*Hypothesis*

# The SOS system of *Escherichia coli* in the regulation of bacteriophage λ development

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**A sequence homologous to the known SOS boxes is found in the $P_o$ promoter of phage λ. It is suggested that the sequence found is a binding site for the LexA repressor. The mechanism of the LexA part in regulation of λ development is discussed. It is based on the competitive transcription of the RNA encoding CII protein and the short OOP-RNA transcribed from the $P_o$ promoter.**

*CII protein* *LexA repressor* *OOP-RNA* *SOS box* *CII-OOP, competitive transcription* *Regulation of λ development*

## 1. INTRODUCTION

After *Escherichia coli* cells are infected with phage λ the response may be either lytic or lysogenic. The regulatory protein CII plays a major part in the processes which determine the choice of one or the other route of phage development [1]. This protein activates transcription from the $P_E$ and $P_I$ promoters [2] thereby ensuring an intensive synthesis of the proteins involved in lysogeny: CI repressor and the Int protein required for the integration of phage DNA in the host chromosome [3,4] (fig.1). The CI repressor inhibits the synthesis of all other phage proteins, including CII, in the lysogenic cell.

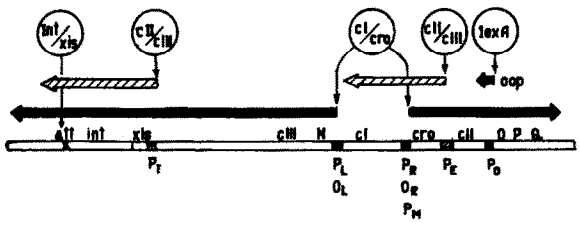


Fig.1. Diagram of regulation of early transcription in phage λ adapted from [7]. Participation of the LexA protein in the regulation of OOP-RNA transcription is shown.

The transition of the prophage to the lytic growth phase may be induced by external factors, such as UV irradiation. The mechanism of this transition is associated with the SOS system of *E. coli*, specifically with the protease activity of the RecA protein with respect to the λ repressor CI [5,6]. The cleavage of the CI protein derepresses the synthesis of the other phage proteins. However, the CII protein shows a low activity in irradiated cells [7], thus favouring the induction of the prophage. The causes of the low CII activity after UV irradiation are unknown.

Prophage induction does not depend on the amount of RecA protein synthesized by the cell [8,9]. Some LexA-controlled process other than RecA synthesis might be involved in the λ induction [9]. Here, we discuss a possible mechanism for the participation of the LexA protein in the regulation of the CII protein synthesis. The mechanism is based on the competitive transcription of the RNA encoding CII and the short OOP-RNA transcribed from the $P_o$ promoter. The regulation of the OOP-RNA transcription is probably carried out through binding between the LexA repressor

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recA        T A C T G T A T G A G C A T A C A G T A
     1      T G C T G T A T A T A C T C A C A G C A
lexA
     2      A A C T G T A T A T A C A C C C A G G G
uvrA        T A C T G T A T A T T C A T T C A G T A
uvrB        A A C T G T T T T T T T A T C C A G T A
ColE1       T G C T G T A T A T A A A A C C A G T G
-------------------------------------------------
CONSENSUS   t a C T G T a T a t a c a t c C A G t a
-------------------------------------------------
            *  * * * *    * * *  * * * * * * * *
oop  λ      T C C T G T T G A T A G A T C C A G T A
            *  * * * *    * * *    * * * * * * *
oop 434     T C C T G T T G A T A G T T C C A G T A
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Fig.2. Sequences of the LexA binding sites. In the consensus sequence capital letters denote nucleotides occurring in these positions in all 6 known sites; lowercase letters denote nucleotides occurring at least three times. Shown underneath are sequences found for the initiation sites of OOP-RNA transcription in phages and 434. Asterisks mark nucleotides occurring in the known SOS box sequences in these positions more often than others.

and supposed operator; which was found by sequence analysis in the region of the $P_o$ promoter.

## 2. ANALYSIS OF THE $P_o$ PROMOTER SEQUENCE

LexA is one of the major proteins of the *E. coli* SOS system; it acts as a repressor on the synthesis of many proteins of the system, including its own synthesis [10,11]. The active repressor is a dimer whose two monomeric units get bound to a DNA sequence known as an 'SOS box' [11]. Fig.2 shows the six known SOS box sequences [12–16].

We looked for SOS boxes by means of a procedure STATSITE developed for the search for functional sites on DNA (in preparation). The method makes use of a statistical function, constructed on the basis of the known LexA binding sites.

Having analysed the region in the vicinity of the $P_o$ promoter, we found a sequence homologous to the known SOS boxes. Fig.2 shows the sequence found: 16 of the 20 nucleotides marked with asterisks occur in these positions in the known SOS boxes more often than others; they include 7 of the 8 most conserved nucleotides.

The above data suggest that the sequence found is a binding site for the LexA repressor. The sequence covers the site of transcription initiation in the $P_o$ promoter (fig.3). Hence, the LexA protein can repress the synthesis of OOP-RNA.

