Identification of genetic factors altering the SOS induction of DNA damage-inducible yebG gene in Escherichia coli

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Abstract

The yebG gene of Escherichia coli is a novel SOS regulon gene, but details of its regulation mechanism and biological function are not yet known. To characterize the regulation of yebG gene as a SOS gene, we identified the genetic factors affecting the SOS induction of yebG gene using yebG-lacZ operon fusion plasmid. We found that the SOS induction of yebG occurs as the cells enter into the stationary growth phase, but its induction is not observed in LB medium in the presence of 1% glucose. A stationary phase SOS induction of the yebG gene does not require the global regulator of stationary phase-specific genes, rpoS, or gyrA functions, but requires cya encoding the adenylate cyclase and hns encoding the histone-like protein H-NS functions. Our results demonstrated that the induction of a DNA damage-inducible yebG gene of E. coli is dependent on cyclic AMP and H-NS. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: SOS regulon; Stationary growth phase; yebG-lacZ operon fusion; cyclic AMP; H-NS

1. Introduction

Exposure of Escherichia coli to agents or conditions that damage DNA or interfere with DNA replication results in a diverse set of physiological changes called SOS responses [1,2]. The SOS response, a regulon of over 20 unlinked genes, many of which are involved in DNA damage tolerance and repair, e.g. recA, lexA, umuDC, recN, sulA, polB, uvrA, uvrB, and uvrD, is induced after a cell encounters DNA-damaging agents [3]. Induction of the SOS response, recombinational repair and damage-induced mutagenesis, the three main functions of RecA, are believed to occur only after RecA is activated by assembling into a spiral filament on single-stranded DNA in the presence of ATP [4,5,6].

Recently, a DNA damage-inducible yebG gene has been newly identified as a novel SOS regulon gene of E. coli [7]. Nucleotide sequence analysis showed that the yebG gene has a putative SOS box, −10 and −35 promoter boxes and a ribosome-binding site. Operon fusion assays demonstrated that its expression is regulated in a recA- and lexA-dependent way. The product of the yebG gene is predicted to be a 96 amino acid residue, 10.7 kDa protein, whose function and details of regulation are not yet known.
In this work, although biological function of yebG gene is not known, we considered the regulation of the SOS induction of yebG as an SOS gene. We identified genetic factors affecting the SOS induction of yebG gene using an E. coli strain carrying yebG-lacZ operon fusion plasmid.

2. Materials and methods

2.1. Bacterial strains, plasmid, bacteriophage and cell growth

The E. coli strains, bacteriophage and plasmid used in this study are described in Table 1. E. coli strains were grown in either M9 medium [8] supplemented with 0.8% glucose, thiamine (1 \( \mu g \) ml\(^{-1} \)) and proline (50 \( \mu g \) ml\(^{-1} \)) or Luria-Bertani broth (LB) at 37°C under aerobic conditions. Transformations were carried out by the method of Sambrook et al. [9]. Antibiotics were used at the following concentrations: ampicillin, 100 \( \mu g \) ml\(^{-1} \); kanamycin, 75 \( \mu g \) ml\(^{-1} \); and tetracycline, 20 \( \mu g \) ml\(^{-1} \).

2.2. Construction of mutant derivatives of E. coli TI21

Several mutant derivatives of E. coli TI21 carrying yebG-lacZ fusion were constructed by generalized transduction with phage P1 as described previously [8]. To introduce rpoS::Tn10 and hns::Tn10 into TI21, transductants were plated onto LB plates containing 5-bromo-4-chloro-3-indolyl-\( \beta \)-d-galactopyranoside (X-gal, 40 \( \mu g \) ml\(^{-1} \)) and tetracycline. Blue, tetracycline-resistant colonies were screened on LB-ampicillin plates containing X-gal to ensure that rpoS and hns had integrated into the E. coli chromosome. In order to avoid the use of strains carrying unrecognized second-site mutations, strains carrying mutant alleles of hns were freshly constructed by P1 transduction for every experiment.

P1 phages grown on \( \Delta \text{cya}:\text{kan} \) and gyrA96 strains were also transduced into E. coli TI21. Transductants were plated onto LB plates containing kanamycin or nalidixic acid. Kanamycin or nalidixic acid-resistant colonies were screened on LB-ampicillin plates containing X-gal to confirm that \( \Delta \text{cya} \) and gyrA had integrated into the E. coli chromosome.

