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Transcription of the halophage ΦH repressor gene is abolished by transcription from an inversely oriented lytic promoter

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Abstract

The temperate phage ΦH of the extremely halophilic archaebacterium *Halobacterium salinarium* encodes a repressor, Rep, which in the immune state represses the production of an early lytic transcript, denoted T4. Rep acts at the transcriptional level by blocking the promoter for T4. The promoter for the *rep* gene itself is positioned back to back to the promoter for T4, in a manner analogous to that of the *cI/cro* genes in bacteriophage λ. Transcription of the *rep* gene does not occur when the phage is growing lytically. We show that this represson of *rep* transcription during lytic growth is due to the transcription per se from the stronger, oppositely oriented promoter for T4, without the need of a phage gene product.

Key words: Halophage ΦH; Archaebacterium; Transcriptional repression; Lysogeny

1. Introduction

The phage ΦH, infecting the extremely halophilic archaebacterium *Halobacterium salinarium* (formerly *H. halobium*), is the best studied halobacterial phage (for reviews, see [1–3]). ΦH grows lytically with a life cycle of about seven hours. Alternatively, the phage can establish lysogeny as a plasmid [4]. The genome consists of a linear 59-kb ds-DNA molecule with terminally redundant ends [5]. The central 12-kb so-called L region can in certain cases recombine out of the phage genome and establish an independent existence as a plasmid, pΦHL ([6,7]; EMBL/GenBank accession no. X65098). The cells carrying the phage genome or the plasmid pΦHL have acquired immunity from phage infection.

Part of this immunity is mediated by a repressor gene, *rep*, encoded on the L region [8,9]. The *rep* gene product, Rep, acts by binding to the DNA upstream of the promoter for the major early lytic transcript, designed T4, thus shutting off transcription. The action of Rep is enhanced by the product of the *per* gene, which is also encoded on the L region [10].

The promoters for *rep* and T4 are situated back to back (see Fig. 1) in a manner analogous to that of *cI* and *cro* in bacteriophage λ [11]. Transcription from the two promoters is mutually exclusive, with only *rep* transcribed in the immune state and only T4 produced during the lytic cycle [7]. These are the only two promoters on the L region which are seen to be switched on or off in the lytic or lysogenic state. All other described promoters are constitutive [3].

Since Rep represses the promoter for T4, it was plausible that the product encoded by T4 would in turn be responsible for switching off *rep* transcription. We decided to investigate in vivo the mechanism of this repression by transforming *H. salinarium* with different DNA constructs.

2. Materials and methods

2.1. Materials

Restriction enzymes, T4 DNA polymerase, Klenow polymerase, T4 DNA ligase, T4 polynucleotide kinase, Taq DNA polymerase and MLV reverse transcriptase were purchased either from Boehringer Mannheim, USB or Pharmacia. Vent DNA polymerase was from New England Biolabs. [γ-^{32}P]ATP was obtained from Amersham, nylon membranes for Southern transfer from Pall and the Bluescript vector from Stratagene. Puromycin was purchased from Sigma.

2.2. Transformation of halobacteria

The DNA constructs were generated in PCR reactions, using the oligonucleotides depicted in Fig. 1, according to Table 1. The PRC products were ligated to the halobacterial shuttle vector pUBP2, which has been described previously [12]. The vector was a gift from F. Pfeifer, Martinsried. The construction of pT4LT6 has been described previously [9]. The oligonucleotide T4L1 hybridises to a sequence present only in the phage variant ΦHL1, lacking the binding sites for the Rep repressor protein (see [9] for this construct). Transformation of halobacteria was performed as described [12–14].

2.3. Standard molecular biological methods

Where nothing else is given, standard methods were followed [15] without additional modifications. PCR and primer extension experi-

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ments were performed as published [16]. The start points for transcription of T4 and *rep* were already known [7]. Since the promoter for T4 is much stronger than the *rep* promoter [7], different amounts of RNA were used in the assays for transcription: 7.5 μg to monitor T4 expression but 15 μg to monitor *rep*. For the puromycin experiments (according to [7]), a concentration of 100μg antibiotic per ml medium was used. The effects of puromycin were seen as a weakening of repression, resulting in T4 transcription. In the monitoring of T4 production from the cells grown under puromycin, 10 μg RNA was used.

3. Results and discussion

The different constructs used in the transformation experiments to study the mutual expression of *rep* and T4 are shown schematically in Fig. 2. In order to study transcription from the *rep* promoter without repressing the promoter for T4, only the first 80 bp of the *rep* gene were included, producing no functional repressor protein. Thus, the promoter for T4 would be transcribed by all transformants. The construct pT64Ko encodes only the first 60 nt of T4, whereas pT64Lå carries the DNA encoding all of T4. In addition, we included in the studies two constructs, pT4LT6 and pT6, which have been described previously [9]. The construct pT6 carries the *rep* gene alone, which is constitutively expressed by the transformants. The plasmid pT4LT6 carries *rep* in inverted orientation, together with a repressor-insensitive T4-producing DNA. The *H. salinarium* transformants P03-pT4LT6 produce both T4 (at an enhanced level) and the *rep* transcript.

