An Endonuclease Specific for Single-stranded DNA Selectively Damages the Genomic DNA and Induces the SOS Response*

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A plasmid carrying the bacteriophage T7.3 endonuclease gene under the control of the lacUV5 promoter could be maintained in the transcriptionally active state only in recA+ strains. In recA+ strains, endonuclease induction resulted in extensive degradation of the genomic DNA and cell death. In sharp contrast, the plasmid DNA remained intact in the supercoiled form. In recA+ strains, the recA protein levels were increased and the SOS functions of the host were activated, as shown by measurements of recA protein synthesis and prophage induction. These results indicate that in non-isogenic to the single-strand-specific endonuclease relative to the plasmid suggests that the two molecules differ in their physiological states and most likely in their degree of single-stranded content.

Several conditions such as exposure to ultraviolet light, alkylating or cross-linking agents, and thymine deprivation which interfere with DNA structure and metabolism are known to induce the complex SOS response in Escherichia coli (1). Recent evidence has established that this phenomenon is mediated and controlled by the recA gene. Damage of DNA induces and activates the recA protein whose proteolytic function inactivates the exA and phage λ repressors (2-4). As a result, many genes previously repressed by lexA, such as the DNA repair system, are activated. In phage lysogens, inactivation of the repressor leads to prophage induction (for reviews, see Refs. 5 and 6).

The nature of the signal which induces and activates the recA protein is not known. The proteolytic activity of this protein is activated in vitro by binding to single-stranded DNA, and the same mechanism has been proposed for the inducing signal in vivo (7). Presumably, single-stranded regions are generated when replication forks or recombination intermediates encounter a DNA lesion or when the recBC or sbcB exo nucleases act at a damaged site (8). However, direct experimental evidence for a causal relationship between the presence of single-stranded DNA regions and recA activation in vivo has been difficult to obtain.

Bacteriophage T7 gene 3 protein is an endonuclease whose major role in vivo appears to be the preferential degradation of genomic DNA. This enzyme is a single-chain protein of 148 amino acids (9) which, in addition to degrading the bacterial DNA, appears to participate, in the maturation of concatemeric phage DNA and genetic recombination (10-14). In vitro, the T7.3 protein exhibits strong specificity for single-stranded DNA (15) and was used to demonstrate the formation of cruciform structures in supercoiled molecules (16).

To study the function of the T7.3 endonuclease in non-infected cells, its gene was introduced and conditionally expressed in E. coli. In infected cells, its gene was introduced and conditionally expressed in E. coli. In infected cells, the DNA degradation which followed induction of this expression could be prevented by the functions controlled by the recA gene.

MATERIALS AND METHODS

General Techniques—Conditions for all enzymatic reactions and other methods used in these studies have been described previously (17-19). Descriptions of the experimental approach are given in the figure legends.

Cloning of the T7.3 Gene—Wild-type bacteriophage T7 DNA was purified as previously described (19). Restriction digestion of this DNA with Drai produced a 4226-bp fragment which was purified by agarose gel electrophoresis. Digestion of this fragment with BstXI produced a 625-bp BstXI-Drai fragment which contained the T7.3 gene and ribosome-binding site sequence (460 bp) flanked by 148 and 17 bp of the upstream and downstream T7 DNA sequences, respectively (9). The 625-bp fragment was rendered blunt with S1 nuclease and inserted at the SfiI site of the pBR322 derivative plasmid pNKS97 (18). Subsequent introduction at the unique NruI site 321 bp upstream of a filled 99-bp BamHI-EcoRI fragment containing the lacUV5 promoter (17) generated plasmids pLAT73 and pALT73 in which the lacUV5 fragment was inserted in the two opposite orientations. pLAT73 synthesizes the synthesis of T7 endonuclease in the absence of active lac repressor, whereas pALT73 serves as a control. The exact coordinates and a few restriction sites of the various DNA segments constituting pLAT73 are shown in Fig. 1. The structures of all DNA molecules used in this work have been verified by extensive restriction mapping and, where necessary, DNA sequencing.

Bacterial Strains—E. coli M15061 (a lac strain), HB101 (a recA strain), and W3110* (a lac repressor-overproducing strain) which were used in previous studies (17). E. coli W3110*? (recA strain isogenic to W3110* except for the recA gene) was generated by K. Hardy (of this Department). E. coli JC4588* (recA strain) and JC4588* (recA strain) which are isogenic except for the recA gene were obtained from J. Clark (University of California). E. coli K12(λ) was obtained from B. Allet (of this Department).

Protein Gel Electrophoresis and Transfer—Samples for gel electrophoresis (24) were prepared by resuspending cell pellets on 0.125 M Tris-HCl, pH 6.8, 15% glycerol, 10 mM β-mercaptoethanol, 4% sodium dodecyl sulfate and boiling for 5 min. Proteins were transferred electrophoretically onto nitrocellulose sheets (20). The sheets were washed twice for 30 min in 100 mM sodium phosphate, pH 7.6, 150 mM NaCl, 0.1% gelatin, 0.01% bovine serum albumin, 0.1% Triton, treated with anti-recA monoclonal antibody (provided by Dr. John Florey, Yale University) for 1 h at 37 °C, and washed twice for 30 min in the same buffer. After exposure to peroxidase-conjugated rabbit immunoglobulins to mouse immunoglobulins (Dakopatts) for 1 h at 37 °C, sheets were washed in 50 mM sodium acetate, pH 5, for 10 min, stained with the addition of 2 ml of 1% (w/v) 3-aminopropyltriethoxysilane in acetone and 0.025 ml of 30% H2O2. Staining was quenched in 50 mM Na2S2O4.

