *dnaC*(Ts) mutants were analyzed; to block elongation, thymine starvation and a *dnaB*(Ts) mutant were analyzed. These mutants were studied under conditions in which their DNA synthesis was inhibited; the presence of the *sfiA sfiC* and *sfiB* mutations prevented SOS-dependent division inhibition (13). Under these conditions, cell division no longer appears strictly coupled to chromosome replication and many anucleate cells accumulate.

This production of anucleate cells requires the presence of cyclic AMP (cAMP) and the cAMP receptor protein (CAP). In the absence of the cAMP-CAP complex, strict coupling between cell division and chromosome replication was restored. This suggests a role for the cAMP-CAP complex in the control of cell division in *E. coli*.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** All bacterial strains used in this study (Table 1) were derived from the K-12 strain AB1157 (17). The *thy* mutants were selected as trimethoprim-resistant clones (except in strain B1673), the *deo* mutants as low (3 μg/ml)-thymine-requiring clones. The *sfiC* mutants were derived from strain GC2480 (9). The *recA1* mutation was introduced by mating with HfrKL16 *recA1*. The thermosensitive AB1157 derivatives *dnaA46*, *dnaC325*, and *dnaB70* were as described previously (7). The *parB* mutation (14) was introduced by P1 transduction (V. Norris, T. Alliote, and R. D'Ari, manuscript in preparation) as were other mutations: *sfiA100*::Tn*5* (9), *sfiB114* (13), *cya* (4), and *crp* (34). The plasmid pXX333 is a pSC101 derivative temperature sensitive for replication and carries the *ccdA* and *ccdB* genes of F plasmid (33); pHA5 is a pBR322 derivative carrying the *crp* gene (2). These plasmids were introduced by DNA transformation. The transformants were cultivated in the

TABLE 1. Bacterial strains

| Strain | Genotype |
|---|---|
| AB1157 | F⁻ *thr leu pro his arg thi lac gal ara xyl mtl supE tsx rpsL* |
| GC2480 | F⁻ *recA441 sfiC thr his arg rpsL* |
| B1654 | as GC2480 *recA*⁺ *sfiA100*::Tn*5 srlC300*::Tn*10 thy deo* |
| GC7035 | as B1654 *sfiB114* |
| B1671 | as B1654 Δ*cya* |
| B1675 | as B1654 Δ*crp* |
| GC7033 | as B1654 *sfiB114* Δ*cya* |
| B1683 | as B1654 *recA1 srl*⁺ |
| GC7016 | as B1654(pXX333) Δ*cya pyrD* |
| GC7048 | as B1654(pHA5) |
| B1673 | as AB1157 *dnaA46 sfiB114 thy*::Tn*5 deo ilv srlC300*::Tn*10 arg*⁺ *leu*⁺ |
| B1674 | as B1673 Δ*cya ilv*⁺ |
| B1691 | as AB1157 *dnaB70 sfiB114 thy deo arg*⁺ *leu*⁺ |
| GC7025 | as B1691 Δ*cya* |
| B1690 | as AB1157 *dnaC325 sfiB114 thy deo thr*⁺ *leu*⁺ |
| GC7024 | as B1690 Δ*cya* |
| GC7026 | as AB1157 *parB sfiB114* Δ*cya thy deo leu*⁺ |

presence of chloramphenicol (pXX333) and ampicillin (pHA5).

**Media.** All experiments were done in minimal 63 medium (32) supplemented with thiamine (10 μg/ml), glucose (0.4%), Casamino Acids (0.4%), thymine (50 μg/ml or 5 μg/ml), and, when needed, uracil (20 μg/ml). Antibiotics were used at the following concentrations: chloramphenicol, 20 μg/ml; ampicillin, 20 μg/ml. cAMP was used at $5 \times 10^{-3}$ M.

**Miscellaneous.** Thymine starvation experiments were carried out at 37°C. Thymine was removed by centrifugation. P1-mediated transduction (32), bacterial conjugation (32), transformation (30), and β-galactosidase assays (32) were as described previously. Particles were counted and their volume distribution determined using a ZB Coulter Counter equipped with a C1000 Channelyzer.

The autoradiography method was as described previously (24). Briefly, it involved prelabelling in [$^3$H]thymine for at least 15 generations. Samples were fixed and stained. The percentage of anucleate cells was evaluated by averaging the direct microscope and photomicrograph counts, using a Zeiss Jenamed microscope.

