The EMBO Journal vol.7 no.1 pp.269-275, 1988

Contacts between the LexA repressor - or its DNA-binding domain - and the backbone of the *recA* operator DNA

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Communicated by G.Dirheimer

Using hydroxyl radical footprinting and ethylation interference experiments, we have determined the backbone contacts made by the entire LexA repressor and its amino-terminal fragment with the *recA* operator DNA. These techniques reveal essentially the same contacts between both proteins and one side of the DNA helix if one assumes that the DNA stays in the normal B-conformation. This result is somewhat unexpected because protection of guanine bases against methylation suggested a somewhat twisted recognition surface. The backbone contacts revealed by both methods are symmetrically disposed with respect to the center of the operator, providing further evidence that the operator binds two LexA monomers. Each half-operator contains seven interfering phosphates. These phosphates are found on both sides of the 5'-CTGT sequence that is believed to be the principal recognition target. On the side close to the center of the operator are found two phosphates, whereas the other five are clustered on the side apart from the dyad axis. We are not aware of such an extended cluster of interfering phosphates for any other DNA-binding protein. A quantification of the hydroxyl radical footprints allowed us to compare further the affinity of the LexA repressor for the *recA* operator with that of its isolated DNA binding domain. We find an only 13-fold higher binding constant for LexA than for its amino-terminal domain, which is in good agreement with our earlier results for the *uvrA* operator using a completely different binding assay.

Key words: ethylation interference/hydroxyl radical footprinting/LexA repressor/*recA* gene/SOS regulatory system

Introduction

Escherichia coli has developed a genetic system called the 'SOS system' consisting of ~20 genes involved mainly in the repair of DNA damage and enhanced mutagenesis. These genes are expressed upon induction of the SOS machinery by chemical and physical carcinogens (for reviews, see Little and Mount, 1982; Walker, 1984). *In vivo* these DNA modifications lead to an 'SOS signal' able to activate the existing pool of RecA protein (RecA) which catalyzes the specific cleavage of the LexA protein (LexA), the common repressor of all the SOS genes including the *recA* and *lexA* genes.

Quantitative DNase I footprinting experiments have revealed that LexA has very different binding affinities for the different SOS operators, ranging from 5×10^9 M^{-1} for the *umuDC* operator (Kitagawa *et al.*, 1985) to 3×10^7 M^{-1} for the *uvrA* operator as determined in our laboratory (Bertrand-Burggraf *et al.*, 1987). At least for a special LexA mutant (LexA 41) this difference in operator affinity may give rise to a 'split phenotype' where the excision repair mechanism (*uvrA, uvrB*) is induced, whereas SOS mutagenesis (*umuDC*) and filamentous growth (*sulA*) are always repressed (Peterson and Mount, 1987).

LexA may be viewed as a two-domain protein with a flexible hinge region linking the two structural domains together (Little and Hill, 1985). Under alkaline pH conditions LexA undergoes a self-cleavage reaction at the Ala-84-Gly-85 bond within the hinge region (Little, 1984), giving rise to the same two fragments as in the RecA-mediated cleavage reaction. The two fragments have been purified and it has been shown by dimethylsulfate (DMS) protection studies that the amino-terminal fragment harbors the DNA-binding site of the protein (Hurstel *et al.*, 1986) and that the carboxy-terminal domain dimerizes essentially in the same way as the entire protein (our unpublished results using equilibrium ultracentrifugation measurements). The equilibrium dimerization constant of LexA (2×10^4 M^{-1}) is itself rather weak (Schnarr *et al.*, 1985). Using a specially designed transcription assay it has been shown that the isolated amino-terminal domain has only a 14-fold lower equilibrium binding constant for the *uvrA* operator than the entire LexA repressor (Bertrand-Burggraf *et al.*, 1987). This rather small difference is nevertheless sufficient to account for SOS induction *in vivo*.

No high-resolution structural information is so far available for LexA, and it is always an open question if LexA harbors the common helix-turn-helix structural motif found in many other regulatory proteins (for a review, see Pabo and Sauer, 1984). A search for homology of LexA with such regulatory proteins done in our laboratory and by others (Ohlendorf *et al.*, 1983; Hurstel *et al.*, 1986) pointed to the amino acids 28-35 for the first α-helix and 39-47 for the second, the 'recognition helix'. It should be stressed that this homology is in fact strong for the first α-helix, whereas only very weak homology is found within the putative recognition helix. In our case the 10 reference proteins were phage λ, 434 and P22 repressors as well as the corresponding cro proteins, *lac* repressor, 434 cII, P22 c1, and the CAP protein. However, using a very extended 'master set' of 37 reference proteins Dodd and Egan (1987) did not find a significant homology of LexA with their set of proteins. In their case, it is possible that useful information is 'diluted out' with this extended master set since even the tryptophan repressor which contains a helix-turn-helix motif does not fit with their reference proteins. On the other hand, as shown by circular dichroism (CD), the amino-terminal fragment of LexA contains a substantial amount of α-helical struc-

