Stationary phase induction of *dnaN* and *recF*, two genes of *Escherichia coli* involved in DNA replication and repair

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The β subunit of DNA polymerase III holoenzyme, the *Escherichia coli* chromosomal replicase, is a sliding DNA clamp responsible for tethering the polymerase to DNA and endowing it with high processivity. The gene encoding β , *dnaN*, maps between *dnaA* and *recF*, which are involved in initiation of DNA replication at *oriC* and resumption of DNA replication at disrupted replication forks, respectively. In exponentially growing cells, *dnaN* and *recF* are expressed predominantly from the *dnaA* promoters. However, we have found that stationary phase induction of the *dnaN* promoters drastically changes the expression pattern of the *dnaA* operon genes. As a striking consequence, synthesis of the β subunit and RecF protein increases when cell metabolism is slowing down. Such an induction is dependent on the stationary phase σ factor, RpoS, although the accumulation of this factor alone is not sufficient to activate the *dnaN* promoters. These promoters are located in DNA regions without static bending, and the -35 hexamer element is essential for their RpoS-dependent induction. Our results suggest that stationary phase-dependent mechanisms have evolved in order to coordinate expression of *dnaN* and *recF* independently of the *dnaA* regulatory region. These mechanisms might be part of a developmental programme aimed at maintaining DNA integrity under stress conditions.

Keywords: *dnaA*–*dnaN*–*recF* operon/DNA polymerase III holoenzyme/DNA replication/RpoS/stationary phase

Introduction

The 40.6 kDa β subunit of DNA polymerase III, the major replicase of the *Escherichia coli* chromosome, is a sliding DNA clamp responsible for tethering the polymerase to DNA and endowing it with high processivity (for a review, see Kelman and O'Donnell, 1995). The structural gene for β , *dnaN*, maps between the *dnaA* and *recF* genes, at 83 min on the *E.coli* chromosome map (Figure 1). The *dnaA* gene product is essential for initiation of DNA

replication at the bacterial chromosomal origin, *oriC*, serving as a major regulator of the timing of initiation (for a review, see Messer and Weigel, 1996). Gene *recF* codes for a DNA-binding protein involved in recombination, repair and in the resumption of DNA replication at disrupted replication forks (Courcelle *et al.*, 1997). Recently, it has been reported that the *dnaN* gene contains an internal in-frame gene, termed *dnaN**, that encodes a smaller form of the β subunit (Skaliter *et al.*, 1996). The novel 26 kDa protein, called β^* , is synthesized in much smaller amounts than the β subunit and it could be involved in a recovery function such as DNA repair.

In exponentially growing cells, *dnaN* and *recF* are expressed predominantly from transcripts starting at the *dnaA* promoters (Pérez-Roger *et al.*, 1991); however, four promoters for *dnaN* (*dnaNp1*–*dnaNp4* in Figure 1) have been detected in the second half of the *dnaA* structural gene (Armengod *et al.*, 1988; Quiñones and Messer, 1988) and two major *recF* promoters (*recFp2p1* in Figure 1) were localized in the middle region of the *dnaN* gene (Armengod and Lambies, 1986). We have previously described that the cumulative transcriptional activity of the *dnaA* promoters, when monitored in logarithmic phase, is 10 and three times higher than that shown by the DNA regions containing the *dnaN* and *recF* promoters, respectively (Macián *et al.*, 1994). However, we observed in our experiments that transcriptional activity of these regions increased during transition from the exponential to the stationary phase of growth.

It is well known that *E.coli* responds to the onset of stationary phase by inducing the synthesis of proteins which are thought to be important for viability during prolonged periods of starvation (for recent reviews, see Hengge-Aronis, 1996a; Huisman *et al.*, 1996). Stationary phase induction of some genes depends on *rpoS*, which encodes an alternative sigma factor, σ^S or σ^{38} , able to recognize a set of promoters that are poorly recognized *in vivo* by RNA polymerase containing the major vegetative σ -factor σ^D , or σ^{70} . On the other hand, the promoter selectivity of RNA polymerase may also be controlled in the stationary phase by modulation of the core enzyme (Ozaki *et al.*, 1992).

In this work, we characterize the expression pattern of the *dnaA* operon genes throughout the growth curve. Interestingly, stationary phase-dependent mechanisms seem to have evolved in order to coordinate expression of the *dnaN* and *recF* genes independently of the *dnaA* regulatory region.