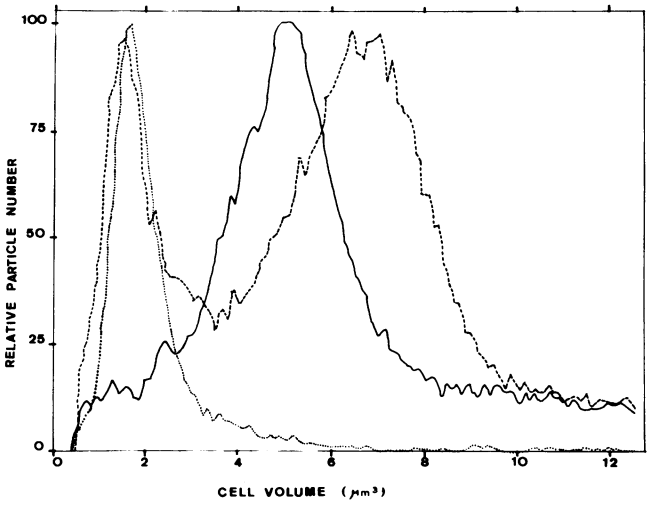


FIG. 1. Cell volume distribution of a *sfiA sfiC* culture during thymine starvation. Symbols: . . . , zero time; ——, 2 h starvation; -----, 4 h starvation.

## RESULTS

**Production of anucleate cells.** To study cell division when DNA synthesis was blocked, *dna*(Ts) mutants and thymine-starved bacteria were analyzed. The thermosensitive mutants studied were affected in the initiation (*dnaA*, *dnaC*) or the elongation (*dnaB*) step of DNA replication. The strains used were essentially isogenic with strain AB1157. In *dna*(Ts) strains, the *sfiB* allele was introduced by P1 transduction. Thymine starvation was performed in *sfiA sfiC* strains. Both the *sfiB* and the *sfiA sfiC* mutations prevent SOS-dependent inhibition of division.

The mass of each mutant studied, as measured by optical density, continued to increase after transfer to nonpermissive conditions. Cell number increased more slowly, resulting in augmentation of mean cell volume, as measured with a Coulter Counter. Cell morphology was filamentous. After about 90 min, normal-sized cells started to accumulate, reaching 20 to 40% of the total cell population by 4 h. Evolution of the filamentous and nonfilamentous populations during thymine starvation is shown in Fig. 1; the evolution of these populations in the case of *dna*(Ts) mutants was similar, although the volume distribution of the filaments was more complex (Norris et al., in preparation). All treatments resulted in a gradual loss of viability.

After 4 h under nonpermissive conditions, the total number of cell particles increased 2.5-fold for thymine-starved cells and the *dnaB*(Ts) mutant and 4- to 5-fold for *dnaA*(Ts) and *dnaC*(Ts) mutants.

To determine the nature of the small, nonfilamentous cells observed by Coulter Counter analysis, autoradiography experiments were performed under the same conditions. Samples were removed at 1-h intervals, prepared for autoradiography, and analyzed under the microscope for the DNA content of individual cells. The percentage of anucleate cells detected at these intervals is summarized in Tables 2 and 3. After 4 h of incubation under nonpermissive conditions, the percentage of anucleate cells in the total population was 10 to 17% compared to <0.2 to 0.7% under permissive conditions (time zero of the experiments). Almost all anucleate cells were rod shaped and of normal size (1.5 to 3 μm long),

TABLE 2. Production of anucleate cells during thymine starvation

| Strain | Relevant markers | Time of starvation (h) | % Anucleate cells (no. of cells analyzed) |
|---|---|---|---|
| B1654 | *sfiA sfiC* | 0 | 0.7 (1,351) |
| | | 1 | 2 (1,291) |
| | | 2 | 7 (483) |
| | | 3 | 13 (701) |
| | | 4 | 15 (786) |
| GC7035 | *sfiA sfiC sfiB* | 0 | 1.9 (1,461) |
| | | 4 | 20 (859) |
| B1671 | *sfiA sfiC cya* | 0 | ≤0.09 (1,111) |
| | | 2 | ≤0.09 (1,105) |
| | | 4 | 0.7 (1,108) |
| | | 6 | 2 (449) |
| B1675 | *sfiA sfiC crp* | 0 | ≤0.2 (500) |
| | | 6 | 2 (495) |
| GC7033 | *sfiA sfiC sfiB cya* | 0 | ≤0.1 (1,000) |
| | | 4 | 0.65 (1,163) |

and less than 0.5% anucleate filaments were detected. Some normal-sized bacteria were also found to contain DNA, and after 4 h this population represented 11, 17, 0.7, and 1.4% of the total population of thymine-starved cells and the *dnaA*, *dnaB*, and *dnaC* thermosensitive mutants, respectively.

