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Interaction of bacteriophage T4 *AsiA* protein with *Escherichia coli* σ^{70} and its variant

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Abstract Bacteriophage T4 produces a small protein *AsiA*, which inhibits transcription from σ^{70}-dependent promoters in *E. coli* by tightly binding to σ^{70} and is therefore termed as anti-sigma factor. We observed that there was no inhibition of single round transcription at *lac UV5* promoter when *AsiA* was added to preformed open complex between RNA polymerase and template DNA. However, transcription was found to proceed normally at 'extended −10' promoters in the presence of *AsiA*. It appears therefore that *AsiA* binds σ^{70} at its 4.2-subdomain or in its close vicinity. Further experiments on immunoprecipitation of σ^{70} and a mutant σ^{70}-V576G with *AsiA* seem to corroborate such conclusion.

Key words: *AsiA*; T4; σ^{70}; Interaction; 4.2 Subdomain

1. Introduction

The primary sigma factor, σ^{70}, of *Escherichia coli* directs promoter specific transcription of most of the bacterial chromosome [1]. The domain-wise activity of σ^{70} has now been well characterized. It appears that the 2.4- and 4.2-subdomains of σ^{70} are responsible for −10 and −35 promoter recognition respectively [2–4]. Various sigma factors from different bacterial species show sequence similarity and high degree of conservation in these promoter recognition elements [5].

A small protein (10 kDa) of bacteriophage T4, *AsiA*, which was first reported several years back [6] inhibits σ^{70} dependent transcriptional activity in *E. coli*. The *AsiA* protein was later termed as anti-sigma factor and it is a part of the T4 bacteriophage regulatory mechanism that shuts off host transcription after infection [7–9]. It was observed that the transcription of T4 genome is regulated in time as infection proceeds. Its middle promoters have excellent matches to the −10 consensus sequence for the σ^{70} subunit, but a binding site for the T4 transcriptional activator *MotA* replaces σ^{70}-35 consensus [10,11]. *AsiA* protein, a product of early RNA of T4, binds σ^{70} tightly and regulates transcription from its middle promoters [12]. This work was motivated with an idea to find out how *AsiA* interacts with σ^{70} and thereby regulates transcription from T4 middle promoters.

2. Materials and methods

The plasmid *pAsiA*, a source for anti-sigma factor was a kind gift from Deborah Hinton, NIH, USA. We followed essentially the same protocol for expression and purification of *AsiA* as reported earlier [12]. *E. coli* RNA polymerase, σ^{70} and the reconstituted holo RNA polymerase were obtained as reported before [13]. All other materials were of purest grade available. A 203-bp long *Eco*RI fragment containing *lac UV5* promoter was a kind gift from Akira Ishihama, NIG, Japan and was used as a template to get a 63-nucleotide long transcript. Similarly, the plasmid carrying the extended −10 promoter (pML-P_{RE}) [14] was a kind gift from Richard S. Hayward, Edinburgh, UK. In vitro transcription was carried out as reported earlier [13]. Quantitation of transcripts was carried out with the help of an image-analyzer (Molecular Dynamics).

3. Results and discussion

Fig. 1 shows single round heparin resistant in vitro transcription at *lac UV5* with different order of addition of *AsiA* and holo RNA polymerase to the template. To observe any effect of *AsiA* on in vitro transcription, it is necessary to use high molar ratio of *AsiA* to *E. coli* RNA polymerase (1 : 25, σ^{70} : *AsiA*, molar ratio) [11].

During in vitro transcription, holo RNA polymerase was incubated with *lac UV5* at 37°C for 15–30 min to allow for open complex formation. Subsequently mixture of NTPs and

