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# Induction of *dnaN* and *dnaQ* gene expression in *Escherichia coli* by alkylation damage to DNA

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**The *dnaN* and *dnaQ* genes encode the β-subunit and the ε-subunit of the DNA polymerase III holoenzyme. By transcriptional fusions to the *galK* gene, translational fusions to *lacZ* and comparative S1 mapping analysis, we investigated the *in-vivo* regulation of *dnaN* and *dnaQ*. We found that DNA damage caused by the alkylating agent methyl methanesulphonate (MMS) leads to a significant induction in *dnaN* and *dnaQ* gene expression suggesting a requirement of increased amounts of at least some DNA polymerase III holoenzyme subunits for recovery from DNA damage caused by MMS. These results are first evidences that subunits of the DNA polymerase III holoenzyme are DNA damage inducible. This MMS induction of *dnaN* and *dnaQ* gene expression is unrelated to the adaptive response. It was not observed in *lexA* and *recA* mutants which abolish the induction of the SOS response.**

*Key words: dnaA*/DNA polymerase III/gene fusions/MMS/S1 mapping/SOS regulon

## Introduction

The DNA polymerase III holoenzyme is the major polymerase responsible for the replication of the chromosome in *Escherichia coli* (Kornberg, 1980; McHenry, 1985). It is a complex multisubunit enzyme containing at least 10 distinct polypeptides (Maki *et al.*, 1988) encoded by genes which map at widely scattered loci in the *E.coli* chromosome. The α subunit is encoded by *dnaE* at 4 min on the *E.coli* genetic map (Welch and McHenry, 1982), *dnaN* at 83 min codes for the β subunit (Burgers *et al.*, 1981), *dnaQ*(*mutD*) at 5 min codes for the ε subunit (Scheuermann *et al.*, 1983) and the *dnaZX* locus at 11 min encodes the subunits τ and γ (Lee *et al.*, 1987; Maki and Kornberg, 1988a). For the subunits θ, σ, σ′, χ and ψ the genetic loci are unknown. It has been estimated that the DNA polymerase III holoenzyme is present in 10–20 copies per cell (Kornberg, 1980). How these genes are regulated resulting in a coordinate expression of the several subunits is unknown.

Little is also known about the role of the DNA polymerase III holoenzyme in DNA repair mechanisms. Participation of DNA polymerase III in excision and post-replication repair of UV damage has been suggested (Bridges and Mottershead, 1976; Sedgwick and Bridges, 1974; Youngs and Smith, 1973; Brotcorne-Lannoye *et al.*, 1985). A major involvement of DNA polymerase III in DNA repair mechanisms was suggested by the observation that *pcbA* mutants, an extragenic *dnaE* suppressor mutation, are sensitive to methyl methanesulphonate and UV nonmutable at *dnaE* restrictive temperature (Hagensee *et al.*, 1987a,b). In an attempt to study the regulation of at least some subunits of the DNA polymerase III holoenzyme in relation to DNA repair mechanisms, we have constructed transcriptional and translational gene fusions of *dnaQ* coding for the ε subunit (Scheuermann *et al.*, 1983) and *dnaN* coding for the β subunit (Burgers *et al.*, 1981) with the *galK* and *lacZ* genes, respectively.

In this paper, we report that in *E.coli*, treatment with the alkylating agent MMS leads to an increase in *dnaN* and *dnaQ* gene expression. The relation of this induction to the adaptive response (for review, see Sekiguchi and Nakabeppu, 1987) and the SOS regulon (for review, see Walker 1984) was analysed.

## Results

### *Transcription from the dnaN and dnaQ promoters is MMS inducible*

Promoter activities were determined using plasmids in which different promoter fragments were cloned in front of the *galK* gene (see Materials and methods). In order to examine whether *dnaQ* and *dnaN* gene expression is inducible by MMS treatment, the level of galactokinase activity was measured in cells of the wild-type strain AB1157 harbouring each of the *galK* fusion plasmids pAQ2 (*dnaQ–galK*) or pAQ31 (*dnaN–galK*). Bacteria carrying the appropriate plasmids were grown in *galK* assay medium to an $OD_{450}$ of 0.25, divided into two cultures and growth was continued for several hours in the absence or presence of 7.38 mM MMS. Samples from treated and untreated cultures were assayed for galactokinase activity at several times. Figure 1 and Table I show that MMS treatment leads to a significant induction in *dnaN* and *dnaQ* transcription. This induction seems to be specific for *dnaN* and *dnaQ* transcription and is not a general induction phenomenon, because several other fusions used as controls showed no induction. This was true for the *galK* fusions of the following promoters (see Table I): *lacUV5*, *tac*, *mioC* (Lother *et al.*, 1985), *rnh* (Quiñones *et al.*, 1987) and *gyrB* (this paper).