The rest of the bacterial population was composed of filaments containing one or sometimes two or more discrete masses of DNA. Generally, the chromosome was seen in a central position in the filament, but occasionally it was at a pole. This latter image might follow a polar division which had given rise to an anucleate cell and a filament with an off-center chromosome. Very few lysed filaments were detected, although these could be observed after longer periods under nonpermissive conditions.

It has been reported that the RecA protein is involved in SOS-independent division inhibition after UV irradiation (5). We compared the response to thymine starvation in *sfiA sfiC* and *recA sfiA sfiC* strains. Mass, particle number, and cell volume distribution were monitored every 15 min for 4 h. The *recA*$^+$ and *recA* strains showed similar evolution. After 4 h, mass increased by a factor of 9.6 and 8.8, particle number by a factor of 2.7 and 2.9, and normal-sized cells represented 28 and 29% of the total population in the *recA*$^+$ and *recA* strains, respectively. Autoradiography experiments were not performed since *recA* bacteria suffer from spontaneous DNA degradation (6). We conclude that the RecA protein is not required for the SOS-independent division inhibition occurring during thymine starvation.

The results described above show that in the absence of Sfi-dependent division inhibition, all means of blocking DNA synthesis produce essentially the same response, namely division inhibition. This inhibition is not absolute and in particular does not prevent the production of anucleate cells.

**Inhibition of anucleate cell formation in the absence of a functional cAMP-CAP complex.** The product of the *cya* gene is adenyl cyclase and that of *crp* is CAP. The cAMP-CAP complex plays a role in the morphology of *E. coli* cells since *cya* and *crp* mutants are smaller than wild-type cells (our observation) and some are spherical (3, 27). Furthermore, the cell division protein FtsZ has been reported to have a higher level of expression in the absence of a functional cAMP-CAP complex (11, 12). Thus it seemed plausible that this complex played a role in cell division regulation and in the SOS-independent mechanism coupling division to DNA replication. To investigate this possibility, Coulter Counter analysis and autoradiography were carried out as described above, using the same conditions of DNA inhibition but in the absence of either adenyl cyclase or CAP. The responses to thymine starvation were analyzed in *cya* and *crp* derivatives of a *sfiA sfiC* strain. The cell volume distribution revealed that there was no longer any accumulation of small, nonfilamentous cells. Autoradiography confirmed that the absence of a functional cAMP-CAP complex abolished the capacity to produce anucleate cells during thymine starvation (Table 2).

The *cya* mutation was also introduced into the *dna*(Ts) *sfiB* strains, and size distribution and autoradiography analysis were carried out at high temperature. In all cases, few or no anucleate cells were found (Table 3).

We have described two other situations leading to the production of anucleate cells. One system operates when the replication of an F plasmid is blocked (31, 33). Under conditions of blocked plasmid replication, the *ccd* functions of F plasmid lead to filament formation and the production of anucleate cells (up to 22% [25]).

Similarly, the *parB*(Ts) mutant produced anucleate cells (27% after 4 h; Norris et al., in preparation); this strain, considered a DNA partition mutant (14), differs from the other *dna*(Ts) mutants in that DNA synthesis is not blocked but only slowed down at a nonpermissive temperature (Norris et al., in preparation). As shown in Table 3, the production of anucleate cells in these two situations was also suppressed in the absence of a functional cAMP-CAP complex. Although the *cya* and *crp* mutants grow slowly, their average cell volume increases by the same factor in 6 h of thymine starvation as that of the *cya*$^+$ *crp*$^+$ strain in 4 h (approximately fivefold).

