Volume 285, number 2, 257–259 FEBS 09893 July 1991

ADONIS 001457939100604A

Heterologous in vitro transcription from two archaebacterial promoters

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Received 22 March 1991; revised version received 3 May 1991

A cell-free extract of *Sulfolobus shibatae* is able to specifically initiate transcription in vitro at the promoter of the plasmid-encoded gene for the major gas vesicle protein of *Halobacterium halobium* and at the promoter for the transcript T4 of the temperate *H. halobium* phage ΦH. The corresponding promoter from the virulent phage mutant ΦHL1 yields enhanced transcription in the heterologous system, in agreement with strongly increased in vivo expression.

Transcription initiation; Cell-free extract; Promoter; Evolution; Phage ΦH; Gas vacuole gene

1. INTRODUCTION

The domain of the Archaea (or archaebacteria) consists of 2 major branches, one termed *Crenarchaeota* comprising extremely thermophilic usually sulfur-dependent organisms of the orders *Thermoproteales* and *Sulfolobales*, while the other, termed *Euryarchaeota*, encompasses the methanogens, the *Thermococcales*, the genus *Thermoplasma* and the extreme halophiles [1]. Recently we described the specific initiation of transcription in vitro at the promoters of 2 rRNA genes by a cell-free extract of *Sulfolobus shibatae* [2]. Using that system we identified 2 sequence elements within an archaebacterial core-promoter region essential for the initiation of transcription. The distal promoter element, DPE, encompasses the 'box A' motif [3,4] which is conserved for the majority of archaebacterial promoters and which resembles the eukaryotic TATA-box of RNA polymerase II promoters. DPE is essential for the efficiency of transcription initiation in the *S. shibatae* system, together with the promoter-specific proximal promoter element, PPE, and is furthermore involved in start-site selection [5]. We have now tested whether promoters from the halophilic branch of archaebacteria are utilised for in vitro t ·:scription in the heterologous system from *S. shibatae*.

2. MATERIALS AND METHODS

2.1. *Materials*

S1 endonuclease, T4 polynucleotide kinase and the Klenow fragment of *E. coli* DNA polymerase I were purchased from Pharmacia, while RNase free DNase I was from Boehringer Mannheim. All radiochemicals were obtained from Amersham.

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2.2. *DNA templates for* in vitro *transcription*

A 936 bp *Hin*dIII fragment from *H. halobium* containing the promoter of the plasmid encoded *p-vac* gene was cloned into pUN121, yielding Pp-vac. The corresponding Pc-vac clone was obtained by inserting a 1.4 kb *Sma*I fragment with the chromosomal encoded *c-vac* promoter into pUN121 [6]. An 800 bp *Mlu*I fragment of *H. mediterranei* containing the *mc-vac* promoter was cloned into pUC19, yielding Pmc-vac [7]. The promoter templates for the transcript T4, PΦH1 and pΦHL1, contained a *Bam*HI fragment of the L-region of *Halobacterium* phage ΦH1 and ΦHL1, respectively, cloned into the pSVcat vector [8].

2.3. *Preparation of a soluble cell-free extract*

The extract was prepared as described previously [2]. Please note that *Sulfolobus* sp. *B12* was renamed *S. shibatae* [9].

2.4. *Preparation of S1-probes and nucleotide sequencing*

Single stranded S1-probe synthesis and nucleotide sequencing were performed as described previously [2]. The primers and templates for S1-probe synthesis were chosen according to Horne and Pfeifer [6], Englert et al. [7] and Gropp et al. [8,10].

2.5. *In vitro transcription experiments and S1 nuclease analysis*

Standard in vitro transcription reactions and S1 nuclease analysis were performed as recently described [2]. Deviations from the standard reaction are outlined in the figure legends.

3. RESULTS AND DISCUSSION

Three promoters of genes coding for the major gas vesicle protein were tested for the initiation of heterologous in vitro transcription by the *S. shibatae* cell-free extract (Fig. 1), the promoter of the plasmid encoded p-*vac* gene and of the chromosomal encoded c-*vac* gene of *H. halobium*, and the promoter of the chromosomal encoded mc-*vac* gene of *H. mediterranei* [6,7]. Initiation occurred at the promoter of the p-*vac* gene of *H. halobium*, specifically at the same nucleotide as in vivo. Neither of the chromosomal *vac* gene promoters was used. A comparison of the promoter structures of these genes to the archaebacterial core promoter structure [3,4,10] revealed a significant dif-

