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Stimulation of transcription from different RNA polymerase II promoters by high mobility group proteins 1 and 2

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High mobility group proteins (HMGs) 1 and 2 are shown to stimulate transcription in vitro from a number of RNA polymerase II promoters. Greatest effects were seen on transcription from the SV40 late promoter, then the SV40 early promoter with similar levels of transcription enhancement being seen for the human metallothionein 2A, adenovirus major late and chicken feather keratin promoters. The results indicate that HMGs 1 and 2 act to increase initiation of transcription in vitro and differential effects on the promoters are consistent with their action being in part to enhance the binding or functional activity of promoter-specific transcription factors.

High mobility group protein 1; High mobility group protein 2; Transcription; RNA polymerase II

1. INTRODUCTION

High mobility group proteins (HMGs) 1 and 2 are abundant chromosomal proteins which have been extensively characterized [1], though their function is still not clear. We have shown, using a HeLa cell in vitro transcription system, that HMGs 1 and 2 can stimulate transcription from both RNA polymerase II and III promoters [2]. Different degrees of stimulation were observed for two RNA polymerase II promoters, the adenovirus major late promoter (AdMLP) and a chicken feather keratin (CFK) promoter. As these experiments involved different preparations of transcription lysate and the transcribed sequences differed we wished to compare the effects of HMGs 1 and 2 on different promoters under more defined conditions. Here we compare the effects of HMGs 1 and 2 on transcription from the AdMLP, the SV40 promoter region and the human metallothionein 2A (MET) and CFK promoters, either in the absence of histones or in the presence of histones, conditions under which we have observed greater levels of stimulation by HMGs 1 and 2 [2].

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2. MATERIALS AND METHODS

2.1. *Plasmids*

The plasmids pHIIIB [3], pSV2CAT [4] and pSVenless [5] have been described previously; pAdMLP was derived from pHIIIB and contains adenovirus major late promoter sequences from −61 to +192 (*Hin*dIII site) relative to the transcription start site inserted between the *Cla*I and *Hin*dIII sites of pBR322. The plasmid pMET CAT was made by ligating, using *Bgl*II linkers, a *Hin*fI fragment incorporating bases −484 to +48 relative to the transcription start site of the human metallothionein 2A promoter [6] into p*Bgl*CAT (derived from pSV2CAT by removal of the SV40 promoter using *Acc*I and *Hin*dIII and insertion of *Bgl*II). For pCFK CAT a *Hin*fI (with *Bgl*II linkers) to *Sau*3A fragment, bases −367 to +56, of chicken feather keratin gene A [7] was cloned into p*Bgl*CAT.

2.2. *In vitro transcription reactions*

Whole cell transcription extracts, preparation of histones and HMGs 1 and 2, transcription assays and analysis of RNAs were as previously described [2]. For incubations containing HMGs 1 and 2 they were pre-incubated with the DNA for 10 min

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before addition of HMGs 1 and 2 with a further 10 min pre-incubation before lysate addition. After a further 30 min nucleoside triphosphates were added and transcription allowed to proceed for 1 h.

3. RESULTS AND DISCUSSION

3.1. *Transcription from different RNA polymerase II promoters*

We have examined transcription from three promoter regions in addition to the AdMLP; the SV40 promoter region, and the MET and CFK promoters were cloned in front of the bacterial chloramphenicol acetyl transferase (CAT) gene (fig.1). The SV40 promoter region and the MET promoter have been extensively characterized and both bind a number of specific *trans*-acting factors, some of which are common to both promoters [8–10]. The CFK promoter is a highly tissue-specific promoter which contains a TATA box but no known binding sites for characterized *trans*-acting factors. The AdMLP utilizes the gene-specific factor, MLTF or USF [11–13]. Transcription reactions received two templates, one containing the AdMLP and producing a transcript of 536 or 538 bases. The second was a test promoter-CAP construct giving a longer transcript, 722 to 728 bases. Transcription from the test promoters relative to the AdMLP could then be compared. As transcripts from the promoter-CAT constructs are almost identical, differences in transcription relate to the initiation phase of transcription. Transcription of the CAT gene under the control of CFK, MET, SV40 early and SV40 early (enhancer deleted) promoters are shown in fig.2, and transcripts of the expected size are seen for all promoter constructs. In addition to the transcript from the SV40 early–early start site a second transcript is seen when pSVenless is the template DNA (lane 6, band A). Analysis of transcription from templates cut with different enzymes demonstrated that this transcript, which is sensitive to 2 μg/ml α-amanitin, originates from the opposite strand to the early transcripts, 44 to 66 bases 5′ to SpI site VI (not shown). We will refer to this as the SV40 late transcript as it has been shown [14] that deletion of the enhancer region, which brings the SV40 late start sites closer to the 21 bp repeats, markedly increases late transcription.

