The catabolite gene activation system of *E. coli* may be directly involved in regulation of bacteriophage λ development

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The primary structure of bacteriophage λ DNA has been searched for the presence of consensus CAP binding sites. Four putative CAP binding sites have been found on the λ genome, indicating that the catabolite gene activation system of *E. coli* may be directly involved in the regulation of λ development. Molecular mechanisms of putative cAMP–CAP-mediated stimulation of lysogenic and lytic responses are discussed.

CAP binding site *Catabolite repression* *λ gene regulation* *Lysis-lysogeny* *λ DNA sequence* *cII/cIII*

1. INTRODUCTION

Temperate phage λ can grow either lytically or lysogenically. The two alternative modes of phage development are determined primarily by functions of cII and cIII gene products, cI and cro repressors, and Int protein [1–3]. The physiological state of the host cell may also influence the lysis-lysogeny decision. Thus, the level of 3′-5′-cAMP was reported to affect the establishment of the lysogenic pathway (see [1]). Phage infection of bacteria grown in the medium with glucose (under catabolite repression), or carrying mutation in CAP (catabolite activating protein) or in gene cya coding for the adenyl cyclase enzyme, leads preferably to the lytic pathway [4–7]. Although the catabolite gene activation system (CGAS) is known to stimulate or repress the function of several bacterial promoters, no such activity has been reported for any phage promoter. It has been suggested that the effect of the cAMP–CAP complex on λ regulation is indirect and mediated through bacterial products (see [1]), like hfl-protein which reduces the activity of cII [8] and himA-hip proteins which are required both for the λ integration [9] and for the synthesis of cII protein [8], or lon gene products which decrease the stability of cII protein [10]. Here we suggest a direct involvement of the CGAS in the λ development and we provide the computer analysis of the primary structure of λ genome supporting this hypothesis.

2. EXPERIMENTAL AND RESULTS

2.1. *Localization of the putative CAP binding sites*

In our recent studies on the expression of cloned λ oL-pL-N DNA conferring a lethal effect in bacteria, we have found that to exhibit the phenotype in the minimal medium, a non-glucose carbon source is required, while in the presence of glucose in the medium, the growth of bacteria appears to be normal (unpublished). Furthermore, we have found that non-glucose carbon sources stimulate the growth of virulent λ phages lacking Int and cI genes (unpublished). These data inspired us to carry out this work.

In accordance with our knowledge on cAMP–CAP function in bacterial cells [11], the CAP binding sites are characterized by a conservative nucleotide sequence located in the promoter

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regions of a number of genes [12]. The consensus sequences for the CAP binding site and for the promoter have been deduced in [13,14], respectively. The CAP binding sites on λ DNA have been sought using a computer and a program developed in the Pushchino Computer Centre (USSR). We have found that several sites are associated with the promoter regions and can be considered putative CAP binding sites (fig.2). First, a DNA segment of extensive homology with the consensus CAP binding structure is located in the vicinity of pL promoter (fig.2). Then, the promoter of ssb gene contains two sites homologous to the consensus (fig.2). Finally, sites of interest have been located in the 3′-end of Ea 22 gene and in the coding region of Ea 59 gene. Both sites are dissociated with a sequence homologous to −35 promoter site (fig.2).

3. DISCUSSION

The cAMP-CAP complex is known to stimulate or repress the initiation of transcription of the catabolite sensitive genes [11] by binding DNA in the promoter region [17–19].

The termination of transcription can also be modulated by this complex [20]. Recently, authors in [21] have suggested an important role for the catabolite effectors in the inhibition of elongation.

The current concept postulates that the CRP-mediated regulation of λ development is indirect and mediated through bacterial gene products. Recent data regarding the primary structure of the λ genome and molecular mechanisms of catabolite repression have allowed us to revise this concept: at least 4 CAP binding sites may be functionally active on the λ genome (fig.1,2).

