BIOCHIMIE, 1982, 64, 565-569.

CNRS symposium, May 1982 - TOULOUSE
Inducible responses to DNA damages

Regulation and autoregulation by *lexA* protein.

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Résumé.

La protéine lexA *réprime de nombreux gènes chez* E. coli. *Lorsque le DNA de la cellule est endommagé, la protéine* lexA *est inactivée et ces gènes sont induits. Nous passons en revue trois aspects de la fonction de répresseur de la protéine* lexA : *comment elle régule les gènes de la réponse SOS, comment elle régule sa propre synthèse, et comment elle reconnaît ses propres sites opérateurs. Des études récentes de la fonction de répresseur de la protéine* lexA *suggèrent que la concentration de protéine* lexA *intacte après induction pourrait déterminer le contrôle minutieux de la réponse SOS.*

Summary.

The lexA *protein represses many genes in* E. coli. *When the cell's DNA is damaged,* lexA *protein is inactivated and these genes are induced. Three aspects of* lexA *protein's repressor function are reviewed : how it regulates genes of the SOS response, how it regulates its own synthesis, and how it recognizes its operator sites. Recent studies of* lexA *protein's repressor function have suggested that the concentration of intact* lexA *protein after induction may determine the detailed control of the SOS response.*

Unless the cell's DNA is damaged, the product of the *lexA* gene of *E. coli* keeps many cellular genes from being transcribed. Repression by *lexA* protein was first proposed for the *recA* gene [1, 2]. Later, genetic experiments established that two other genes were repressed by *lexA* protein — the *lexA* gene itself [3, 4] and a gene necessary for the filamentous growth observed after DNA damage [3]. Repression of *recA* and *lexA* by purified *lexA* protein was soon demonstrated *in vitro* [5, 6]. A set of many genes induced during the SOS response (one of which is the DNA repair gene *uvrA* [7]) was identified by the use of Mu : lacZ fusions [8]. Subsequent application of the genetic test used in reference 3 established that *lexA* protein also represses these genes [7, 9], a fact confirmed by experiments *in vitro* [9, 11]. Other cellular genes : *himA, uvrB, sfiA,* and *umuC,* have since been shown by similar analysis to be repressed by *lexA* protein [10, 11, 12, 13, 14, 15].

During the SOS response, *lexA* protein is cleaved by the *recA* gene product [16]. The effective concentration of *lexA* protein drops drastically, allowing induction of *lexA*-repressed genes. If the cell survives, *lexA* protein concentration eventually returns to its normal level [see the report by J. Little in this volume].

In this review I discuss three aspecs of *lexA* protein's action as a repressor : how it regulates other genes induced during the SOS response, how it regulates its own synthesis, and how it recognizes its operator sites.

lexA *regulation of SOS functions.*

Construction *in vitro* of plasmids which direct the synthesis of large amounts of *lexA* protein has facilitated purification of the protein [5, 6, unpublished]. The mechanism by which *lexA* protein represses its target genes is similar to that employed by other known repressor proteins. *I.exA* protein binds to operator sites in front of the genes whose transcription it represses [5, 6, 11, 13]. For two *lexA*-repressed genes so far examined, binding of *lexA* protein excludes binding of RNA polymerase to the promoter [5, 6]. *LexA* protein operator sites differ in their sequences [5, 6, 11, 13] and affinities for *lexA* protein [5, unpublished).

Affinity of operator sites for *lexA* protein can be measured by a variant of the DNase protection or « footprinting » technique [19]. Affinity is known for operator sites in front of four *lexA*-repressed genes : *recA, uvrB, sfiA,* and the *lexA* gene itself [5, unpublished]. *LexA* protein binds most tightly to the operator site in front of the *recA* gene, and less tighly to the operator sites in front of *uvrB, sfiA,* and *lexA*.

After major DNA damage, large reductions in *lexA* protein concentration occur and every *lexA*-repressed gene becomes derepressed [17, J. Little, this volume, R. Brent and J. Douhan III, unpublished]. In contrast, intermediate *lexA* protein concentrations occur after mild DNA damage (« subinduction » [18]), at the beginning and at the end of an SOS response to drastic DNA damage [John Little, this volume, R. Brent, unpublished], and after DNA damage in *rec*A430 mutant cells deficient in *recA* protease activity [R. Brent, unpublished]. Because different operators have different affinities for *lexA* protein, it has been proposed that at intermediate *lexA* protein concentrations many *lexA*-repressed genes might be induced, but that the *recA* gene, whose operator binds *lexA* protein tightly, would not be induced [5]. It is possible that differential induction of *lexA*-repressed genes may allow a degree of fine control of the SOS response (see last section).

Some *lexA*-repressed genes are also subject to other forms of regulation. *UvrB* maintains a high uninduced level of expression with a second, *lexA* independent promoter [13]. *HimA*, a gene whose product is required for the integration of bacteriophage lambda [20], seems to be repressed both by *lexA* protein and by a complex of its own gene product and another host protein (called *hip* or *himD* product) [10]. It is possible that some SOS-induced genes may also be regulated in more complex ways. For example, it is possible that some genes with long lags in the onset of their induction [8] require for their transcription a *lexA*-repressed positive regulatory protein.

