Molecular Microbiology (2000) **36**(4), 973–981

Cell division, guillotining of dimer chromosomes and SOS induction in resolution mutants (*dif*, *xerC* and *xerD*) of *Escherichia coli*

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Summary

We have studied the growth and division of *xerC, xerD and dif* mutants of *Escherichia coli*, which are unable to resolve dimer chromosomes. These mutants express the Dif phenotype, which includes reduced viability, SOS induction and filamentation, and abnormal nucleoid morphology. Growth was studied in synchronous cultures and in microcolonies derived from single cells. SOS induction and filamentation commenced after an apparently normal cell division, which sheared unresolved dimer chromosomes. This has been called guillotining. Microcolony analysis demonstrated that cell division in the two daughter cells was inhibited after guillotining, and microcolonies formed that consisted of two filaments lying side by side. Growth of these filaments was severely reduced in *hipA*$^+$ strains. We propose that guillotining at *dif* destroys the expression of the adjacent *hipBA* genes and, in the absence of continued formation of HipB, HipA inhibits growth. The length of the filaments was also affected by SfiA: *sfiA dif hipA* mutants initially formed filaments, but cell division at the ends of the filaments ultimately produced a number of DNA-negative cells. If SOS induction was blocked by *lexA3* (Ind$^-$), filaments did not form, and cell division was not inhibited. However, pedigree analysis of cells in microcolonies demonstrated that lethal sectoring occurred as a result of limited growth and division of dead cells produced by guillotining.

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Introduction

Guillotining was initially observed by Niki *et al*. (1991) in *mukB* cells of *Escherichia coli*. These partitioning mutants produced about 5% anucleate cells and, in some cells, septum formation was observed that transected the nucleoid and resulted in products with unequal amounts of DNA. A comparable phenotype has been observed in *smc* mutants of *Bacillus subtilis* (Moriya *et al*., 1998; Britton and Grossman, 1999). In some situations, guillotining can occur at a substantial fraction of the septa. Cook and Rothfield (1999) have studied septum formation at the poles of filamentous cells of *E. coli* in which cell division and DNA replication had been transiently inhibited and, in those conditions, the frequency of guillotining was over 25%.

A specialized form of guillotining might be expected to occur in cells that cannot resolve dimer chromosomes. Resolution of dimers requires the *dif* site (Kuempel *et al*., 1991) and XerC and XerD resolvase proteins (Blakeley *et al*., 1991, 1993), as well as cell division and the FtsK cell division protein (Steiner and Kuempel, 1998a; Steiner *et al*., 1999). This indicates that resolution occurs late in the cell cycle, when there is an intimate interaction between the *dif* site, the resolvase proteins and the septum-located FtsK protein. In mutants that are unable to resolve dimers, it seems likely that the septum proceeds through (i.e. guillotines) the DNA that connects the incompletely partitioned nucleoids. This is suggested by the observations that SOS is induced in *dif* mutants (Kuempel *et al*., 1991; Tecklenburg *et al*., 1995), in *xerC and xerD* mutants (unpublished experiments) and in *ftsK* mutants (Liu *et al*., 1998). As SOS induction is an indicator of DNA damage (Sassanfar and Roberts, 1990), dimer chromosomes are apparently damaged during growth and division. The damage appears to require cell division, as SOS induction is blocked if cell division is inhibited in *dif*, *xerC* or *xerD* mutants (unpublished experiments), and also in *ftsK* mutants (Liu *et al*., 1998).

In examining the growth and division of *dif*, *xerC and xerD* mutants, we have observed some unusual patterns that are unique to these mutants. These observations were made by examining the growth of single cells into microcolonies, and they demonstrate that guillotining

