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Differences in the organization of adenovirus E1A promoters are not important for their full activity

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The 5'-E1A control regions of adenovirus (Ad) types 2, 3 and 12 were cloned upstream from the *cat* gene. Each serotype was characterized by unique organization of the 5'-E1A non-coding region. All three 5'-non-coding E1A regions were able to stimulate the *cat* gene transcription in HeLa cells. Hybrid plasmids between Ad2/Ad3 and Ad2/Ad12 revealed the same level of CAT activity as the native plasmids but the optimum activity of promoter was achieved only when its organization was not modified. These observations suggest that various nuclear factors take part in the activation process of each promoter.

Adenovirus subgroup; Gene regulation; Promoter; Enhancer; *cat* Gene; Transcription activation

1. INTRODUCTION

Adenovirus (Ad) provides a useful model system to study the control of eukaryotic gene expression. In human cells infected with Ad2 or Ad5, a set of five early viral promoters (E1A, E1B, E3, E2A and E4) are coordinately expressed [1-3]. The E1A gene is one of the first transcription units to be expressed. Then, after infection, the products of that gene are required to activate transcription of other early viral genes [1-3]. Primary E1A transcript is differentially spliced to produce five mRNAs: 13 S, 12 S, 11 S, 10 S and 9 S [4,5]. The 13 S and 12 S messengers encode polypeptides of 289 and 243 amino acids, respectively, they differ one from the other only by an internal addition of 46 amino acids to the larger protein [1-3]. Both 13 S and 12 S products have been reported to be important in: transcription activation of several cellular genes transcribed by RNA polymerase II or III; inhibition of the expression of certain viral and cellular genes; and transformation and immortalization of primary cells [1-3,6].

Several enhancer elements have been described in the 5'-E1A control regions of only the related serotypes 2 and 5 of subgroup C [7-9]. Analysis of DNA sequences reveals that these enhancer elements are partially conserved in the Ad3 and Ad12 sequences upstream to E1A cap site but their organization is different (fig.1).

The aim of the present study has been to compare the upstream sequences of E1A cap site of Ad2, Ad3 and Ad12 fused to the *cat* gene and confirm the hypothesis that different nuclear factors recognize each E1A promoter.

2. MATERIALS AND METHODS

2.1. *Cells*

HeLa cells were grown as monolayers in Dulbecco's medium (GIBCO) containing 5% newborn calf serum.

2.2. *HeLa cells transfection and CAT assay*

All transfections were carried out by using the calcium phosphate coprecipitation technique [10,11]. The CAT assay was described previously [10,11].

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2.3. *Plasmids*

pKH47 is a pBR322-derivate plasmid and it was supplied by Bethesda Research Laboratories. The pVM214, p2E1Acat and p3E1Acat were described previously [10,11]. The hybrid plasmids: Ad2/Ad3 (−498 to −45/−46 to +30 = pVM261), Ad2/Ad12 (−498 to −45/−45 to +49 = pVM291), Ad3/Ad2 (−510 to −46/−45 to +40 = pVM315), Ad12/Ad2 (−445 to −45/−45 to +40 = pVM1210) were constructed using a *Pvu*II site which conserved 13 nucleotides (nt) upstream from the TATA box of E1A promoters. The nucleotide sequence (−498 to −306) containing the enhancer 'A' [7] was deleted (pVM268) from p2E1Acat. The plasmid pVM293, without the first sequence of enhancer 'B' [8], was obtained after eliminating the sequence between the *Acc*I (−306) and *Bal*I (−229) from p2E1Acat. The pVM295 was obtained by deletion of the fragment extending between *Sal*I (651) and *Nde*I (2387) restriction sites from p2E1Acat (pKH47 sequence); this fragment contained a silencer sequence [12]. The pVM296 and pVM297 were constructed by insertion of an *Xba*I linker or a fragment of 50 nt, respectively, in the *Bal*I site present at −229 of pVM295 (fig.1). The p12E1Acat contained the fragment 0–494 nt from the pAd12E1A, upstream from the encoding sequence for the *cat* gene.