Autoregulation of lexA *synthesis.*

lexA protein represses transcription of its own gene, and thus controls its own concentration in the cell [3, 4]. Derivatives of bacteriophage lambda that fuse the *lexA* promoter to the *lacZ* gene allow measurement of *lexA* transcription *in vivo* [3, unpublished]. The *lexA* promoter is about 80 per cent repressed in a undamaged cell [3, unpublished], and is measureably induced after even minor DNA damage [unpublished]. The *lexA* gene has two operator sites for *lexA* protein [5, 6], in contrast with the single operator site found for the other six *lexA*-repressed genes so far examined. The protein binds to the two operator sites in front of *lexA* coordinately [5, 6], and, as mentioned above, with an affinity weaker than that for operator sites of the three other *lexA* repressed genes for which affinity has been measured [5, unpublished].

A set of mutations affecting the two operator sites in the *lexA* control region has recently been isolated [unpublished]. Studies *in vitro* of *lexA* protein binding to wild-type operator regions and to these mutants have revealed that the binding is cooperative [unpublished]. Three lines of evidence demonstrate this point. First, the intrinsic affinities of the isolated operators differ from each other by a factor of two, but if both sites are intact on a wild-type template their affinity for *lexA* protein is the same. Second, a mutation in one site that is cryptic if the adjacent site is wild-type weakens binding by a factor of five if the adjacent site is destroyed by a drastic mutation. Third, binding of *lexA* protein to both operator sites in the wild-type control region is weakened two to eightfold if one of the sites has methylated cytosines in the CCAGG sequence embedded within it. The probable consequences of *lexA* protein's weak affinity and cooperative binding to the operator sites in front of its own gene are considered later in this article.

As mentioned above, the affinity of *lexA* protein for both operators in its control region is diminished if one of the sites has methylated cytosines within it. It is thus possible that the basal level of *lexA* protein is lower in strains of *E. coli* that do not methylate cytosine (*eg.* B strains [22], *dcm* mutants [23]), and that SOS functions might be easier to induce in these strains. Other possible effects of methylation-dependent differences in *lexA* protein affinity can be imagined. For example, since there are plasmids which carry *lexA*-repressed genes for colicins, cloacins, and mutagenesis functions [24, 25, 26, G. Walker, pers. com.], and since these plasmids can pass from strain to strain of bacteria, one variable affecting the expression of these plasmid-borne functions should be the known difference in cytosine methylation in different hosts.

Physical aspects of lexA *protein binding.*

All eight *lexA* protein operator sites which have been sequenced possess approximate twofold rotational symmetry [5, 6, 13, 24, 25, 26, 27]. The center of symmetry passes between two bases in the operator sites. Genetic, chemical, and nuclease probe experiments with mutant and wild-type operators have revealed a great deal about the nature of the binding interaction. Dimethyl sulfate protection experiments with *lexA* protein and five wild-type operators show that *lexA* protein protects the N7 position of two loose clusters of roughly symmetrically disposed G's in the operator sites [5, unpublished]. *lexA* protein protects no A's, which means that the only functional groups *lexA* protein protects from dimethyl sulfate attack lie in the major groove of the DNA. Protected G's are

found as far as 9 bases out from the center of symmetry, suggesting that the operator covers at least 18 basepairs of DNA [5, unpublished]. More recent probe experiments with the *lexA* control region suggest that each operator is exactly 18 basepairs long [unpublished].

At concentrations of 10^{-5} - 10^{-6} *M*, *lexA* protein runs at a position on sizing columns that suggests that it is a dimer [5]. The carboxyl terminal portion of *lexA* protein shows substantial amino acid homology with the carboxyl termini of many bacteriophage repressors [24, 28]. Plasmids that direct the synthesis of amino terminal fragments of *lexA* protein comprising about 60 per cent and 80 per cent of the length of the intact molecule repress *lexA*-repressed genes if and only if the protein fragments are produced in large amounts [unpublished, see also 24]. These and other data suggest that *lexA* protein contacts DNA with groups in its amino terminus, but requires an intact carboxyl terminus in order to dimerize and bind DNA strongly. The bacteriophage lambda and P22 repressors show a similar division of their functions into amino terminal and carboxy terminal domains [29, 30].

Three mutations in the operators in front of the *lexA* gene have unexpectedly small effects on repressor binding. Binding is diminished in one case by a factor of 5, in the other cases by a factor of 40 [unpublished]. The mutations are multiple substitutions which drastically alter the operator sequence. If the *lexA* control region is visualized so that the *lexA* gene itself is on the right, these mutations lie in the right half of either *lexA* operator site, while analogous mutations which abolish measurable repressor binding change the left half of either site. It is possible that the contacts that *lexA* protein makes with one half of an operator site are much more important than the contacts the protein makes with the other half.