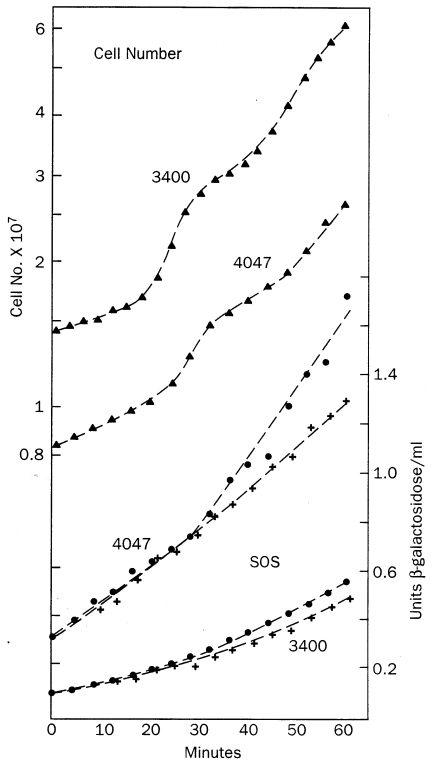


Fig. 1. Induction of SOS at cell division. Strains PK3400 (*sfiA::lacZ*) and PK4047(*xerD sfiA::lacZ*) were synchronized by the 'baby machine' procedure (Helmstetter *et al*., 1992). Cell number (▲) was determined by Coulter counter. Cephalexin (12 μg ml^{-1}) was added to half of each culture at 0 min, which completely blocked cell division (data not shown). β-Galactosidase activity is presented as units of enzyme ml^{-1} culture (Pardee *et al*., 1959), plotted on linear co-ordinates. For comparison of PK3400 with PK4047, units of enzyme ml^{-1} have been normalized for an initial cell density of 10^7 cells ml^{-1}. β-Galactosidase activity for cells without cephalexin (●); β-galactosidase activity for cells with cephalexin (+).

occurred at cell division in more than 10% of the cells. Furthermore, these observations have enabled us to demonstrate that the *hip* locus, which is adjacent to *dif*, can influence the filament elongation of cells in which guillotining has occurred. We report here our results on these unusual growth patterns, as well as experiments on induction of SOS during the cell cycle of synchronous cultures.

Results

The Dif phenotype and SOS expression

The relationship between cell division and SOS induction in *xerC*, *xerD and dif* mutants was first observed in synchronous cultures. Cells were synchronized by the 'baby machine' procedure (Helmstetter *et al*., 1992), and Fig. 1 shows results obtained with PK3400, which contains a *sfiA::lacZ* SOS reporter gene, and PK4047, which is a *xerD* derivative. Typically, the division synchrony for PK4047 was not as concise as that produced by PK3400, and fewer cells were obtained from the 'baby machine'. In addition, the cell number for PK4047 increased less than twofold at division in the synchronous cultures, and the increase in the number of cells over the course of the experiment was less. This results from the fraction of cells that commence filamentation after each cell division. It should also be mentioned that, although PK4047 grew more slowly in batch cultures (37 min mass doubling time) than PK3400 (27 min mass doubling time), both strains normally exhibited a 27 min cell cycle in synchronous cultures. This indicates that *xerD* cells have a normal length cell cycle, until filamentation is initiated.

Figure 1 also shows data for the induction of SOS, as monitored by the *sfiA::lacZ* reporter. These data are presented as total units of β-galactosidase activity ml^{-1} culture (Pardee *et al*., 1959), normalized to an initial cell concentration of 10^7 ml^{-1}, and they are plotted with linear co-ordinates. The level of SOS expression in PK4047 was 3.6-fold higher than that in PK3400, although size analysis with the Coulter counter demonstrated that the 'baby cells' were the same size. This indicates that SOS was already induced in a fraction of the PK4047 cells as they divided and were collected from the 'baby machine'. The constant slope of the line suggests that β-galactosidase was produced from the reporter gene at a constant rate during the first cell cycle. The rate of expression then increased at cell division, as a result of SOS induction that occurred at that time. To test whether this induction required cell division, half of each culture received cephalexin at the start of the experiment. This completely blocked cell division and had virtually no effect on mass increase (data not shown). It did, however, block the increased rate of SOS expression, which otherwise occurred at cell division (Fig. 1). Similar results have been obtained in analyses of *xerC and dif* mutants.

If SOS induction was caused by shearing, by the septum, of DNA that extended between partially separated nucleoids (i.e. guillotining), this would be expected to produce double-strand breaks. If so, the RecBC enzyme might be required to generate the signal that induced SOS. The induction could be similar to that observed in cells treated with quinolone inhibitors of DNA gyrase. That treatment produces double-strand breaks (Snyder and Drlica, 1979; Chen *et al*., 1996), but SOS is not induced in treated cells if they are *recBC* mutants (Chadhury and Smith, 1985). Similarly, we have observed that RecBC is required for SOS induction in *xerD* mutants. Whereas a *xerD sfiA::lacZ* mutant (PK4047) growing in batch culture produced 196 Miller units of enzyme activity (Miller, 1992), a *recBC xerD sfiA::lacZ* derivative (PK4129) only produced 26 units. The $xerD^+$ *sfiA::lacZ*

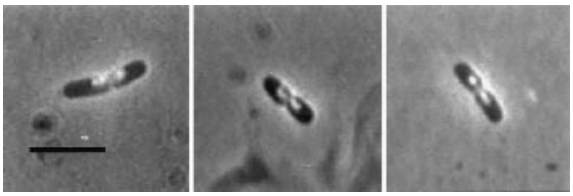


Fig. 2. Examples of guillotining at cell division in a *dif* deletion mutant (Δ*dif-hipA::cam*, PK3946). Cells were spread onto microscope slides coated with agar medium, grown for 15 min at 37°C, fixed with methanol and then stained with DAPI. The scale bar indicates 5 μm.

strain (PK3400) produced 36 units. Induction of SOS did not require RecD, however, as a *recD xerD sfiA::lacZ* strain (PK4145) produced 258 Miller units. Comparable results have also been observed for the SOS induction that occurs in *xerC*, *dif* and *ftsK* mutants (unpublished experiments).

