Stereospecific relationships between elements in an SV40/Adenovirus-2 heterologous promoter

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ABSTRACT

Spacing mutations have been constructed between an SV40 72 bp enhancer element and the Adenovirus-2 major late promoter upstream element (Ad2MLP-UE), the Ad2MLP-UE and the Ad2MLP TATA-box and the SV40 72 bp enhancer and the TATA-box in the absence of the Ad2MLP-UE. A clear periodic pattern of transcription from the Ad2MLP was obtained by inserting multiples of half of a DNA turn between the SV40 enhancer and the Ad2MLP-UE. We interpret this as a stereospecific requirement for the spatial alignment between the enhancer element and either the Ad2MLP-UE or the TATA-box. Insertions between the upstream element and the TATA-box did not give a periodic transcription pattern, though insertion of half of a DNA turn increased the steady-state level of specific RNA. Unexpectedly, spacing mutations between the enhancer and TATA-box in which the upstream element had been point mutated gave indications of stereospecificity, whereas the same insertions with a deleted upstream element showed little requirement for a precise stereoalignment.

INTRODUCTION

Transcription of eukaryotic RNA polymerase B (II) genes is controlled in part by the binding of DNA sequence-specific proteins to various promoter elements (see ref.1 for review and references therein). The adenovirus-2 major late promoter (Ad2MLP) and the SV40 early promoter are used as model systems to study the mechanisms of transcription of RNA polymerase B (II) genes. They each contain a TATA-box element which bind TATA-box binding proteins [BTFI (2), TFIID (3)]. Further upstream to the TATA-box, Sp1 factor binds to the 21 bp repeats of the SV40 early promoter(5-7) and the upstream element factor (UEF, also called USF and MLTF) binds to the Ad2MLP upstream element [Ad2MLP-UE (2, 4, 8-10)]. The SV40 early promoter also contains, further upstream, an enhancer which binds a complex array of DNA sequence-specific factors (1, 11).

Many of these transcription factors are likely to function as signals to direct correct and efficient initiation of transcription by RNA polymerase B (II) via protein-protein interactions. Establishment of transcription preinitiation complexes are facilitated by interactions of upstream element binding factors such as UEF (2), ATF (12) or Gal4 derivative (13) with the TATA factor. The interactions between transcription factors in an active transcription complex are thought to be facilitated by the precise alignment of their binding sites along the DNA double helix. By inserting odd or even multiples of half a DNA turn between promoter elements, the importance of stereospecific alignment of transcription factors has been demonstrated for several prokaryotic, eukaryotic and viral systems (14-21).

We have previously reported the bidirectional properties of the Ad2MLP upstream element (Ad2MLP-UE) using a heterologous system comprised of a SV40 72 bp enhancer and the Ad2MLP (9). It was therefore of interest to determine the stereospecific relationships between the enhancer, upstream element and TATA-box elements in this recombinant.

MATERIALS AND METHODS

The recombinant pML15 (9, see Fig. 1A) contains a SV40 72 bp enhancer element from EcoRI (346) to BamHI (101) inserted upstream to the Ad2MLP at a Hind III site (–86) using a BamHI-Hind III adaptor.

Insertion of 5, 10, 15, 21 and 25 bp DNA between the SV40 enhancer and the Ad2MLP-UE of pML15 were constructed by ligating the KpnI/BamHI fragments from the pSE series of recombinants (19), which contain the SV40 enhancer and the spacer sequences, to the large KpnI (294)/BamHI (101) fragment from pML15 to generate pML15.5A/B, .10A/B, .15A/B, .21A/B and .25A/B. Inserts of 2, 3 and 4 bp were generated by ligating synthetic oligonucleotides of BamHI/HindIII adaptors of the required lengths to a BamHI (101)/HindIII (–86) fragment from pML15. The sequences in increasing length were: 5'GATCCACTCGA, 5'GATCCACTGCGA and 5'GATCCACTTGCGA, resulting in pML15.2, .3, and .4, respectively.

Spacing mutants of 10, 15 and 21 bp between the Ad2MLP-UE and the TATA-box were constructed by ligating oligonucleotides with ends complementary to XhoI into pML16 (9) cut at –42 by XhoI. The sequences used, in increasing length, were: 5'TCGAACTAGG, 5'TCGACCTAGT, 5'TCGAACTAGGTCAAG, 5'TCGACTTGACCTAGT, 5'TCGAACTAGGTCAACCGTTCG, 5'TCGACGAACGGTTGACCTAGT, creating pML16.10A/B, .15A/B and .21A/B, respectively in which the XhoI site was retained. A 4 bp insert was generated by blunt-end ligating a cut and repaired XhoI site in pML16 [pML16.4B]. Shorter inserts of 1, 2, 3, 5 and 7 bp and an alternative sequence for the 4 bp spacer [pML16.4A] were constructed by ligating oligonucleotides, covering the Ad2MLP-UE from HindIII (–86) to XhoI (–42) and including the spacer sequences, to the XhoI cut pML16 vector. The inserted sequences, in increasing length, were: 5'A, 5'AG, 5'AGT, 5'AGTT, 5'AGTTG, 5'AGTTGGA creating pML16.1, .2, .3, .4A, .5 and .7, respectively, in which the XhoI site was destroyed.