*dnaN* expression seems to be regulated not only by the own promoters which reside entirely within the reading frame of the preceding *dnaA* gene (Quiñones and Messer, 1988) but also by both *dnaA* promoters, because of the operon organization of *dnaA* and *dnaN* (Sakakibara *et al.*, 1981; Ohmori *et al.*, 1984). Therefore, we included in our analysis a *dnaA–galK* promoter fusion. As seen in Table I transcription from these promoters is also MMS inducible. These transcripts reach *in vivo* into the *dnaN* gene (Quiñones and Messer, 1988), and are thus apparently involved in the observed MMS induction of *dnaN*. The poor

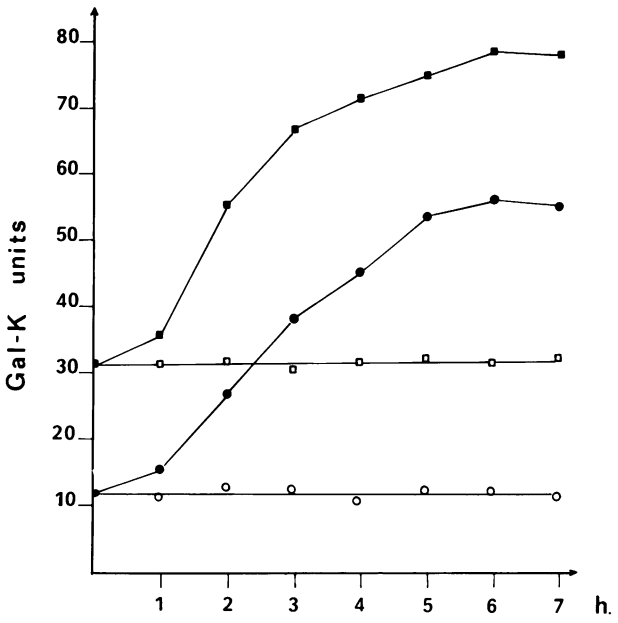


**Fig. 1.** *In-vivo* induction of *dnaN* and *dnaQ* transcription by MMS. Cultures of either AB1157 (pAQ2) or AB1157 (pAQ31) were grown in the presence or absence of MMS as described in Materials and methods and assayed for galactokinase activity at the times indicated. *dnaN–galK* fusion (○, ●); *dnaQ–galK* fusion (□, ■); open symbols, untreated; filled symbols, MMS treated cultures.

**Table I.** Influence of MMS induction on *dnaN* and *dnaQ* transcription

| Strain[a] | Vector | Promoter fusion | GalK units[b] | |
|---|---|---|---|---|
| | | | −MMS | +MMS |
| AQ198 | pUTE13 | – | 0.5 | 0.4 |
| AQ111 | pAQ2 | *dnaQ–galK* | 31.1 | 78.5 |
| AQ199 | pAQ31 | *dnaN–galK* | 10.5 | 56.2 |
| AQ112 | pAQ4 | *rnh–galK* | 11.7 | 13.0 |
| AQ192 | pLSK1-1 | *lacUV5–galK* | 86.2 | 82.3 |
| AQ280 | pLSK34-1 | *tac–galK* | 181.5 | 170.0 |
| AQ281 | pAQ62 | *gyrB–galK* | 18.1 | 18.5 |
| AQ282 | pLSK92-7 | *mioC–galK* | 155.0 | 161.7 |
| AQ197 | pLSK88-1 | *dnaA–galK* | 23.5 | 68.1 |

[a]All strains were constructed in AB1157 background.
[b]Cells for the galactokinase assay were grown to an $OD_{450}$ of 0.2 and divided into MMS treated (7.38 mM) and untreated cultures. After 6 h samples (1 ml) were frozen in liquid nitrogen and assayed for galactokinase activity.

translation level of the *dnaN* transcripts in comparison to *dnaQ* (see Figure 2) might require additional longer transcripts starting at the *dnaA* promoters in order to achieve an *in vivo* ratio between β and ϵ subunits of 2:1 (Maki *et al.*, 1988).

### *MMS inducibility of translational fusions of dnaQ and dnaN to lacZ*

To examine the behaviour of the translational fusions upon MMS treatment the intracellular level of β-galactosidase activity was measured in cells of the wild type strain CSH-26 (Miller, 1972) carrying each of the *lacZ* fusion plamids pAQ180 (*dnaQ–lacZ*), pAQ184 (*rnh–lacZ*) or pAQ47 (*dnaN–lacZ*). MMS treatment was done as described for the *galK* fusions. These results which are summarized in Figure 2 show that MMS induction also results in an