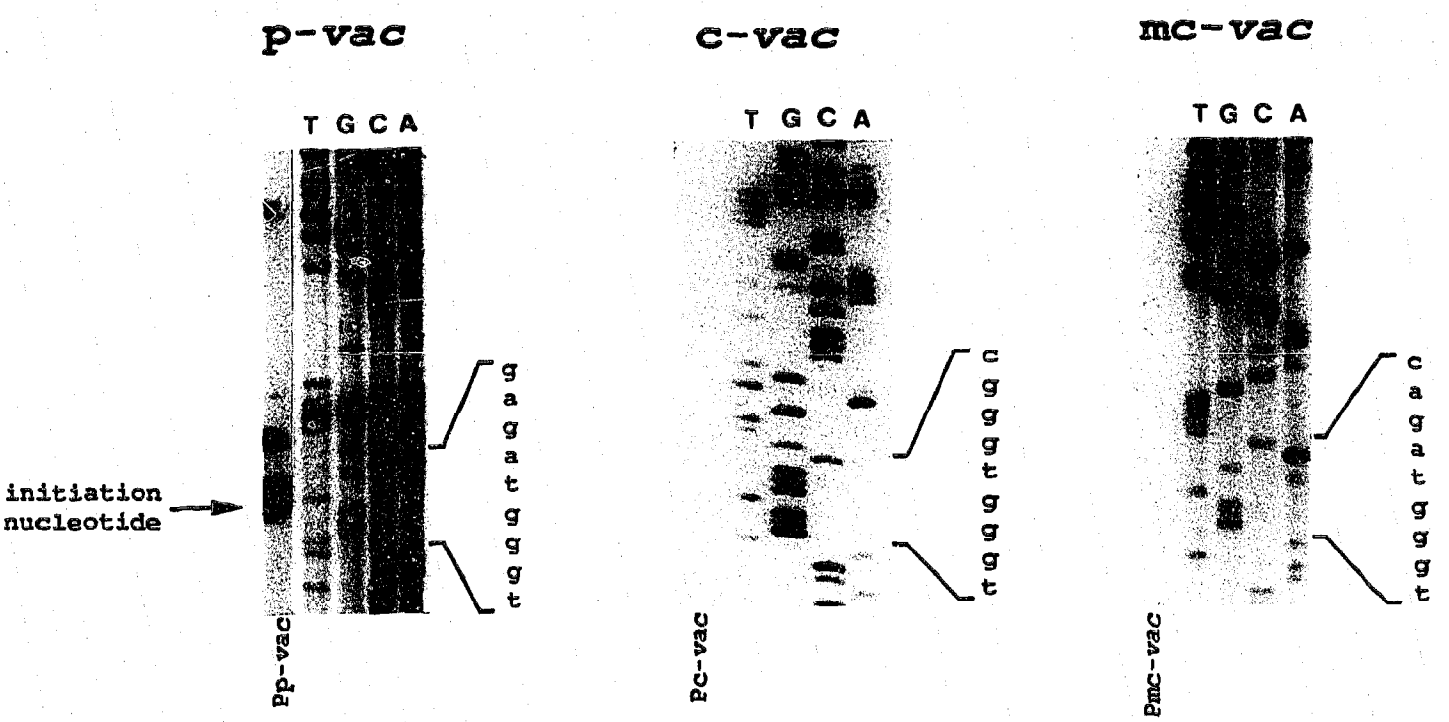

Fig. 1. S1 nuclease analysis of in vitro transcription products obtained with 1.5 μg of DNA template, each one specified below the corresponding lane, and 8 μl of *S. shibatae* extract. The sequence context around the in vivo used initiation site (compare Fig. 2) is shown by the respective sequencing ladder (lanes A, C, G, T).

ference between the box A motifs within the DPEs. The box A of the p-*vac* promoter conforms to the archaebacterial promoter consensus, both in its distance from the initiation site (24 nucleotides) and in its sequence (Fig. 2), while in the case of the c-*vac* gene promoters no box A-like motif could be found in the expected region. In contrast to the constitutive expression of the p-*vac* gene, the expression of the c-*vac* and mc-*vac* genes is regulated in vivo [6,7]. The promoter for the UV-inducible SSV1 transcript T_{ind} [3] and the promoter of the bacterio-opsin related protein (*brp*) gene

H. halobium		
	p-*vac*	acacatcc**TTATGT**gatgcccgagtatagttagagatgggt
	c-*vac*	aacggcggttttccggacactccctgtagttgcgggtgggt
	brp	gtctttttttgatgctcggtagtgacgtgtgtattcatatg
H. mediterranei		
	mc-*vac*	acgaatgattttgttacttgccaacacgttttcagatgggt
phage ØH		
	T4	gaatagat**ATAAGT**tagacccctcgtaaagtccagactgac
S. shibatae		
	16S/23S rRNA	agttagat**TTATAT**gggatttcagaacaatatgtataatgc
SSV1		
	T ind	gtcgactctgtgtatcttatgtatcttatacaaaaaatatg
box A consensus sequence		T T TTA N A A

Fig. 2. Promoter regions of archaebacterial genes. The box A motifs are shown in uppercase letters, all in vivo used initiation sites are marked by black boxes above the sense DNA strands.

of *H. halobium* [11] are examples of 2 other regulated archaebacterial promoters which also show no box A motif (Fig. 2). One mode of archaebacterial gene-regulation could therefore involve modification of the box A motif concomitant with the requirement for corresponding specific transcription factors. The *S. shibatae* system appears only to be able to utilise those heterologous promoters which are constitutively expressed.

