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Interaction of the LexA repressor and the *uvrC* regulatory region

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We have studied the in vitro interaction of the LexA repressor protein and the *uvrC* regulatory region. We find that there is specific binding to two regions, the region we have defined as *lexA1* and the *lexA2-lexA3* region. Our findings support the possibility of an inducible regulation for this complex operon.

LexA repressor; *uvrC*

1. INTRODUCTION

The *E. coli uvrABC* genes encode a DNA repair nuclease which recognizes bulky adducts in the DNA and in an ATP-dependent fashion incises both 5′ and 3′ to the lesion, producing a fragment of approximately 12 nucleotides. The resulting single-strand region is acted upon by DNA polymerase I, DNA helicase II and polynucleotide ligase to re-establish the covalent structure of the helix. (For review see [1].)

It has been demonstrated that the *uvrAB* genes are under regulation of the LexA repressor and are inducible [2-5]. The *uvrAB* genes have been shown to have regulatory regions which encode LexA protein binding sites [5,6]. The regulation of the *uvrC* gene as a component of the DNA repair system of *E. coli* is less clear. We have cloned and sequenced the 5′ regulatory region of the *uvrC* gene and have noted multiple promoters [7,8] both by sequence analysis and by RNA polymerase binding. The proximal promoter region is not adequate for normally regulated expression of the *uvrC* gene [9]. Van Sluis et al. [10] noted that the *uvrC* gene cannot be shown to be induced by DNA damage. However, studies with fusion constructs in plasmids with reporter genes gave conflicting suggestions of inducibility [10] or non-inducibility [11]. We noted that the 6 carboxy terminal amino acids of *uvrC* are not needed for DNA repair, since cloned structural genes lacking these carboxy terminal elements were adequate for complementation [7,9]. Structural genes containing the complete 5′ terminal element gave slightly better complementation, however [7].

We have defined two open reading frames 5′ to *uvrC*, one encoding a 23 kDa protein and one encoding a 28 kDa protein (fig.1). The 23 kDa protein was predicted to be a regulatory protein by us [7] and we have recently noted that it contains elements placing it in the OmpR category of proteins [12]. The 23 kDa protein overlies two promoters (P3 and P4) proximal to the *uvrC* structural gene and also overlies two potential LexA binding sites, neither of which is canonical. The carboxy terminus of the more distal 28 kDa protein lies close to an additional LexA binding site which is canonical. The complex arrangement of the promoter regions and the potential LexA binding sites as well as the overlapped regulatory regions are reminiscent of the macromolecular synthesis operon [13]. Our observations support the conclusion that most transcription through *uvrC* originates at P2, with termination near the terminus of *uvrC* or inside of *uvrC* [7] (fig.1). The pattern is suggestive of a steady state level of synthesis for the *uvrC* protein, perhaps for a 'housekeeping' function, with an induced transcription from the more proximal P3 promoter. Because of this and because of the inducibility of the rest of the *uvrABC* repair enzyme system we have studied binding of the LexA protein to the regulatory region in vitro. We report here that as demonstrated by gel retardation analysis we observed interaction with both the *lexA1* and the *lexA2-lexA3* regions.

2. MATERIALS AND METHODS

2.1. *Plasmids*

Plasmid constructions have been previously reported [7]. Plasmid preparations were performed as previously reported.

2.2. *LexA protein*

LexA protein was a gift from Dr. J.W. Little.

2.3. *Electrophoretic analysis of the interaction of LexA protein with DNA*

The regulatory region binding was studied by polyacrylamide gel electrophoresis as described by Fried and Crothers [14].