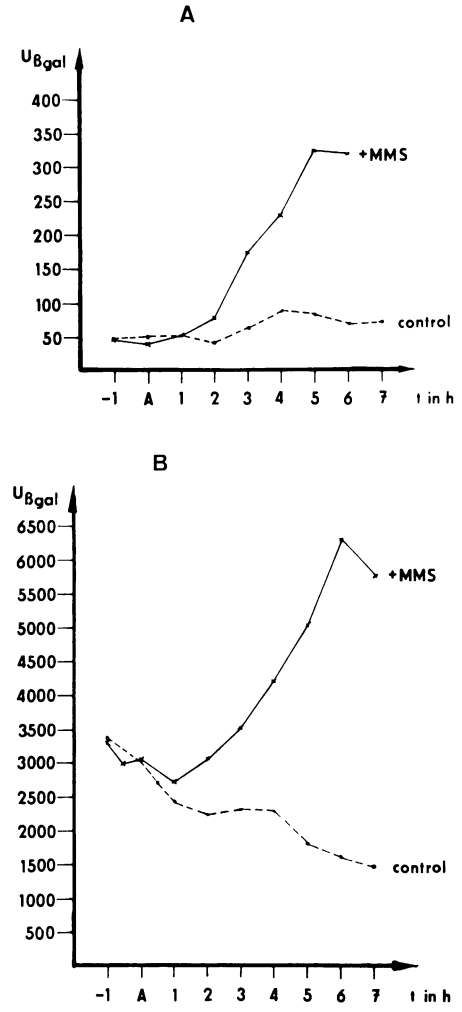


**Fig. 2.** (**A**) *In-vivo* induction of *dnaN* gene expression by MMS. Cultures of CSH-26 carrying pAQ47 (*dnaN'–'lacZ* translational fusion) were grown to an $OD_{600}$ of 0.2–0.3 (A, time 0) and the test culture was treated with 7.38 mM MMS. The control culture continued to grow in the absence of MMS. Samples from treated and untreated cultures were assayed for β-galactosidase activity at times indicated. (**B**) *In-vivo* induction of *dnaQ* gene expression by MMS. Cultures of CSH-26 carrying pAQ180 (*dnaQ'–'lacZ* translational fusion) were grown in the presence or absence of MMS as described above and samples from treated and untreated cultures were assayed for β-galactosidase activity at times indicated.

increased translation of *dnaQ* and *dnaN* as expected from the higher transcription observed with the *galK* fusions. While for the *dnaN–galK* fusion in untreated bacteria a constant expression level was measured during several generations, for the *dnaQ–lacZ* fusion a slow decrease of the basal level was observed. Furthermore, a comparison of the β-galactosidase activities of the translational *lacZ* fusions and the galactokinase level of the transcriptional *galK* fusions for *dnaN* and *dnaQ* (Table I and Figure 2) shows an unexpectedly low β-galactosidase activity for the *dnaN–lacZ* fusion which does not correlate with the promoter strength, probably due to the absence of a good ribosome binding site immediately upstream of the translation start.

### *S1 nuclease mapping of the MMS inducible promoters*

Because the genes *dnaA*, *dnaN* and *dnaQ* which are MMS inducible possess several promoters, comparative S1