1 The abbreviation used is: bp, base pair.

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RESULTS

Cloning and Expression of the T7.3 Endonuclease Gene—Plasmid pLAT73 carries the T7.3 endonuclease gene and ribosome-binding sequences under the transcriptional control of the inducible lacUV5 promoter (Fig. 1). Since expression of this gene in *E. coli* was expected to destroy the genomic DNA, pLAT73 was introduced in *E. coli* W3110(iqrecA-) cells which overproduce the *lacI* repressor (21).

To measure the production of endonuclease in this strain, a growing culture was induced with lactose, and, at various times, aliquots were removed and processed through the streptomycin/ammonium sulfate step (22) of the T7.3 enzyme purification procedure (16). Incubation of these DNA-free extracts with purified plasmid DNA and analysis by gel electrophoresis revealed that the supercoiled DNA was converted into nicked and linear forms (Fig. 2) in a reaction typical of the T7.3 enzyme and of nucleases specific for single-stranded DNA in general (16). Since the activity appeared at increasing levels after induction, it was concluded that cells harboring the pLAT73 plasmid can indeed express the T7.3 endonuclease in active form.

Endonuclease Expression Inactivates the Host Cell—To probe the effect of the T7.3 nuclease in vivo, cell viability was studied before and after induction of W3110(iqrecA-) containing pLAT73 with lactose. As a control, cells harboring a plasmid (pALT73) which carries the lacUV5 promoter fragment inserted in the opposite orientation were used in parallel.

For approximately 90 min after induction, both cultures continued to grow at similar rates. However, the cells containing pLAT73 stopped growing and remained at the same optical density overnight although no signs of cell lysis were evident over this period of time. On the other hand, the control culture continued to grow for a few more hours before reaching saturation.

At various times before and after the addition of inducer, samples were removed, diluted ten-million-fold in media without lactose, and plated on nonselective and on ampicillin-containing plates. Growth in the presence of lactose for at least 200 min had no effect on the plating efficiency of cells harboring the control plasmid, pALT73 (Fig. 3). In contrast, only 50% of cells harboring pLAT73 survived after exposure to lactose for 30 min and few cells survived exposure for 90 min. These results indicated that temporary induction of the T7.3 nuclease gene is lethal. Although cultures continue to grow until 90 min after the addition of lactose, the amount of endonuclease that is synthesized over this period of time is sufficient to eventually kill the cells. In addition, the fact that the same plating efficiency was obtained in the presence as in the absence of ampicillin suggests that cell death is more likely due to disruption of the cellular functions rather than...
damage of the plasmid which carries the ampicillin resistance gene.

Primary Target of the Endonuclease Is Genomic Rather Than Plasmid DNA—Since the T7.3 nuclease was expected to attack DNA in vivo, W3110\((\text{id} \text{recA}^-)\) cells harboring pLAT73 were induced with lactose and the nucleic acids in the total lysates were analyzed directly by gel electrophoresis. The results in Fig. 4 show that 1 h after the addition of lactose the intensity of the band corresponding to genomic DNA begins to decrease, indicating that part of the DNA is being degraded. By 7 h, most of the genomic DNA has already been reduced to smaller fragments. In sharp contrast, most of the plasmid DNA remains intact in the supercoiled from. Only after 5 h of induction does a faint band corresponding to the position of nicked circular DNA appear.

These results indicate that the T7.3 endonuclease which was shown above to be recoverable in active form from induced cells is in fact active in vivo. The primary target of this endonuclease activity is the genomic DNA whose degradation is one of the physiological functions of the T7 enzyme in phage-infected cells. On the other hand, the plasmid is resistant to nucleolytic attack, and, in this sense, it resembles T7 DNA in phage-infected cells.

recA\(^+\) Strains Survive T7.3 Gene Expression—In the experiments described above, a bacterial strain which overproduces the lacI repressor was used to ensure minimal expression of the T7.3 gene in the unduced state. Upon induction, genomic DNA was degraded and cell viability was lost. Consequently, it was expected that a strain such as MC1061 which does not produce lacI repressor and allows constitutive expression of the lacUV5 promoter would not produce viable colonies upon transformation with pLAT73. On the other hand, a strain such as HB101 which contains normal levels of lacI repressor should produce fewer or similar numbers of colonies than W3110\(^+\) cells which overproduce the lacI repressor. Surprisingly, it was found that a few hundred transformants were obtained with either MC1061 or W3110\(^+\) cells, whereas HB101 cells produced no transformants. The control plasmid pALT73 transformed all three strains with similar efficiency.