3.2. *Stimulation of transcription by HMGs 1 and 2*

In the absence or presence of histones, HMGs 1

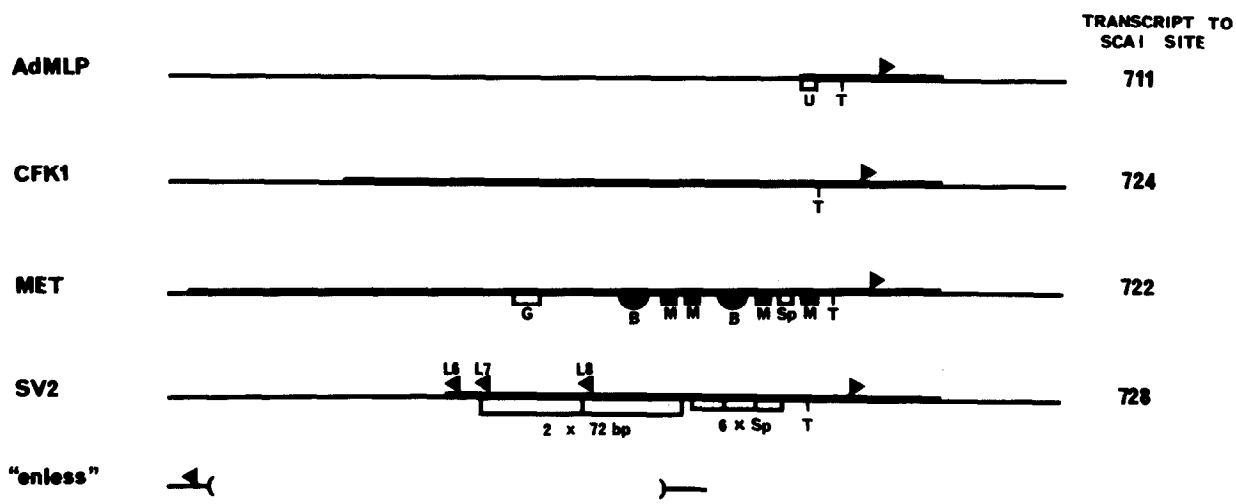

Fig.1. Maps of promoter-CAT constructs. The promoter regions contained in the plasmids pAdMLPCAT, pCFK1CAT, pMETCAT, pSV2CAT and pSVenless are shown as thick lines. Transcription start sites are indicated (►) and the length of the transcripts to the *ScaI* site in the CAT gene indicated. Recognized promoter sequence elements are shown below. T, TATA box; U, MLTF or USF binding site; G, glucocorticoid response element; B, basal level element; M, metal response element; Sp, SpI binding site; 2 × 72 bp, SV40 enhancer [9–14]. The bracketed region is deleted in pSVenless.