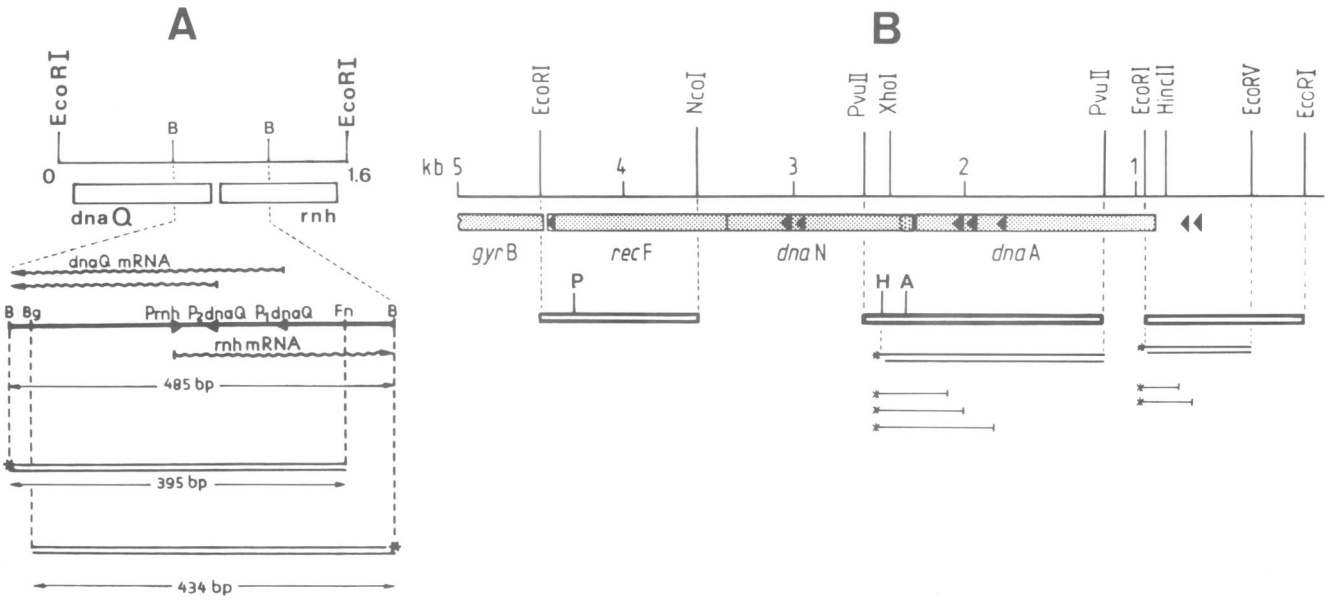


**Fig. 3.** (**A**) Structure of the *dnaQ*–*rnh* chromosomal region of *E.coli*. The genes are indicated by boxes. The fragment carrying the overlapping convergent promoter region was amplified for clarity. Black arrowheads show the promoters. The 5′ end-labelled sites in the fragments used as hybridization probe are indicated by stars. Abbreviations: B, *Bam*HI; Bg, *Bgl*I; Fn, *Fnu*4HI. (**B**) Structure of the *dnaA*–*gyrB* of the *E.coli* chromosome. The genes are indicated by dotted boxes. The fragments carrying regulatory regions which were fused to the *galK* and or *lacZ* gene are indicated by open boxes. Promoters are symbolized by black arrowheads. The double stranded DNA probes used for S1 mapping and the resulting S1 protected fragments are shown. The 5′ end-labelled sites are indicated by stars. Relevant restriction sites are given. In addition, H, A and P signify *Hin*fI, *Ava*II and *Pvu*I, respectively.