TABLE 3. Production of anucleate cells in *dna*(Ts) strains at 42°C

| Strain | Relevant markers | Time at 42°C (h) | % Anucleate cells (no. of cells analyzed) |
|---|---|---|---|
| B1673 | *dnaA sfiB* | 0 | ≤0.2 (500) |
| | | 1 | 0.7 (559) |
| | | 2 | 2.8 (564) |
| | | 3 | 4.7 (565) |
| | | 4 | 10 (906) |
| B1691 | *dnaB sfiB* | 0 | ≤0.2 (500) |
| | | 1 | 0.2 (390) |
| | | 2 | 5.4 (276) |
| | | 3 | 11 (906) |
| | | 4 | 12 (748) |
| B1690 | *dnaC sfiB* | 0 | ≤0.2 (500) |
| | | 1 | 0.6 (570) |
| | | 2 | 8 (249) |
| | | 3 | 17 (556) |
| | | 4 | 17 (848) |
| B1674 | *dnaA sfiB cya* | 0 | 0.2 (681) |
| | | 4 | 0.3 (507) |
| GC7024 | *dnaB sfiB cya* | 0 | ≤0.1 (800) |
| | | 5 | 1.4 (697) |
| GC7025 | *dnaC sfiB cya* | 0 | 0.1 (800) |
| | | 5 | 1.3 (770) |
| GC7026 | *parB sfiB cya* | 0 | ≤0.1 (600) |
| | | 5 | ≤0.1 (980) |
| GC7016 | *sfiA sfiC*(pXX333) *cya* | 0 | ≤0.2 (500) |
| | | 8 | ≤0.1 (1,000) |

In conditions of balanced growth in which DNA synthesis is not perturbed (the initial points in the above experiments), the percentage of anucleate cells produced spontaneously is also lower in the absence of the cAMP-CAP complex (Tables 2 and 3). In perturbed and unperturbed growth conditions, the SOS-independent coupling of DNA replication and cell division appears to be stricter in the absence of the cAMP-CAP complex.

**Effect of exogenous cAMP on anucleate cell production in *cya* mutants.** The effects of the *cya* mutation are generally reversed by adding cAMP to the growth medium. Cell volume distributions were analyzed in *cya* strains to determine to what extent exogenous cAMP could restore the production of nonfilamentous normal-sized cells under conditions in which DNA synthesis was inhibited.

An *sfiA sfiC cya* strain was analyzed during thymine starvation. cAMP ($5 \times 10^{-3}$ M) was added either at the start of thymine starvation or 2 h later. In both cases, the accumulation of normal-sized cells after 4 h of thymine starvation was similar to that obtained in the *cya*$^+$ strain (data not shown). This normal-sized population included anucleate cells, as confirmed by autoradiography (Table 4). In contrast, no normal-sized cells were detected with

TABLE 4. Production of anucleate cells during thymine starvation in the presence of exogenous cAMP

| Strain | Relevant markers | Moment of cAMP addition (h)[a] | Time of starvation (h) | % Anucleate cells (no. of cells analyzed) |
|---|---|---|---|---|
| B1671 | *sfiA sfiC cya* | | 0 | ≤0.2 (350) |
| | | 0 | 4 | 19 (336) |
| | | 2 | 4 | 17 (515) |
| GC7033 | *sfiA sfiC sfiB cya* | | 0 | ≤0.1 (1,000) |
| | | 0 | 4 | 0.65 (1,163) |
| | | −24 | 0 | 0.12 (800) |
| | | −24 | 4 | 13 (500) |
| GC7035 | *sfiA sfiC sfiB* | | 0 | 2.2 (496) |
| | | 0 | 4 | 32 (1,554) |
| B1654 | *sfiA sfiC* | −24 | 0 | ≤0.2 (500) |
| | | −24 | 2 | 12 (516) |
| | | −24 | 4 | 32 (1,796) |
| GC7048 | *sfiA sfiC* (pHA5) | −24 | 0 | ≤0.2 (500) |
| | | −24 | 2 | 27 (907) |
| | | −24 | 4 | 50 (950) |

[a] In hours with respect to the time at which thymine was removed (time 0). −24 indicates cultures grown overnight in the presence of cAMP before thymine removal; cAMP was maintained during thymine starvation.

*dna*(Ts) *sfiB cya* mutants when cAMP was added at the time of transfer to 42°C (data not shown).