2.3. The SOS induction assay by mitomycin C-treatment

Overnight cultures of E. coli cells were diluted 100-fold in fresh medium and were grown to an optical density at 600 nm (OD\(_{600}\)) of 0.2 to 0.4 at 37°C with shaking. Cultures were divided into two portions, and one portion was treated with mitomycin C (1 \( \mu g \) ml\(^{-1} \)), while the other was left untreated.

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH26</td>
<td>( \Delta ) (pro-lac) ara thi</td>
<td>[8]</td>
</tr>
<tr>
<td>SP850</td>
<td>Hfr (Hayes) ( e14^{-} ) relA1 spoTI ( \Delta ) (cyaI400):\text{kan} thi-1</td>
<td>CGSC(^a)</td>
</tr>
<tr>
<td>JM109</td>
<td>recA1 endA1 supE44 hsorR17 gyrA96 thiA relA1 ( \Delta ) (lac-proAB):F(^{+}) (traD36 proAB+ lacB(\Delta) lacZ(\Delta)M15)</td>
<td>[24]</td>
</tr>
<tr>
<td>CU211</td>
<td>CSH26 but hns::\text{neo}</td>
<td>[25]</td>
</tr>
<tr>
<td>LB133</td>
<td>araD139 ( \Delta ) (argF-lac)U169 deoC1 relA1 rpoL150 ptsF25 rbsR rpoS359::Tn10 attB::Pchb-lacZ</td>
<td>[26]</td>
</tr>
<tr>
<td>TI21</td>
<td>CSH26/pDA22</td>
<td>This study</td>
</tr>
<tr>
<td>TI23</td>
<td>CU211/pDA22</td>
<td>This study</td>
</tr>
<tr>
<td>TI31</td>
<td>TI21 but rpoS359::Tn10</td>
<td>TI21XPi(LB133)(^b)</td>
</tr>
<tr>
<td>TI40</td>
<td>TI21 but ( \Delta ) (cyaI400)::\text{kan}</td>
<td>TI21XPi(SP850)(^b)</td>
</tr>
<tr>
<td>TI44</td>
<td>TI21 but gyrA96</td>
<td>TI21XPi(JM109)(^b)</td>
</tr>
<tr>
<td>P1(_{vir})</td>
<td>Lab. collection</td>
<td></td>
</tr>
<tr>
<td>Plasmid</td>
<td>yebG-lacZ operon fusion, Amp(^r)</td>
<td>[7]</td>
</tr>
</tbody>
</table>

\(^a\)CGSC, Coli Genetic Stock Center, School of Medicine, Yale University.

\(^b\)P1\(_{vir}\) phage cultivation and generalized transduction were performed according to the method described by Miller [8].
Aliquots of cultures were taken every hour and the β-galactosidase activity was measured.

To examine the glucose effect on yebG induction, overnight cultures of E. coli cells were diluted 100-fold in fresh medium and were grown to an OD₆₀₀ of 0.2 to 0.4 at 37°C with shaking. Cultures were separated into three portions: one portion was treated with mitomycin C only; the second portion was treated with mitomycin C and glucose (1%) at the same time; and the third portion was left untreated. Aliquots of cultures were taken every hour and β-galactosidase assay was done.

2.4. Measurement of β-galactosidase activity

The β-galactosidase activity was measured as described by Miller [8]. Units of activity were calculated with the following formula: 

\[(\text{OD}_{420} - 1.75 \times \text{OD}_{550})/(\text{time of incubation (min)} \times \text{volume (ml)} \times \text{OD}_{600})\]

and were expressed as Miller units. All SOS induction experiments have been repeated three or more times and representative results are shown.

3. Results

3.1. The SOS induction of yebG gene by mitomycin C-treatment

To determine the induction of the yebG gene by mitomycin C-treatment, we measured the specific activities of β-galactosidase from strain TI21 carrying the yebG-lacZ operon fusion plasmid grown in either LB or minimal medium (Fig. 1).

In LB medium, the β-galactosidase-specific activity of TI21 increased about 7-fold and the SOS induction of yebG gene occurred when the culture entered the stationary phase (Fig. 1A). However, in the minimal medium, the β-galactosidase activity of TI21 was not induced. The increased β-galactosidase activity in LB medium did not occur in the presence of 1% glucose (Fig. 2), indicating that the SOS induction of yebG gene is sensitive to catabolite repression, known to be mediated by low cAMP levels [10].