If the T4 gene product were responsible for the repression of the *rep* promoter, the transformants carrying pT64Ko (strain P03-pT64Ko) would show transcription from both promoters, but the cells containing pT64Lå (strain P03-pT64Lå) would show T4 production only. Infecting the P03-pT64Ko cells, thus supplying the T4 product in *trans* from the infecting phage DNA, would be a means to distinguish between possible *cis* and *trans* action of the T4 product.

We used primer extension with the oligonucleotides OT660 and OT455, (Fig. 1), to monitor transcription from the promoters for *rep* and T4, respectively. Production of T4 by the transformants was taken as a proof of successful introduction of the DNA constructs into the *H. salinarium* cells.

The result of the experiment is shown in Fig. 2. In the event, neither the cells carrying the pT64Ko nor those with the pT64Lå construct showed significant transcription from the *rep* promoter (Fig. 2c and d), though they

Fig. 1. DNA sequence of the regions encoding the *rep* and T4 transcripts, showing the promoter structures as well as the sequences of the oligonucleotides used in generating the DNA constructs used in the transformation studies. The repressor binding sites are shown in boldfaced, dotted underlined type. Start points of transcription are shown in boldfaced underlined type and the direction of transcription indicated. Consensus 'Box A' sequences for archaebacterial promoters [19] are underlined. Oligonucleotides used in PCR reactions and primer extension assays are shown under the sequence. Note that sequence has been omitted between the hybridisation point for OT6P1 and O64P as well as between the hybridisation points for OT64B and OT4L2. The numbering is according to the published sequence of the L plasmid [7].

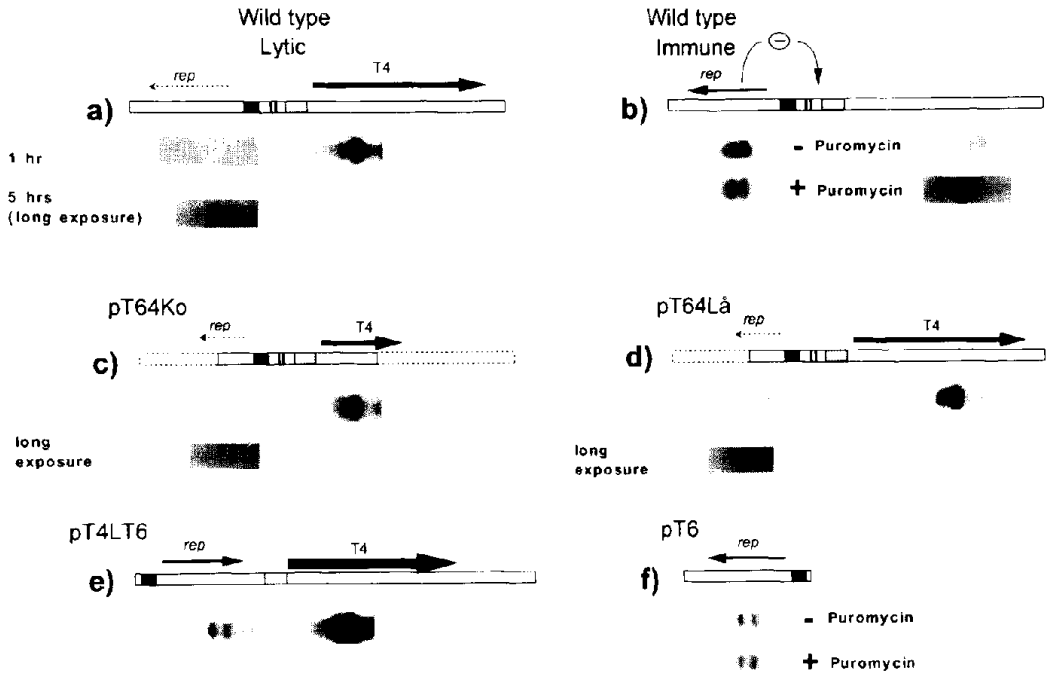

Fig. 2. Schematic map of the DNA constructs used in this assay as well as the transcription signals from T4 and *rep* detected by primer extension. Transcription is represented by arrows, the thickness of the arrows roughly indicating the intensity of the primer extension signal. A dashed arrow indicates an almost undetectable transcription signal. The *rep* promoter is represented by a shaded box, the T4 promoter is hatched and the Rep binding sites are shown as two black bars (not present on the constructs pT4LT6 and pT6). The dashed boxes indicate DNA which is not present on the pT64Ko and pT64Lå constructs, respectively. Primer extension signals from transcription are shown under each diagram. Note that different amounts of RNA were used in the puromycin experiments than in the others (see main text).

transcribed the T4 promoter, confirming the succesful transformation. Infecting strain P03-pT64Ko with ΦH had no effect on transcription. In contrast, the *rep* gene cloned on pT6 and pT4LT6 is constitutively transcribed (Fig. 2e and f). Thus, changing the relative positions of the *rep* and the T4-producing DNA is sufficient for derepression of *rep* transcription, even when T4 is still produced from the same vector, as in P03-pT4LT6.

