Effect of the DNA-binding domain of poly(ADP-ribose)synthetase on accurate transcription initiation in a HeLa cell lysate

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The effect of papain-digested fragments of poly(ADP-ribose)synthetase on accurate transcription initiation by RNA polymerase II was studied using a HeLa cell lysate. It was found that the DNA-binding domain of the enzyme, like the intact enzyme, preferentially suppressed random transcription initiation, whereas the automodification domain had no effect on transcription.

Accurate transcription initiation *Poly(ADP-ribose)synthetase* *HeLa cell lysate* *DNA-binding domain*

1. INTRODUCTION

The mechanism of control of the initiation of transcription catalyzed by RNA polymerase II is important for regulation of eukaryotic gene expression. With the use of HeLa cell and KB cell lysates, in which accurate transcription initiation takes place [1,2], it has been demonstrated that in addition to RNA polymerase II, several protein factors are essential for this reaction [3–6]. We suggested that one of these proteins is a stimulatory protein of RNA polymerase II, named S-II, since antibody against S-II significantly repressed the synthesis of run-off RNA initiated from a correct initiation site on truncated DNA in a HeLa cell lysate [7]. Authors in [8] fractionated a nuclear extract of HeLa cells and identified poly(ADP-ribose)synthetase as one of the factors that is indispensable for accurate transcription initiation. They showed that this enzyme suppressed nick-induced random transcription initiation in the absence of NAD, which is a substrate for the synthesis of poly(ADP-ribose) [8]. Thus, S-II and poly(ADP-ribose)synthetase are the only proteins so far known to participate in accurate transcription initiation in vitro and they have been purified to homogeneity.

Recently, authors in [9,10] developed a method to cleave poly(ADP-ribose)synthetase into a DNA-binding domain and automodification domain (catalytic domain) by digestion with papain. This paper demonstrates that the DNA-binding domain, but not the autocatalytic domain of poly(ADP-ribose)synthetase is responsible for suppression of nick-induced random transcription initiation in a HeLa cell lysate, resulting in the production of run-off RNA initiated from the correct initiation site on truncated DNA.

2. MATERIALS AND METHODS

2.1. *Assay of accurate transcription initiation*

A HeLa cell lysate for RNA synthesis was prepared as in [1]. The final preparation contained 21 mg/ml of protein in buffer consisting of 20 mM Hepes (pH 7.9), 100 mM KCl, 12.5 mM $MgCl_2$, 0.1 mM EDTA, 2 mM dithiothreitol, and 17% (v/v) glycerol. The standard reaction mixture for

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accurate transcription, in a total volume of 25 μl, consisted of 12 mM Hepes (pH 7.9), 7.5 mM $MgCl_2$, 60 mM KCl, 0.2 mM EDTA, 1.3 mM dithiothreitol, 10% glycerol, 500 μM ATP, CTP and GTP, 5 μM UTP containing 10 μCi of [α-^{32}P]UTP (Amersham, 410 Ci/mmol), 60 μg/ml of *Sma*I-digested pSmaF of adenovirus serotype 2 DNA (11.6–18.2 map units) and 4–9 μl of a HeLa cell lysate. The reaction mixture was incubated at 30°C for 60 min. RNA was extracted from the reaction mixture as in [1] and analyzed by 7 M urea–5% polyacrylamide gel electrophoresis followed by autoradiography.

2.2. *Poly(ADP-ribose)synthetase and its functional domains*

Poly(ADP-ribose)synthetase was purified from calf thymus as in [11]. The DNA-binding domain (M_r 46000) and automodification domain (M_r 74000) of poly(ADP-ribose)synthetase were prepared by mild digestion with papain as in [9]. The resulting polypeptides each gave a single band on SDS-polyacrylamide gel electrophoresis.

3. RESULTS AND DISCUSSION

Poly(ADP-ribose)synthetase was shown to be one of the factors necessary for accurate transcription initiation in vitro [8]. Since this enzyme is known to modify various proteins resulting in change in their biological activity, it seemed interesting to determine whether the catalytic activity of this enzyme is indispensable for accurate transcription initiation in vitro.