We also studied the early lytic gene encoding transcript T4 of the *Halobacterium* phage ΦH. The box A of the T4 promoter conforms well to the consensus sequence and we found specific initiation of transcription by the *Sulfolobus* extract (Fig. 3). This gene is located in the L-region of the phage ΦH DNA. The presence of this region as a plasmid confers resistance to ΦH infection to the halobacterial host [12]. However, a phage mutant carrying an insert in its L-region, ΦHL1, is able to multiply in this immune host. Although the mechanism of this escape is not known in detail, it involves a strongly enhanced production of transcript T4. The difference between the T4 promoter of ΦHL1 to the T4 promoter of ΦH is the insertion of the sequence element ISH23/50 [13,14] immediately upstream of the box A motif. We found that the heterologous system also shows enhanced transcription from a promoter construct (PΦHL1) which carries the insertion element, compared to transcription from the wild-type promoter (Fig. 3). A possible explanation would be that the inser-

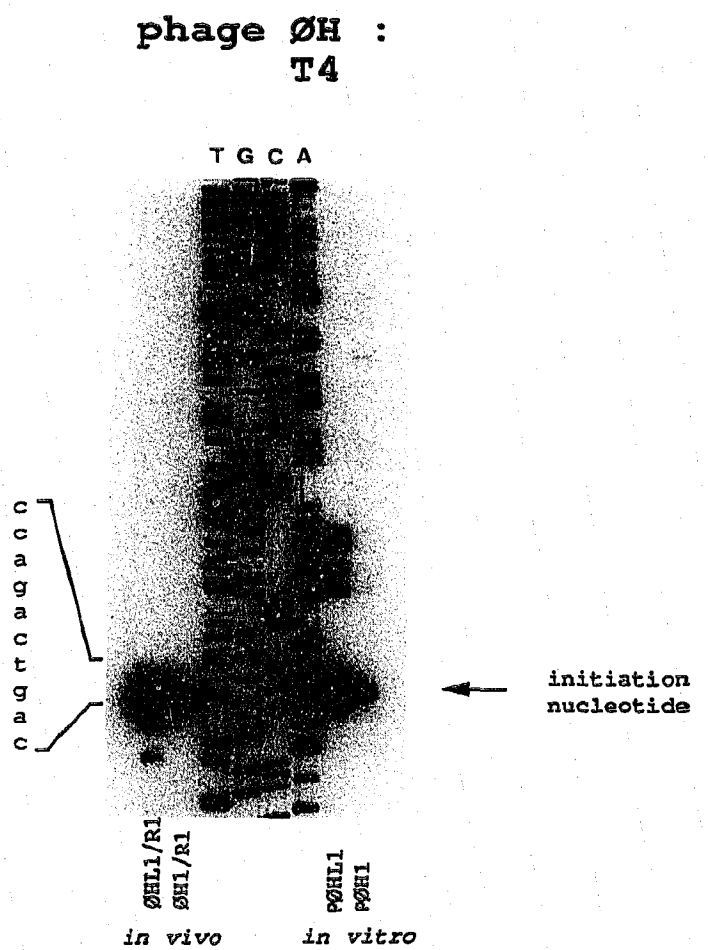

Fig. 3. S1 nuclease analysis of 1 μg in vivo RNA [8] and of in vitro transcription products, obtained with 1 μg DNA template and 20 μl *S. shibatae* extract.

tion element carries an enhancer-like sequence which is acting in the in vitro system. This hypothesis would require that the sequence and its cognate DNA-binding protein are conserved within both branches of archaebacteria. Since detailed investigation of the archaebacterial transcription mechanism just started [2,15,16], nothing is known so far about the existence of archaebacterial enhancers. A second possibility would be that a sequence involved in negative control and located immediately upstream of the promoter for transcript T4 is destroyed or moved away by insertion of the ISH23/50 element. This hypothesis seems to be true for the increased expression in vivo, since several repressor sites have been mapped in this region [17]. Although we have identified a sequence with a negative control function upstream of the promoter of the 16 S/23 S rRNA operon of *S. shibatae* [5], we found no obvious similarity to the sequence upstream of the T4 promoter. Alternatively, the increased transcription rate from the ΦHL1-T4 promoter may have different causes in vivo and in the heterologous in vitro system.

We have shown that an *S. shibatae* extract is able to transcribe 2 halobacterial genes. This result indicates that the basal transcription apparatus is conserved throughout all archaebacteria. In particular, the TATA-like box A motif seems to be of primary importance for the interaction with the heterologous RNA polymerase or putative transcription factors and therefore reflects most likely an ancestral promoter motif.

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