Results

Transcriptional activity of the *dnaN* and *recF* promoter regions is dependent on growth phase but independent of growth rate

To study the activity of the *dnaA*, *dnaN* and *recF* promoter regions throughout the growth curve, we constructed *lacZ*

Fig. 1. Schematic diagram of *dnaA*, *dnaN*, *dnaN** and *recF* organization. Coordinate 0 is the middle of the *EcoRI* site located at the beginning of the *dnaA* structural gene (Ohmori *et al.*, 1984). Structural genes *dnaA*, *dnaN*, *dnaN** and *recF* start at -63, 1346, 1748 and 2446, respectively. The main transcriptional start points are at -297 and -214 (for *dnaA* promoters *p1* and *p2*), 934, 1077, 1105 and 1319 (for *dnaN* promoters *p1*–*p4*), 1377 (for promoter *p5*), 1718 (for *dnaN** promoter) and 1806 and 1826 (for *recF* promoters *p2* and *p1*, respectively). T1, T2 and T3 are transcription termination sites responsible for transcriptional polarity of the *dnaN*–*recF* operon (Armengod *et al.*, 1991).

transcriptional fusions in single copy on the chromosome as previously described (Macián *et al.*, 1994). Figure 2A and B shows that the β -galactosidase activity of fusions containing the *dnaN* and *recF* promoter regions increased 100- and 44-fold, respectively (from 1 to 116 and from 5 to 220 Miller units), during transition into stationary phase. Interestingly, this induction is dependent mostly on *rpoS* in the case of the *dnaN* promoter region but not in the case of the DNA region containing the *recF* promoters. Figure 2C also shows that the β -galactosidase activity of the fusion carrying the *dnaA* promoters only increases ~4-fold during transition into the stationary phase (from 60 to 250 Miller units) and that this small induction is not dependent on *rpoS*.

Since expression of some stationary phase-inducible genes is inversely correlated with growth rate (Aldea *et al.*, 1990; Yamagishi *et al.*, 1993), we examined the transcriptional activity of the *dnaN* and *recF* promoter regions during exponential growth in various media. Thus, we measured the β -galactosidase production of MC1000 cells lysogenized with λ IC482 (*dnaN::lacZ*), λ IC451 (*recF::lacZ*) and, as a control, λ MAV103 (*bolA::lacZ*) during exponential growth in LBT (doubling time: 25 min), M9Caa (doubling time: 60 min) and M9LT (doubling time: 120 min) media. The results obtained (data not shown) indicated that the activity of the *dnaN* and *recF* promoter regions is independent of growth rate, whereas expression of *bolA* increases with decreasing growth rates, as described previously by Aldea *et al.* (1990). Therefore, it can be concluded that the growth phase induction of the *dnaN* and *recF* promoter regions (Figure 2A and B) is not a consequence of the gradual reduction in growth rate that takes place during the growth phase transition.

Dissectional analysis of the *dnaN* regulatory region

Four promoters for *dnaN* (*p1*–*p4*) have been detected previously by using transcriptional fusions, S1 mapping of mRNAs, deletion analysis and *in vivo* *dnaN* complementation tests (Armengod *et al.*, 1988). In the same work, the presence of a weak promoter, named *p5*, at the beginning of the *dnaN* structural gene was also reported. In order to identify promoters controlled by RpoS at the *dnaN* regulatory region, we have studied the growth phase

Fig. 2. Growth phase-dependent induction of the *dnaA*, *dnaN* and *recF* promoter regions. Strains were MC4100 (wild-type, $\square$) and RH90 (*rpoS359::Tn10*, $\blacksquare$) carrying *lacZ* transcriptional fusions λ IC482 (A), λ IC451 (B) and λ IC549 (C). Optical densities (thin lines) and β -galactosidase activities (thick lines) were determined along the growth curve.

induction of *dnaN* transcripts using the S1 nuclease protection assay. Results from Figure 3 indicate that the same start sites of transcription are detected when using total cellular RNA obtained during both exponential and stationary phases of growth. Therefore, stationary phase induction of *dnaN* depends on some of the previously detected promoters. The autoradiograph shown in Figure 3

also suggests that the activity of the *dnaNp1* and *dnaNp2* promoters is growth phase inducible and RpoS dependent. It is relevant to point out here that any growth phase effects on transcripts started at *dnaNp4* might be underestimated from results such as those shown in Figure 3 since these transcripts seem to be particularly reluctant to S1 protection analysis; other authors have been unable to detect them by this method (Quiñones and Messer, 1988; Tadmor *et al.*, 1994).