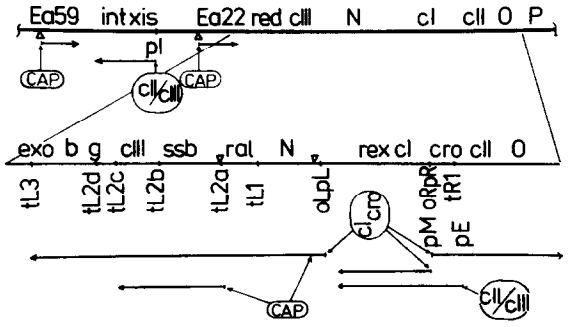

Fig.1. The organization, transcription and regulation of early genes, according to [3]. The region between Ea22 and P is enlarged in the lower line. Below this line, relevant promoter and terminator sites are indicated; below both lines transcripts are shown. Putative catabolite sensitive transcripts are labeled CAP; CAP binding sites are indicated by ▽.

Regarding the CAP-binding site in the vicinity of the pL promoter, it is conceivable that the cAMP–CAP compex facilitates the binding of the N gene product to RNA polymerase at nutL site, thus promoting an antitermination function of N protein, rather than the initiation of transcription itself. This suggestion is deduced from the finding that the discussed putative CAP binding site is located on the antisense strand within 93–108 nucleotides downstream from the transcription startpoint (fig.2). Normally, CAP binding sites involved in modulation of initiation of transcription are closely linked with the promoter specific sequences and lie on the sense strand of DNA [21].

In an early stage of phage infection, when the amount of N protein in the cell is low, a number of terminators identified in the region between N and exo λ genes (fig.1) hamper the transcription from the pL promoter. Two first terminators (tL1 and tL2a) are 92% efficient together. The next one, tL2b, is 59% efficient alone [22]. Hence, the pL transcription downstream tL2b decreases to a minimal level. The CGAS function at nutL site can eventually result in an increase in the efficiency of ssb and cIII gene expression (fig.1). Stimulation of λ cIII function is relevant to the observations on the effect of the catabolite repression in the lysogenic pathway [4–7] (see section 1); it leads to an increase in the lysogenization frequency.

The promoter region of ssb gene contains two sites of catabolic regulation with 6 and 9 nucleotides homology to the consensus structure (fig.2). Recently, authors in [22] identified a terminator tL2a in this region (fig.2). Since the region contains promoter-specific sequences we suggest that the CGAS mediates the positive regulation of the transcription of gene ssb. We can imagine that the cAMP–CAP complex binds to the promoter-specific sequences and stimulates the interaction of RNA-polymerase with −35 and −10 promoter sequences. However, cAMP–CAP-mediated modulation of transcription termination at tL2a site is also conceivable, although tL2a is a ϱ-independent signal [22]; normally, catabolite-sensitive termina-