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TABLE I
Prophage induction by T7.3 endonuclease

<table>
<thead>
<tr>
<th>Indicator bacteria</th>
<th>IPTG</th>
<th>K12(λ)</th>
<th>K12(λ) + pALT73</th>
<th>K12(λ) + pLAT73</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>170 col</td>
<td>130 col</td>
<td>200 col</td>
<td></td>
</tr>
<tr>
<td>ND</td>
<td>5 plq</td>
<td>55 plq</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td>161 plq</td>
<td></td>
</tr>
<tr>
<td>ND</td>
<td>0.25</td>
<td>ND</td>
<td>300 plq</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>2 plq</td>
<td>1 plq</td>
<td>300 plq</td>
<td></td>
</tr>
</tbody>
</table>
of plaques produced by the K12(λ) or the K12(λ)/pALT73 cells did not increase, whereas the number of plaques generated by the K12(λ)/pALT73 cells increased to approximately 300 (Table I). (It is not surprising that the number of plaques is higher than the number of viable cells since at the time of plating some cells were about to release plaque-forming phage but no viable colonies.) These results indicated that activation of the T7.3 gene product causes λ prophage induction and implicated activation of the SOS response and the DNA repair system as the most likely mechanism by which recA+ cells overcome the activity of the T7 endonuclease.

Induction of recA Protein—If the observed prophage induction upon expression of the T7.3 gene is indeed a consequence of activation of the SOS response, then the levels of recA protein should also be increased.

To test this possibility, total protein extracts were prepared from W33110(iqrecA+) cells harboring pLAT73 before and after induction with lactose. As a measure of the recA levels in uninduced cells, extracts were also prepared from plasmid-free cells.

Comparison of the intensity of the recA protein bands in lanes 1 and C of Fig. 5 revealed that before addition of lactose, the levels of recA protein in cells harboring pLAT73 were increased relative to the control. Apparently, low level transcription of the T7.3 gene escapes repression and partially induces the recA protein. This is consistent with the results of the prophage induction experiments which showed that cells harboring pLAT73 generated more plaques than the control even before addition of lactose (Table I).

Induction of T7.3 gene expression leads to increased levels of recA protein synthesis. This increase is already evident 20 min after addition of lactose and becomes severalfold higher than the control 120 min later (cf. lanes 4 and C, Fig. 5). The same conclusions can be drawn whether one compares band intensities of the recA protein either of directly stained gels or of Western blots of the same gels (Fig. 5, A and B, respectively).

These results are consistent with the idea that the DNA repair system of the host is activated in response to the damage inflicted on the genomic DNA by the T7 endonuclease. Here also, as with other DNA-damaging agents, the repair system is activated as part of the SOS response as evidenced by the observed induction of the recA protein and λ prophage and the fact that the recA protein enables the cells to overcome expression of the T7.3 gene.

DISCUSSION

Selective Degradation of Genomic DNA—When expression of the T7.3 gene carried by plasmid pLAT73 was induced in E. coli, the genomic DNA was rapidly degraded, leading to cell death. In this respect, the recombinant T7.3 enzyme served the same function as in T7-infected cells. Since the restriction fragment carrying the endonuclease gene does not code for any other intact gene (9), we conclude that neither phage infection nor any other function carried by T7 is necessary for this T7 endonuclease activity.

In phage-infected cells, the genomic DNA is degraded but the newly synthesized phage DNA remains intact (10, 11). In this respect, the observed resistance of the pLAT73 plasmid to nucleolytic attack resembles that of T7 DNA. As in the case of T7, the reason for this selectivity is unclear. The T7.3 enzyme is an endonuclease with strong specificity for single-stranded DNA (15). Even a few bases of a locally exposed strand are sensitive to this activity, as shown by the specific cleavage of cruciform structures in supercoiled DNA in vitro (16). If the single-strand specificity of the T7.3 enzyme is responsible for the preferential degradation of the genome, then the latter must exhibit a higher degree of exposed single-
stranded regions in vivo than either the supercoiled plasmid or the linear T7 DNA. This assumption is presently under investigation.

Activation of the SOS Response—The selective degradation of the genomic DNA and the subsequent inactivation of the cellular functions which follow induction of the T7 endonuclease were studied in cells deficient in recA protein. In such strains, the T7.3 gene could be maintained only in the transcriptionally inactive state. In contrast, cells proficient in recA protein were able to tolerate constitutive expression of the endonuclease. These results indicated that the recA gene product and/or the functions controlled by it can overcome the T7 endonucleolytic activity.

Studies with recA+ cells demonstrated that induction of the T7 enzyme induces the recA levels and the SOS response of the cell, leading to prophage induction. One of the functions activated as part of the SOS response is the DNA repair system of the host. Conceivably, activation of this system could repair the damage inflicted by the T7 enzyme and enable recA+ cells to tolerate its nucleolytic function.

A number of agents which interfere with DNA metabolism are known to induce the SOS response. To our knowledge, however, induction of the SOS response by the product of the recombinant T7.3 gene is the first example of SOS induction by enzymatic nucleolytic activity in normal, undisrupted cells.

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REFERENCES