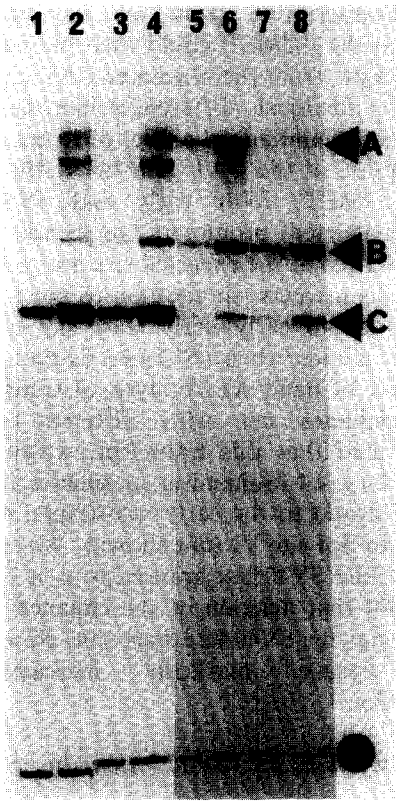

Fig.2. Effects of HMGs 1 and 2 on transcription from different promoters. Reactions for lanes 1 to 4 and 5 to 8 contained 30 and 40 ng, respectively, of control template *Bam*HI-cut pAdMLP. Lanes 1 and 2 received 70 ng of *Sca*I-cut pCFKCAT; lanes 3 and 4, 70 ng *Sca*I-cut pMETCAT; lanes 5 and 6, 40 ng *Sca*I-cut pSVenless and lanes 7 and 8, 40 ng *Sca*I-cut pSV2CAT. Even numbered lanes received 0.3 μg of HMGs 1 and 2. A, SV40 late transcript; B, transcript initiating from test promoter; C, transcript initiating from the AdMLP; •, labelled DNA marker fragment. Lanes 1–4 and 5–8 differ in exposure times.

and 2 stimulated transcription from all the tested RNA polymerase II promoters (figs 2 and 3). Further, transcription from some promoters was preferentially stimulated by HMGs 1 and 2. Enhancement by HMGs 1 and 2 for the CFK promoter was consistently slightly less and for the MET promoter slightly greater than for the AdMLP (table 1).

Stimulation of transcription caused by HMGs 1 and 2 from the SV40 early promoter (plus enhancer) was greater than the major late promoter (table 1). Deletion of the enhancer resulted in less stimulation of transcription from this promoter though it was still 2- to 3-fold greater than that of the AdMLP (table 1). Whether this effect is due to loss of enhancer function or competition for transcription components between the SV40 early and late promoters is not known.

Most notably stimulation by HMGs 1 and 2 of the SV40 late promoter, both in the absence and presence of histones, was significantly greater than that of both the SV40 early (enhancer deleted) promoter and the AdMLP. As noted above, the degree of stimulation of transcription from the SV40 early promoter and AdMLP was reduced in reactions containing pSVenless compared with those containing pSV2CAT, though the control level of transcription was not substantially altered. This effect was consistently observed using two HeLa lysate preparations. The SV40 late promoter thus becomes a relatively stronger promoter in the presence of HMGs 1 and 2 and the decreased stimulation of the AdMLP and SV40 early promoter suggests that there is a competition for transcription factors (or RNA polymerase II) between the different promoters. HMGs 1 and 2 may directly and differentially enhance association of a common transcription component to initiation complexes or differences in stimulation may be a consequence of enhancement by HMGs 1 and 2 of the binding of promoter-specific factors which in turn results in increased utilization of common components of the transcription machinery.

The results demonstrate that HMGs 1 and 2 can stimulate transcription in vitro from a number of RNA polymerase II promoters. In addition, a differential stimulation of promoters is seen and it is possible that this has relevance in determining promoter activity in vivo within a chromosomal environment. We have found that HMGs 1 and 2 have minimal effects on chain elongation in vitro and that their major effect is on the rate of formation of active initiation complexes (Tremethick, D.J. and Molloy, P.L., in preparation). Differences in transcription enhancement by HMGs 1 and 2 of the promoter-CAT constructs where the transcribed region is essentially identical also indicate that HMGs 1 and 2 primarily act to stimulate initiation of transcription. This is probably also true for the SV40 late transcription, though the transcribed region is different. Transcription stimulation may be caused at least in