To investigate further which promoters at the *dnaN* regulatory region are RpoS dependent, we have carried out a dissectional analysis of this region so that the transcriptional activity of each one of the *dnaN* promoters

Fig. 3. Transcription initiation sites of the *dnaN* promoters. Total cellular RNA was isolated from MC4100 (*rpoS*⁺) and RH90 (*rpoS*⁻) cells during exponential (Ex) or stationary (St) growth in LBT. The DNA probe was a 1401 bp *EcoRI*–*RsrII* fragment (extending from nucleotide –3 to 1399 in Figure 1) labelled at the 5' end of the *RsrII* site. In lane 1, a labelled *HaeII* digest of pBR322 DNA is shown as a reference marker, and the sizes (in base pairs) of some fragments are indicated on the left. Lane 2 shows the DNA probe without S1 treatment. Samples were run on a 5% polyacrylamide, 8 M urea gel.

could be monitored independently by using transcriptional fusions to the *lacZ* reporter gene. Data from Table I indicate that P1 (λ IC715), P2 (compare λ IC687 and λ IC695) and P4 (compare λ IC693 and λ IC699) are strongly induced by entry into the stationary phase and that this induction is mostly RpoS dependent. Note that exclusion of the –35 region of P1, P2 and P4 in λ IC797, λ IC695 and λ IC699 respectively, greatly reduces the RpoS induction of these constructs. This suggests that nucleotides in the –35 regions of these promoters play a prominent role in RpoS-dependent transcription.

Recently, a promoter consensus for σ^{38} consisting of the –10 sequence CTATACT and an upstream region with intrinsic DNA curvature has been proposed (Espinosa-Urgel *et al.*, 1996). Although we have not tested whether the *dnaN* promoters are recognized *in vitro* by σ^{38} , we have examined these promoters for the presence of a curved DNA structure since homology to the consensus –10 can be found. To do this, we have analysed, according to Espinosa-Urgel and Tormo (1993), the electrophoretic mobility of DNA fragments encompassing the *dnaA* and *dnaN* promoters on polyacrylamide gel electrophoresis at low temperature, because it is well established that, under such conditions, DNA fragments containing curved DNA show anomalous electrophoretic mobility. Curiously, we have found that fragments containing the *dnaA* promoters exhibit anomalous mobility at 4°C but that those including the *dnaN* promoters do not (data not shown). Computer predictions of DNA bending performed according to Espinosa-Urgel and Tormo (1993) support the conclusion that the *dnaA* promoter region contains a curved DNA structure whereas the *dnaN* promoters seem to be located in DNA regions without static bending.

Accumulation of RpoS is not sufficient for induction of *dnaN*

The failure to induce a *dnaN::lacZ* fusion in the *rpoS* mutant (see Figure 2A) was complemented by the presence of a wild-type allele of *rpoS* *in trans* on plasmid pIC747 (data not shown). This agrees with the conclusion that the *rpoS* gene is directly or indirectly required for the growth phase-dependent activation of the *dnaN* promoter region. However, it should be pointed out that *rpoS* is under control of the *tac* promoter in pIC747 and that *rpoS*

Table I. Dissectional analysis of the *dnaN* promoter region

Phage	Regulatory elements ^a	β -Galactosidase activity (Miller units) ^b			
		<i>rpoS</i> ⁺		<i>rpoS</i> ⁻	
		OD ^c <0.3	OD >1.7	OD <0.3	OD >1.7
λ IC482	P1–P5	1.3	116	1.0	18
λ IC715	P1	0.6	34	0.3	3
λ IC797	(–10)P1	0.3	3	0.3	1
λ IC687	P2, (–35)P3	0.7	26	1.0	4
λ IC695	(–10)P2, P3, (–35)P4	0.2	5	0.4	3
λ IC693	P4, P5	0.9	58	0.6	9
λ IC699	(–10)P4, P5	0.8	7	0.9	6
λ IC701	P5	0.2	4	0.5	4

^a*dnaN* promoters present on the chromosomal fragment fused to *lacZ*. Some of the DNA fragments inserted into the promoter probe vector contain a truncated promoter; the –35 or –10 box remaining in the cloned fragment is shown in parentheses.

^b β -Galactosidase activity was measured in MC4100 (*rpoS*⁺) and RH90 (*rpoS*⁻).

^cOptical density at 600 nm.

overexpression mediated by isopropyl- β -D-thiogalactopyranoside (IPTG) was not able to induce activity of the *dnaN* promoter region during the exponential phase. This result might be due to the fact that the RpoS protein is degraded rapidly by the ClpXP protease during exponential growth (Schweder *et al.*, 1996); degradation slows down significantly during stationary phase, allowing RpoS to accumulate (Muffler *et al.*, 1996; Pratt and Silhavy, 1996). Since it has been shown recently that RpoS accumulation also occurs at low temperature during exponential growth, and that this accumulation produces induction of the *bolAp1* promoter (Sledjeski *et al.*, 1996), we decided to test whether the RpoS-dependent activity of the *dnaN* promoter region was also induced under such conditions. We have found (data not shown) that, as expected, the exponential phase activity of a *bolA::lacZ* transcriptional fusion (λ MAV103) increased with decreasing temperatures (from 45 to 325 Miller units) in an RpoS-dependent manner; however, the β -galactosidase activity of a *dnaN::lacZ* transcriptional fusion (λ IC482) during exponential growth was similar at 20 and 37°C (~2 Miller units). Therefore, RpoS accumulation is not sufficient for induction of the *dnaN* promoter region, and some additional factor or condition may be required to activate the *dnaN* promoters.