An alternative explanation for the effect of *recBC* on SOS induction in these strains is that dimer chromosomes do not form in *recBC* mutants. If dimers did not form, there would be no guillotining, and no DNA damage would occur. However, the amount of recombination at the *dif* locus in *recBC* mutants demonstrates that dimers do form (Steiner and Kuempel, 1998b).

Guillotining in DAPI-stained cells

If guillotining occurs, it should be possible to observe it in cells stained with DAPI. Figure 2 shows cells in which it appears that the septum is about to shear the DNA that extends between the nucleoids. Ideally, we should have been able to observe such events in 10–15% of dividing cells, but cells of the type shown in Fig. 2 were rare, and their actual frequency was less than 0.5%. Other types of guillotining have been somewhat easier to identify by microscopy (Niki *et al*., 1991; Moriya *et al*., 1998; Cook and Rothfield, 1999). In those situations, the septum proceeded through the bulk of the nucleoid. The guillotining of dimer chromosomes is probably more difficult to observe, as the two almost completed nucleoids are only connected by a thin stem of DNA.

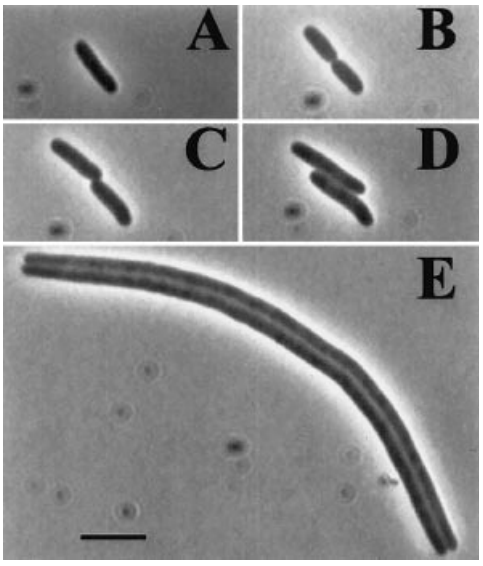


Fig. 3. Twin filaments formed by division and elongation of a *dif* deletion mutant (Δ*dif-hipA::cam*, PK3946). Cells were spread onto a microscope slide coated with agar medium, and pictures were taken at intervals during growth at 37°C. The scale bar indicates 5 μm for all figures.A. The initial cell.B. After 15 min.C. After 30 min.D. After 45 min.E. After 120 min.

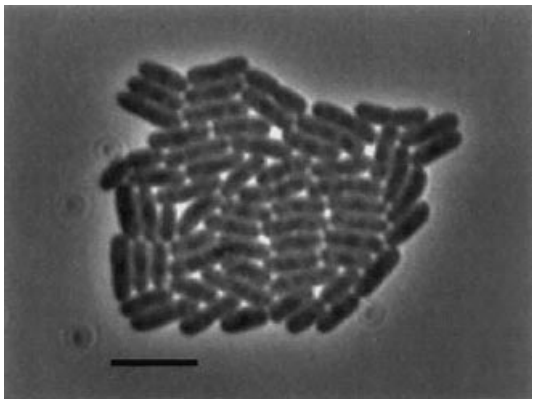


Fig. 4. Formation of a wild-type microcolony by a wild-type strain (PK3872). The initial cell grew and divided for 2 h at 37°C on a microscope slide coated with agar medium. The scale bar indicates 5 μm.

Formation of twin filaments

The evidence obtained from synchronous cultures and DAPI-stained cells does not, by itself, provide convincing evidence of guillotining. However, in examining the growth pattern of cells exhibiting the Dif phenotype, we have observed a pattern that is unique to these mutants and is most readily explained by guillotining. These observations were made by spreading cells from exponentially growing cultures onto agar-coated slides and examining their growth into microcolonies (Stewart and D'Ari, 1992). The unusual growth pattern that we observed consists of two filamentous cells of similar length lying side by side. Figure 3 shows the formation of twin filaments by a *dif* mutant in which 1 kb has been removed from this region (Δdif-hipA::cam; PK3946). We propose that this pattern results from guillotining of a dimer chromosome at the first cell division, DNA damage and SOS induction in both daughter cells, and inhibition of subsequent cell division in the two cells. This inhibition is primarily the result of induction of the SOS system (see below), but other

Table 1. Formation of twin filaments.