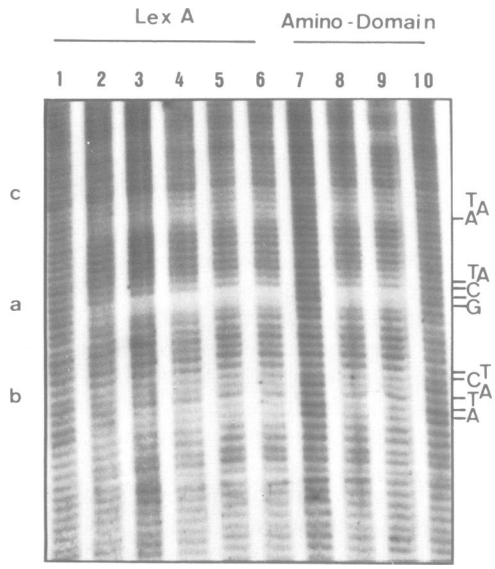

Fig. 1. Effect of the entire LexA repressor or its DNA-binding domain on the hydroxyl radical cleavage of the non-coding strand (upper strand) of the *recA* regulatory region. Both proteins show qualitatively the same pattern consisting of three protected regions denoted a, b and c. Positions protected by >25% at a LexA repressor concentration of 30 nM are exhibited at the right side of the autoradiogram. The 175-bp *recA* DNA, labeled at its 5′-*Hin*dIII end (~0.5 nM), was submitted to hydroxyl radical cleavage with increasing amounts of LexA repressor: 2 nM (**lane 2**), 6 nM (**lane 3**), 30 nM (**lane 4**), 60 nM (**lane 5**), 120 nM (**lane 6**); or its DNA-binding domain: 500 nM (**lane 8**), 5.3 μM (**lane 9**). **Lanes 1, 7** and **10** are control lanes without protein.

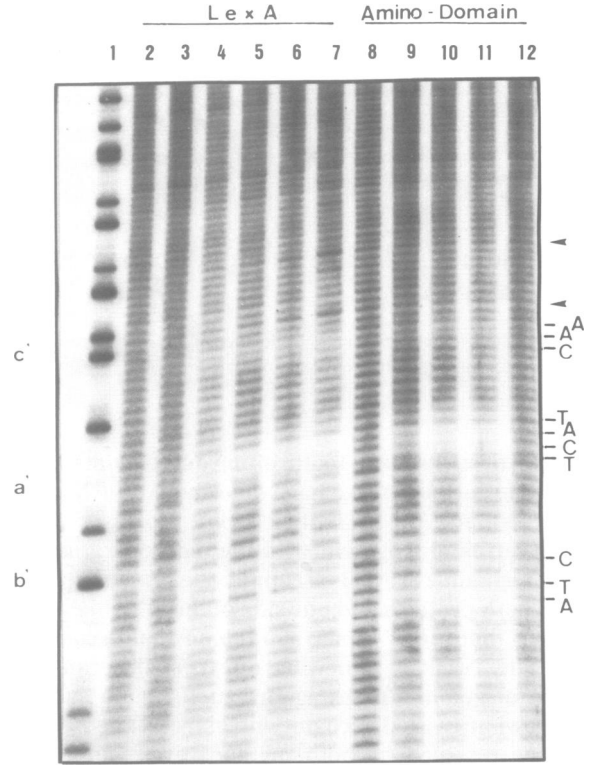

Fig. 2. Same type of experiment as in Figure 1 displayed for the 5′-*Eco*RI end-labeled strand (lower, coding strand). Protected regions are denoted a′, b′ and c′. On this strand, binding of the protein enhances the hydroxyl radical cleavage at the positions shown by arrows (see text). LexA repressor concentrations were 2 nM (**lane 3**), 6 nM (**lane 4**), 30 nM (**lane 5**), 60 nM (**lane 6**), 120 nM (**lane 7**). The concentrations of the DNA-binding domain were: 50 nM (**lane 9**), 500 nM (**lane 10**), and 5.3 μM (**lane 11**). **Lanes 2, 8** and **12** are control lanes without protein. **Lane 1** shows a sequencing ladder of the guanine bases.

ture (Hurstel *et al.*, 1986) and the Chou and Fasman method predicts α-helical segments approximately in the regions found to be homologous with our master set of reference proteins (see below).