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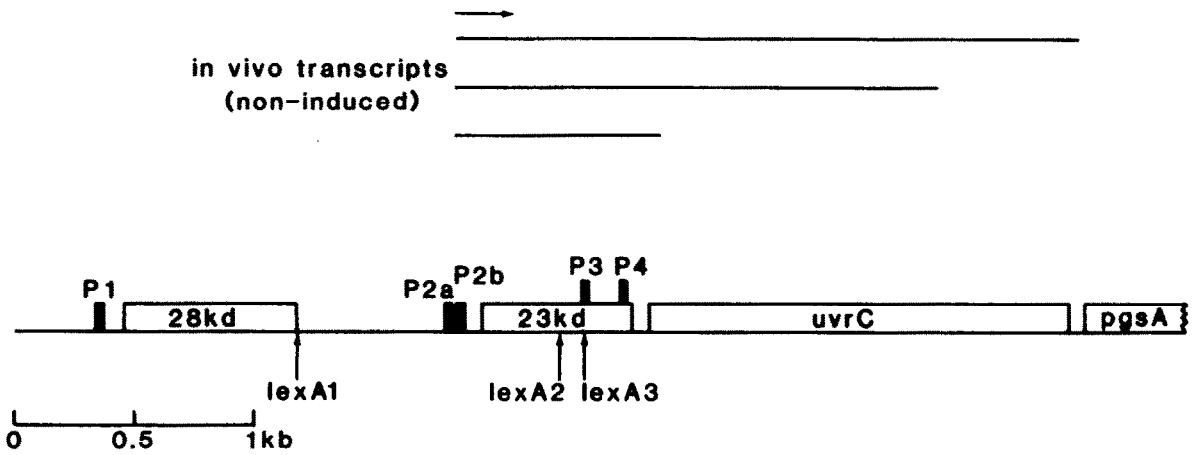

Fig.1. *uvrC* regulatory region structure.

2.4. *End-labeling of the DNA fragments*

Plasmid DNA was purified as previously described [9] and digested with restriction enzymes as described in the text. After isolation of the initial cleavage product the DNA was end-labeled with ^{32}P using Klenow fragment of DNA polymerase I [15]. The end-labeled fragment was digested with the second restriction nuclease and then isolated on a 5% polyacrylamide gel. The location of the band was identified by a preliminary autoradiogram, the band was cut out and macerated and the DNA extracted into buffer.

2.5. *LexA binding reactions*

The complex was formed in a 10 μl reaction mix containing the DNA fragment at 0.1–1.0 ng/μl, LexA protein at 1–10 μM, 20 mM Tris, pH 7.4, 0.15 mM EDTA, 20% glycerol, 25 mM NaCl and 0.1% BSA. Reactions were incubated 10 min at 7°C and loaded onto the gel for electrophoresis.

3. RESULTS

For the present study we isolated two regions of DNA to test LexA binding, a 364 base fragment (*Bgl*II to *Hin*cII) containing both *lexA2* and *lexA3* sites [7] and a 430 base pair fragment (*Pst*I to *Dra*I) containing the *LexA1* site. In preliminary experiments we observed that band retardation could be detected for the *lexA2-lexA3* region at LexA protein concentrations as low as 2 μM. In the study shown in fig.2, the specificity of the binding is shown by competition with a *Hin*fI cut of pBR325. Only at 1000-fold excess of the competitor DNA is decreased binding noted. This indicates there is a high degree of specificity in the interaction of the LexA protein with the fragment of DNA containing the putative LexA binding sites, *LexA2* and *LexA3*.

As a comparative result we investigated the interaction of LexA protein with the *cea* region of the ColE1 origin, a fragment of DNA displaying specific high affinity for LexA protein binding [16]. We observed specific interaction of the LexA protein with the *cea* region with LexA concentrations as low as 200 nM. Thus, the binding to the *lexA2-lexA3* regions shows