Strain	Relevant genotype	Twin filament frequency[a]	Length of twin filaments[b]
PK3872	Wild type	0.3% (3/1000)	Medium
PK3946	*Δ dif-hipA::cam*	10.6% (166/1570)	Long
PK3879	*xerC*	13.5% (163/1209)	Medium
PK3880	*xerD*	10.4% (113/1088)	Medium
PK3881	*Δ dif::tet*	12.8% (93/726)	Medium
PK4159	*xerC hipA*	13.3% (123/925)	Long
PK4174	*xerC* pHM418	13.1% (140/1073)	Long
PK4183	*Δ dif-hipA::cam uvrD*	22.6% (135/597)	Long

a. See Experimental procedures.
b. Medium and long twin filaments are 7–15 and greater than 30 μm long respectively.

means of cell division control could also be involved (Hill *et al*., 1997).

Analyses of cells of this *dif* deletion mutant forming microcolonies indicated that, of the single cells that divide at least once on the slide, 10.6% of them gave rise to the twin-filament pattern (Table 1). The other 89.4% of the dividing cells formed microcolonies with varying numbers of cells. We have limited our analyses to cells that divided at least once on the microscope slides. We have not attempted to analyse the dead and dying cells, which arise from guillotining events that occurred in the broth culture before spreading the cells. Of course, twin filaments can also form in a microcolony after the first cell division, but these instances were included among the cells that divide more than once.

As mentioned above, the twin-filament growth pattern was unique to mutants expressing the Dif phenotype. Figure 4 shows a microcolony produced by a wild-type strain (PK3872), in which the cells show the normal range of sizes. Analysis of wild-type cells demonstrated that only 0.3% formed twin filaments (Table 1).

We have also tested the growth pattern of a *xerC* mutant (PK3879), and 13.5% of the cells formed twin filaments (Table 1). However, these filaments were considerably shorter (Fig. 5A) than those formed by the *Δ dif-hipA::cam* mutant (Fig. 3). We have also tested a *xerD* mutant (PK3880), as well as a *dif* deletion (*Δdif::tet*; PK3881) in which only 58 bp has been removed. These also gave 10–13% twin filaments, and the cell lengths were similar in length to those observed with the *xerC* mutant (Table 1).

A possible explanation for the extensive growth of the filaments produced by the 1 kb *Δ dif-hipA::cam* deletion, compared with that observed in the *xerC*, *xerD* and 58 bp *dif::tet* deletion, is the absence of *hipA*. Consistent with this, we have observed that twin filaments produced by a *xerC hipA* mutant (PK4159) have a length similar to those produced by the 1 kb *dif-hipA* deletion (Fig. 5B). The frequency of twin filaments was not affected (Table 1). The inhibition of growth by HipA protein was an additional effect of guillotining, and that will be considered further in the *Discussion*.

The experiments described above indicate that the formation of twin filaments resulted from guillotining of dimer chromosomes at cell division. A prediction of this is that, if the frequency of formation of dimer chromosomes were increased, the frequency of twin filaments would also be increased. We have described previously that the frequency of recombination at *dif* is 14.6% in PK3872, and that this increased to 24% in a *uvrD* mutant (Steiner and Kuempel, 1998b). In strains that are unable to resolve dimer chromosomes, the frequency of twin filaments should show a comparable increase. We have tested a *Δ dif-hipA::cam uvrD* strain (PK4183), and the frequency of twin filaments was 22.6% (Table 1).

We have also tested the growth of *ftsK* mutants in microcolonies. Those strains produced filamentous cells, but the pattern of growth and division was more

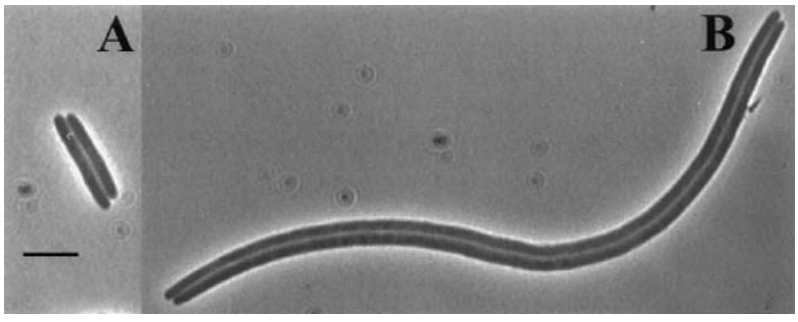


Fig. 5. Twin filaments formed by division and elongation of a *xerC* mutant, and the effect of inactivating *hipA*. The scale bar indicates 5 μm for both figures.A. Short twin filaments formed by a *xerC hipBA*$^+$ cell (PK3879) incubated for 2 h at 37°C. Cells are 9 μm long.B. Long twin filaments formed by a *xerC hipA* cell (PK4159) incubated for 2 h at 37°C.