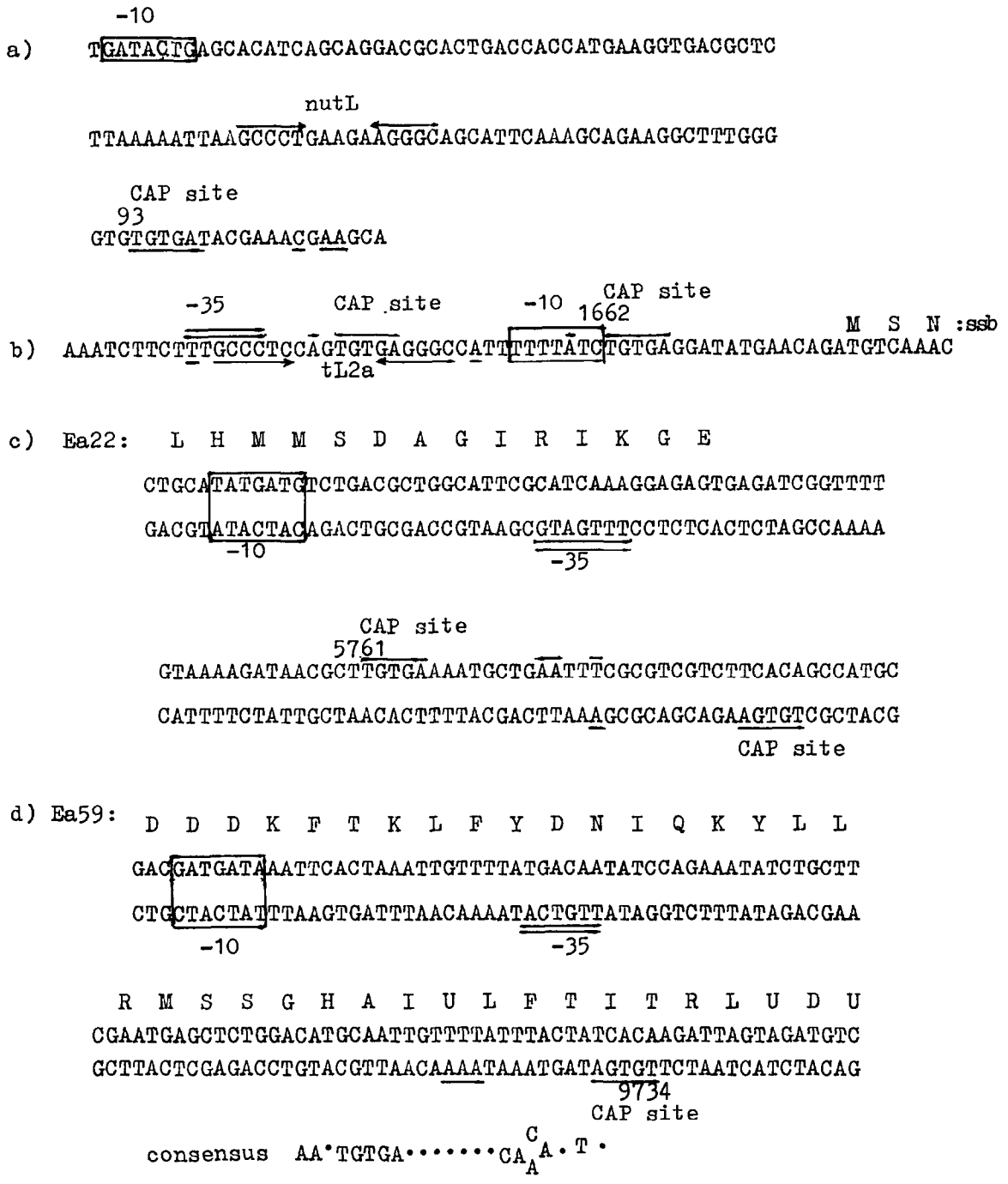

Fig.2. The sequence of the λ DNA segments containing putative CAP binding sites: (a) the DNA sequence flanking the 3′-end of the pL promoter [15]; (b) the DNA sequence flanking the 5′-end of the ssb gene [16]; (c) the DNA sequence of the Ea22, and (d) Ea59 gene regions [12]. Numbers indicate the distance from the startpoints of the transcription. Double lines show the −35 promoter sequence; the −10 region is boxed. The terminator tL2a and nutL regions are shown by arrows. Codons for proteins are indicated by one-letter amino acid code. The putative CAP binding sites are outlined; the consensus sequence is given at the bottom.

tions are ϱ-dependent [21]. The function of ssb gene has not yet been determined. The only data we are aware of are by authors in [23], who reported that the gene product is responsible for the tro phenotype and stimulates the lytic growth. As a working model, we propose that the CGAS directly modulates both modes of phage development of wild-type (temperate) λ phages, however, the stimulation of the lysogeny appears to be stronger.

The stimulation of the lytic functions has been revealed in the experiments with the virulent λ phages incapable of cIII-cII-cI mediated lysogenizing the cell (see section 2).

The role of the putative CAP binding sites associated with genes Ea59 and Ea22 in the regulation of λ development is unclear, since the functions of these genes have not yet been determined.

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