Table 1

CAT activity in HeLa cells transfected with plasmids containing the *cat* gene under the control of E1A promoter of Ad2, Ad3 or Ad12

Plasmids	CAT activity		
	+ pKH47	+ pVM214	b/a
p2E1Acat	1.00[a]	2.26[b]	2.26
p3E1Acat	0.47	2.26	4.80
p12E1Acat	0.84	2.00	2.30
pVM261	0.47	2.25	4.78
pVM291	0.95	2.10	2.21
pVM315	0.94	2.35	2.50
pVM1210	0.82	1.39	1.70
pVM268	0.44	0.96	2.18
pVM293	0.71	2.13	3.00
pVM295	23.30	46.50	1.99
pVM296	6.30	12.10	1.92
pVM297	3.50	9.10	2.60

CAT activity was assayed as previously described [10,11]. The relative CAT activities are expressed in arbitrary units; CAT activity: 1.00 corresponds to 1700 cpm [^{14}C]Ac-Cm/min per 10^6 cells at 37°C. All values are averages from three separate experiments

3. RESULTS

To test the role of upstream sequences of E1A promoters, we inserted the 5'-control regions of the E1A early gene of Ad2, Ad3 and Ad12 upstream to the *cat* gene. The *cat* gene was reported as the best to study the complex regulation mechanism of the adenoviral gene in the absence of other viral gene products. Hybrid E1A promoters were constructed between Ad2 and Ad3, and Ad2 and Ad12. The enhancer sequences A [7] or B [8] were deleted from p2E1Acat. These plasmids were introduced into HeLa cells and the activity of each promoter was readily assayed by measuring the extent of conversion of chloramphenicol to its acetylated forms.

The CAT activity appeared at low-level in HeLa cells transfected with the plasmids: p3E1Acat (native), pVM261 (hybrid), pVM268 and pVM293 (deleted) (table 1). The same level of activity was observed for HeLa cells transfected with the plasmids: p2E1Acat, pVM291, pVM315, p12E1Acat and pVM1210 (table 1). A high level of CAT activity was observed when plasmids pVM295, pVM296 and pVM297 (table 1) without a silencer sequence were transfected, however, that activity decreased when the distance between two sequences of enhancer B [8] increased (pVM296, pVM297). The Ad2 E1A gene products (pVM214) stimulated the native or modified E1A promoters of Ad2, Ad3 and Ad12 (table 1).

4. DISCUSSION

The organization of E1A non-coding sequences of Ad2, Ad3 and Ad12 is very different (fig.1). The distance between two sequences of enhancer B [8] is distinct among Ad2 (88 nt), Ad3 (97 nt) and Ad12 (119 nt). The enhancer A sequence [7] is included between two sequences of enhancer B [8] in Ad12 but not in Ad2 and Ad3 (fig.1). This organization, although different, is not important for the activity of E1A promoters, because the CAT activity of hybrid plasmids: pVM291, pVM315 and pVM1210 is similar to p2E1Acat and p12E1Acat, and higher than pVM261 and p3E1Acat (table 1).

Both enhancer elements described A and B [7,8] are required for full activity of the Ad2 E1A promoter (pVM268, pVM293) and the distance between two sequences of enhancer B [8] is very important to the promoter activity (pVM295, pVM296, pVM297) (table 1).

Two sequences recognized by the E2F cellular factor [13] are present between two sequences of