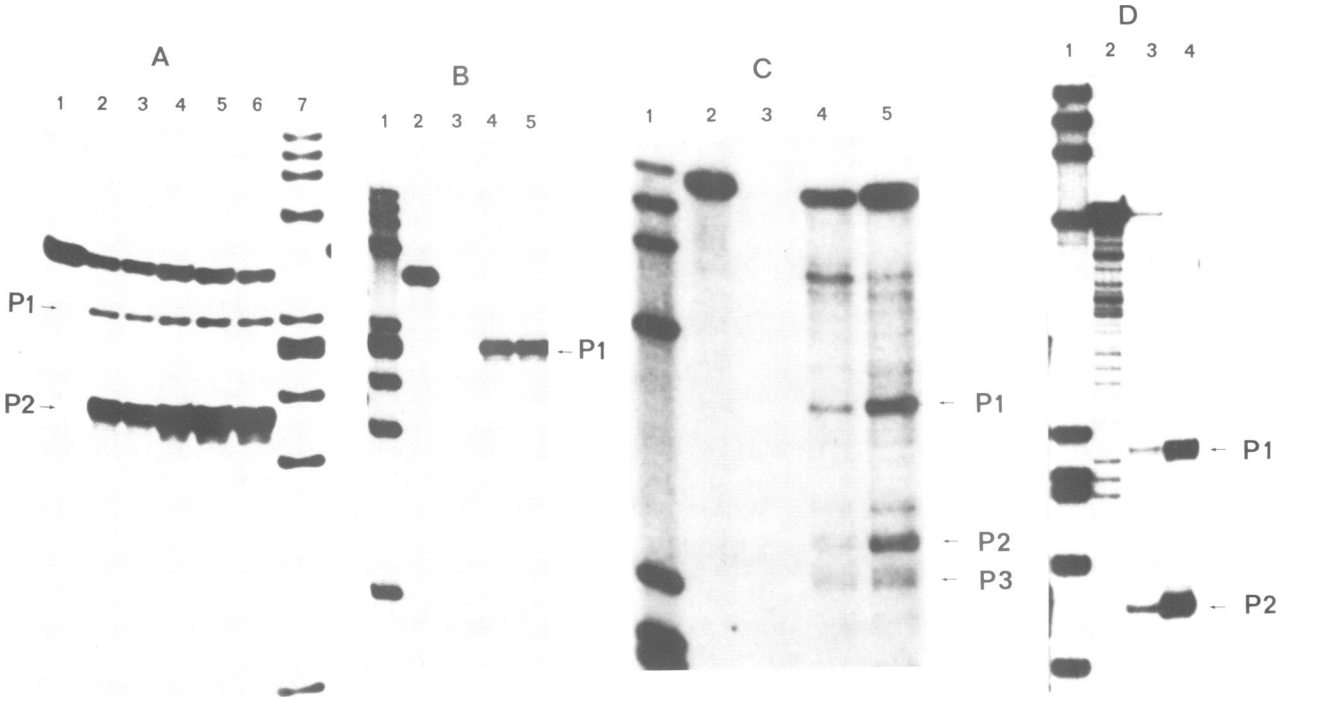


**Fig. 4.** Comparative S1 nuclease mapping of the *dnaQ*, *rnh*, *dnaN* and *dnaA* transcripts after MMS induction. (**A**) S1 nuclease analysis of the *dnaQ* transcripts from the two *dnaQ* promoters was performed with a 395-bp *Fnu*4HI–*Bam*HI DNA fragment 5′ end-labelled at the *Bam*HI site as specific hybridization probe (Figure 3A). **Lane 1**, *dnaQ* probe; **lane 2**, RNA from the wild-type strain AB1167 without MMS; **lane 3**, RNA from the *recA*56 mutant treated with MMS; **lane 4**, RNA from the *ada*10::*Tn*10 mutant treated with MMS; **lane 5**, RNA from strain AB1157 treated with MMS; **lane 6**, RNA from the mutant *lexA*::*Tn*5 *recA*730 treated with MMS; **lane 7**, ϕX174/*Hae*III standard. (**B**) S1 mapping of the *rnh* transcripts as control was done with a 434-bp *Bam*HI–*Bgl*I DNA fragment (Figure 3A). **Lane 1**, ϕX174/*Hae*III standard; **lane 2**, *rnh* probe; **lane 3**, tRNA control; **lane 4**, RNA from strain AB1157 without MMS; **lane 5**, RNA from strain AB1157 treated with MMS. (**C**) S1 mapping of the *dnaN* transcripts starting from the three *dnaN* promoters was performed with a 1272-bp-*Hin*fI–*Pvu*II DNA fragment 5′ end-labelled at the *Hin*fI site as hybridization probe (Figure 3B). **Lane 1**, ϕX174/*Hae*III standard; **lane 2**, *dnaN* probe; **lane 3**, tRNA control; **lane 4**, RNA from strain AB1157 without MMS; **lane 5**, RNA from strain AB1157 treated with MMS. (**D**) S1 analysis of the transcripts starting from the two promoters of the *dnaA*–*dnaN* operon was performed using a 624-bp *Eco*RI–*Eco*RV DNA fragment labelled at the 5′-end of the *Eco*RI site (Figure 3B). **Lane 1**, ϕX174/*Hae*III standard; **lane 2**, *dnaA* probe; **lane 3**, RNA from the uninduced strain AB1157; **lane 4**, RNA from the MMS induced strain AB1157.

nuclease mapping of the different chromosomal transcripts was done to localize the inducible promoters. The experiments were carried out using specific 5′ terminally labelled DNA fragments as hybridization probes for the *dnaQ*, *dnaN*, *dnaA* transcripts, and for the *rnh* transcript as control, as described previously (Quiñones *et al.*, 1988; Quiñones and Messer, 1988; Kücherer *et al.*, 1986 and Quiñones *et al.*, 1987). The hybridization probes used are described in Materials and methods and visualized in Figure 3. Figure 4 summarizes the results of such experiments. Following MMS treatment a clear induction of all specific transcripts of *dnaN*, *dnaQ* and *dnaA* can be seen. Moreover, from Figure 4C it can be seen that induction of *dnaN* gene expression is based on the activation of the three *dnaN* promoters and on transcripts originating from the *dnaA* promoters, seen as a full length protected probe fragment in Figure 4C. The starts of these transcripts are shown in Figure 4D which also demonstrates a clear increase of *dnaA* transcription in MMS treated cells. Both *dnaA* promoters are significantly induced. Figure 4A shows that the MMS induction of *dnaQ* is based essentially on the activation of the principal promoter *dnaQP2*. Corroborating the *galK* and *lacZ* experiments, the MMS induction of *dnaQ* transcription can be seen in the *ada*10::*Tn*10 and *lex*A::*Tn*5 *recA*730 mutant, but it is abolished in *recA*56 bacteria.

### *Influence of DNA repair mutants on MMS induction of dnaN and dnaQ gene expression*

MMS is an alkylating agent which causes the formation of several methylated bases in DNA (Singer and Kusmierek, 1982). This DNA damage leads to the induction of (i) the adaptive response (Samson and Cairns, 1977; Jeggo *et al.*, 1977; Volkert and Nguyen, 1984) which is positively regulated by the *ada* gene product (Jeggo, 1979; Lemotte and Walker, 1985; Nakabeppu *et al.*, 1985; Teo *et al.*, 1986; Nakabeppu and Sekiguchi, 1986), and (ii) the SOS responses (Bagg *et al.*, 1981; Boiteux *et al.*, 1984; Otsuka *et al.*, 1985; Costa de Oliveira *et al.*, 1986) which are controlled by the *lexA* and *recA* gene products (for reviews, see Little and Mount, 1982; Walker, 1984). Therefore, effects of *E.coli* DNA repair mutations affecting the adaptive and the SOS response on the expression of the *dnaN* and *dnaQ* genes were studied. As shown in Table II, the *ada*10::*Tn*10 mutation, which completely blocks the induction of the adaptive response (Lemotte and Walker, 1985; Volkert *et al.*, 1986), has no effect on the MMS induction of *dnaN* and *dnaQ* gene expression. These results suggest that this MMS induction is not related to the principal DNA repair system directed against alkylation damage, the adaptive response. On the contrary, the *lexA*1 and *recA*56 mutations which block the induction of the SOS regulon abolish completely the MMS induction of *dnaN* and *dnaQ* gene expression, suggesting that this MMS induction might be a delayed SOS function. These results were corroborated with the *lacZ* fusions (data not shown). Therefore, in order to examine this hypothesis we measured *dnaN* and *dnaQ* gene expression in the SOS constitutive double mutant *lexA*::*Tn*5 *recA*730 which is deficient in LexA repressor and synthesizes increased amounts of the activated form of RecA protein constitutively. Table II shows that in this mutant no constitutively enhanced (formerly derepressed) *dnaN* and *dnaQ* gene expression is observed.