## 3. THE PART OF THE LexA PROTEIN IN THE REGULATION OF BACTERIOPHAGE λ DEVELOPMENT

Available data [2–9] and our sequence analysis of the early transcription region of phage λ suggest an additional mechanism for the involvement of the *E. coli* SOS system in the regulation of phage λ development during the infection or prophage induction by external agents.

The choice between the lytic and lysogenic routes of phage development depends on the synthesis ratio of the CI and CRO repressors [17]. To allow integration of the phage into host chromosome, the Int protein must also be actively synthesized [18]. After phage DNA has entered the cell, the *E. coli* RNA polymerase starts transcription from the $P_L$ and $P_R$ promoters, thereby ensuring the synthesis of the anti-terminator protein N and the subsequent synthesis of the proteins CII and CIII (fig.1). CII plays a special role at the early stage: it activates transcription from the $P_E$ and $P_I$ promoters [2] and ensures an intensive expression

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      80                  90                  97
cII:ThrAsnLysLysArgProAlaAlaThrGluArgSerGluGlnIleGlnMetGluPheTER        SOS box
5'-ACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCATTATGACA
3'-TGGTTATTTTTTGCGGGCCGCCGTTGGCTCGCAAGACTTGTTTAGGTCTACCTCAAGACTCCAGTAATGACCTAGATAGTTGTCCTCAGTAATACTGT

AUUUUUUGCGGGCCGCCGUUGGCUCGCAAGACUUGUUUAGGUCUACCUCAAGACUCCAGUAAUGACCUAGAUAGUUGppp 5' OOP-RNA
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Fig.3. A fragment of the phage λ DNA sequence. The sequence of OOP-RNA is shown underneath. The amino acid sequence of the 3′-end of the CII protein is shown above the DNA sequence. The sequence of the hypothetical binding site for the LexA repressor is framed. It covers the point of transcription initiation from the $P_o$ promoter. Hence the LexA protein may serve as a repressor of OOP-RNA synthesis. The fact that the CII gene and OOP-RNA overlap suggests a mechanism for the inhibition of CII expression based on competitive transcription.

of the CI and Int genes. The CII protein is synthesized from $P_R$-RNA. Short OOP-RNA, which partially overlaps the CII gene [19], is transcribed from the $P_o$ promoter. The role of OOP-RNA is as yet unclear, although a number of hypotheses have been advanced.

The above sequence analysis suggests that there is a LexA binding site in the area of the $P_o$ promoter, which covers the point of transcription initiation (fig.3). Thus the transcription of OOP-RNA will be blocked in λ-infected *E. coli* cells containing an active LexA repressor in sufficient amounts. If there is a shortage or lack of active LexA repressor, OOP-RNA will be transcribed towards the RNA from the $P_R$ promoter. This competitive counter-transcription may cause a partial premature termination of the $P_R$-RNA transcription. The resulting abortive RNA will give rise to an incomplete and probably inactive CII protein. By contrast, in those cells where the SOS system functions normally under physiological conditions the LexA protein blocks the transcription of OOP-RNA, thereby ensuring an effective expression of the CII protein. This must increase the probability of lysogenization. If for some reason the cell has a shortage of active LexA repressor, the competitive transcription of OOP-RNA will inhibit the synthesis of CII and increase the probability of a lytic response.

Prophage induction by external DNA-damaging agents (e.g., UV irradiation, mitomycin C) also involves the SOS system of *E. coli*. Until recently it was believed that only the RecA protein is involved in the prophage induction [20], because it could cleave the repressor CI.

However Quillardet et al. [9] have shown that prophage induction does not depend on the level of expression of the RecA gene but depends essentially on whether or not an active LexA repressor is present. They supposed that it was not an increase of the RecA synthesis that underlay prophage induction but another process controlled by the LexA protein. We suggest that this process may be the synthesis of the λ OOP-RNA.

After an activated RecA protein cleaves the CI repressor, RNA transcription starts from the $P_L$ and $P_R$ promoters. The CII protein synthesized may, as mentioned above, stimulate the synthesis of the CI repressor, thereby hindering prophage induction. Yet, as soon as the RecA protein cleaves the LexA repressor the synthesis of OOP-RNA is switched on. The competitive transcription of OOP-RNA and the $P_R$-RNA inhibits the synthesis of CII and consequently raises the probability of prophage induction. This mechanism is quite consistent with the experimental data on prophage induction in LexA mutants [9,21]. The derepression of OOP-RNA synthesis may play an important role not only in inhibiting the synthesis of the CII protein but in other processes essential to prophage induction, for instance phage DNA replication. Thus the LexA protein may be directly involved in the regulation of phage λ development.

Phage 434, which is related to λ, has a sequence in the region of $P_o$ promoter that is homologous to the SOS boxes and differs from the analogous region of phage λ by one base substitution only (fig.2) [22]. Since this difference leaves the most conserved nucleotides untouched, we can presume that the mechanism proposed above may also operate in phage 434.

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