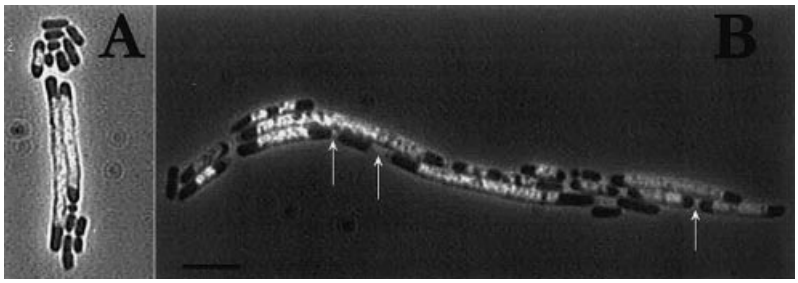


Fig. 6. The effect of *sfiA* on twin filaments. Slides spread with *Δdif-hipA::cam sfiA* (PK4171) were incubated for 2 h, then fixed and stained with DAPI. The scale bar indicates 5 μm for both figures.A. Colony in which division occurred at ends of twin filaments, which lacked DNA.B. Colony in which divisions occurred at ends as well as in the central region of twin filaments. Arrows indicate some divisions that occurred in nucleoid-free regions.

complicated than that observed in the strains described above. Those experiments will be described in a separate publication.

Inhibition of cell division

We have also investigated the basis of the cell division inhibition that leads to the formation of twin filaments. As suggested above, a simple explanation is that division damages the DNA and induces SOS. This leads to production of the SfiA (SulA) protein, which then blocks subsequent cell division by means of interaction with FtsZ (for a recent review, see Walker, 1996). As a test of this, we have examined the growth of a *sfiA Δdif-hipA::cam* mutant (PK4171). Twin filaments still formed after cell division, and there was no further cell division until these filaments had achieved a length equivalent to eight or so cells. In contrast to the *sfiA*$^+$ cells, however, cell division then occurred. This is shown in Fig. 6, in which the DNA has been stained with DAPI. The divisions in these filaments were often at the ends of the cells, which lacked DNA (Fig. 6A). Divisions also sometimes occurred through the middle region of the filament, but these appeared to occur in regions that lacked DNA (Fig. 6B). It should be noted that, although these *sfiA* filaments divided, the divisions took place after most of the growth had already been completed. We never observed that cells in these microcolonies resumed normal growth and division.

In spite of the cell divisions that occurred, primarily at the ends of the *sfiA Δdif-hipA::cam* twin filaments, filamentous cells were still present. One possible source of this residual inhibition of cell division could be the SfiC protein (D'Ari and Huisman, 1983). Similarly to SfiA, SfiC blocks division by interacting with FtsZ, and its synthesis is induced by DNA damage. SfiC synthesis is not controlled by LexA, however, and it is encoded in the excisable element e14 (Maguin *et al*., 1986). Tests based on a polymerase chain reaction (PCR) assay, as well as Southern hybridizations, have demonstrated that PK3872, and hence all its derivatives, lack e14 (unpublished experiments). Consequently, the residual inhibition of division cannot be caused by SfiC.

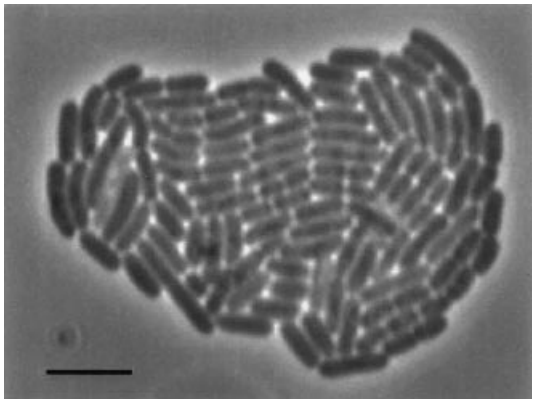
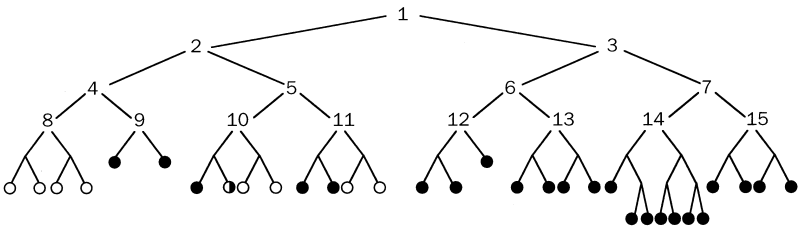


Fig. 7. Formation of a microcolony in which lethal sectoring occurred, by *Δdif-hipA::cam lexA3* cells (PK4172) incubated for 225 min. The scale bar indicates 5 μm. See Fig. 8 for pedigree of cells in colony.