These findings indicate that the product encoded on the DNA transcribed as T4 has no effect on *rep* transcription, but that it is transcription per se of the T4 promoter that represses *rep* expression.

The possibility remained that the *rep* product were necessary for efficient transcription of its own promoter, i.e. that the truncation of the *rep* gene in the construct, and not the transcription of T4, were responsible for the lack of *rep* transcription. This was considered unlikely, since the gene is constitutively expressed by the transformants P03-pT6 and P03-pT4LT6, which carry *rep* genes where only one repressor-binding site is present (see Fig. 1). Nevertheless, we tested this possibility by growing the *H. salinarium* strain P03-pT6, expressing the *rep* gene, in a medium containing a potent inhibitor of translation, puromycin [7]. The Rep protein confers only a limited degree of immunity to transformants [9], which could be taken as an indication that the protein is unstable. If the Rep protein has a positive influence on *rep* transcription, abolishing protein synthesis should result in lowered expression of the gene. As a control of the effects of puromycin, we grew the immune strain R_1L under the same conditions. The strain R_1L carries the plasmid pΦHL, expresses *rep* but not T4 and is immune from phage infection [7].

Under puromycin, neither the P03-pT6 transformants nor the strain R_1L showed diminished transcription of the *rep* gene (Fig. 2b and f). (The double bands seen from the *rep* transcript were present in all RNA preparations used; double signals are a common phenomenon in mapping ΦH promoters [7]).

In the RNA from strain R_1L, a weak signal from emerging T4 transcription could be observed one hour

Table 1
Oligonucleotides employed in amplifying the DNA fragments inserted in the halobacterial shuttle vector pUBP2 to generate the different constructs described in this work

DNA construct generated	Oligonucleotides employed
pT6	OT6P1, OT6P2
pT4LT6	OT6P1, OT6P2, OT4L1*, OT4L2
pT64Ko	O64P, OT64B
pT64Lå	O64P, OT4L2

*The oligonucleotide OT4L1 hybridises to a part of an IS element which is present in the L region only in the phage variant ΦHL1. See [9] for the construction of this DNA.

after puromycin addition (Fig. 2b; 10 μg RNA used). This indicated that the repression indeed was weakening, due to the puromycin. This low level of T4 transcription has no appreciable effect on the *rep* promoter. The immunity conferred by the *rep* gene alone has been reported as fairly weak [9] and the weakening of repression under puromycin indicates that the *rep* gene product indeed is unstable, though it does not regulate its own expression.

A rigorous proof of the lack of *rep*-autoregulation would be to introduce a truncated *rep* gene into *H. salinarium* and check for expression. We did however further observe, in long exposure of the primer extension films, a very faint signal from the *rep* gene in the case of lytically growing phage, were the entire *rep* gene is present (lower part of Fig. 2a). (The signal was present as well in the RNA from the P03-pT4Ko and P03-pT4Lå transformants). Thus the *rep* gene is actually transcribed at a very low level during lytic growth, but the repressor produced has no effect on its own synthesis, nor on T4 transcription.

The situation where two promoters are oriented back to back is very common in eubacterial phages (reviewed in [17]) but the case of inhibition of transcription from one promoter by transcription from another is an unusual mechanism which as far as we know has mostly been reported for eucaryotes and not been encountered in the archaeacteria. It contrasts with the situation for example in coliphage λ, where C1 repressor binding to the operators O_{R1} and O_{R2} represses *cro* transcription and stimulates its own synthesis, while Cro binding to O_{R3} in turn represses *cI* transcription [11]. One instance of mutually exclusive transcription has been described for the *Salmonella* phage P22, where the *arc ant* and *mnt* promoters are located back to back and down-mutations in the *arc ant* promoter result in increased *mnt* transcription (McClure et al., cited in [18]). We note however, that the distance between the start points of transcription for *rep* and T4 is 127 bp, which is substantially longer than the 36 bp separating the *mnt* and *arc ant* start points. The steric effects of strong T4 production must be quite pronounced in order to inhibit transcription over such a distance. It seems possible that the inhibitory effects are due to the presence of transcription factors binding upstream of the T4 'Box A' region [19] (Fig. 1), though the molecular details of *H. salinarium* transcription have been notoriously difficult to unravel (see for example [20]).

Transcription from the T4 promoter is not seen until 30 min post infection, whereas the earliest lytic ΦH transcription becomes observeable after 10 minutes [16]. The fact that *rep* needs to produce an (unstable) protein before repression can take place but the T4 promoter only needs to attract enough polymerase to excert its inhibitory effect may be one reason why lysogeny is very seldom established by ΦH ([21]; Stolt, unpublished).

The indications that the Rep repressor is unstable also suggests a possible role for the product of the *per* gene, which enhances the effiency of *rep* [10]. Clearly, gene expression in ΦH is more subtle than the simple switching on or off of different genes.

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