Fig. 1. Growth curves (A, C) of strain TI21 and the SOS induction (B, D) of yebG gene by mitomycin C-treatment (1 μg ml⁻¹). Strain TI21 carrying yebG-lacZ operon fusion plasmid was grown to an OD₆₀₀ of 0.2 to 0.4 in LB medium (A) or minimal medium (C), and cultures were distributed into two portions. One portion was immediately treated with mitomycin C (closed circles) and the other was left untreated (open circles), and then β-galactosidase activity was measured.

Fig. 2. Effect of glucose (1%) on the SOS induction of yebG gene by mitomycin C-treatment. Strain TI21 was grown to an OD₆₀₀ of 0.2 to 0.4 in LB medium, and cultures were distributed into three portions. One portion was immediately treated with mitomycin C (square); the second portion was treated with 1% glucose and mitomycin C at the same time (triangle); and the third portion was left untreated (circle).
3.2. The stationary phase SOS induction of the yebG gene requires cya and hns functions

A number of genes induced in the stationary phase require cAMP and the cAMP receptor protein (CRP) for their expression [11,12]. Taddei et al. [13] reported that cAMP controls the SOS induction. Therefore, to assess the cAMP-dependence of yebG induction, we constructed a set of isogenic strains, TI21 (cya+‡) and TI40 carrying a Δcya mutation, and measured the β-galactosidase activities at various phases of growth in LB medium. The specific activity of β-galactosidase in the mutant was lower than that in the isogenic control strain TI21, indicating that the SOS induction of yebG gene is dependent on the intracellular cAMP level (Fig. 3).

The histone-like protein H-NS is also an important player in the complex regulatory network that controls the expression of numerous stationary phase-induced and osmotically regulated genes in

E. coli [14,15]. To ensure whether the stationary phase SOS induction of yebG is dependent on hns, we constructed an isogenic strain TI23 carrying the hns mutation, and measured the β-galactosidase activity at various growth phases grown in LB medium. The results showed that the specific activity of β-galactosidase in the mutant is significantly repressed compared to that in the isogenic control strain TI21 (Fig. 4), indicating that the SOS induction of yebG gene in the stationary growth phase is dependent on H-NS.

3.3. The SOS induction of the yebG gene does not require rpoS and gyrA functions

The increase in yebG induction in stationary phase, especially during growth in rich medium, is

Fig. 3. Effect of cya mutation on the induction of yebG gene by mitomycin C-treatment (1 μg ml⁻¹). A: Growth curves of strains TI21 (circles) and TI40 (squares). B: β-galactosidase activities of strains TI21 (circles) and TI40 (squares). Cultures of strains TI21 (cya+) and TI40 (Δcya) were grown to an OD₆₀₀ of 0.2 to 0.4 in LB medium and immediately separated into two portions. One portion was immediately treated with mitomycin C (closed symbols) and the other was left untreated (open symbols).

Fig. 4. Effect of hns mutation on the induction of yebG gene by mitomycin C-treatment (1 μg ml⁻¹). A: Growth curves of strain TI21 (hns+) and TI23 (hns). B: β-galactosidase activities of strains TI21 (circles) and TI23 (squares). Cultures were grown to an OD₆₀₀ of 0.2 to 0.4 in LB medium and immediately separated into two portions. One portion was immediately treated with mitomycin C (closed symbols) and the other was left untreated (open symbols).
characteristic of genes regulated by $\sigma^S$, the stationary phase sigma factor encoded by rpoS [16–18].

To confirm that the SOS induction of yebG requires rpoS function, we prepared the isogenic mutant TI31 (rpoS), and measured the $\beta$-galactosidase activity in cells grown in LB medium. The results showed that the yebG induction of the mutant was nearly identical to that of the isogenic control strain (Fig. 5A), indicating that the SOS induction of yebG gene in LB medium is induced during the stationary phase of growth unlike other SOS genes (Fig. 1). Some of the stationary phase-induced genes require the cAMP-CRP complex for their expression, whereas others are hyperinduced in mutants unable to make this complex [11]. In addition, it is already known that SOS induction and mutagenesis are cAMP-dependent [13]. To ensure the cAMP-dependence of yebG induction, we measured the specific activity of $\beta$-galactosidase from cells grown in LB medium in the presence of glucose (1%). The results showed that glucose completely repressed the yebG induction (Fig. 2), suggesting that the SOS induction of yebG gene is dependent on cAMP level. To confirm this possibility, we introduced the cya mutation into the isogenic control strain TI21. The cya mutant strain showed decrease in the SOS induction of yebG gene by mitomycin C-treatment. The results are compatible with a glucose effect on yebG expression and demonstrate that the SOS induction of yebG gene is regulated by intracellular cAMP levels.