To verify that cAMP was effective in these experimental conditions, we measured the inducibility of the lactose operon in the *dnaA*(Ts) *sfiB cya* mutant. A culture containing the *lac* inducer isopropyl-β-D-thiogalactopyranoside ($5 \times 10^{-3}$ M) was transferred to 42°C, cAMP was added, and the level of β-galactosidase was measured at different times. Within 15 to 30 min the level of β-galactosidase in the *cya* strain was similar to that in the *cya*$^+$ strain. Thus, under the above conditions, the action of cAMP quickly followed its addition.

The inability of exogenous cAMP to restore a Cya$^+$ phenotype in *dna*(Ts) *sfiB cya* strains might be due to a difference in genetic background between these strains and the *sfiA sfiC cya* strain used in the thymine starvation experiment: the *dna*(Ts) mutants carried a *sfiB* mutation, while the thymine-starved strain did not. We therefore introduced the *sfiB* mutation into the *sfiA sfiC cya* strain and analyzed the response to exogenous cAMP during thymine starvation. In contrast to the results obtained with the *sfiA sfiC cya sfiB*$^+$ strain, addition of cAMP at the onset of thymine starvation did not restore production of normal-sized cells in the *sfiA sfiC sfiB cya* strain, and after 4 h of starvation essentially no anucleate cells were detected (Table 4). However, the production of anucleate cells was restored in this strain by cultivating it overnight in the presence of cAMP and maintaining the cAMP level throughout the experiment.

When DNA synthesis is blocked, the ability of exogenous cAMP to reverse the *cya* mutation with respect to anucleate cell production is affected by the *sfiB* mutation: the cAMP must be present much longer in *sfiB* cultures to be effective.

**Higher production of anucleate cells in *cya*$^+$ strains in the presence of exogenous cAMP.** The fact that the absence of a functional cAMP-CAP complex completely abolished anucleate cell production led us to attempt to increase the internal concentration of this complex in *cya*$^+$ strains.

cAMP was added to *sfiA sfiC* and *sfiA sfiC sfiB* strains; cAMP was also added to the *sfiA sfiC* strain harboring a multicopy plasmid coding for the CAP protein. An analysis of anucleate cell production during thymine starvation of the different strains is presented in Table 4. The addition of cAMP to plasmid-free *sfiA sfiC* and *sfiA sfiC sfiB* strains results in frequencies of anucleate cells up to twice as high as those obtained when cAMP was not added (Table 2). Even more anucleate cells were produced by the *sfiA sfiC* strain when the multicopy CAP plasmid was present and cAMP was added; after 4 h of thymine starvation, the cell population comprised 50% anucleate cells and 50% DNA-containing filamentous cells (Table 4, Fig. 2). Some of the anucleate cells were twice as long as normal-sized cells. In the absence of exogenous cAMP, this plasmid-bearing strain lysed during thymine starvation. Some of the anucleate cells had definite constriction; this analysis cannot determine whether these constrictions were formed in the anucleate cell itself or in the original DNA-containing filament.

Under conditions in which the internal concentration of functional cAMP-CAP complex is probably increased, more anucleate cells are produced when DNA synthesis is blocked. Thus the SOS-independent mechanism coupling