To compare the overall positioning of LexA on the DNA double helix with that of repressors with a known three-dimensional structure we have undertaken protection and interference studies revealing contacts of the protein with the DNA backbone. These studies are complementary to the DMS protection experiments (Hurstel *et al.*, 1986) that revealed a strongly and a weakly protected guanine in each *recA* half-operator upon binding of both the amino-terminal fragment and the entire LexA repressor. As reported below, the backbone contacts are detected by phosphate ethylation interference experiments for LexA (Siebenlist and Gilbert, 1980), and by hydroxyl-footprinting for both LexA and its amino-terminal fragment (Tullius and Dombroski, 1986). These footprints are very similar, if not identical, for the two protein species, suggesting further that LexA contacts the DNA mainly or exclusively via its amino-terminal domain, whereas the carboxy-terminal domain would be only involved in dimerization. In agreement with our previous results on the *uvrA* operator (Bertrand-Burggraf *et al.*, 1987), we find an ~13-fold weaker binding affinity to the *recA* operator for the isolated amino-terminal domain than for the entire protein.

Both methods—hydroxyl radical footprinting and ethylation interference—indicate nearly identical contact points for LexA, lying on one half-side of the DNA helix. The observed patterns are discussed in comparison with those obtained with regulatory proteins containing the helix—turn—helix motif, especially the phage λ repressor.

Results

Hydroxyl radical footprinting

The use of hydroxyl radicals to monitor specific contacts between proteins and nucleic acids has been introduced recently by Tullius and Dombroski (1986). The hydroxyl radicals are generated by reduction of hydrogen peroxide by iron(II) complexed with EDTA. Sodium ascorbate is used to regenerate the iron(II) during the reaction, making it possible to produce footprints with micromolar concentrations of iron. In these studies no intercalating agent is attached to the EDTA as done earlier by Dervan and collaborators to study the interaction of DNA with small molecules using methidiumpropyl—EDTA (Van Dyke *et al.*, 1982). Those deoxyriboses that are occluded by the bound protein are cut with decreased efficiency by the radical, resulting in a set of bands of reduced intensity in the sequencing gel pattern. These bands correspond thus to the points of contact of the protein with the DNA backbone.

Figures 1 and 2 show the cleavage pattern of a 175-bp DNA fragment labeled either at the *Hin*dIII or the *Eco*RI end of the fragment. Figure 1 represents the non-coding strand (upper strand in Figure 4) and Figure 2 the lower

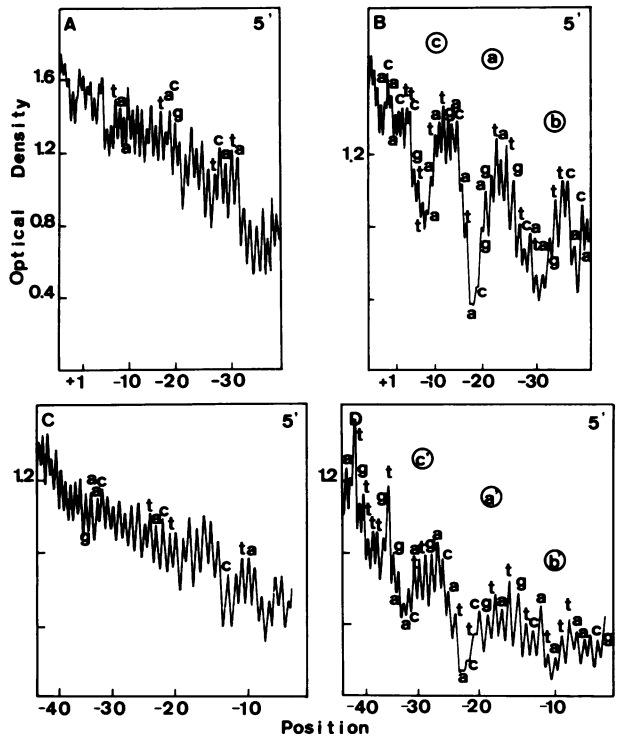

Fig. 3. Densitometer scans of the autoradiograms shown in Figures 1 and 2 for the control lanes without protein (**panels A** and **C** for the upper and the lower strand respectively) and in the presence of 30 nM LexA repressor (**panel B** for the upper and **panel D** for the lower strand). Those bases protected by >25% are indicated in the **panels A** and **C**, whereas the complete sequence figures in **panel B** and **D**.