The pML16X series was constructed by replacing the pML16 series (pML16, .5A/B, .10A/B, .15A/B, .21A) from HindIII (–86) to XhoI (–42) with synthetic oligonucleotides of the same sequence except for two point mutations at –60 (C to T) and –62 (G to A). This mutation in the Ad2MLP-UE is equivalent to the mutation in pM1 (22).

To insert oligonucleotides between the SV40 enhancer and the TATA-box, the Ad2MLP-UE was deleted from BamHI (101) to XhoI (–42) in pML16 which was then religated with BamHI/XhoI adaptors of appropriate size from 8 to 13 bp. The sequences used, in increasing length, were: 5'GATCCACA, 5'GATCCACGA, 5'GATCCACGAA, 5'GATCCACGAGA, 5'GATCCACGAGTA, 5'GATCCACGAGTTA to give pML16δ.4, .5, .6 (pML16δ in reference 9), .7, .8 and .9, respectively. Longer sequences were inserted by replacing the SV40 enhancer of pML16δ.6 from EcoRI (346) to BamHI (101) with enhancer plus 5, 10, 15 and 21 bp spacer sequences [EcoRI (346) to BamHI (101)] from the previously described pSE series (19), thereby generating pML16δ.11A/B, .16A/B, .21A/B and .27A/B.

Oligonucleotides were synthesised by the simultaneous method (23) or on a Gene Assembler (Pharmacia). All recombinants were confirmed by dideoxy sequencing of the

double stranded plasmid DNA (23).

Plasmids (3–5 μ g) were transfected into HeLa cells as previously described (9) using the calcium phosphate procedure. After 48h cytoplasmic RNA was prepared and 5 μ g of RNA was analysed by quantitative S1 nuclease-mapping (24). The probe used was that described in reference 23 which was prepared by primer extension of an M13mp18 clone containing the Ad2MLP fused to the rabbit β -globin gene with the 32 P-end-labelled oligonucleotide 5'GCACCATTCTGTCTGTTTGGG. The results were quantitated by densitometry scanning.

RESULTS

1. Insertions Between the SV40 Enhancer and the Adenovirus Major Late Promoter Upstream Element.

The SV40 enhancer has previously been shown to function in a heterologous system with the Ad2MLP (9, 25, 26). It was therefore of interest to determine whether or not this enhancer element would demonstrate the 'cyclic' dependence of multiple half turns of DNA observed for the SV40 early promoter. DNA sequences of 2, 3, 4, 5, 10, 15, 21 and 25 bp were inserted between the SV40 enhancer and the Ad2MLP-UE (Fig. 1A) in the recombinant pML15, previously described (9), thereby generating the pML15 series pML15.2 to pML15.25 A/B. These recombinants contain an internal reference β -globin gene with its own promoter to allow balanced comparisons of the test promoter with the 'wild-type' response of pML15. For the 5, 10, 15, 21 and 25 bp insertions, the sequences were placed in both directions A and B to assess any cis- acting effects of the synthetic 'random' sequences.

Insertions of 2, 3, 4 and 5 bp DNA resulted in a steady decrease in transcription from the Ad2MLP until 35% of wild type (pML15) transcription was reached with pML15.5A/B (in which a near half turn of DNA had been inserted, Fig.2, lanes 1 to 6, Fig.6A). Introduction of nearly one full turn of DNA (10 bp) produced constructions (pML15.10A/B) that transcribed RNA from the Ad2MLP to 65% of the level obtained with pML15 (Fig. 2, lanes 7 and 8, Fig. 6A), reflecting a return towards 'wild-type' levels of transcription. Introduction of 15 bp caused a decrease in the steady state level of RNA from the Ad2MLP to 35% of transcription from pML15, whereas transcription from pML15.21A/B (21 bp insertions) was 60% of the level observed with pML15 (Fig. 2, lanes 9, 10 and 11, 12; Fig.6A). Recombinants with insertions of 25 bp DNA (pML15.25A/B) expressed RNA from the Ad2MLP at 38% the steady-state level of pML15 (Fig.2, lanes 13 and 14, Fig.6A). In all cases the orientation of the insert had only a small effect on the transcriptional response. The B series consistently gave rise to transcripts at a slightly greater level than the A series (Fig.6A). This 'cyclic' response in transcription from the Ad2MLP by increasing the distance between the enhancer and the upstream element allows us to conclude that a factor(s) that binds to the SV40 enhancer can interact in a stereospecific manner with factors which operate on a further downstream element in this heterologous system. This element is most likely to be the Ad2MLP-UE and/or the Ad2MLP TATA-box. The insertions used for the pML15 series that were derived from the pSE series (see Materials and Methods) do not create additional protein binding sites as determined by DNase1 footprinting (A. Wildeman, unpublished data).