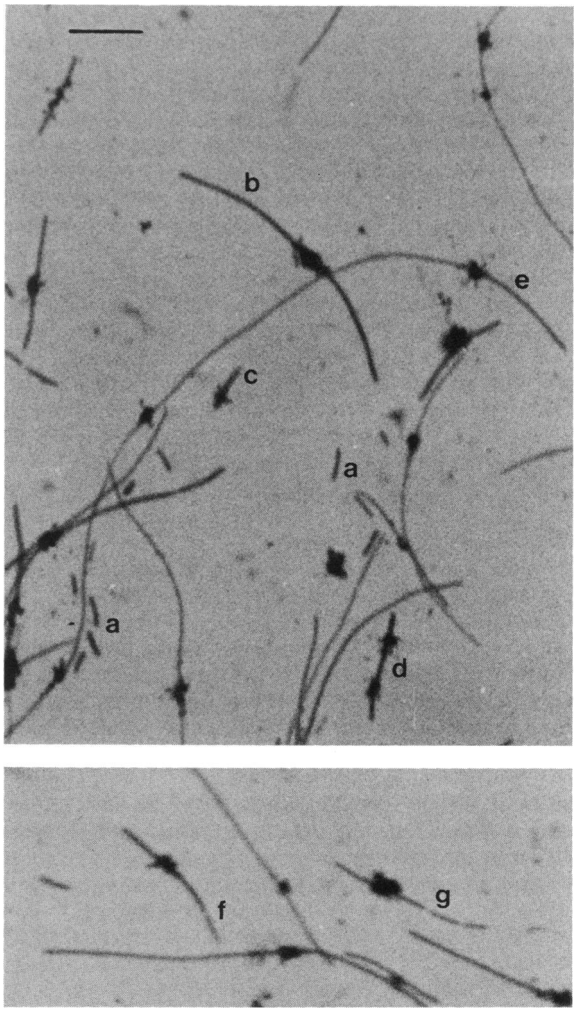


FIG. 2. Autoradiography of thymine-starved (4 h) *sfiA sfiC* (pHA5) culture in the presence of $5 \times 10^{-3}$ M cAMP. The culture contains normal-sized anucleate cells (a), and filaments with one centered chromosome (b), one off-centered chromosome (c), two chromosomes (d), three chromosomes (e), one constriction (f), or two constrictions (g). The bar represents 10 μm.

cell division to DNA synthesis is less strict under these conditions.

## DISCUSSION

When DNA synthesis is blocked in *E. coli*, cell division is rapidly inhibited by at least two coupling mechanisms (5, 20). The first involves the SOS division inhibitors SfiA and SfiC and their target FtsZ (SfiB). In the present study we further characterized the SOS-independent coupling mechanism in various mutants carrying either *sfiA sfiC* or *sfiB* mutations. Whether DNA synthesis was blocked in initiation or elongation, the response was essentially the same: filamentation and production of anucleate cells at a much higher level than that observed in $sfi^+$ strains (24) or in unperturbed growth conditions.

Other researchers have observed anucleate cell production when DNA synthesis was blocked under conditions that did not induce the SOS response: *lexA* ($Ind^-$) and *recA* strains during thymine starvation (18, 23) or a *dnaA*(Ts) strain at 42°C (15, 35). It was claimed that the temperature-sensitive *dnaA46* mutant required a second mutation, *divA*, to produce anucleate cells under restrictive conditions (15). We have no evidence for the existence of a $div^+$ allele in our strains.

Anucleate cells could be produced either by the cell division process or by DNA degradation. DNA breakdown occurs during thymine starvation (18). The following observations, however, support a significant production of anucleate cells by the cell division process: (i) during thymine starvation, the whole bacterial population forms filaments before the new population of normal-sized nonfilamentous cells appears; (ii) very few anucleate filaments could be detected; and (iii) partial septa were observed at the poles of filaments by phase-contrast microscopy as well as by autoradiography (Fig. 2).

The anucleate cells observed are different from the anucleate "minicells" produced spontaneously by the *minB* mutant (1, 10). The residual division occurring when DNA synthesis is inhibited appears to take place at normal division sites to produce normal-sized cells. Interestingly, the length distribution of anucleate cells revealed that in the presence of exogenous cAMP and multicopy CAP plasmid some of the anucleate cells were longer. This may reflect a capacity conferred by the cAMP-CAP complex to activate secondary division sites in conditions of perturbed growth.

A functional cAMP-CAP complex seems to be required for anucleate cell production in all cases studied: initiation blocks (*dnaA*, *dnaC*), elongation blocks (thymine starvation, *dnaB*), and perturbations (*parB*, $ccd^+$ plasmid). This suggests that the cAMP-CAP complex may be a regulatory factor in cell division.

The morphological anomalies displayed by *cya* and *crp* mutants may also reflect a role of the cAMP-CAP complex in cell division during unperturbed growth. Similarly the *cha* mutation, probably in the *crp* gene, causes inability to divide and lethality (11).

Our results show that in conditions of perturbed DNA synthesis the cAMP-CAP complex promotes cell division. It is known to regulate the transcription, positively or negatively, of many operons (36). It may be a positive regulator of a key cell division protein or, alternatively, a negative regulator of a division inhibitor.

One key cell division protein is the product of the *ftsZ* gene (15, 37). It is involved in the early stages of septation and is the target (SfiB) of the SOS-dependent division inhibitors SfiA and SfiC. It is also subject to autoregulation (11, 12). It may therefore be significant that the cAMP-CAP complex exerts negative transcriptional control on the *ftsZ* gene (11, 12).