To test this, we prepared the DNA-binding domain and the automodification domain containing the catalytic site from calf thymus poly(ADP-ribose)synthetase by limited digestion with papain followed by column chromatography on nicked DNA–cellulose as in [9] and examined the effect of each domain on accurate transcription initiation in a HeLa cell lysate using the *Sma*I fragment of adenovirus 2 DNA containing the major late promoter. Fig.1 shows that the DNA-binding domain, like the intact enzyme, specifically suppressed random transcription initiation, whereas the automodification domain had no effect. When transcription was performed in a HeLa cell lysate under optimum conditions with the *Sma*F fragment as template, a run-off product of 536 base

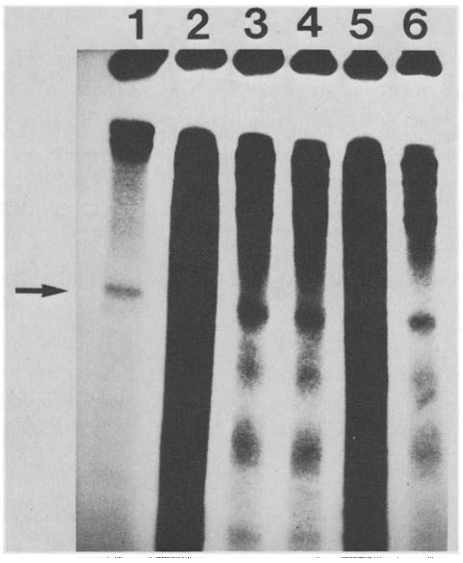

Fig.1. Effect of poly(ADP-ribose)synthetase and its papain-digested fragments on transcription in a HeLa cell lysate. Transcription was performed using 7.6 mg/ml (lane 1) or 3.3 mg/ml (lanes 2–6) of HeLa cell lysate with 0.8 μg poly(ADP-ribose)synthetase (lane 3), 0.8 μg DNA-binding domain (lane 4), 0.8 μg automodification domain (lane 5) and 0.8 μg each of DNA-binding and automodification domain (lane 6). The position of the specific run-off transcript of 536 base RNA is indicated by the arrow.

RNA, which was sensitive to α-amanitin, was synthesized (lane 1). However, when the amount of HeLa cell lysate was reduced, the product RNA gave a big smear (lane 2), although overall RNA synthesis was greatly increased (table 1). These results indicate that nonspecific transcription initiation takes place under these conditions. When purified poly(ADP-ribose)synthetase was added to this reaction mixture, the smear disappeared and the specific transcript of 536 base RNA was clearly visible (lane 3), indicating that this enzyme suppresses random transcription initiation. Overall RNA synthesis was also suppressed under these conditions (table 1).

Then we tested the effect of the DNA-binding domain (46 kDa protein) and automodification domain (74 kDa protein) of poly(ADP-ribose)synthetase produced by papain digestion. When the 46 kDa protein was added to the reaction mixture in which random transcription initiation takes place, the resulting product gave a discrete band of

Table 1

Effect of papain-digested fragments of poly(ADP-ribose)synthetase on overall RNA synthesis in a HeLa cell lysate

Lane no.	Condition	RNA synthesis (cpm/0.025 ml)
1	HeLa lysate alone (7.6 mg/ml)	15800
2	HeLa lysate alone (3.3 mg/ml)	48500
3	+ poly(ADP-ribose)-synthetase	29000
4	+ DNA-binding domain	25000
5	+ automodification domain	51600
6	+ DNA-binding and auto-modification domain	18000

Lane no., in fig.1. After the reaction, samples of 4 μl were taken from each mixture, and their acid-insoluble radioactivity was measured

536 base RNA, whereas when the 74 kDa protein was added, the product gave a smear, as shown in lanes 4 and 5, respectively. Overall RNA synthesis was also suppressed by the 46 kDa protein, but was not affected by the 76 kDa protein (table 1). When the 46 and 74 kDa proteins were added simultaneously, the resulting RNA gave a discrete band (lane 6). The results clearly indicate that the DNA-binding domain of poly(ADP-ribose)synthetase contained activity to suppress random transcription initiation, whereas the automodification domain had no effect on transcription. Thus, it was concluded that the catalytic activity of poly(ADP-ribose)synthetase is not essential for accurate transcription initiation in vitro.

It is known that poly(ADP-ribose)synthetase preferentially binds to nicks on DNA [12]. Authors in [8] showed that the enzyme suppressed nick-induced random transcription initiation by RNA polymerase II. Here, we demonstrate that the DNA-binding domain of poly(ADP-ribose)synthetase preferentially inhibits random transcription initiation but not accurate initiation. Thus, it is clear that the effect of poly(ADP-ribose)synthetase on accurate transcription initiation in vitro is to suppress random initiation and that its effect is due to the DNA-binding domain of the enzyme. Since this enzyme was also effective for accurate transcription initiation by RNA polymerase I in vitro [13], its effect seems to be rather nonspecific. Probably, other proteins having affinity to nicks on DNA would have the same effect on accurate transcription initiation in vitro.

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