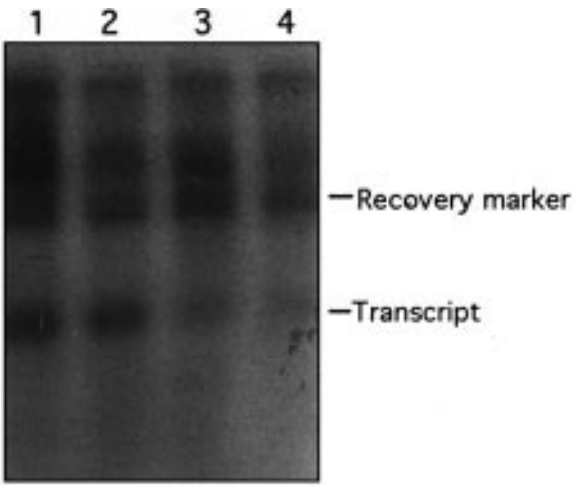

Fig. 1. Single round in vitro transcription at *lac UV5* promoter in the presence of *AsiA* protein. 0.2 pmol of linear template was incubated with 2 pmol of reconstituted holo *E. coli* RNA polymerase at 37°C for 15–30 min prior to the addition of NTP mix and heparin. Subsequently the reaction was allowed to proceed for 5 min at 37°C and the reaction was stopped with EDTA. A recovery marker was added along with the stop solution. Lane 1: control (*lac UV5*+RNA polymerase); lane 2: *lac UV5* was incubated with RNA polymerase for 15 min at 37°C and then 50 pmol of *AsiA* was added and the complex was further incubated for 15 min prior to the addition of NTP and heparin mix. Lane 3: Holo RNA polymerase (2 pmol) was incubated with 50 pmol of *AsiA* at 37°C for 15 min prior to the addition of *lac UV5*. Lane 4: σ^{70} (2 pmol) was incubated with 50 pmol of *AsiA* at 37°C for 15 min, incubated further with core RNA polymerase (2 pmol) at 37°C for 5 min before the addition of *lac UV5*.

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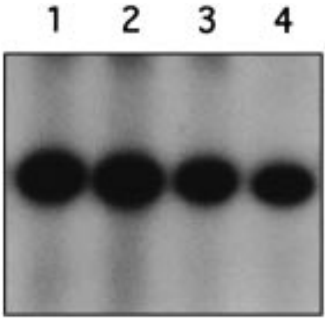

Fig. 2. Single round in vitro transcription at 'extended −10 promoter' in the presence of *AsiA* protein. Order of addition of RNA polymerase and *AsiA* to the template DNA in lanes 1–4 was same as in Fig. 1.

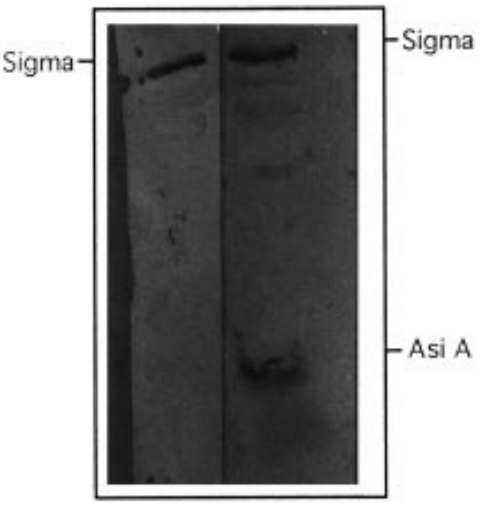

Fig. 3. Western blot analysis of *E. coli* RNA polymerase-*AsiA* complex with anti-σ^{70} and anti-*AsiA* antibodies. The complex between holo RNA polymerase containing σ^{70} or σ^{70}-V576G were incubated with excess of *AsiA* in a normal transcription buffer. To each polyclonal antirabbit σ^{70}-antibody was added prior to immunoprecipitation with protein A-Sepharose beads. The precipitated complex was electrophoresed on a 13% SDS-polyacrylamide gel and the gel was subjected to Western analysis with anti-σ^{70} and anti-*AsiA* antibodies. Left lane: σ^{70}-V576G; right lane: σ^{70}.

heparin were added and transcription was allowed to proceed for 5 min before the reaction was terminated. It can be seen from Fig. 1, lane 2, that once the open complex between enzyme and promoter was formed, *AsiA* has no effect on single round transcription. However, when RNA polymerase or σ^{70} alone were incubated with *AsiA* prior to the addition of template, 70–80% inhibition of transcription was noticed (lanes 3 and 4, and Table 1). A 104 base-pair long *Bam*HI fragment of T7A1 promoter was used as a recovery marker to control any error during recovery of the RNA product. To quantitate the degree of inhibition of transcription, at least three independent experiments were performed and the autoradiograph was subsequently scanned with the help of a laser scanner (Table 1). Thus, the value of 80% inhibition represents an average value.

In a subsequent experiment a *Eco*RI-*Pvu*II fragment of plasmid pML-P_{RE} was used as a template. In this DNA, normal −35 sequence is replaced with a synthetic extended −10 sequence TGTTATAAT [14]. The product in such a template is 174 long RNA as characterized before [14,15]. It can be seen from Fig. 2 that very little inhibition of transcription, if any, was observed in this case when *AsiA* was incubated with free σ^{70} or RNA polymerase prior to the addition of the template. Here the value of inhibition of transcription was always less than 20% in different independent experiments (Table 1).

The above two sets of experiments taken together indicate that (a) *AsiA* protein does not inhibit transcription once the open complex between promoter and RNA polymerase is formed and (b) if the −35 sequence in the promoter is altered then *AsiA* bound σ^{70} or hole RNA polymerase is capable in making the full length RNA chain. Thus, it appears −35 promoter recognition domain of σ^{70}, i.e. 4.2-subdomain or its vicinity is the site of action of *AsiA*. Therefore, *AsiA* bound RNA polymerase is oblivious about the presence of −35 box at extended −10 promoter. On the other hand, when both −35 and −10 promoter consensus were present, inhibition of transcription occurs in the presence of *AsiA*. Presumably the nature of interaction between extended −10 promoter and RNA polymerase is different from that with a normal promoter.

