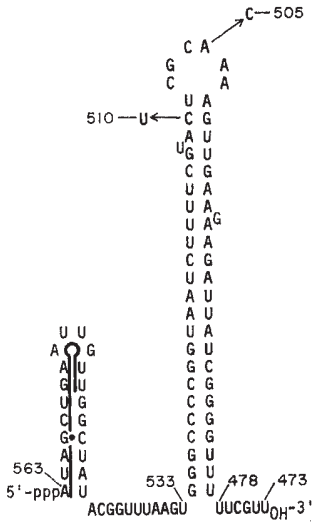

Fig. 4 Possible secondary structure of RNAI and homology to the origin of pSM1. The two arrows at the top of the loop indicate the differences between the sequence of RNAI and pSM1 and pTR1 at positions 505 (A→C) and 510 (C→U). The line within the first small hairpin loop indicates the region complementary to the origin region between nucleotides 2,367 and 2,380 of the pSM1 replication region sequence (see Fig. 3 and ref. 1).

RNA polymerase reaches the termination point the secondary structure would be disrupted and, analogously to the pattern of transcription in attenuation systems[8,9], transcription might proceed beyond the termination point reported here. It is difficult to predict if RNAI is actually translated. A small open reading frame begins at the start codon GUG (535–533) just before the large stem structure seen in Fig. 4 and continues until the termination codon UAG (384–382) (see Fig. 3). This reading frame is preceded by the sequence GGU (542–540), which may serve as a ribosome binding site[10]. The protein which could be translated from the extended RNA would have a MW of ~5,300, and as this is the region encoding copy number control and incompatibility functions, both of which are probably controlled by a repressor from this region, the 5,300-MW polypeptide is a possible candidate. However, because the base-pair change between pSM1 and R1 would not cause an amino acid change if the extended RNAI were translated, this small polypeptide would be identical in the wild-type and mutant plasmids. The two modes of transcription of RNAI may, however, correspond to the repressed and derepressed states of replication. Thus, the rate of plasmid replication could be tied, deterministically, to other aspects of cellular metabolism by the average rate of initiation for RNAI translation. This larger RNA species may no longer be effective either because of steric problems in binding at the origin or in forming a duplex with nascent RNAII or because of improper or altered processing of the molecule.

Recent data obtained in the ColE1 plasmid system shows a very similar pattern of transcription in the region required for replication to that seen in pSM1 and pTR1 (ref. 11), although the ColE1 plasmid does not require any of its own proteins for replication and has no transcript corresponding to RNAIII. A small RNA which may be analogous to RNAI is produced in the direction opposite to the direction of replication and ~450 bases upstream from the replication origin[12]. A small insertion mutation that maps in this RNA increases the copy number of the plasmid, indicating that this RNA is an important control element[13]. Furthermore, it is transcribed in the direction opposite that of a second, larger transcript required *in vitro* as a primer for DNA synthesis[11]. We do not know whether RNAII in our system can be used as both an mRNA and a primer for R-factor replication. It is reasonable to propose that RNAI, by interacting at the origin region, is involved in copy number control, based on its location in the replication region, the changes found in pSM1 and pTR1 that appear to cause the increased copy number phenotype and by partial analogy to the ColE1 system. Further genetic and biochemical analyses to determine the nature of this control system are under way.

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Note added in proof: In recent attempts to shorten the replication region we have been unable to delete the region coding for RNAI and RNAII but the region coding for RNAIII can be eliminated. Thus RNAIII is unlikely to be a primer for DNA synthesis but the possibility that its normal function includes some other aspect of replication control is not excluded.