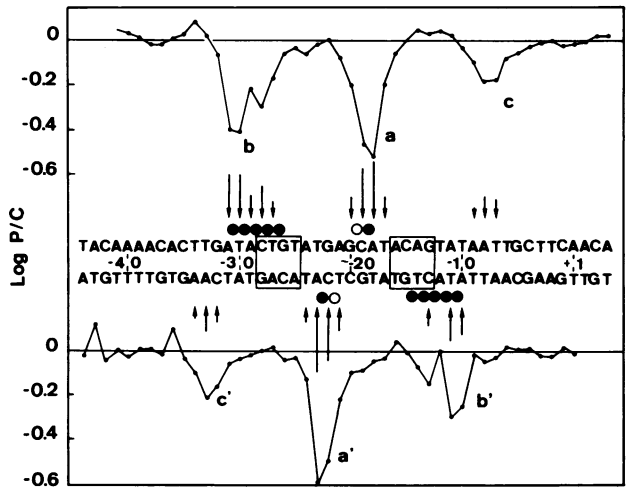

Fig. 4. Plot showing the hydroxyl cleavage perturbation pattern under conditions corresponding to maximal effects produced by the LexA repressor (30 nM). These effects are given as the decimal logarithm of the averaged optical density ratio P/C, P and C being the normalized level of hydroxyl cleavage in the presence and absence respectively of protein. A positive value of $\log_{10}$ P/C denotes an enhancement of cleavage by hydroxyl radicals, whereas a negative value corresponds to protection. The arrows along each strand indicate protection by >25%, their length being proportional to the strength of the effect. Filled dots indicate the phosphates of the *recA* regulatory region which interfere strongly with LexA binding. Open dots indicate phosphates interfering to a weaker extent. The boxed base pairs constitute the primary SOS consensus sequence. The dyad of the *recA* palindrome between base pairs −20 and −21 is indicated by ~.

strand of the *recA* gene. In the absence of repressor the cleavage pattern is virtually sequence-independent (see lane 1 in Figure 1) under the conditions used for these experiments. At lower pH values and ionic strength this sequence independence is partly lost (see below).

Upon addition of LexA or of its amino-terminal domain, both DNA strands exhibit three protected regions, denoted a, b and c on Figure 1 and a′, b′ and c′ on Figure 2. The protection against hydroxyl-radical cleavage is stronger in the center of the operator (regions a and a′) than in the regions further apart from the dyad axis (b, b′ and c, c′). The amino-terminal domain of LexA produces qualitatively the same protection pattern as the whole repressor but an ~13-fold higher molar concentration of the amino-terminal fragment is necessary to produce a similar degree of protection. This finding reflects its weaker equilibrium binding constant due to the lack of the carboxy-terminal domain (see below).

Optical scanning along each lane of the gel reveals the three protected regions more clearly (see Figure 3). Furthermore it allows the calculation of the average optical density ratio P/C for each band of the protein containing lanes as described in Materials and methods. These averaged optical density ratios were determined, particularly for the threshold repressor concentration (30 nM) above which a maximal protection of both strands was observed. Figure 4 shows these values plotted along the sequence of DNA surrounding the *recA* regulatory region. The deoxyriboses for which the protection is > 25% upon binding of the protein are visualized by arrows. As already seen by visual inspection of the gels, the regions a and a′ are dominated by the quite strong protection—up to 75%—of two symmetry-related positions (A-18, C-19 in a, and C-22, A-23 in a′). Backbone contacts on the outer sides of the recognition grooves are defined by the regions b and b′ in the protection pattern. Both regions may be considered as a cluster of about five protected deoxyriboses with a much weaker protected sugar in the center of the cluster (A-29 in b, and A-12 in b′). Again these clusters are symmetrically disposed with respect to the dyad. The occurrence of these symmetrical backbone protections shows that the LexA repressor occupies both half-sides of the *recA* operator. Further, since the observed protection exceeds 50% it can be concluded that the interacting species is likely to be a LexA dimer, because 50% would be the maximal expected protection in the case where LexA would be bound to each half-operator separately as a monomer. This result is not trivial since LexA was found to be largely monomeric in the concentration range used for these experiments.

The regions c and c′ represent additional weak contacts located even further outside on both half-operators. The protection of these regions shows the same dependence on the concentration of repressor as the internal ones. This suggests that they should be due to specific binding of LexA rather than to an additional non-specific binding (Schnarr and Daune, 1984) starting co-operatively from the bound specific complex. Similar far-outside protections against hydroxyl radical cleavage have been observed by Tullius and Dombroski (1986) in the case of the phage λ repressor (see below).

Beside these protections, the histogram in Figure 4 reveals at least two deoxyribose positions which are more easily

cleaved in the presence of LexA (those belonging to the thymines −36 and −43). This enhanced cleavage is also detectable by eye in Figure 2, lanes 5 and 6, just above the c′ region. The symmetry-related position on the top strand does not reveal this enhancement, either because there are no thymines in the corresponding positions, or because there is no TTTT-stretch on the right-hand side of the operator. Such stretches may give rise to local deformations of the DNA-helix (Koo *et al.*, 1986).