Lethal sectoring in lexA3 *mutants*

The continued inhibition of cell division in the *sfiA sfiC* mutants suggested that an additional pathway blocked cell division in these filaments. Interestingly, this pathway is apparently controlled by LexA. As we reported previously (Kuempel *et al*., 1991), *lexA3*(Ind$^-$) *Δdif* cells growing in liquid cultures do not form filaments. The SOS pathway remains repressed in these cells, as the LexA3 repressor is not inactivated by DNA damage. We have tested the effect of LexA3 further by analysing the growth of microcolonies of a *lexA3 Δdif-hipA::cam* strain (PK4172). There were many dead and dying cells present in cultures of this strain, and many microcolonies produced fewer than 10 cells. The pattern of growth and division in larger colonies (Fig. 7) was different from that observed in the *sfiA sfiC Δdif-hipA::cam* strain (Fig. 6), and these colonies appeared to be similar to those produced by a wild-type strain (Fig. 4).

Tracking the growth of cells in larger colonies of the *lexA3 Δdif-hipA::cam* mutant demonstrated that 'lethal sectoring' was occurring (Haefner, 1968; Capaldo *et al*., 1974). Figure 8 shows the pedigree of the individual cells in the colony shown in Fig. 7. Some cell divisions gave



● dead cell
○ cells from which all descendants in subsequent generations in the microcolony were viable
◑ cells that produced some dead cells in subsequent generations

Fig. 8. Lethal sectoring during growth of *Δdif-hipA::cam lexA3* cells (PK4172) in microcolony shown in Fig. 7. Pedigree was constructed from digitized images taken during growth of colony. Not all divisions that produced the 109 cells in the microcolony are shown; most cells were descendants of cells 8, 10 and 11.

viable progeny that continued to grow, whereas other divisions produced progeny that ultimately ceased growth and division. Division of cell 4, for example, produced daughter cells with different fates. Cell 8 divided, and its progeny produced many of the cells in the colony. Cell 9 divided and gave two daughters, which immediately ceased growth and division. This suggests that guillotining occurred during the division of cell 9. However, guillotining did not always lead to an immediate halt in growth and division. All the progeny derived from cell 3 ultimately stopped growth and division, which suggests that guillotining occurred during the division of cell 3 that produced daughters 6 and 7. Some of the 'mortally wounded' progeny divided up to four times before division ceased. An alternative explanation is that guillotining occurred during the division of cell 6 as well as cell 7, and some progeny then divided three times. These patterns of growth were unique to the *lexA3 Δdif-hipA::cam* strain; a *lexA3 dif*$^+$ strain did not exhibit lethal sectoring.

Discussion

Guillotining

The results reported here demonstrate that guillotining, which is shearing of DNA by the septum at cell division, occurs in mutants displaying the Dif phenotype. Our first indication of this was provided by the observation that SOS induction occurred at cell division in synchronous cultures of *xerD* (Fig. 1), as well as *xerC and dif* mutants (data not shown), and that SOS induction was blocked if cell division was inhibited (Fig. 1). This led to a model in which the ingrowing septum interacted with the *dif* region, which was proposed to be in the region that connected the partially segregated but still unresolved chromosomes. The *dif* site, as well as the XerC and XerD resolvase proteins, were proposed to interact at this time to effect resolution. If resolution failed to occur, as in a resolution mutant, cell division would still be completed, and the DNA would be guillotined. It should be mentioned that this model led to our demonstration, by means of a density label assay, that resolution in wild-type cells requires cell division (Steiner and Kuempel, 1998a). More recently, we demonstrated that the FtsK septum protein was an additional component of the resolution complex (Steiner *et al*., 1999).

Although SOS induction (Fig. 1) and DAPI-stained cells (Fig. 2) indicated that guillotining occurred in resolution mutants, analysis of microcolonies provided an alternative procedure to characterize guillotining. The frequency at which we observed these twin filaments is consistent with the proposal that they are the result of guillotining. We demonstrated previously that resolution at the *dif* locus normally occurs at a frequency of 15% in strain PK3872 (Steiner and Kuempel, 1998a), and the frequency was increased to 24% in a *uvrD* derivative (Steiner and Kuempel, 1998b), which exhibits a hyper-recombination phenotype. The mutants that we have studied here were all derived from PK3872 and, in derivatives that lack a functional resolvase system, guillotining should replace resolution. The frequencies of twin filaments shown in Table 1 for the resolution mutants, as well as the *uvrD* derivative, are consistent with this prediction.

Interaction between dif *and* hip

An unexpected finding from these experiments was the role that *hipBA* played in the elongation of the filaments. The *dif* site is only 77 bp downstream from the translation stop for *hipA* (Black *et al*., 1991), but there has never been any evidence of a functional interaction between these genes. As shown here, the filaments were considerably longer in *hipA* mutants (*Δdif-hipA::cam*, Fig. 3; *xerC hipA*, Fig. 5B) than if wild-type *hipA* was present (*xerC*, Fig. 5A; also *xerD* and *Δdif::tet*, data not shown). Studies by Black *et al*. (1994) have demonstrated that the expression of *hipBA* is autoregulated by HipB protein, that HipA protein is present in a tight complex with HipB, and that free HipA is toxic to cells. Recently, Falla and Chopra (1999) have noted that genes similar to *hipBA* are present in a low-copy *Rhizobium* plasmid, and they proposed that these plasmid genes could function as a post-segregation killing

system that stabilizes maintenance of the plasmid. They suggested that, if the HipB analogue was unstable, loss of the plasmid would ultimately lead to unbound HipA analogue, which would then kill or inhibit the growth of these cells.