2. Insertions Between the Major Late Upstream Element and the TATA-Box.

Using DNase 1 footprinting, a cooperative interaction for the binding of a TATA-box factor to the Ad2MLP with binding of the UEF has been reported (3). Cooperativity in the binding

of factors to DNA probably involves direct protein-protein interactions. We therefore inserted DNA sequences between the Ad2MLP-UE and the TATA-box, using the engineered XhoI site at position -42 of pML16 (9), to investigate the stereospecific alignment of the UEF and the TATA-box factor when bound to our heterologous promoter. The resulting series of recombinants contained inserts of 1, 2, 3, 4, 5, 7, 10, 15 and 21 bp in the two orientations, called A and B (pML16.1 to pML16.21A/B, Fig. 1B). The sequence of pML16 is essentially that of pML15 except for the presence of the XhoI site at -42 in pML16, which we have previously shown to have no effect on transcription from the Ad2MLP or the binding of UEF, when compared to pML15 [Fig. 3, lanes 3 and 4] (9).

Introduction of 10, 15 and 21 bp DNA of two different sequences A and B (approximately 1, 1.5 and 2 turns of DNA) between the Ad2MLP-UE and the TATA-box resulted in a steady and undramatic decrease in transcription from the Ad2MLP (Fig 3, lanes 12 to 17; Fig. 6B), reaching 66% of the transcription from pML16 with pML16.21A/B. We have to note that *in vitro* as well as *in vivo* assays established the stimulating effect of the UE of the Ad2MLP to be 3–6 fold and 20 fold, respectively (22, 25). We may expect that absence of the UE in such experiments may result in a decrease to 30% at least. Most surprisingly, since it is generally expected that the wild-type arrangement of promoter elements would be the most efficient for transcription from a promoter, insertion of four or five base pairs of DNA (pML16.4A/B and pML16.5, Fig. 3, lanes 8, 9 and 10) resulted in an increase in transcription from the Ad2MLP when compared to the wild-type level of pML16 (Fig. 6B). In this case, the stimulation of transcription was about 2 fold as obtained from 3 independent experiments. *Cis*-acting DNA sequence effects are unlikely to explain this stimulation since the four base pair insertions were achieved either by ligation of a repaired XhoI site (pML16.4B) or by using synthetic oligonucleotides (pML16.4A). This unexpected marked increase in transcription from the Ad2MLP when a half turn of DNA was inserted between the Ad2MLP-UE and the TATA-box occurred sharply as the length of insert increased [the transcription levels from spacing mutations of one, two and seven base pairs (pML16.1, .2 and .7; Fig. 3, lanes 5, 6 and 11) were close to pML16]. The 'down' effect of pML16.3 relative to pML16, was most likely due to an effect of the particular sequence inserted. A degree of sequence effect is evident when comparing pML16.10A with B, pML16.15A with B and pML16.21A with B. The A series of insertions allowed slightly more efficient transcription than the B series except for pML16.4A/B in

Figure 1. Recombinants containing insertions between: the SV40 enhancer and the Ad2MLP upstream element (Ad2MLP-UE), pML15 series (A); Ad2MLP-UE and the TATA-box, pML16 series (B); the SV40 enhancer and a mutated Ad2MLP-UE, pML16X series (C); and the enhancer and the TATA-box in which the Ad2MLP-UE was deleted, pML16 δ series (D). Restriction sites destroyed during cloning are in parentheses. Also the XhoI site at -42 was not retained in pML16.4B, .10A/B, .15A/B and .21A/B. The filled triangles represent the Ad2MLP TATA box. The crosses in C represent the double point mutation within the Ad2MLP-UE (UE) at -60 and -62. The probe used for S1-mapping is indicated in D and extends from +60 in the β -globin gene to the EcoRI (346) site in the SV40 enhancer.

These schematic representations are not to scale. Insertions above the central axes in A, C and D were constructed from the pSE series of recombinants (19,23) using a BamHI/HindIII (A and C) or a BamHI/XhoI (D) adaptor. Insertions below the central axes in A and D were constructed from BamHI/HindIII (A) or BamHI/XhoI (D) adaptors of increasing size. See Materials and Methods for details of these constructions.