Footprinting experiments allowed us to estimate the equilibrium dissociation constant from the repressor concentration necessary to produce a half-maximal protection of the DNA against cleavage at the specific binding site. In the case of LexA, the situation is slightly more complex because we have to deal with a coupled equilibrium. Indeed free LexA is expected to be fully monomeric in the solution under the conditions used for the footprinting experiments. The two possible binding pathways have been discussed in detail earlier (Bertrand-Burrgraf *et al.*, 1987). The second-order equilibrium dissociation constant may thus be written as $K = (R^2 \times O)/R_2O$. The repressor concentration (R) at half-maximal operator protection ($R_2O = O$) represents in this case the square root of K, corresponding to a kind of 'mean association constant' $K_A = 1/\sqrt{K} = 1/R$. In the case of the entire LexA repressor, half-protection is achieved at ~4 nM and for the isolated amino-terminal domain at ~50 nM. The corresponding K_A values are respectively 2.5×10^8 M^{-1} and 2.0×10^7 M^{-1}. A comparable 14-fold lower affinity of the amino-terminal domain with respect to the entire protein has already been found in the case of the *uvrA* operator (Bertrand-Burggraf *et al.*, 1987). The rather small influence of a lacking carboxy-terminal domain is likely to reflect the weak dimerization of LexA and thus the small additional gain of energy of the entire protein with respect to the isolated DNA-binding domain.

Hydroxyl radical cleavage is not always uniform. In an earlier work (Granger-Schnarr *et al.*, 1986) we have shown, using a gel-retardation assay, that the relative balance between specifically and non-specifically bound LexA is pH dependent: lowering the pH to slightly acidic values favors non-specific binding. Hydroxyl-radical footprinting gives similar results in that the footprints are weaker at pH 6.2 than at pH 7.9, conditions used for the experiments shown in Figures 1 and 2.

Whereas the cleavage pattern at pH 7.9 is essentially uniform in the absence of repressor, lower pH and ionic strength give rise to some rare cleavage 'hot spots'. The most pronounced ones are those where cleavage occurs at a cytosine deoxyribose within a 5′-GCA context. Throughout the *recA* regulatory region this holds for the cytosines at positions −5 and −19. To our knowledge the hypersensitivity of this sequence to hydroxyl-radical cleavage has not been reported earlier. Tullius and Dombroski (1985) have found that purine residues 3′ to a pyrimidine are slightly less cleaved than other deoxyriboses. In our case the cleavage at the adenine and guanine deoxyriboses within the GCA motif seem quite normal. The enhanced cleavage at the cytosine deoxyribose is probably linked to a particular local DNA structure of the GCA sequence.

Ethylation interference experiments

Interference experiments differ from protection experiments in that the DNA is chemically modified prior to complex formation, whereas protection experiments are done in the presence of the protein during the modification reaction. Interference experiments necessitate a sufficiently stable complex to separate the complexed DNA from the free DNA.

We have used the ethylation of the DNA backbone to identify those phosphates for which ethylation interferes with binding of LexA to the *recA* operator. The labeled DNA fragments were ethylated as described in Materials and methods in order to obtain statistically less than one modification per fragment. The complex of the modified DNA with LexA was formed under conditions where an unmodified DNA fragment is fully bound (~10^{-7} M of LexA). The small amount of free modified DNA is thus essentially due to DNA molecules ethylated at a site interfering negatively with the protein binding. Free and bound DNA species were then separated on a 5% polyacrylamide gel, eluted from the gel and submitted to cleavage proceeding specifically at ethylated phosphates.

Panel A of Figure 5 shows the cleavage products of the non-coding strand of the *recA* regulatory region for the free and bound DNA fractions run in parallel with Maxam−Gilbert sequencing products. Panel B (lane 7) shows that the cluster which is unresolved in panel A (lane 5) corresponds to five successive phosphates which interfere destructively with LexA binding. Such a broad cluster of interfering phosphates is quite unusual and to our knowledge no other sequence-specific binding protein has shown such a pattern. Two clusters of five phosphates are found on both outer ends of the operator sequence overlapping partly with the consensus recognition sequence 5′-CTGT found in all the SOS operators. Two other interfering phosphates, a rather weak and a very strong one, are found on both operator half-sides close to the center of symmetry of the operator.

As seen in Figure 4, there is a very good agreement between deoxyribose positions protected against hydroxyl radical cleavage and interfering phosphates. The two clusters of five interfering phosphates coincide with the protected regions b and b′ and the two strong central interfering phosphates are situated in the middle of the protected regions a and a′. As in the case of phage λ repressor and cro proteins (Tullius and Dombroski, 1986) there are no interfering phosphates in the weakly protected regions c and c′, suggesting that contacts in these positions are fairly loose.