Two other observations are consistent with this idea. First, if all sequenced *lexA* protein operators are aligned so that the genes they repress are transcribed from left to right, comparison of the operator sequences shows that bases in the right half of an operator site are less likely to be conserved among all the sites. Second, the strongest o^c mutation known for the *recA* gene lies in the left half of the site [A. J. Clark, pers. comm.].

While both bacteriophage lambda and *lac* repressors probably make stronger contacts with one half of an operator site than with the other [31, 32], it is possible that the binding of *lexA* protein to its operator sites represents an extreme example of an imperfectly symmetric interaction. Although the effect of multiple substitution operator mutations on lambda and *lac* repressor binding has not been examined, it would be quite surprising to find a mutation in operator sites of either protein that changed 5 out of 9 bases in one half of a site but dimiminished binding by only fivefold.

Another line of evidence suggests that *lexA* protein might bind DNA differently than other repressor proteins. Solution of the crystal structure of several DNA binding proteins has revealed a common supersecondary structure [28, 33, 34]. Two alpha helices are held at a conserved angle, and one of the two helices fits into the major groove of the DNA. Comparisons of the sequence of phage repressor proteins, phage cro proteins, other bacterial repressors, and even *matal,* a regulatory protein in yeast [D. Ohlendorf and B. Matthews, pers. comm.] have made plausible the existence of these two conserved alpha helices in all of the above proteins. But although there is carboxy terminal homology between *lexA* protein and other repressor proteins, nothing in the *lexA* sequence predicts a helix which corresponds to the helix in the other proteins which fits into the major groove [28, 34]. It is possible that the amino terminus of *lexA* protein interacts with DNA by a different mechanism.

Fine control of the SOS response.

lexA protein represses genes of the SOS response. While under some circumstances all *lexA*-repressed genes are induced, it is likely that in other circumstances control of the amount of *lexA* protein allows the cell to make a graduated response to DNA damage.

Recent experiments have revealed details of *lexA* protein's repressor function. *lexA* protein binds weakly to operators of some genes like *uvrB* and *sfiA* and tightly to the *recA* operator. In a normal cell, *lexA* protein keeps the promoter of its own gene under partial repression by binding weakly to two adjacent operator sites. *lexA* protein binding is cooperative, which tends to steepen the repression curve at this locus at most *lexA* protein concentrations. Measurements of the amount the *lexA* promoter is repressed *in vivo* suggest that cooperativity makes the *lexA* promoter easier to derepress than it otherwise would be. These facts have led to a picture of how the concentration of *lexA* protein can affect the response of a cell to DNA damage.

Consider three different amounts of DNA damage a cell may endure. First, it must sometimes

respond to very minor DNA damage for which normal, constitutive amounts of SOS gene products are adequate. Second, it must sometimes respond to massive DNA damage in which all *lexA* repressed genes are induced fully. Third, it must sometimes sustain an intermediate level of DNA damage. This third case may involve efficient induction of some *lexA*-repressed genes but not of others.

1) If damage to the cell's DNA is minor, then constitutive amounts of DNA repair enzymes will suffice to correct it. *lexA* protein concentration will decrease only a little if it decreases at all. If *lexA* protein concentration decreases, elevated transcription from the *lexA* promoter will rapidly restore normal levels of the protein.

2) If damage to the cell's DNA is drastic then the rate of *lexA* protein cleavage will vastly exceed its rate of new synthesis. Since *lexA* protein binds tightly to the operator in front of the *recA* gene, production of large amounts of *recA* protein should depend on the nearly-complete destruction of *lexA* protein. Production of large amounts of *recA* protein may be necessary for efficient induction of lambda-like prophages and for efficient recombinational repair of DNA.

3) If the amount of damage to the cell's DNA falls between the above two extremes, then *lexA* protein's self-repression, as described above, makes possible an intermediate *lexA* protein concentration. If DNA damage is prolonged, then the cell will maintain some functional *lexA* protein only if the rate of *lexA* protein synthesis exceeds the rate of cleavage. The self repression of the *lexA* promoter outlined above makes it easy to fulfill this constraint. As noted above, operators of some *lexA*-repressed genes bind *lexA* protein weakly, while other operators bind it tightly. An intermediate *lexA* protein concentration may allow efficient induction of DNA repair genes like *uvrB* without inducing genes like *recA* whose full expression can sometimes kill the cell.

Confirmation of the above outline of how fine control of the SOS response might work awaits further experiments *in vivo*. It is interesting to note that if this picture of the system that *lexA* protein uses to regulate its own concentration is correct, then cooperativity and autogenous regulation act to make it difficult to induce the SOS system maximally, while the same ways of controlling genes work to make some bacteriophages easy to induce [21].

Acknowledgments.

I thank Mark Ptashne for experimental support and many valuable discussions. I thank Ann Hochschild, Robin Wharton, Mitchell Lewis, Anne Ephrussi, Carl Pabo and Rog Yocum for comments on the manuscript.

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