Osmotic induction of the *dnaN* promoters

The cellular level of σ^{38} also increases during the exponential growth phase in response to high osmolarity, and this produces induction of many σ^{38} -dependent genes (for a review, see Hengge-Aronis, 1996b). Therefore, we have analysed whether osmotic accumulation of RpoS is able to activate the *dnaN* promoters. As shown in Figure 4, *dnaNp1*, *dnaNp2* and *dnaNp4* are induced in exponentially growing cells in response to moderate hyperosmotic conditions that only slightly reduce the growth rate (the doubling time changes from 40 to 45 min). The same kind of experiments performed in strain RH90 (*rpoS359::Tn10*) revealed that *rpoS* is required for osmotic induction of the *dnaN* promoters (data not shown). The *bolAp1* promoter was used as a positive control in our experiments (data not shown) because osmotic induction of this σ^{38} -dependent promoter has been reported previously (Hengge-Aronis *et al.*, 1993).

Taken together, our results indicate that the RpoS-dependent induction of *dnaN* may be triggered by both stationary phase and hyperosmotic conditions but not by incubation at low temperature.

dnaN promoters are used to accomplish discoordinate regulation of the *dnaA* operon genes

Transcription initiated at the *dnaA* promoters is controlled by a complex network of positive and negative regulatory elements. Several sequences have been found in the *dnaA* promoter region which are recognized by specific proteins capable of repressing or activating transcription from the *dnaA* promoters (Froelich *et al.*, 1996; Lee *et al.*, 1996; Messer and Weigel, 1996). In addition, termination of transcription occurs along *dnaA* (Wende *et al.*, 1991; Perez-Roger *et al.*, 1995; compare also λ IC549 and λ IC638 in Figure 5). In spite of this, expression of *dnaN* depends mainly on transcription started at the *dnaA* promoters in exponentially growing cells because, under such condi-

Fig. 4. Osmotic induction of the *dnaN* promoters. Cells were grown overnight at 37°C in rich A medium with or without 15% sucrose, subcultured 1:100 into the same medium, and grown until they reached the cell densities indicated on the abscissa. The strain used was MC4100 lysogenized with λ IC482 (A), λ IC715 (B), λ IC687 (C) and λ IC693 (D). $\square$: A media; $\blacksquare$: A media + 15% sucrose.

tions, the activity of the *dnaN* promoters is very low (Macián *et al.*, 1994). As shown in Figure 5, this situation changes drastically when cells enter the stationary phase. At that time, the transcriptional activity of the *dnaN* promoter region increases 100-fold and becomes higher than that produced from the *dnaA* promoters (compare λ IC482 and λ IC638). This suggests that in stationary phase cells, expression of *dnaN* is largely dependent on transcription started at its own promoters.

Fig. 5. The *dnaN* promoters are used to accomplish discoordinate regulation of the *dnaA* operon genes. The horizontal bars below the map represent the chromosomal fragment fused to *lacZ* in λ RZ5 derivatives. ^aExpressed as Miller units. The strain used was MC1000. ^bOptical density at 600 nm.

Translational expression of the *dnaN* and *recF* genes

To analyse further the response of the *dnaA* operon distal genes to entry into stationary phase, we have made use of translational fusions to *lacZ*. As shown in Table II, the β -galactosidase activity of pIC785 and pIC371, two translational *dnaN*::*lacZ* fusions, increases >60-fold in starved (stationary) cells. Note that pIC371 is a late translational *dnaN*::*lacZ* fusion containing most of the *dnaN* open reading frame (ORF); therefore, it could synthesize both DnaN::LacZ and DnaN*::LacZ proteins (see Figure 1). Since it has been reported that accumulation of β^* occurs during stationary phase (Skaliter *et al.*, 1996), we have examined induction of protein fusions by Western blot analysis using polyclonal antibodies against β -galactosidase; no DnaN*::LacZ protein could be detected from extracts of pIC371-bearing cells in conditions which allowed clear detection of the DnaN::LacZ protein (data not shown). This indicates that most of the β -galactosidase activity exhibited by pIC371 is due to the DnaN::LacZ protein. Therefore, considering that the induced activities of the early and late translational DnaN::LacZ fusions are similar (compare pIC785 and pIC371 in Table II), and assuming that the level of β -galactosidase activity reflects the translation of β , it may be concluded that accumulation of the processivity factor of DNA polymerase III holo-enzyme, the β subunit, occurs in cells whose metabolism is slowing down.