Chou and Fasman prediction for the DNA binding domain of the LexA repressor

The Chou and Fasman predictive method (Chou and Fasman, 1974) as implemented in the software package of the University of Wisconsin Genetics Computer Group (version 4, April 1986) has been applied to the DNA-binding domain of LexA. This algorithm showed that four segments along the sequence of this domain (positions 1−16, 29−35, 42−51 and 68−74) were predicted to be in an α-helical conformation. These segments have calculated mean helical potentials of respectively 1.17, 1.25, 1.33 and 1.37. To test the significance of these numbers we have calculated the corresponding values for the helical segments of the phage λ and the phage 434 repressors. It turned out that our values were within the range of the mean helical potentials calculated for these helices.

The only portion within LexA predicted to contain two closely related α-helices is the one corresponding to the

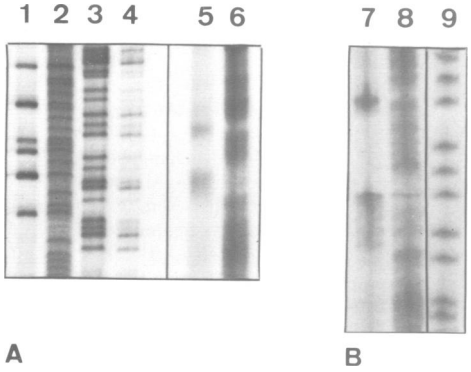

Fig. 5. Identification of those phosphates for which ethylation interferes with binding of the entire LexA repressor to the *recA* operator on a 8% polyacrylamide sequencing gel. **Lanes 5** and **7** show those bands eluted from the free ethylated DNA fraction on a 5% polyacrylamide gel (see Materials and methods) and **lanes 6** and **8** those from the corresponding bound fraction. **Lanes 1–4** and **lane 9** are Maxam–Gilbert sequencing products (G, G+A, T+C, C and G+A). **Lane 7** shows on bottom the cluster of five interfering phosphates and two other phosphates on top of the lane. Alignment of a 5′-labeled sequencing band with a 5′-labeled ethylation cleavage product identifies the phosphate 5′ to that base (Bushman *et al.*, 1985).

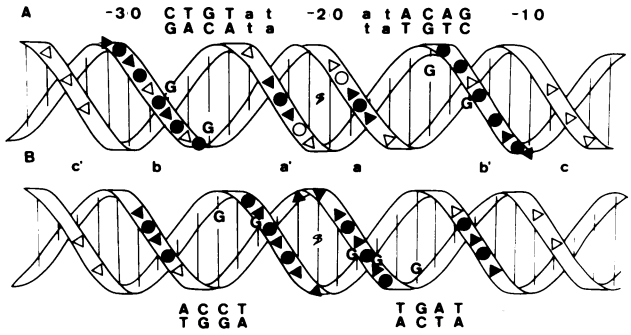

Fig. 6. The contacts found for the LexA repressor (this work) are monitored on a projection of the DNA helix (**panel A**) and compared with those found for the interaction of the phage λ repressor with the OR1 operator (Ptashne *et al.*, 1980; Tullius and Dombroski, 1986) (**panel B**). Triangles indicate protected deoxyribose positions, dots interfering phosphates. Open symbols mean weak protection or interference. Those base pairs being crucial for site-specific recognition are indicated in capital letters, as are the guanine bases protected against methylation.

putative helix–turn–helix motif emerging from sequence homologies with other repressors. Particularly, the predicted location of the first helix of this fold (from position 29–35) matches quite nicely the one deduced from sequence homologies. The location of the third predicted helix (42–51) coincides only partly with that of the putative recognition helix which is assumed to run from residues 39 to 47.

Discussion

The contacts between the LexA repressor and the backbone of the *recA* operator have been monitored by hydroxyl-radical footprinting and ethylation-interference experiments. Both methods are complementary in that hydroxyl radicals attack primarily the deoxyribose entities (Hertzberg and Dervan, 1984; Tullius and Dombroski, 1986), whereas ethylnitrosourea reacts mainly with the phosphate groups of the DNA backbone (Siebenlist and Gilbert, 1980). Thus non-identical, but closely related parts of the DNA backbone are probed by the two methods.