The role of *hipBA* in inhibiting formation of extensive filaments after guillotining can be explained on the basis of these properties of the HipA and HipB proteins. The experiments reported here indicate that DNA in the terminus region is damaged by cell division and, in direct support of this, we have observed that guillotining produces DNA degradation at *dif*, as well as cell division-dependent DNA turnover (unpublished experiments). Also consistent with this is the recent report by Niki *et al*. (2000) that *dif* is located at mid-cell, before septum formation. Owing to degradation of the *hipBA* genes, which are immediately adjacent to *dif*, HipB protein would no longer be produced. Degradation of HipB would lead to release of previously bound HipA protein, which would in turn inhibit cell growth. Based on the lengths of the filaments produced by resolution mutants that are *hipBA*$^+$ (*xerC*, *xerD* or *Δdif::tet* mutants), elongation ceased within one generation after cell division.

As an additional test of the role of *hipBA* in filament formation, we have examined a *xerC* mutant that contains pHM418 (Moyed and Broderick, 1986). This plasmid provides additional copies of *hipBA* in the cell, which would not be subject to guillotining and DNA damage. Adequate levels of HipB would now be maintained, because of autoregulation (Black *et al*., 1994). Consequently, there would always be sufficient HipB to bind any free HipA. We observed that 13.1% of the dividing cells of the *xerC* mutant containing pHM418 formed twin filaments (Table 1), with lengths similar to those formed by the *Δdif-hipA::cam* (Fig. 3) or *xerC hipA* mutants (Fig. 5B).

Inhibition of cell division

Cell division is inhibited after guillotining, and our data demonstrate that the SOS system is involved at two different levels. The major cause of inhibition is the SfiA protein, and twin filaments that are over 50 μm long are formed. In *sfiA* mutants, a rather unusual growth pattern was observed. Division was inhibited after guillotining, but only temporarily. After substantial elongation had occurred, cell divisions then occurred, which were primarily at the ends of the filaments (Fig. 6A). We propose that guillotining produces abnormal nucleoids that remain at the centre of the cell, and divisions only start occurring when regions of the cell are produced that lack DNA. This is primarily at the ends of the filaments, although septa sometimes formed between the masses of DNA in the central region of these cells (Fig. 6B). As described in *Results*, SfiC cannot be responsible for any inhibition of division in the strains used here, as they lacked the excisable element e14, which contains this gene (Maguin *et al*., 1986).

The inhibition of division in the nucleoid-containing regions of the filaments occurred by a pathway that was independent of SfiA and SfiC. As division in this region was not inhibited in *lexA3* mutants (Fig. 7), this inhibition was apparently controlled by LexA. This SfiA SfiC-independent pathway of cell division inhibition is probably similar to that recently characterized by Hill *et al*. (1997). Inhibition of cell division by that pathway required DNA damage, which was provided by exposure to mitomycin C. For the experiments reported here, the DNA damage was caused by guillotining. A simple explanation for this inhibition, at least for resolution mutants, is that LexA controls a process similar to nucleoid occlusion (Mulder and Woldringh, 1989; Woldringh *et al*., 1991), which blocks septum formation through nucleoid-containing regions.

It should be stressed that, in the mutants described here, neither the SfiA SfiC-dependent nor SfiA SfiC-independent pathways were capable of halting the initial division that guillotined the dimer chromosomes. Dimer chromosomes were not detected by the cells, which lacked a mechanism to halt or retard the impending division. We have examined the lengths of the cells that ultimately produce twin filaments, and there is no indication that division was retarded to allow additional time for attempted resolution (for example, see Fig. 3). Guillotining induced SOS, but there was insufficient time for the impending cell division to be inhibited by SfiA. These results suggest that *E. coli* lacks a system to detect unresolved dimers. An alternative possibility, however, is that such a system is normally present, but it is absent in *dif*, *xerC and xerD* mutants.

The *lexA3 Δdif-hipA::cam* mutant, which lacks the SfiA SfiC-dependent and -independent pathways of division inhibition, exhibited some unusual properties. It did not form filaments, and it should be noted that this was not because of the absence of dimer chromosomes. We have demonstrated that *lexA3* mutants exhibit the typical amount of recombination at *dif* (15%; Steiner and Kuempel, 1998b). Although the *lexA3 Δdif-hipA::cam* strain did not form filaments, it showed extensive lethal sectoring. The pattern of cell division indicates that, once guillotining occurred, many cells did not immediately stop division (Fig. 8). Filaments were not formed, as a result of this continued division, and these cells presumably guillotined their DNA repeatedly. Some of these 'mortally wounded' cells could divide up to four times.