During the course of our investigation on the structure-function relationship in σ^{70}, we reported a mutant σ^{70}, where site-specific alteration was carried out in the conserved 4.2-subdomain (σ^{70}-V576G) [15]. This protein has normal core RNA polymerase binding ability, however, upon reconstitution with core RNA polymerase was found to be inefficient in promoter recognition. To ensure that the 4.2-subdomain of σ^{70} is the binding site of *AsiA*, we incubated holo RNA polymerase containing σ^{70} and σ^{70}-V576G with excess of *AsiA* and subsequently immuno-precipitated the complex with anti-σ^{70}-antibody. Fig. 3 shows that with normal σ^{70}, *AsiA* is also co-precipitated whereas it remained unbound to σ^{70}-V576G mutant. It should be mentioned here that σ^{70}-V576G appears to be conformationally similar to native σ^{70} [15], however, with an altered promoter recognition ability. The variation in the intra-helix distance at the 4.2-subdomain of the mutant σ^{70} in comparison to that of wild type σ^{70} seems to be responsible for the change in DNA binding.

It has been shown recently that in the presence of *AsiA*, *Mot A* dependent transcription ensues from T4 middle pro-

Table 1
Degree of inhibition of 63-nucleotide long *lac UV5* transcript in the presence of *AsiA*

Sample	Normalized intensity of the transcript	% Inhibition
(DNA+RPase)	5134	–
(DNA+RPase)+*AsiA*	4630	10
DNA+(RPase+*AsiA*)	1396	73
DNA+[core RPase+(σ^{70}+*AsiA*)]	1150	78

[a]RPase stands for RNA polymerase.
[b]Degree of inhibition was normalized with respect to background and recovery marker.

moter by unmodified RNA polymerase [9]. However, unmodified RNA polymerase recognises middle promoter of T4 as extended −10 promoters [11]. Hinton et al. [11] suggested that holo RNA polymerase in the presence of *AsiA* interacts with the T4 middle promoter *Puvsx* weakly, although the '−35 region' of *Puvsx* resembles extended '−10 region'. We do not know the reason for this discrepancy with our result which shows no effect of *AsiA* on the transcription from a classical 'extended −10 promoter'. However, one should note that transcription at T4 middle promoter requires the presence of two accessary factors like *AsiA* and *Mot A*.

The work reported in this study suggests that in vitro, when *AsiA* is present, *E. coli* RNA polymerase can transcribe only extended −10 promoters whereas normal promoters with −35 and −10 consensus remain inhibited.

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References

[1] Helmann, J.D. and Chamberlin, M.J. (1981) Ann. Rev. Biochem. 57, 839–872.
[2] Gardella, T., Moyle, H. and Susskind, M.M. (1989) J. Mol. Biol. 206, 579–590.
[3] Siegele, D.A., Hu, J.C., Watter, W.A. and Gross, C.A. (1989) J. Mol. Biol. 206, 591–603.
[4] Waldburger, C., Gardella, T., Wong, R. and Susskind, M.M. (1990) J. Mol. Biol. 215, 267–276.
[5] Gross, C.A., Lonetto, M. and Losick, R. (1992) in: Transcription Regulation (Mcknight, S.L. and Yamamoto, K.R., Eds.) pp. 129–176, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[6] Stevens, A. (1976) in: RNA Polymerase (Losick, R. and Chamberlin, M., Eds.) pp. 617–627, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[7] Brown, K.L. and Hughes, K.T. (1995) Mol. Microbiol. 16, 397–404.
[8] Kassavetis, G.A. and Geiduschek, E.P. (1984) Proc. Natl. Acad. Sci. USA 81, 5101–5105.
[9] Stitt, B. and Hinton, D. (1994) in: Molecular Biology of Bacteriophage T4 (Karam, J.D. et al., Eds.) pp. 142–160, American Society for Microbiology, Washington, DC, .
[10] Guild, N., Gayle, M., Sweeney, T., Hollingsworth, T., Modeer, T. and Gold, L. (1988) J. Mol. Biol. 199, 241–258.
[11] Hinton, D.M., Amegadzie, R.M., Gerber, J.S. and Sharma, M. (1996) J. Mol. Biol. 256, 235–248.
[12] Hinton, D.M., Amegadzie, R.M., Gerber, J.S. and Sharma, M. (1996) Methods Enzymol. 274, 43–57.
[13] Gopal, V., Ma, H.W., Kumaran, M.K. and Chatterji, D. (1994) J. Mol. Biol. 242, 9–22.
[14] Kumar, A., Malloch, R.A., Fujita, N., Smillie, D.A., Ishihama, A. and Hayward, R.S. (1993) J. Mol. Biol. 232, 406–418.
[15] Reddy, B.V.B., Gopal, V. and Chatterji, D. (1997) J. Biomol. Struct. Dyn. 14, 407–419.