**Table II.** Influence of DNA repair mutants on MMS induction in *dnaN* and *dnaQ* transcription

| Strain[a] | Relevant genotype | Plasmid | Promoter fusion | GalK units[b] | |
|---|---|---|---|---|---|
| | | | | −MMS | +MMS |
| AQ111 | wild-type | pAQ2 | *dnaQ−galK* | 32.0 | 77.3 |
| AQ199 | wild-type | pAQ31 | *dnaN−galK* | 10.7 | 55.8 |
| AQ123 | *lexA*1 | pAQ2 | *dnaQ−galK* | 30.5 | 30.8 |
| AQ284 | *lexA*1 | pAQ31 | *dnaN−galK* | 11.0 | 10.2 |
| AQ131 | *recA*56 | pAQ2 | *dnaQ−galK* | 31.5 | 30.1 |
| AQ285 | *recA*56 | pAQ31 | *dnaN−galK* | 10.1 | 8.5 |
| AQ283 | *ada*10::*Tn*10 | pAQ2 | *dnaQ−galK* | 32.2 | 78.2 |
| AQ286 | *ada*10::*Tn*10 | pAQ31 | *dnaN−galK* | 11.5 | 61.0 |
| AQ287 | *lexA*::*Tn*5 | pAQ2 | *dnaQ−galK* | 30.4 | 75.3 |
| AQ278 | *lexA*::*Tn*5 | pAQ31 | *dnaN−galK* | 11.2 | 57.0 |
| AQ298 | *lexA*::*Tn*5, *recA*730 | pAQ2 | *dnaQ−galK* | 33.1 | 83.0 |
| AQ290 | *lexA*::*Tn*5, *recA*730 | pAQ31 | *dnaN−galK* | 11.5 | 59.5 |

[a]All strains were constructed in AB1157 background.
[b]Cells of mutant strains carrying relevant promoter fusions were grown to an $OD_{450}$ of 0.2 and divided into MMS treated and untreated cultures. After 6 h growth at 37°C samples were assayed for galactokinase activity.

## Discussion

In this paper we studied the *in-vivo* gene expression of *dnaN* and *dnaQ* which code for the β and the ε subunit of DNA polymerase III holoenzyme using transcriptional and translational gene fusions and by visualizing their transcripts. Our results show that following MMS treatment a clearly enhanced gene expression for *dnaN* and *dnaQ* can be observed. This implies that the expression of these genes is inducible by DNA damage caused by alkylating agents and suggests that not only the β and the ε subunit might be synthesized in larger amounts after MMS induction but also the other subunits of DNA polymerase III holoenzyme, because all the subunits but β exist in equimolar ratios (Maki *et al.*, 1988).

Recently, Hagensee *et al.* (1987b), using the *pcbA* mutant, have given genetic evidence that a functional DNA polymerase III is required for optimal repair of DNA damage caused by MMS. The *pcbA* allele is a *dnaE* extragenic suppressor mutation which permits experiments with *dnaE* mutants under conditions of inactive DNA polymerase III holoenzyme. Our results are in agreement with their finding and additionally show that enhanced amounts of at least some subunits of the DNA polymerase III holoenzyme may be necessary for DNA damage caused by MMS.

The β subunit of DNA polymerase III seems to be responsible for the high processivity and for stable initiation complex formation and for the dissociation and reassociation of the holoenzyme from one template to another (LaDuca *et al.*, 1986; Kwon-Shin *et al.*, 1987; McHenry *et al.*, 1987; Maki *et al.*, 1987; Lasken and Kornberg, 1987; Maki and Kornberg, 1988b). The ε subunit possesses the 3′→5′ proofreading exonuclease function (Scheuermann and Echols, 1984) which by cooperative interaction with the α subunit (the *dnaE* gene product) results in the high fidelity of DNA polymerase III holoenzyme (Maki and Kornberg, 1987). Our finding that MMS induces *dnaN* and *dnaQ* gene

expression suggests a requirement for increased amounts of polymerase III holoenzyme for repair and recovery of MMS induced DNA damage, in agreement with the small number of polymerase molecules per cell.

This increased synthesis of at least some subunits of DNA polymerase III holoenzyme after MMS treatment is not related to the adaptive response, the principal repair mechanism directed against alkylation damage, because no change in the MMS induction of *dnaN* and *dnaQ* gene expression was observed in the *ada*10::*Tn*10 mutant, which completely blocks the induction of the adaptive response.