pIC356 is a translational *recF*::*lacZ* fusion where both the *dnaN* and *recF* promoters are present. As shown in Table II, synthesis of RecF::LacZ protein from pIC356 is induced during entry into the stationary phase of growth. Although we have found that the transcriptional activity of the *recF* promoter region is induced in starved cells (Figure 2B), induction of the RecF::LacZ protein from pIC356 seems to be entirely dependent on the *dnaN* promoters (compare pIC356, which contains the *dnaN* and *recF* promoters, with pIC361, which only includes the *recF* promoters). This is because terminators T1, T2 and T3 (see Figure 1), which drastically reduce transcription started at the *recF* promoters (Armengod *et al.*, 1991), remain active during transition into the stationary phase (data not shown). On the other hand, it should be noted that the β -galactosidase activity mediated by the *dnaN* promoters is ~50- to 300-fold lower when determined with pIC356 (the *recF*::*lacZ* protein fusion) than when using pIC785 or pIC371 (the *dnaN*::*lacZ* protein fusions).

Phage	Promoter region	β -galactosidase activity ^a	
		OD ^b <0.3	OD>1.7
λ IC549	<i>dnaA</i>	51	130
λ IC638	<i>dnaA</i>	33	55
λ IC482	<i>dnaN</i>	1	100

Table II. Growth phase effects on expression of *dnaN*::*lacZ* and *recF*::*lacZ* translational fusions

Plasmid	Fragment ^a	Fusion ^b	β -Galactosidase/ β -lactamase ^c	
			OD ^d <0.3	OD >1.7
pIC785	(-3)-1725	<i>dnaN</i>	25	1700
pIC371	(-3)-2419	<i>dnaN</i> , <i>dnaN</i> *	23	1600
pIC356	(-3)-2586	<i>recF</i>	0.5	6
pIC361	1490-2586	<i>recF</i>	0.03	0.03

^aChromosomal fragment inserted into pMLB1034. The numbering system is that of Figure 1.

^bGene translationally fused to *lacZ*.

^cThe ratio of β -galactosidase units to β -lactamase units. The strain used was MC4100 (*rpoS*⁺).

^dOptical density at 600 nm.

This suggests that the RecF and β (DnaN) proteins are differentially expressed from transcripts started at the *dnaN* promoters and that additional regulatory mechanisms modulate the synthesis of the *recF* gene product in both the exponential and stationary phases of growth.

Discussion

In the present work, we have characterized the pattern of expression of the *dnaA*-*dnaN*-*recF* operon throughout the growth curve, and demonstrate that the *dnaN* promoters are used to accomplish discoordinate regulation of the operon genes.

We began this investigation with the observation that the transcriptional activity of the *dnaN* and *recF* promoter regions increased during transition from the exponential to the stationary phase of growth. Our previous results indicated that, in exponentially growing cells, the *dnaA* promoters are ~10- and 4-fold stronger than the promoters for the *dnaN* and *recF* genes, respectively (Macián *et al.*, 1994). This was in agreement with the assessment that, under such conditions, expression of *dnaN* and *recF* depends mostly on transcription started at the *dnaA* promoters (Pérez-Roger *et al.*, 1991). Here we show that transcriptional activity of the *dnaN* promoter region increases 100-fold during transition into stationary phase, which has important effects on the expression pattern of the *dnaA* operon genes. Dissectional analysis of the *dnaN* regulatory region indicates that the *dnaNp1*, *dnaNp2* and *dnaNp4* promoters are induced strongly during entry into

the stationary phase (Table I) or under hyperosmotic conditions (Figure 4) and that this induction is mostly RpoS dependent. Our results are consistent with the observation that although RpoS accumulation is necessary for expression of target promoters, it is not always sufficient (for references, see Sledjeski *et al.*, 1996; Muffler *et al.*, 1997). We propose that some specific factor(s) and/or condition(s) that are generated by osmotic stress or starvation (stationary phase), but not by incubation at low temperature, are needed in addition to RpoS accumulation in order to stimulate the activity of the *dnaN* promoters. It is noteworthy that trehalose and K^+ glutamate, which accumulate under stationary phase and hyperosmotic conditions, specifically stimulate the activity of σ^{38} -containing RNA polymerase at σ^{38} -dependent promoters (Ding *et al.*, 1995; Kusano and Ishihama, 1997). The induction of the *dnaN* gene reported here suggests that the β clamp, or the DNA polymerase III holoenzyme, is required for maintenance of DNA integrity under stationary phase and high osmolarity conditions.