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An inducible DNA replication–cell division coupling mechanism in *E. coli*

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Cell division is a tightly regulated periodic process. In steady-state cultures of Enterobacteriaceae, division takes place at a well defined cell mass[1] and is strictly coordinated with DNA replication[2]. In wild-type *Escherichia coli* the formation of cells lacking DNA is very rare[3], and interruptions of DNA replication arrest cell division[4–6]. The molecular bases of this replication–division coupling have been elusive but several models have been proposed. It has been suggested, for example, that the termination of a round of DNA replication may trigger a key event required for cell division[4–8]. A quite different model postulates the existence of a division inhibitor which prevents untimely division and whose synthesis is induced to high levels when DNA replication is perturbed[9]. The work reported here establishes the existence of the latter type of replication–division coupling in *E. coli*, and shows that the *sfiA* gene product is an inducible component of this division inhibition mechanism which is synthesized at high levels after perturbations of DNA replication.

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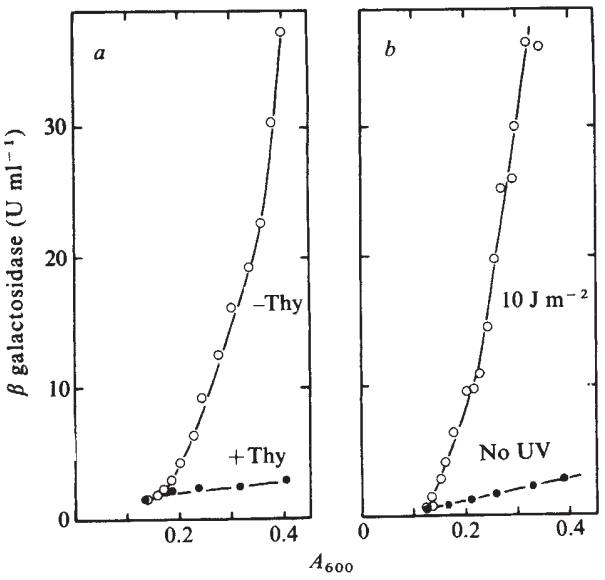

Fig. 1 Inducibility of *sfiA* gene expression by thymine starvation and UV-ray irradiation. Bacteria were grown at 37 °C in M63 minimal salts medium[35] supplemented with glucose, casaminoacids and growth requirements. At an absorbance A of ~0.4, the cultures were adjusted to A 0.1, a part of each was induced (*a*, centrifuged and washed twice, then resuspended in medium without thymine; *b*, irradiated with 10 J m^{-2} UV ray) and incubation was continued at 37 °C. Samples were withdrawn every 5 (induced cultures) or every 15 (uninduced cultures) min for absorbance measurements and β-galactosidase assays (carried out as in ref. 35). Enzyme units were calculated as described in ref. 37. Strain construction is described in Table 1 legend.

Much evidence points to the induction by DNA damaging treatments of a number of biological phenomena, collectively termed the SOS response (reviewed in refs 10–12). These phenomena include the appearance of new DNA repair and mutagenic activities, massive synthesis of the recA protein, lytic development of certain prophages in lysogenic strains and division inhibition (filamentous growth) in non-lysogens. The *recA* and *lexA* functions regulate the expression of all these phenomena. Mutant alleles of either locus can entirely prevent induction of the SOS response (*recA*$^-$, *lexA*$^-$) or cause its gratuitous expression at 42 °C (*tif* at the *recA* locus, *tsl* at the *lexA* locus)[10–12].

Incubation of non-lysogenic *tif* or *tsl* strains at 42 °C arrests cell division, resulting in the formation of long, multinucleate filaments. This filamentous growth is completely suppressed by *sfiA* mutations, whereas other aspects of the SOS response are unaffected[13–15]. The *sfiA* locus thus defines an element directly involved in the division inhibition process.

One model of replication–division coupling predicts the existence of an inducible division inhibitor. We thus sought to determine whether synthesis of the *sfiA* product is induced when DNA replication is perturbed.