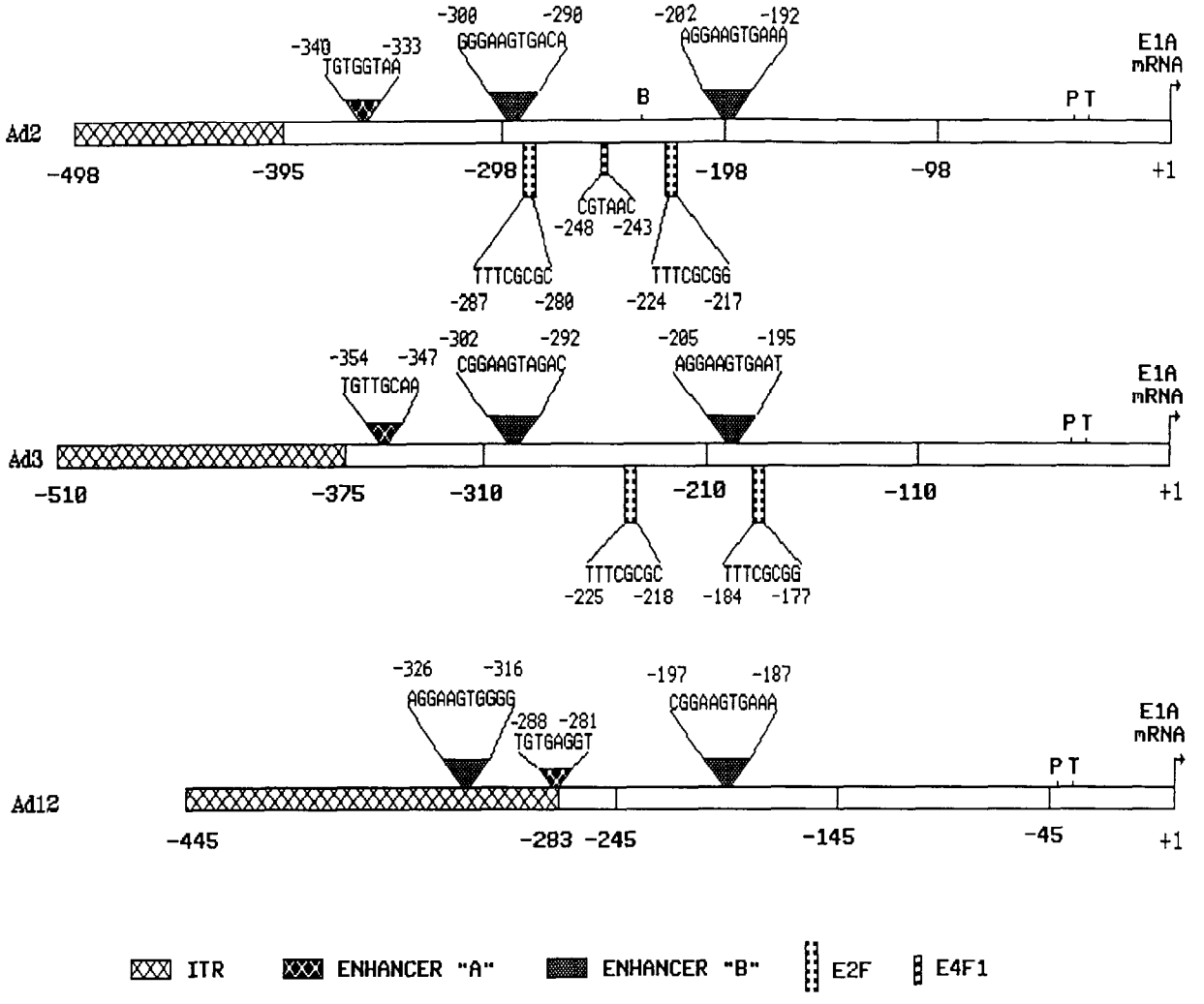

Fig.1. Organization of 5'-non-coding region E1A of adenovirus serotypes 2, 3 and 12. The enhancers A [7] and B [8], the sequences recognise for cellular factors E2F [13] and E4F1 [15], and ITR, inverted terminal repeat, are represented using the corresponding symbols. B, *Bal*I; P, *Pvu*II; T, TATA box.

enhancer B [8] in Ad2 E1A (fig.1). The E2F binds to the E1A enhancer as well as to the E2A-early promoter, but does not interact with regulatory sequences of any other E1A-inducible genes (E1B, E3, E4 and 70 kDa hsp) [13]. The activation of the E2F factor probably results in a large stimulation of E2A transcription and a smaller stimulation of E1A transcription. It is likely that other proteins are important for stimulation of other promoters [14]. One of two sequences recognized by the E2F factor is present between the sequences of enhancer B [8] in Ad3 and both sequences recognized by this factor are absent in Ad12 E1A (fig.1). The binding site for E4F1 cellular factor [15] is localized between the two sequences of enhancer B [8] in Ad2 E1A (fig.1). The binding sites for this factor are localized upstream E2A, E3 and E4 cap site of Ad5 (Ad2) [15]. However, this sequence is absent in Ad3 and Ad12 E1A promoters. The Ad2 E1A gene products can stimulate the cellular factor(s) that recognize(s) these different promoter organizations. The cellular factor present in non-infected HeLa cells [16] recognize the 'consensus' core of Ad2 enhancer A [7], but

this consensus varies between Ad2/Ad3, Ad2/Ad12 and Ad3/Ad12 (fig.1).

Why is the different organization of undamaged E1A 5′-non-coding sequences not essential for the full activity of promoters which control the E1A gene expression?

It is possible that the different cellular factors recognize each E1A organization. The undamaged upstream sequences are probably required to form a transcriptional initiation stable complex and the TATA box is the critical sequence of this formation [14,17,18].

The isolation of those cellular factors and the study of their competition in the different E1A regulatory sequences will be required for a better understanding of the still obscure process leading to the formation of the transcriptional complex.

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