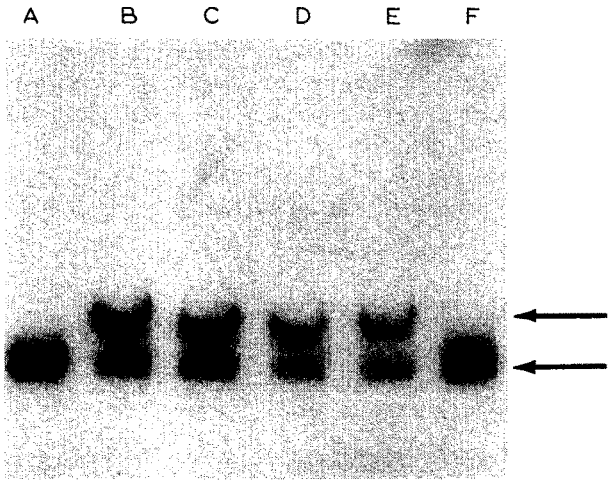

Fig.2. Competition for LexA binding to *lexA2-lexA3* sites of the *uvrC* gene. The concentration of the 364 base pair DNA fragment was 100 ng/ml (1 ng/reaction). The samples were prepared and analyzed as described in section 2. A contains no LexA protein and B–F contain 2 μM LexA protein. Competitor DNA: C contains 1 ng of competitor DNA; D, 10 ng; E, 100 ng and F, 1000 ng.

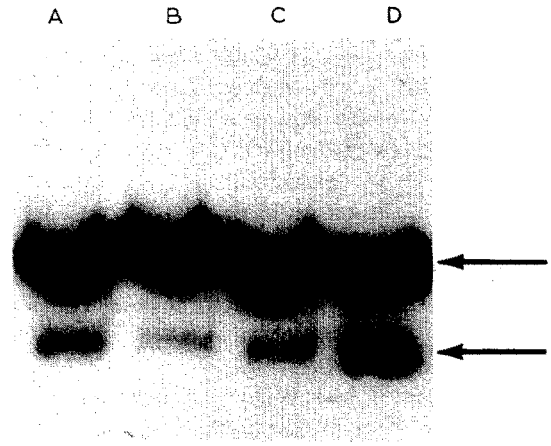

Fig.3. Competition for LexA binding to *lexA1* of the *uvrC* regulatory region. Conditions were as in fig.2 except that the 430 base pair *lexA1*-containing fragment was used. A contains no competitor DNA, B contains 10 ng of *Hin*fI cut pBR325 DNA; C, 100 ng and D, 1000 ng.

somewhat lower affinity than to *cea*, but a high degree of specificity. Due to autocatalytic cleavage of the LexA protein with time, the concentration of active subunits is uncertain with any preparation, but the relative affinity can be assessed for DNA fragments.

Fig.3 shows the binding of the LexA protein to the *lexA1* site of the *uvrC* regulatory region. In preliminary experiments we observed retardation of the *lexA1*-containing DNA fragment at approximately the same concentration of LexA protein as for the *lexA2-lexA3* DNA fragment, 2 μM. Fig.3 contains data supporting the specificity of this interaction; a 5000-fold excess of competitor DNA did not eliminate the retardation on electrophoresis.

4. DISCUSSION

The results presented support the conclusion that LexA protein interacts with potential binding sites in the *uvrC* regulatory region. It must be remembered that demonstrated in vitro interactions do not conclusively argue for in vivo regulatory roles. Nevertheless our results indicate that a specific interaction can be demonstrated for the LexA protein at two different sites in the *uvrC* regulatory region.

Our prior studies have indicated that most of the transcription through the *uvrC* region begins at the P2 promoter. For the regulation of *uvrC* gene expression, it might be anticipated that *lexA2-lexA3* binding sites would be of greatest importance. The results presented here are in contrast to those presented by another group [17] which failed to detect specific binding of the LexA protein in the *uvrC* regulatory region. We have used a slightly larger nucleic acid fragment. It may be that there is an interaction of the LexA protein with *lexA2* and *lexA3* which are approximately 90 nucleotides apart. It is also possible that the binding requires the interaction of two sites because neither *lexA2* nor *lexA3* fits the canonical LexA binding sequence. The *lexA1* site was not studied in the previous report.

The *lexA1* site appears to be a normal LexA binding site and displays approximately the same characteristics in our experiments as the *lexA2-lexA3* binding site. Therefore we conclude that there is a specific binding of the LexA protein to both the *lexA1* and the *lexA2-lexA3* sites. We do not have a suggested role for the *lexA1* binding site. Although there are several weak promoters in the region, we have been unable to establish a significant open reading frame in either direction on the basis of sequence analysis [7].

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