To obtain accurate quantitative measurements of the rates of *sfiA* gene expression in various conditions we chose the powerful method of operon fusion, using the phage Mu d(Ap, *lac*)[16] to link the structural gene for β-galactosidase (*lacZ*) to the promoter of the *sfiA* operon. Near one extremity of the phage genome are the *lacZ* and *lacY* genes, lacking a promoter. The phage genome is so constructed that, when inserted in a host operon in the proper orientation, the *lac* genes of the prophage can be expressed from the adjacent bacterial promoter: their level of expression then reflects the level of expression of the bacterial operon in which the prophage is inserted.

Previous studies have shown that *sfi*-dependent filamentation occurs early during thymine starvation[17]. The effect of thymine starvation on *sfiA* expression was investigated in a *thyA* *sfiA*::*lacZ* fusion strain. The differential rate of β-galactosidase synthesis in these conditions quickly increases 20–40-fold (Fig. 1*a*, Table 1) reaching ~0.3% of total protein synthesis. Thus the *sfi*-dependent filamentation normally observed during thymine starvation is correlated with a large increase in the rate of *sfiA* gene expression. A UV-ray dose of 10 J m^{-2} similarly causes a rapid 20–40-fold increase in the differential rate of β-galactosidase synthesis (Fig. 1*b*, Table 1). Nalidixic acid, a specific inhibitor of DNA gyrase, and methyl methanesulphonate, a DNA alkylating agent, also induce high rates of *sfiA* gene expression (Table 1). A Mu d(Ap, *lac*) prophage inserted in the *gal* operon does not lead to UV-ray inducible β-galactosidase synthesis (Table 1), showing that the inducibility observed in *sfiA*::*lacZ* strains depends on the *sfiA* location of the prophage. We conclude that perturbations of DNA replication induce a high level of expression of the *sfiA* gene.

We next studied the effect on *sfiA* gene expression of mutations known to suppress or enhance *sfi*-dependent filamentation. *recA*$^-$ and *lexA*$^-$ mutations completely suppress the SOS

Table 1 Induction of *sfiA* gene expression

Strain	Inducing treatment	β-galactosidase specific activity (U per mg dry weight) Un-induced	Induced 60 min	Dose
sfiA::*lacZ thyA*	Thymine starvation	50	410	
sfiA::*lacZ*	UV-ray irradiation	30	140	1 J m^{-2}
			440	10 J m^{-2}
sfiA::*lacZ*	Nalidixic acid	30	110	2 μg ml^{-1}
			710	40 μg ml^{-1}
sfiA::*lacZ*	Methyl methanesulphonate	30	360	0.025%
sfiA::*lacZ*	Nitrofurantoin	30	90	2μg ml^{-1}
sfiA::*lacZ recA*	UV-ray irradiation	10	10	1 J m^{-2}
			10	10 J m^{-2}
sfiA::*lacZ lexA*	UV-ray irradiation	10	10	1 J m^{-2}
			10	10 J m^{-2}
sfiA::*lacZ tif*	42 °C	80	700	
sfiA::*lacZ tsl*	42 °C	200	450	
sfiA::*lacZ tsl recA*	42 °C	200	400	
sfiA::*lacZ lon*	UV-ray irradiation	20	400	10 J m^{-2}
gal::*lacZ*	Galactose	10	50	0.4%
	UV-ray irradiation	10	10	10 J m^{-2}
gal::*lacZ recA*	Galactose + UV rays	10	25	0.4%, 10 J m^{-2}
gal::*lacZ lexA*	Galactose + UV rays	10	30	0.4%, 10 J m^{-2}
gal::*lacZ tif*	42 °C	10	10	