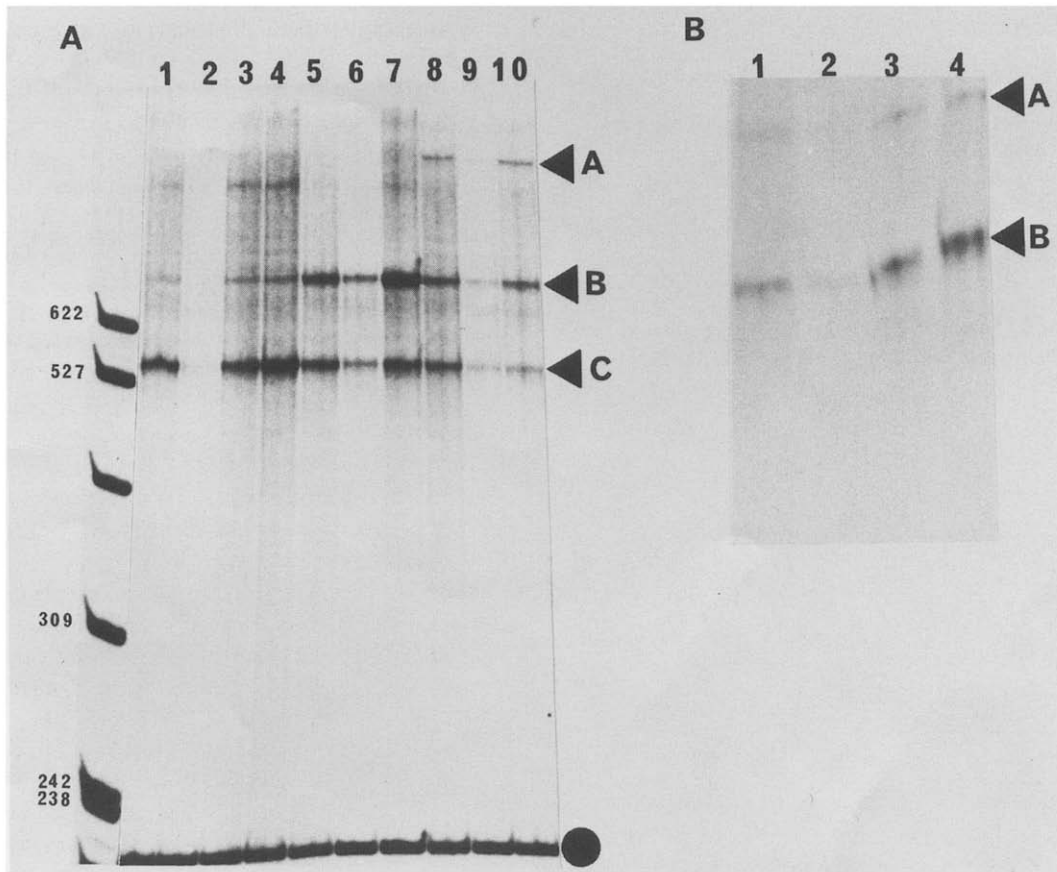

Fig.3. Effects of HMGs 1 and 2 on transcription in the presence of histones. (Panel A) Reactions for lanes 1 to 4 contained 70 ng of *Sca*I-cut pMETCAT and 30 ng of *Bam*HI-cut pAdMLP; lanes 5 to 7, 50 ng of *Sca*I-cut pSV2CAT plus 50 ng *Bam*HI-cut pAdMLP; lanes 8 to 10, 50 ng *Sca*I-cut pSVenless plus 50 ng *Bam*HI-cut pAdMLP. Lanes 1, 5 and 8 contained no histones or HMGs 1 and 2; lanes 2, 3, 4, 6, 7, 9 and 10 contained 0.3 μg of histones; lane 3 received 0.15 μg of HMGs 1 and 2 and lanes 4, 7 and 10, 0.3 μg. A, late SV40 transcript; B, transcript arising from test promoter; C, major late transcript; •, labelled DNA fragment. (Panel B) Reactions contained 0.24 μg of *Sca*I-cut pCFKCAT and 0.16 μg of *Sma*I-cut pHIIIB. Lane 1 contained no histones, lanes 2 to 4 0.9 μg histones; lanes 1 to 4 contained 0, 0, 0.8 and 1.6 μg of HMGs 1 and 2 respectively. A, transcript initiating from the CFK promoter; B, AdMLP transcript.

part by enhanced binding of gene-specific transcription factors in the presence of HMGs 1 and 2 as this has been demonstrated for one factor, MLTF [15]. Differences observed in transcription stimulation could be caused by differences in the nature or number of interactions between HMGs 1 and 2 and gene-specific transcription factors or different affinities of HMGs 1 and 2 for promoter sequences. Interaction of HMGs 1 and 2 with 'general' transcription factors or alteration of interactions between specific and general transcription factors is also possible.

Table 1

The effect of HMGs 1 and 2 on in vitro transcription

Promoter	Degree of stimulation	
	Minus histones	Plus histones
Chicken feather keratin	2.9	7.3
Adenovirus major late	3.5	11.4
Human 2A metallothionein	4.6	>20
Adenovirus major late	3.4	22
SV40 early	>8.0	6.1
Adenovirus major late	7.2	3.6
SV40 late (minus enhancer)	11.5	8.0
SV40 early (minus enhancer)	6.5	3.6
Adenovirus major late	3.6	1.3

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