The $\sigma^S$ subunit of RNA polymerase acts as a master regulator in a regulatory network that controls the expression of numerous stationary phase-induced genes in E. coli [15,20,21]. rpoS, the structural gene for $\sigma^S$, is induced during entry into the stationary phase [16,17]. Recently, it was demonstrated that the histone-like protein H-NS is also a component on strains grown in LB medium. The specific activity of $\beta$-galactosidase in the mutant was the same as that in the isogenic control strain TI21 (Fig. 5B), indicating that yebG promoter is not dependent on DNA supercoiling.

4. Discussion

In the present study, we characterized the regulation of a newly identified SOS regulon gene, yebG of E. coli. In order to characterize the regulation of the yebG gene, we screened the genetic factors altering the yebG expression against the DNA-damaging agent, mitomycin C.

To assess the SOS induction of yebG gene, we employed a yebG-lacZ operon fusion plasmid. The yebG induction by mitomycin C-treatment occurred only in LB medium, but not in the minimal medium and we found that the SOS induction of yebG in LB medium is induced during the stationary phase of growth unlike other SOS genes (Fig. 1). Some of the stationary phase-induced genes require the cAMP-CRP complex for their expression, whereas others are hyperinduced in mutants unable to make this complex [11]. In addition, it is already known that SOS induction and mutagenesis are cAMP-dependent [13]. To ensure the cAMP-dependence of yebG induction, we measured the specific activity of $\beta$-galactosidase from cells grown in LB medium in the presence of glucose (1%). The results showed that glucose completely repressed the yebG induction (Fig. 2), suggesting that the SOS induction of yebG gene is dependent on cAMP level. To confirm this possibility, we introduced the cya mutation into the isogenic control strain TI21. The cya mutant strain showed decrease in the SOS induction of yebG gene by mitomycin C-treatment. The results are compatible with a glucose effect on yebG expression and demonstrate that the SOS induction of yebG gene is regulated by intracellular cAMP levels.

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Fig. 5. Effects of rpoS (A) and gyrA (B) mutations on the expression of yebG gene by mitomycin C-treatment (1 $\mu$g ml$^{-1}$). Cultures of strains TI21 (wild-type, circles), TI31 (rpoS, squares) and TI44 (gyrA, triangles) were grown to an OD$$_{600}$ of 0.2 to 0.4 in LB medium and separated into two portions. One portion was immediately treated with mitomycin C (closed symbols) and the other was left untreated (open symbols).
of this network. H-NS is a small, abundant non-basic DNA-binding protein with a preference for AT-rich curved DNA regions [22]. Moreover, the expression of \( \sigma^8 \) itself is derepressed in an \( hns \) mutant background by a mechanism that acts at the post-transcriptional level [15]. To ensure that \( \sigma^8 \) and H-NS control the expression of a stationary phase-induced \( yebG \), we introduced \( rpoS \) and \( hns \) mutations into the wild-type T121 strain and assessed the SOS induction of \( yebG \) gene employing the \( \beta \)-galactosidase assay. Our results demonstrated that \( rpoS \) function is not required for the SOS induction of \( yebG \) during the stationary phase of growth, but \( hns \) function is required. In particular, the regulation of \( yebG \) by H-NS suggested the possibility that H-NS is involved in the induction of other SOS genes upon changes in chromatin structure.

It was previously reported that \( recA \), a global regulator of SOS regulon, whose expression is influenced by DNA topological changes [19], is also affected by DNA gyrase [23]. In addition, \( yebG \) itself is regulated by the \( recA-lexA \) system [7]. To confirm the effect of DNA gyrase on the SOS induction of \( yebG \) gene, we constructed an isogenic strain carrying the \( gyrA \) mutation, and measured the SOS induction of \( yebG \) in a stationary phase. According to our results, \( gyrA \) function is not required for the SOS induction of \( yebG \) gene, indicating that \( yebG \) expression is not dependent on DNA supercoiling.

In this work, we demonstrated that the SOS induction of \( yebG \) gene occurs at the onset of the stationary growth phase and its induction requires \( cya \) and \( hns \) functions. However, to elucidate the details of \( yebG \) regulation and its biological function, further studies at the molecular level are needed.

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References