The establishment of a consensus structure for promoters recognized by σ^{38} is still a controversial matter. In contrast to what has been described for other RpoS-dependent promoters (Espinosa-Urgel *et al.*, 1996), we have not found static bending associated with the *dnaN* promoters. Moreover, the fact that the same S1 nuclease-resistant DNA fragments were detected when using total RNA obtained from both *rpoS*⁺ and *rpoS*⁻ strains (Figure 3) indicates that the *dnaNp1*, *dnaNp2* and *dnaNp4* promoters can be recognized *in vivo* by $E\sigma^{70}$. Curiously, DNA fragments including the *dnaN* promoters exhibited very weak binding to RNA polymerase containing σ^{70} (Armengod and Lambies, 1986), probably because they deviate ~50% from the consensus sequences recognized by this holoenzyme (Armengod *et al.*, 1988), and one of them, *dnaNp4*, has been shown to be unable to direct *in vitro* transcription in the presence of 100 mM KCl (Ohmori *et al.*, 1984). Therefore, it is possible that $E\sigma^{70}$ requires ancillary factors and/or specific conditions in order to initiate transcription at the *dnaN* promoters. Since we have not tested whether the *dnaN* promoters are recognized *in vitro* by $E\sigma^{38}$, we cannot exclude the possibility that RpoS plays an indirect role in regulation of the *dnaN* promoters; thus, a gene(s) under the control of RpoS might encode a transcriptional factor(s) able to help $E\sigma^{70}$ to initiate transcription at the *dnaNp1*, *dnaNp2* and *dnaNp4* promoters. It is noteworthy in this respect that deletion of the -35 consensus region, which is usually needed for the recognition of promoters by $E\sigma^{70}$, greatly reduces the RpoS induction of the *dnaN* promoters. Alternatively, $E\sigma^{38}$ might recognize the *dnaN* promoters in spite of their lack of static bending. In relation to this, it has been shown that the presence of upstream curved sequences is not essential for recognition of the *galP1* promoter by $E\sigma^{38}$ and that DNA curvature probably contributes to promoter strength rather than to σ^{38} -specific recognition (Kolb *et al.*, 1995).

Our results also show that *recF* expression is induced during entry into stationary phase and that such an induction is dependent on the *dnaN* promoters (Table II). This coordinate induction of *dnaN* and *recF* suggests that both genes are involved in the same process. Recently, Courcelle *et al.* (1997) have shown that when replication

is disrupted by UV lesions, *recF* is required for the resumption of replication at DNA replication forks. These authors propose that the replisome disintegrates at a DNA lesion and then *recF* is required to re-assemble a replication holoenzyme at the site of a DNA replication fork. Since re-assembly of a processive holoenzyme should require the participation of β , coordinate induction of *dnaN* and *recF* might facilitate recovery of DNA replication at stalled replication forks. Interestingly, expression of β has been shown to be induced by DNA-damaging agents (Kaasch *et al.*, 1989; Quiñones *et al.*, 1989; Tadmor *et al.*, 1994). On the other hand, there are some indications that a replication fork stalls at some frequency and disintegrates during a normal course of replication (Bierne and Michel, 1994; Canceill and Ehrlich, 1996). Moreover, Courcelle *et al.* (1997) have presented results suggesting that replication can be disrupted in the absence of DNA damage. Thus, disruption appears more frequent in a *thy*⁻ background, presumably because the efficiency of processing and delivery of thymine nucleotides to the replication machinery is compromised in the auxotrophs. Also here, *recF* is required for the re-assembly of a replication holoenzyme at a DNA fork. If stalling of DNA replication was more frequent during entry into the stationary phase, then coordinate induction of *dnaN* and *recF* would be required to guarantee that the round of replication already under way can be completed. Kolter *et al.* (1993) have postulated that the cessation of growth could halt key metabolic processes, DNA replication in particular, at stages at which severe and irreparable damage could occur. Then, in order to ensure their survival, *E.coli* cells should be able to make an orderly transition into stationary phase such that the cell cycle would not be arrested randomly. If this were the case, coordinate induction of *dnaN* and *recF* could be a part of a developmental programme aimed at maintaining cell viability during prolonged periods of starvation. Therefore, from a teleological point of view, it makes sense to have evolved regulatory circuits for coordinate expression of *dnaN* and *recF* independent of the *dnaA* regulatory region.