We show here that the division-promoting action of the cAMP-CAP complex is severely retarded after the addition of exogenous cAMP to *cya sfiB* mutants, in which the FtsZ protein, although still functional, is altered. It is thus possible that the FtsZ protein itself can act as a division inhibitor, as previously proposed (16, 38), preventing the production of anucleate cells in the absence of a functional cAMP-CAP complex when DNA synthesis is perturbed. Alternatively, FtsZ may be activated to promote division by a protein regulated positively by the cAMP-CAP complex.

## ACKNOWLEDGMENTS

We wish to thank Agnès Ullman, Willie Donachie, Barry Holland, and Larry Rothfield for valuable discussions and Emmanuelle Maguin and Philippe Bouloc for support during this project. We are grateful to Sota Hiraga and Hiraji Aiba for providing plasmids, Antonia Kropfinger for secretarial help, Richard Schwartzmann for printing the micrographs, and Michel Lejour for preparing the figures.

This work was supported in part by a grant from the Centre National de la Recherche Scientifique ATP "Microbiologie." V.N. is a recipient of grant 83400 from the Science and Engineering Research Council.

## LITERATURE CITED

1. **Adler, H., W. Fisher, A. Cohen, and A. Hardigree.** 1967. Miniature *E. coli* cells deficient in DNA. Proc. Natl. Acad. Sci. USA **57**:321–326.
2. **Aiba, H., S. Fujimoto, and N. Ozaki.** 1982. Molecular cloning and nucleotide sequencing of the gene for *E. coli* cAMP receptor protein. Nucleic Acids Res. **10**:1345–1361.
3. **Aono, R., M. Yamasaki, and G. Tamura.** 1979. High and selective resistance to mecillinam in adenylate cyclase-deficient or cyclic adenosine 3′,5′-monophosphate receptor-deficient mutants of *Escherichia coli*. J. Bacteriol. **137**:839–845.
4. **Brickman, E., L. Soll, and J. Beckwith.** 1973. Genetic characterization of mutations which affect catabolite-sensitive operons in *Escherichia coli*, including deletions of the gene for adenyl cyclase. J. Bacteriol. **116**:582–587.
5. **Burton, P., and I. B. Holland.** 1983. Two pathways of division inhibition in UV-irradiated *E. coli*. Mol. Gen. Genet. **190**:309–314.
6. **Capaldo, F. N., and S. D. Barbour.** 1975. DNA content, synthesis and integrity in dividing and non-dividing cells of $Rec^-$ strains of *Escherichia coli* K12. J. Mol. Biol. **91**:53–66.
7. **Casaregola, S., R. D'Ari, and O. Huisman.** 1982. Role of DNA replication in the induction and turn-off of the SOS response in *Escherichia coli*. Mol. Gen. Genet. **185**:440–444.
8. **Cohen, S. S., and H. D. Barner.** 1954. Studies on unbalanced growth in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **10**:885–893.
9. **D'Ari, R., and O. Huisman.** 1983. Novel mechanism of cell division inhibition associated with the SOS response in *Escherichia coli*. J. Bacteriol. **156**:243–250.
10. **Davie, E., K. Sydnor, and L. I. Rothfield.** 1984. Genetic basis of minicell formation in *Escherichia coli* K-12. J. Bacteriol. **158**:1202–1203
11. **Donachie, W. D., K. J. Begg, and N. F. Sullivan.** 1984. Morphogenes of *Escherichia coli*, p. 27–62. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Donachie, W. D., N. F. Sullivan, D. J. Kenan, V. Derbyshire, K. J. Begg, and V. Kagan-Zur.** 1983. Genes and cell division in *Escherichia coli*, p. 28–33. *In* J. Chaloupka, A. Kotyk, and E. Streiblova (ed.), Progress in cell cycle controls. Czechoslovak Academy of Sciences, Prague.
13. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations in *sfiA* and

*sfiB* restore division in *tif* and *lon* strains and permit expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.