Surprisingly, the increase of *dnaN* and *dnaQ* gene expression seems to be connected to the SOS response, because it is not observed in both the *recA*56 and the *lexA*1 mutants. Nevertheless, we consider that this MMS induction does not represent a true SOS function because of the following reasons. (i) It is a delayed induction phenomenon in comparison to the fast induction of the SOS response by MMS as measured by the kinetics of increase in gene expression of a *sfiA*::lacZ and a *umuD/C−galK* gene fusion (data not shown; Bagg *et al.*, 1981; Otsuka *et al.*, 1985). (ii) *dnaN* and *dnaQ* are not components of the SOS regulon, because there is no SOS box (i.e. LexA repressor binding site; Wertman and Mount, 1985) in their promoter regions. (iii) In the double mutant *lexA*::*Tn 5 recA*730 expressing the SOS regulon constitutively there is no significantly enhanced (i.e. derepressed) *dnaN* and *dnaQ* gene expression. (iv) In this mutant the MMS induction of *dnaN* and *dnaQ* was still observed. This might suggest that the inducibility of the SOS regulon may be a condition and not the reason for the MMS induction of *dnaN* and *dnaQ*. The partial *lexA recA* dependence may, therefore, indicate an indirect interaction between the SOS regulon and the mechanism(s) of genetic control of DNA polymerase III holoenzyme.

Evidences for the existence of such relationships between several global control networks come from the observation that some SOS inducing agents can also induce some genes of the heat-shock regulon (Krueger and Walker, 1984). The block of cell division triggered by the induction of the SOS regulon results from the induction of the *sfiA* gene, repressed by the LexA protein (Huisman and d'Ari, 1981; Cole, 1983). The *sfiA* gene product is proteolytically regulated by the gene product of *lon* (Mizusawa and Gottesman 1983) which is a member of the heat-shock regulon (Neidhardt *et al.*, 1984). The heat-shock response can be also partially induced by treatments which trigger the stringent control (Grossman *et al.*, 1985), another global regulatory system in *E.coli* (Lamond and Travers, 1985). Furthermore, overproduction of the *ada* gene product causes a partial inhibition of the SOS response (Vericat *et al.*, 1988) despite the fact that some alkylating agents (e.g. MMS or MNNG) also induce the SOS regulon (Volkert, 1988) and that in *tagA alkA* mutants the failure of 3-methyladenine glycosylase I and II sensitizes the strain for SOS inducibility, showing that the persistence of 3-methyladenine in DNA most likely leads to SOS induction (Boiteux *et al.*, 1984; Costa de Oliveira *et al.*, 1986). Furthermore, a similar *lexA recA* dependence in the induction of *phr* gene expression has been reported (Ihara *et al.*, 1987), although the *phr* gene is not a component of the SOS regulon. The implications of all these observations are to date not completely understood.

Our results support the finding that the DNA polymerase III holoenzyme is required for repair of DNA damage caused by MMS and suggest that increased amounts of at least some subunits of DNA polymerase III holoenzyme are required for recovery from DNA damage caused by MMS. This paper and our previous work concerning inducibility of the *dnaQ* gene by 2-aminopurine (Quiñones *et al.*, 1988) are the first evidence that subunits of the DNA polymerase III holoenzyme are DNA damage inducible.

**Table III.** Bacterial strains used.

| Strain | Genotype | Source |
|---|---|---|
| AB1157 | *thr thi proA his*-4 *argE rpsL*31 *sup*-37 *lacY*1 *galK*2 | laboratory stock |
| CSH-26 | Δ (*lac−pro*) *thi* | Miller (1972) |
| AQ57 | as AB1157, but *lexA*1 *zjb*::*Tn*10 | Quiñones *et al.* (1988) |
| AQ79 | as AB1157, but *recA*56 *srl*::*Tn*10 | Quiñones *et al.* (1988) |
| AQ256 | *argE his*-4 *leu*-6 *proA thr*-1 *ara*-14 *galK*2 *lacY*1 *mtl*-1 *xyl*-5 *thi*-1 *rpsL*31 *supE*44 *tsx*-33 *rfa*-55 *ada*10::*Tn*10 | Volkert *et al.* (1986) |
| AQ259 | as AB1157, but *ada*10::*Tn*10 | P1(AQ256) × AB1157 |
| HB101 | *ara*-14 Δ(*gpt−proA*) *galK*2 *lacY*1 *hsdS*20 *leu*-6 *rpsL*20 *thi*-1 *recA*13 | laboratory stock |
| 9396 | *Hfr recA*1 *srl*::*Tn*10 *thi relA*1 | laboratory stock |
| KA10 | as CH26, but *rpsL* | laboratory stock |
| KA20 | as CH-26, but *recA*1 *srl*::*Tn*10 | KA10 × 9396 |
| KA30 | as CSH-26, but *lexA*1 *zjb*::*Tn*10 | P1(AQ57) × KA10 |
| KA40 | as CH-26, but *ada*10::*Tn*10 | P1(AQ256) × KA10 |
| JM30 | as AB1157, but *lexA*::*Tn*5 *recA*730 | E.Witkin |
| AQ56 | as AB1157, but *lexA*::*Tn*5 | P1(JM30) × AB1157 |

## Materials and methods

### *Bacterial strains, media and genetic procedures*

The *E.coli* strains used are listed in Table III. P1 transduction for strain construction, growth media, X-gal and MacConkey plates are described by Miller (1972). Transformations were done as described (Maniatis *et al.*, 1982).