There are 10–20 copies of DNA polymerase III holoenzyme per *E.coli* cell and ~300 dimers of β . This number of β dimers is considered to be high enough to processively extend thousands of Okazaki fragments on the lagging strand, in a model predicting a stoichiometric consumption of one β clamp per Okazaki fragment (Kelman and O'Donnell, 1995). A β dimer does not assemble onto DNA by itself, and the ATP-dependent clamp-loading activity of the γ complex is needed for the numerous reinitiation events on the lagging strand. Enigmatically, addition of a large excess of β to the holoenzyme circumvents the need for ATP in forming a processive polymerase. Since ATP levels decrease as cells go from the exponential to the stationary phase of growth (for references, see Siegele and Kolter, 1992), it is also possible that the induction of β reported here would be required to guarantee processive extension of Okazaki fragments in starved cells because of progressive lowering of ATP levels.

Finally, since DNA polymerase III holoenzyme also functions in repair and mutagenesis and, in addition, β may interact with other proteins involved in DNA repair, such as DNA polymerase II (Kelman and O'Donnell,

Table III. *E. coli* strains, plasmids and bacteriophages used in this study

Strain, plasmid or phage	Description ^a	Reference
<i>E. coli</i> strains		
MC1000	F ⁻ <i>araD139 Δ(araABOIC-leu)7679 galUK Δ(lac)X74 rpsL thi</i>	Silhavy <i>et al.</i> , 1984
MC4100	F ⁻ <i>Δ(arg-lac)U169 araD139 rpsL 150 ptsF25 fibB5301 rbsR deoC relA1</i>	Silhavy <i>et al.</i> , 1984
RH90	MC4100 <i>rpoS359::Tn10</i>	Muffler <i>et al.</i> , 1996
Plasmids		
pIC449	parent for <i>lacZ</i> transcriptional fusions	Macián <i>et al.</i> , 1994
pIC552	parent for <i>lacZ</i> transcriptional fusions	Macián <i>et al.</i> , 1994
pIC451	<i>Sau3A</i> fragment 1486–1923 inserted into <i>SmaI</i> -cut pIC449	Macián <i>et al.</i> , 1994
pIC482	<i>EcoRI</i> – <i>XhoI</i> fragment from –3 to 1490 inserted into <i>SmaI</i> -cut pIC449	Macián <i>et al.</i> , 1994
pIC549	<i>Clal</i> – <i>EcoRI</i> fragment from –389 to –3 inserted into <i>SmaI</i> -cut pIC449	Macián <i>et al.</i> , 1994
pIC638	<i>Clal</i> – <i>HaeIII</i> fragment from –389 to 865 inserted into <i>SmaI</i> -cut pIC449	Pérez-Roger <i>et al.</i> , 1995
pIC687	<i>RsaI</i> fragment 935–1093 inserted into <i>XhoI</i> -cut pIC552	this work
pIC693	<i>RsaI</i> fragment 1093–1465 inserted into <i>SmaI</i> -cut pIC552	this work
pIC695	<i>BstEII</i> – <i>EcoRV</i> fragment 1056–1296 inserted into <i>SmaI</i> -cut pIC552	this work
pIC699	<i>EcoRV</i> – <i>XhoI</i> fragment 1296–1490 inserted into <i>SmaI</i> -cut pIC552	this work
pIC701	chromosomal DNA fragment 1324–1670 inserted into <i>SmaI</i> -cut pIC552	this work
pIC715	<i>NciI</i> – <i>TaqI</i> fragment 890–1004 inserted into <i>SmaI</i> -cut pIC552	this work
pIC797	chromosomal DNA fragment 921–1039 inserted into <i>SmaI</i> -cut pIC552	this work
pMLB1043	parent for <i>lacZ</i> translational fusions	Silhavy <i>et al.</i> , 1991
pIC356	<i>lacZ</i> translational fusion of DNA fragment from –3 to 2586	Pérez-Roger <i>et al.</i> , 1991
pIC361	<i>lacZ</i> translational fusion of DNA fragment 1490–2586	Pérez-Roger <i>et al.</i> , 1991
pIC371	<i>lacZ</i> translational fusion of DNA fragment from –3 to 2419	Pérez-Roger <i>et al.</i> , 1991
pIC785	<i>lacZ</i> translational fusion of DNA fragment from –3 to 1725	this work
pKK223-3	vector from overexpression of proteins under the <i>tac</i> promoter	Pharmacia Biotech
pIC669	<i>lacI^q</i> gene inserted into the <i>PvuII</i> site on pKK223-3	this work
pIC747	<i>rpoS⁺</i> gene under the <i>tac</i> promoter in pIC669	this work
Phages		
λRZ5		Slauch and Silhavy, 1991
λIC451	λRZ5×pIC451	
λIC482	λRZ5×pIC482	
λIC549	λRZ5×pIC549	
λIC638	λRZ5×pIC638	
λIC687	λRZ5×pIC687	
λIC693	λRZ5×pIC693	
λIC695	λRZ5×pIC695	
λIC699	λRZ5×pIC699	
λIC701	λRZ5×pIC701	
λIC715	λRZ5×pIC715	
λIC797	λRZ5×pIC797	
λMAV103		Aldea <i>et al.</i> , 1990