Figure 6A shows that both methods reveal similar contacts between the two macromolecules. All the backbone contacts are essentially on one side of the DNA helix (assumed to be in the normal B-conformation). This finding is not completely expected in view of the repartition of the guanine bases protected against methylation in the presence of LexA (Hurstel *et al.*, 1986). In an earlier work we argued that it is difficult to imagine that LexA recognizes a single face of the DNA because the protected bases are too far apart. The greatest distance between two protected guanines is 18 bp which corresponds to >1.5 turns of the DNA. One way to reconcile the two data sets (backbone and major groove contacts) is a model where the bulk of the protein body sticks to one face of the DNA cylinder through contacts with the DNA backbone, and a protruding 'reading head' probes the bottom of the major groove (where recognition takes place) slightly sideways.

One may ask further how the backbone contacts are disposed with respect to the bases recognized directly by the protein through van der Waals or hydrophobic contacts. An answer to this question necessitates a tentative identification of the recognized base pairs which is facilitated by the great number of different SOS operators and their striking homology (Walker, 1984). In fact all the known SOS operators contain a consensus 5′-CTGT sequence, whereas the center of the different operators is rather variable with some preference for an alternating $(AT)_4$-stretch (Wertman and Mount, 1985). It thus seems likely that this CTGT sequence contains most of the information read by the LexA repressor upon its interaction with operator DNA. Furthermore, for each base pair within this CTGT sequence, operator-constitutive (O^c) mutants have been found either in the *recA* or in the *lexA* operator (Wertman *et al.*, 1984; Wertman and Mount, 1985). On the contrary, the central 6 bp seem to be of minor importance for the site-specific recognition in that neither O^c mutants nor protected guanine bases have been found in this region. One may suppose that these central base pairs play only an indirect role in allowing the two half-operators to accommodate more or less easily the optimal structure of a LexA dimer. Strong evidence for such a 'phasing' of two operator half-sites through central A–T base pairs exists in the case of the phage 434 repressor–operator complex (Koudelka *et al.*, 1987)

Figure 6A shows that backbone contacts are found on both sides of the major groove at the CTGT loci. A comparable, but not identical, relative disposition between backbone contacts and recognized base pairs has been found in the case of the phage λ repressor (Figure 6, panel B). In this case the sequence of 4 bp forming the principal target of the recognition is bracketed by the interfering phosphates (Ptashne *et al.*, 1980; Hochschild *et al.*, 1986). In the case of LexA, two interfering phosphates of the outer clusters b and b′ overlap with the CTGT recognition sequence. In both cases the 'window' between the interfering phosphates of regions b and a′ is 4 bp, a spacing which has also been reported for *lac*, P22 repressors and CAP protein (Majors, 1975; Barkley and Bourgeois, 1978; Poteete and Ptashne, 1982). Ebright (1985) reported that such a 4-bp window is quite common for regulatory proteins harboring the helix–turn–helix motif. Obviously this similarity is far from sufficient to conclude that LexA contains this structural

motif, but it is certainly an argument in favor of this possibility.

Another minor difference between the backbone contact patterns of the two proteins concerns the relative 'weight' of the protected regions. LexA has a greater number of contacts in the outer part of each half-operator (regions b and b′) and less contacts in the center (regions a and a′), whereas the phage λ repressor shows just the opposite behavior. This relative preponderance of the outer operator segments in the case of LexA agrees well with a similar observation as far as DMS protection is concerned (Hurstel *et al.*, 1986). It is tentative to speculate at this stage that, if LexA contained the common helix−turn−helix motif, the roughly inverted backbone contact pattern might arise from an ~180° rotation of the helix−turn−helix motif upon insertion into the major groove. Such a rotation has been suggested recently by Kaptein and co-workers (Boelens *et al.*, 1987) in the case of the *lac* repressor.

A final point that merits discussion is the apparent discrepancy between the total number of interfering phosphates and the number of ionic interactions between LexA and operator DNA. In this work we determine a total of 14 interfering phosphates, whereas in an earlier work (Bertrand-Burrgraf *et al.*, 1987) we found only 5.5 ± 1 ionic interactions. It should be pointed out that this latter value was obtained for the interaction of LexA with the *uvrA* operator. However we do not expect that the nature of the contacts of LexA with the DNA backbone would change dramatically from one SOS operator to the other. In fact negative interference may occur, not only upon disruption of a salt bridge upon phosphate ethylation, but at least upon disruption of a hydrogen bond between a protein residue and a phosphate group as well. As shown recently by the refinement of the structure of the 434 repressor−operator complex, most of the ethylated phosphates interfering with repressor binding appear to form hydrogen bonds with peptide amino groups of the phage 434 repressor (Anderson *et al.*, 1987). It is thus not surprising that the number of ionic interactions determined from equilibrium studies is smaller than the number of interfering phosphates, since hydrogen-bonded phosphates are not expected to have a strong salt dependence.