Bacteria were grown at 37 °C in M63 salts medium[35] supplemented with glucose, casaminoacids and growth requirements (except for the experiments with *tif* and *tsl* strains, grown at 30 °C) to a value of A_{600} of ~0.4, then diluted to A 0.1 (the thymine-starved culture was first centrifuged and washed twice to remove exogenous thymine). One part of the culture was induced (by removal of thymine, UV-ray irradiation, temperature shift or addition of the appropriate factors), the cultures were further incubated for 60 min and β-galactosidase specific activities measured[35]. Adenine (100 μg ml^{-1}) was added to the *tif* cultures at the time of the temperature shift. To isolate Mu d(Ap, *lac*) insertions at the *sfiA* locus, we selected for a Sfi$^-$ phenotype[13,25,26] in a *lon* strain[24], taking advantage of the hypersensitivity of the strain to low concentrations of nitrofurantoin[36]. Lysogenic Sfi$^-$ clones were selected from a Mu d(Ap, *lac*)-infected *lon* culture on plates containing ampicillin (50 μg ml^{-1}) and nitrofurantoin (2 μg ml^{-1}). These were purified, checked for UV-ray resistance (to confirm the Sfi$^-$ character) and analysed genetically. All Sfi$^-$ clones carried a *sfiA* mutation, about 50% co-transducible with *pyrD* by P1[13,15,25,26]. For most isolates the introduction by transduction of the Sfi$^+$ character was invariably accompanied by loss of the Mu d(Ap, *lac*) prophage; the strains thus harboured a single Mu d(Ap, *lac*) prophage, located at the *sfiA* locus and conferring a Sfi$^-$ phenotype. Strains of this type having a significant basal level of β-galactosidase were considered to have the *lac* genes fused to the *sfiA* promoter. One such fusion was transduced with P1 *vir* (selection Ap^R) into a strain carrying a chromosomal *lac* deletion (to prevent extraneous synthesis of β-galactosidase) and a Mu c^+ prophage inserted in the *trp* operon (to prevent thermoinduction of the Mu d(Ap, *lac*) prophage, whose repressor is thermolabile). One Ap^R transductant, verified to have a Sfi$^-$ phenotype, was used for this study; all other *sfiA*::*lacZ* fusion strains were derived from this transductant. The *gal*::*lacZ* strains were constructed in a similar way using a strain carrying Mu d(Ap, *lac*) inserted in the *galT* gene as donor in the transduction to Ap^R.

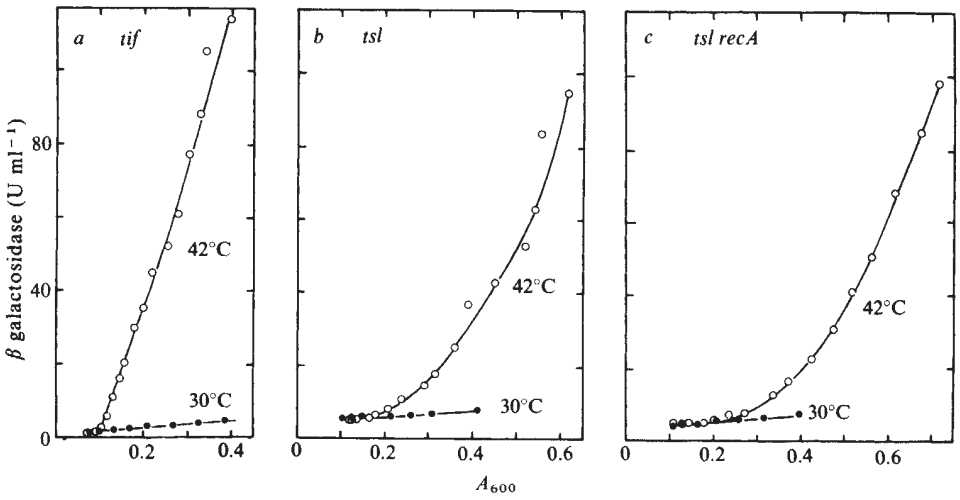

Fig. 2 Regulation of *sfiA* gene expression in *tif*(*recA*) and *tsl*(*lexA*) mutants. Bacteria were grown at 30 °C (*a*, in M63 minimal salts medium[35] supplemented with glucose, casaminoacids, growth requirements and—at 42 °C—adenine; *b* and *c*, in LB broth). At *A* 0.4, the cultures were diluted to *A* 0.1 in medium prewarmed to 42 °C(open symbols) or 30 °C (closed symbols) and incubation was continued. Samples were withdrawn every 5 (42 °C cultures) or every 15 (30 °C cultures) min for absorbance measurements and β-galactosidase assays[35,37]. Strain construction is described in Table 1 legend.