One possible explanation for the SfiA SfiC-independent pathway was that it acted through the UmuC and UmuD proteins, whose syntheses would remain repressed in a *lexA3* mutant. It has been suggested that the UmuC/UmuD proteins might provide a damage checkpoint that

Table 2. Strains used in these studies.

Strain number	Relevant genotype	Source of strain or mutant allele
PK3400	*lacIZ Δ(Mlu)* λ *sfiA::lacZ*	Strain LJ24; Rasmussen *et al.* (1991) *sfiA::lacZ* reporter; Lin and Little (1988)
PK4047	PK3400 *xerD2::*Tn*10–9kan*	Blakeley *et al.* (1993)
PK4129	PK4047 *recB21 recC22*	A. J. Clark
PK4145	PK4047 *recD1903::*mini-*tet*	Biek and Cohen (1986)
PK3872	e14$^-$ parent of all strains listed below	Steiner and Kuempel (1998a)
PK3879	*xerC::cam* Y17	Colloms *et al.* (1990)
PK3880	*xerD2::*Tn*10-9kan*	Blakeley *et al.* (1993)
PK3881	*Δdif::tet*	Cornet *et al.* (1994)
PK3946	*Δdif-hipA::cam2771*	Kuempel *et al.* (1991)
PK4159	*xerC::cam* Y17 *hipA::kan*	This laboratory
PK4171	*Δdif-hipA::cam2771 sfiA*::Tn*5*	Dimpfl and Echols (1989)
PK4172	*Δdif-hipA::cam lexA3 zja-505*::Tn*10*	DM7012, David Mount
PK4173	*xerC2::kan*	Colloms *et al.* (1990)
PK4174	*xerC2::kan* pHM418	Moyed and Broderick (1986)
PK4183	*Δdif-hipA::cam2771 uvrD::kan*	Washburn and Kushner (1991)

inhibits growth until damaged DNA has been repaired (Opperman *et al.*, 1996; 1999). As a test of this, we have examined a *umuDC* derivative of a *sfiA sfiC Δdif-hipA::spc* strain. This strain exhibited a growth pattern similar to that shown in Fig. 6, in which cell division remained inhibited in the central region of filaments. Consequently, UmuC and UmuD are not responsible for the LexA3-repressed, SfiA SfiC-independent inhibition of division described here.

Experimental procedures

Bacterial strains

The strains used in these studies are shown in Table 2. The derivatives of the indicated parental strains were constructed by P1 transduction, with selection for the relevant antibiotic resistance and scoring for the pertinent phenotype.

The *hipA::kan* insertion was constructed by inserting the kanamycin resistance gene of pUC4-K (Pharmacia) into the *Hpa*I site in *hipA* in pHM418 (Moyed and Broderick, 1986) and integrating this into the chromosome by homologous recombination.

Microcolony analysis

Slides were prepared as described by Stewart and D'Ari (1992), using LB agar (Miller, 1992). After preparation, the slides were stored at 37°C in a covered slide box containing water-soaked paper. Cultures grown overnight in LB medium were diluted 1000 ×, grown to mid-log phase and spread onto coated slides. A coverslip was placed over the slide, and it was returned to the humid chamber. To determine the frequency of twin-filament formation (Table 1), slides were incubated for 120 min. Cells that had divided at least once were then scored; twin filaments were cells that divided only once. Single filaments and single cells that had not grown or divided were not scored. Lethal sectoring (Figs 7 and 8) was characterized by photographing growing colonies at 15 min intervals to determine the pedigrees of the cells. To analyse cells by fluorescence microscopy (Figs 2 and 6), cells were grown in the humid chamber in the absence of a coverslip. Slides were removed and dried, fixed over methanol vapours and treated as described previously (Kuempel *et al.*, 1991). Pictures were taken with a Cohu 4912 monochrome digital camera on a Zeiss Axioskop, using a 100 × Plan-Fluar objective. Image Pro Plus was used for data management.

Synchrony

Synchronous cultures were obtained by the procedure described by Helmstetter *et al.* (1992), using M9 medium supplemented with 0.2% glucose and 1.0% casamino acids (Miller, 1992). Cell number was determined with a Coulter counter, using a 50 μm orifice. Cephalexin (12 μg ml^{-1}) was used to inhibit cell division in part of each culture. β-Galactosidase activity was determined by standard procedures (Pardee *et al.*, 1959; Miller, 1992).

Acknowledgements

This work was supported by grant GM32968 from the National Institutes of Health. We thank Chad Roline for excellent technical assistance.

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