14. **Hirota, Y., M. Ricard, and B. Shapiro.** 1971. The use of thermosensitive mutants of *E. coli* in the analysis of cell division, p. 13–31. *In* L. A. Manson (ed.), Biomembranes. Plenum Press, New York.
15. **Hirota, Y., A. Ryter, and F. Jacob.** 1968. Thermosensitive mutants of *E. coli* affected in the process of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. **33**:677–694.
16. **Holland, I. B., and C. Jones.** 1985. The role of the FtsZ protein (SfiB) in UV-induced division inhibition and in the normal *Escherichia coli* cell division cycle. Ann. Microbiol. (Paris) **136A**:165–171.
17. **Howard-Flanders, P., and L. Theriot.** 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics. **53**:1119–1136.
18. **Howe, W. E., and D. W. Mount.** 1975. Production of cells without deoxyribonucleic acid during thymidine starvation of *lexA*$^-$ cultures of *Escherichia coli* K-12. J. Bacteriol. **124**:1113–1121.
19. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication–cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
20. **Huisman, O., R. D'Ari, and J. George.** 1980. Inducible *sfi*-dependent division inhibition in *Escherichia coli*. Mol. Gen. Genet. **177**:629–636.
21. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4494.
22. **Huisman, O., M. Jacques, R. D'Ari, and L. Caro.** 1983. Role of the *sfiA*-dependent cell division regulation system in *Escherichia coli*. J. Bacteriol. **153**:1072–1074.
23. **Inouye, M.** 1971. Pleiotropic effect of the *recA* gene of *Escherichia coli*: uncoupling of cell division from deoxyribonucleic acid replication. J. Bacteriol. **106**:539–542.
24. **Jaffé, A., and R. D'Ari.** 1985. Regulation of chromsome segregation in *Escherichia coli*. Ann. Microbiol. (Paris) **136A**:159–164.
25. **Jaffé, A., T. Ogura, and S. Hiraga.** 1985. Effects of the *ccd* function of the F plasmid on bacterial growth. J. Bacteriol. **163**:841–849.
26. **Jones, C. A., and I. B. Holland.** 1984. Inactivation of the essential division genes *ftsA*, *ftsZ*, suppresses mutation at *sfiB*, a locus mediating division inhibition during the SOS response in *E. coli*. EMBO J. **3**:1181–1186.

26a. **Jones, C., and I. B. Holland.** 1985. Role of the SulB (FtsZ) protein in division inhibition during the SOS response in *Escherichia coli*: FtsZ stabilizes the inhibitor SulA in maxicells. Proc. Natl. Acad. Sci. USA **82**:6045–6049.

27. **Kumar, S.** 1976. Properties of adenyl cyclase and cyclic adenosine 3′,5′-monophosphate receptor protein-deficient mutants of *Escherichia coli*. J. Bacteriol. **125**:545–555.
28. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
29. **Lutkenhaus, J.** 1983. Coupling of DNA replication and cell division: *sulB* is an allele of *ftsZ*. J. Bacteriol. **154**:1339–1346.
30. **Maniatis, R., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Miki, T., K. Yoshioka, and T. Horiuchi.** 1984. Control of cell division by sex factor F in *Escherichia coli*. I. The 42.84-43.6 F segment couples cell division of the host bacteria with replication of plasmid DNA. J. Mol. Biol. **174**:605–625.
32. **Miller, J. M.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Ogura, T., and S. Hiraga.** 1983. Mini-F plasmid genes that couple host cell division to plasmid proliferation. Proc. Natl. Acad. Sci. USA **80**:4784–4788.
34. **Sabourin, D., and J. Beckwith.** 1975. Deletion of the *Escherichia coli crp* gene. J. Bacteriol. **122**:338–340.
35. **Tang, M.-S., and C. E. Helmstetter.** 1980. Coordination between chromosome replication and cell division in *Escherichia coli*. J. Bacteriol. **141**:1148–1156.
36. **Ullmann, A., and A. Danchin.** 1983. Role of cyclic AMP in bacteria. Adv. Cyclic Nucleotide Res. **15**:1–53.
37. **Walker, J. R., A. Kovarik, J. S. Allen, and R. A. Gustafson.** 1975. Regulation of bacterial cell division: temperature-sensitive mutants of *Escherichia coli* that are defective in septum formation. J. Bacteriol. **123**:693–703.
38. **Ward, J. E., Jr., and J. F. Lutkenhaus.** 1984. A *lacZ-ftsZ* gene fusion is an analog of the cell division inhibitor *sulA*. J. Bacteriol. **156**:815–820.