response[10–12]. In *recA* or *lexA* derivatives of the *sfiA*::*lacZ* fusion strain no UV-ray induction of β-galactosidase synthesis is observed in the dose range 1–10 J m^{-2} (compare with Table 1). These same *recA* and *lexA* mutations introduced into a *gal*::*lacZ* fusion strain do not affect the inducibility of β-galactosidase synthesis by galactose, and the capacity of these highly UV-ray sensitive strains to synthesize β-galactosidase is not seriously reduced by 10 J m^{-2} UV-ray irradiation (Table 1).

The *tif* and *tsl* mutations in the *recA* and *lexA* genes cause constitutive expression of some SOS functions at 42 °C (refs 18, 19), including *sfi*-dependent filamentation[13,14,17]. At this temperature in *tif sfiA*::*lacZ* (Fig. 2*a*, Table 1) and *tsl sfiA*::*lacZ* (Fig. 2*b*, Table 1) fusion strains, a high rate of β-galactosidase synthesis is induced, but no similar induction is observed in a *tif gal*::*lacZ* fusion strain (Table 1). Furthermore, division inhibition by expression of the *tsl* mutations, unlike all other known inducing treatments, does not depend on $recA^+$ activity[20] and in a *tsl recA sfiA*::*lacZ* fusion strain a high level of β-galactosidase synthesis is still observed at 42 °C (Fig. 2*c*, Table 1).

The $recA^+$ and $lexA^+$ gene products are thus regulators of *sfiA* gene expression, probably at the transcriptional level. The *recA* and *din* genes are similarly inducible in a $recA^+$–$lexA^+$-dependent manner[19,21], and in the case of the *recA* gene this regulation has been shown to be at the transcriptional level[22,23].

The *lon* mutation[24] amplifies *sfi*-dependent filamentation[13,25,26], sensitizing the cell to small doses of DNA damaging treatments. At least seven different enzymes involved in cell-wall biosynthesis are present at higher levels in *lon* strains than in lon^+ strains[27], and in one case, that of the *gal* operon, the *lon* mutant has been reported to have increased transcription rates[28]. The induction of *sfiA* gene expression by UV-ray irradiation was identical in *lon* and lon^+ *sfiA*::*lacZ* fusion strains in basal level and induction kinetics (see Table 1). Thus lon^+ function does not seem to be a transcriptional regulator of the *sfiA* operon. As *lon* mutants are also deficient in Deg protease activity[29], it is tempting to speculate that this protease is normally involved in eliminating excess sfiA protein, allowing rapid resumption of cell division as soon as normal DNA replication is restored.

Current models accounting for the regulation of the SOS response postulate that DNA damaging treatments result in the formation of a signal molecule by the DNA replication complex[30–32]. This molecule activates the recA protein to a protease form which, in turn, cleaves certain cellular repressors, in particular the *c*I repressor of λ prophage[33] and the lexA protein[34]. The results reported here show that a high rate of *sfiA* gene expression is part of the SOS response. The *sfiA* product is directly involved in SOS division inhibition and its synthesis is induced very rapidly during thymine starvation or after UV-ray irradiation (Fig. 1). The *sfiA* product thus provides a highly sensitive link coupling cell division to DNA replication by the following probable sequence of events: perturbations or alterations of the DNA replication complex cause the generation of a signal molecule which activates the recA protein to its protease form. The recA protease then cleaves the *lexA* repressor, leading to derepression of the *sfiA* operon. Finally, the high concentration of sfiA protein thus produced results in an inhibition of cell division. In this sense the *sfiA* gene product can be considered an inducible division inhibitor, although its precise role in division inhibition remains to be elucidated.

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