^aThe numbering system used to define *E. coli* chromosomal fragments present in some plasmids is that used in Figure 1. Fragments with 5' protruding ends were inserted, unless stated otherwise, into the *SmaI* site of *lacZ* fusion vectors after filling in the ends with PolIK.

1995), the stationary phase induction of β could be associated with a DNA repair process.

Materials and methods

Strains, plasmids, phages and DNA manipulations

Bacterial strains, plasmids and phages used in this study are listed in Table III. For DNA manipulations, standard procedures were followed (Silhavy *et al.*, 1984; Sambrook *et al.*, 1989). The chromosomal fragment carried by pIC701 was obtained from pIC350 (Armengod *et al.*, 1988) by digesting with *HindIII* and *AccI*. pIC687 was constructed by inserting a 158 bp *RsaI* fragment, after ligation to *XhoI* linkers (CCCTCGAGGG), into the *XhoI* site of pIC552. To construct pIC797, a 200 bp PCR fragment was amplified by using oligonucleotides CGATCCTGATG-AAAAAGGCCGACG and TTGCGGCCGCGCAGCAAGTCGCGCA-GCG, and then digested with *HaeIII* and *AccI*; the resulting 120 bp fragment was inserted, after filling in the protruding ends with PolIK, into *SmaI*-cut pIC552. A 350 bp PCR DNA fragment was prepared by using oligonucleotides CCGCTACAACAGGTGAGCGGTCC and GGCAGGGAAGCTTCGACTTCACTCTG and, after digestion with *RsrII* and *HindIII*, inserted between the *RsrII* and *HindIII* sites of pIC340 (Pérez-Roger *et al.*, 1991) to produce pIC785. A 1400 bp *BamHI*–*EagI* fragment containing the *lacI^q* gene was obtained from pMM40 (Kleiner *et al.*, 1988) and, after treatment with PolIK, was inserted into the *PvuII*

site of pKK223-3 to produce pIC669. In this plasmid, the multiple cloning site of pKK223-3 has been replaced by a new one containing sites for *EcoRI* and *XhoI*. pDEB2 (Bohannon *et al.*, 1991) was digested with *BamHI* and *EcoRI* to obtain a 2300 bp fragment containing the *rpoS⁺* gene. This fragment was inserted between the *EcoRI* and *XhoI* sites of pIC669, after filling in the protruding ends with PolIK, to produce pIC747. We transferred fusions from pIC449 and pIC552 derivatives to λ phage intermediates via homologous recombination with λ RZ5 and then transferred these to the *E. coli* chromosome as previously described (Macián *et al.*, 1994).

Media, growth conditions and enzyme assays

LBT and A media and YM9 buffer have been described previously (Armengod and Lambies, 1984; Pratt and Silhavy, 1996). M9Caa and M9LT are YM9 supplemented with 0.4% glycerol, 0.05% casamino acids and 1 μ g/ml thiamine or with 0.4% glycerol, 0.01% casamino acids, 1 μ g/ml thiamine, 40 μ g/ml leucine and 40 μ g/ml thymine, respectively. Antibiotics were added as recommended (Sambrook *et al.*, 1989). Growth was monitored by measuring the optical density at 600 nm (OD₆₀₀) or by determining living cell numbers by plating aliquots diluted in YM9 buffer onto LBT plates. For determination of enzymatic activities, unless otherwise stated, cells were grown overnight at 37°C in LBT, subcultured 1:100 into the same medium and grown until OD₆₀₀ ~0.4. Cultures were then diluted 10-fold into fresh medium to prolong the exponential phase of growth. Samples were removed at appropriate

intervals for OD₆₀₀ and enzymatic activity measurements. β -Galactosidase assays were performed on SDS–chloroform-permeabilized cells as described by Miller (1992). All β -galactosidase activities are expressed as Miller units. To estimate the relative copy numbers of the plasmids, we determined the β -lactamase activity of the plasmid-bearing cells as previously reported (Macián *et al.*, 1994). Values of enzymatic activities represent the averages of at least three independent experiments with the standard deviation usually not exceeding 10%.

S1 mapping assay

Preparation of total RNA and S1 mapping experiments were essentially as described previously (Armengod *et al.*, 1988).

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