### *Molecular cloning and DNA manipulations*

Restriction enzyme digests, isolation of DNA fragments, ligations and agarose gel electrophoresis were done as described by Maniatis *et al.* (1982). For routine and rapid examination of plasmid DNA, the boiling method of Holmes and Quigley (1981) was used. Highly purified plasmid DNA was prepared by caesium chloride gradient centrifugation (Maniatis *et al.*, 1982). Dephosphorylation of DNA fragments was performed with alkaline phosphatase from Boehringer as recommended by the supplier. The 5′ ends of DNA fragments were labelled with [$\gamma$-$^{32}$P]ATP (Amersham) and T4 polynucleotide kinase as described (Maniatis *et al.*, 1982).

### *RNA isolation*

Total cellular RNA was isolated from MMS treated and untreated bacteria according to Brosius *et al.* (1982) as described by Quiñones *et al.* (1987) with the minor modification that the MMS induced bacteria were grown to a density of 2 × $10^9$ cells per ml in L-broth with ampicillin at 37°C in the presence of 7.38 mM MMS.

### *S1 nuclease mapping*

S1 nuclease mapping of the specific *dnaQ*, *rhn*, *dnaN* and transcripts were performed according to Berk and Sharp (1977) as described (Quiñones *et al.*, 1988; Quiñones and Messer, 1988; Kücherer *et al.* 1986). The following 5′ end-labelled fragments were used as specific hybridization probe: for S1 nuclease mapping of the *dnaN* transcripts starting from the three promoters residing entirely in the reading frame of the preceding *dnaA* gene a 1.272 bp *Pvu*II−*Hin*fI, labelled at the 5′-end of the *Hin*fI site; for the mapping of the *dnaA* transcripts which start from the two autogeneously regulated promoters a 624 bp *Eco*RV−*Eco*RI fragment which was 5′ end-labelled at the *Eco*RI site; for the mapping of the *dnaQ* transcripts a *Bam*HI−*Fnu*4HI fragment carrying both *dnaQ* promoters, labelled at the

5'-end of the *Bam*HI site. RNA starting from the *rnh* promoter the hybridization probe was a 434-bp *Bam*HI−*Bgl*I fragment labelled at the 5'-end of the *Bam*HI site. For orientation concerning the several specific hybridization probes used see Figure 3.

### *Galactokinase assay*

The intracellular galactokinase activity of bacteria carrying promoter−*galK* fusion plasmids was determined as described by Quiñones *et al.* (1987). Cells for galactokinase assays were grown in M9 medium containing 0.25% casaminoacids, 0.2% fructose, 10 μg thiamine and 50 μg ampicillin per ml. Units are expressed as nmol galactose phosphorylated/min/ml cells/$OD_{450}$. The copy number of the promoter fusion plasmids was not significantly different as judged by agarose electrophoresis and determination of the β-lactamase activity according to Sargent (1968).

### *β-galactosidase assay*

The intracellular β-galactosidase activity of Δ (*lac−pro*) bacteria carrying translational fusions to the *lacZ* gene (Minton, 1984) was determined as described by Miller (1972).

### *Construction of the transcriptional promoter fusions to the galK gene*

Gene fusions to the *galK* gene (McKenney *et al.*, 1981) were constructed using the promoter probe vectors pFD51 (Rak and von Reutern, 1984) and pUTE13 a derivative of pFD51 in which the polylinker region (*Eco*RI to *Sma*I) was replaced by the polylinker of M13 mp19. In this system fusions to *galK* are transcriptional fusions and have the advantage that a translational coupling of the cloned fragment with the *galK* gene can be excluded, because of the presence of translation stop codons in each reading frame preceding the *galK* gene. The fusion of the regulatory sequences of the *dnaQ*, *rnh*, *dnaA* and *dnaN* genes to the *galK* gene which have been previously described (Quiñones *et al.*, 1987; Quiñones and Messer, 1988) are shown in Figure 3. The *gyrB−galK* fusion was constructed as follows: A 967-bp *Nco*I−*Eco*RI fragment carrying the *gyrB* promoter was isolated from the 3.55-kb *Eco*RI fragment containing the *dnaA−gyrB* region of the *E.coli* chromosome (Figure 3; for DNA sources see Quiñones and Messer, 1988). This fragment was made blunt ended with Klenow polymerase and cloned into the *Sma*I site of pUTE13. Galactokinase positive clones were isolated following transformation in strain HB101 and the correct orientation of the insert was confirmed by mapping the *Pvu*I site. The resulting plasmid with the *gyrB* promoter towards the *galK* gene was designated pAQ62 and used as additional control in some MMS induction experiments.

### *Construction of translational fusions to the lacZ gene*

In order to construct translational gene fusions to *lacZ* the vectors developed by Minton (1984) were used. The 5' regulatory sequences including the N-terminal part of the reading frame of the genes *dnaQ*, *rnh* and *dnaN* were fused in frame to *lacZ* are described in Quiñones *et al.* (1987) and Quiñones and Messer (1988) resulting in the plasmids pAQ180, pAQ184 and pAQ47 respectively.

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