Materials and methods

Protein purification

The LexA repressor of *E.coli* was purified as described elsewhere (Schnarr *et al.*, 1985). The specific breakdown of the LexA repressor in its amino-terminal DNA-binding domain and its carboxy-terminal domain was achieved using the autocleavage pathway under basic conditions described by Little (1984). The amino-terminal domain was purified from the mixture of the cleavage products as described by Hurstel *et al.* (1986).

Preparation of labeled DNA fragments

The pJL5 plasmid (Little *et al.*, 1981) containing the regulatory region of the *recA* gene was purified as described in Clewell and Helinski (1970) and Katz *et al.* (1973). It was digested either with *Eco*RI or *Hind*III restriction enzymes, labeled with ^{32}P at its 5′ ends and further cut with the other enzyme. The resulting 175-bp fragments were purified by electrophoresis on a non-denaturing 8% acrylamide gel, and eluted with a 500 mM sodium acetate buffer.

Ethylation interference experiments

The labeled DNA fragments were ethylated essentially as described by Shanblatt and Revzin (1986). The 175-bp fragments (~1 pM) were diluted in 100 μl of 50 mM sodium cacodylate pH 8.0, 1 mM EDTA, and the same volume of ethanol saturated with ethylnitrosourea (ENU, from Sigma) was added. Ethylation was allowed to occur during 20 min at 50°C. Finally the DNA was precipitated, washed and resuspended in 10 mM Tris−HCl, pH 7.2, 1 mM EDTA.

Binding reaction with LexA repressor and protein interaction electrophoresis. LexA (125 nM) was incubated for at least 10 min with the ethylated fragments (~5 nM) at 0°C in 12.5 mM sodium phosphate, 0.1 mM EDTA, 40 μg/ml BSA in a total volume of 24 μl. Another 6 μl (50% glycerol, 0.5% bromophenol blue and xylene cyanol) were added before loading the sample on a 5% acrylamide gel to separate free and bound DNA fragments. The running buffer was 10 mM Tris−HCl, pH 7.4, 1 mM EDTA, and the electric field was 9 V/cm. The DNA fragments were recovered from the gel by elution with a 500 mM sodium acetate buffer at 37°C for 24 h. Potentially contaminating LexA repressor was removed by phenol extraction. The cleavage reaction of the ethylated fragments was performed as in Shanblatt and Revzin (1986).

Hydroxyl radical footprinting experiments

Protein-binding conditions. The labeled DNA fragments were diluted into a buffer (50 mM Hepes, pH 7.9, 50 mM NaCl, 50 μg/ml BSA) at a final concentration of ~0.5 nM. The appropriate amount of LexA repessor was added to the labeled DNA and allowed to bind for ~20 min at 37°C. Final protein concentrations typically ranged from 2 to 120 nM for the LexA protein, and from 10 nM to 5.3 μM for its DNA-binding moiety.

The cleavage reaction was done as described by Tullius and Dombroski (1986). Samples were suspended in the sequencing buffer (80% formamide and 20% of 10 mM NaOH, 1 mM EDTA, 0.1% bromophenol blue, 0.1% cyanol blue buffer).

Some experiments were done at lower pH values and ionic strengths: binding buffer was 10 mM Hepes, pH 6.2, 5 mM NaCl, 50 μg/ml BSA.

Optical density scanning and footprint quantification. The relative extent of protection of a band (i) along the DNA backbone in presence of a given amount of protein has been expressed as the ratio of its optical density $OD_p(i)$ (measured at 800 nm on a Shimadzu densitometer) and the optical density $OD_c(i)$ of the same band in the control lane (without protein). In order to account for the slight variability of the total amount of radioactivity loaded on each lane of the gel and that of the overall radioactivity along each lane (see panels A and C of Figure 3), this ratio has been further normalized with respect to a set of N unprotected bands (j) by the following relationship:

$$P/C(i,j) = [OD_p(i).OD_c(j)]/[OD_c(i).OD_p(j)]$$

The final extent of protection of band (i) was taken as the mean value of the N numbers calculated by this relationship.

Sequencing gel electrophoresis. The cleavage products of the 5′-*Eco*RI or 5′-*Hind*III end-labeled fragments were run on a 8% polyacrylamide sequencing gel for 3 or, respectively, 4 h at 50 V/cm.

Acknowledgements

We thank Dr John Little for the plasmids used in this work and Professor Guy Dirheimer for his comments and criticism of the manuscript. This work was supported by a fellowship of the Ministère de la Recherche et de la Technologie to S.H., by an INSERM grant (871 007) to M.G.-S., by a research grant of the European Community (ST2J-0291) to M.S. and by the Ligue Nationale de la Lutte contre le Cancer.

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Received on November 2, 1987