Figure 2. Insertions between the SV40 enhancer and the Ad2MLP upstream element. The pML15 series of recombinants were transfected into HeLa cells. Cytoplasmic RNA was isolated and analysed by S1-mapping as described in Materials and Methods. +1 β -GLOB and +1 Ad2MLP indicate the major start sites of transcription of the reference rabbit β -globin gene and from the Ad2MLP, respectively.

which no sequence effect was observed (Fig. 6B).

DNase 1 'footprinting' experiments of HeLa extracts on the pML16 series of insertion recombinants show that in each case, the UEF protects exactly the same sequence site on the Ad2MLP as the wild-type promoter, and UEF binding is not affected by flanking sequences. This protection spans from nucleotide -49 to -67 on the coding strand and from nucleotide -50 to -66 on the non coding strand (data not shown and see ref. 9). Furthermore these data indicate that the insertions do not generate new binding sites for proteins.

3. Insertions Between the SV40 Enhancer and the Adenovirus Major Late Promoter TATA-Box in the Absence of the Ad2MLP Upstream Element.

The SV40 72 bp repeat enhancer has been shown to stimulate correct transcription from the Ad2MLP start site in the absence of any upstream element (25). In such constructions interactions may occur between the enhancer complex and the general transcription machinery located around the TATA-box and the start site. Furthermore, the 'cyclic' effect of insertions between the enhancer and upstream element, described above for transcription from the Ad2MLP, could be due to the requirement for a specific stereoalignment between factors that bind to the enhancer and the upstream element and/or the TATA-box. We therefore investigated the effects of inserting DNA sequences between the SV40 enhancer and the Ad2MLP TATA-box in which the upstream element was either mutated such that it no longer binds the UEF (pML16X series, Fig. 1C) or deleted (pML16 δ series, Fig. 1D). The recombinant pML16X contains a double point mutation within the Ad2MLP-UE which has previously been shown not to bind the UEF in vitro and drastically reduces transcription from the Ad2MLP both in vitro and in vivo (8). Surprisingly, in the context of our heterologous system, mutation of the Ad2MLP-UE had no effect on the transcription

Figure 3. Insertions between the Ad2MLP upstream element and the TATA-box. The pML16 series of recombinants were transfected into HeLa cells. Cytoplasmic RNA was isolated and analysed by S1-mapping as described in Materials and Methods. +1 β -GLOB and +1 Ad2MLP indicate the major start sites of transcription of the reference rabbit β -globin gene and from the Ad2MLP, respectively. Lanes 1 and 2 are controls of the probe hybridised to 5 μ g of total yeast RNA and incubated in the absence (lane 1) or presence (lane 2) of S1-nuclease.

from the promoter when compared to transcription from the wild-type Ad2MLP-UE in pML15 (Fig. 4, compare lanes 1 and 2; Fig. 6C). Furthermore, insertion of 5, 10, 15 and 21bp DNA between the SV40 enhancer and the mutated Ad2MLP-UE, gave an essentially quantitatively identical pattern of transcription to the pML15 series of constructions (compare Fig. 2 and Fig. 4). Insertions of 5 or 15bp DNA (equivalent to 0.5 and 1.5 turns of DNA respectively, Fig. 4, lanes 3, 4 and 7, 8, respectively) were more severely deleterious than insertions of 10 or 21 bp (equivalent to 1 and 2 turns of DNA respectively, Fig. 4, lanes 5, 6 and 9, respectively).

In agreement with Hen et al. (25) deletion of the SV40 enhancer element from pML16 reduced transcription from the Ad2MLP TATA-box to a level undetectable by S1-nuclease mapping [(25), our unpublished data]. Deletion of the upstream element from pML16 to generate pML16 δ .4 caused a five fold decrease in transcription from the Ad2MLP when compared to transcription from pML15, pML16 (Fig. 5, lanes 1, 2 and 3). Such a decrease was also observed in *in vitro* transcription experiments when the UE was deleted (9). As discussed previously the close proximity of the enhancer to the Ad2MLP (25) in pML16 probably results in the reduction in the effect of deleting the Ad2MLP-UE from 20 fold to five fold. Insertions of 4, 5, 6, 7, 8 and 9 bp DNA (pML16 δ .4 to .9, Fig. 5, lanes 3 to 8) between the BamHI and XhoI sites had little effect on transcription from the Ad2MLP when compared to pML16 δ .4 (Fig. 6D). Larger insertions of 11, 16, 21 and 27 bp DNA, equivalent to nearly 1, 1.5, 2 and 2.5 DNA turns respectively (pML16 δ .11 to .27, Fig. 5, lanes 10 to 17), gave a slow decrease in transcription to 60% of the level of RNA obtained

Figure 4. Insertions between the SV40 enhancer and a mutated Ad2MLP upstream element. The pML16X series of recombinants were transfected into HeLa cells. Cytoplasmic RNA was isolated and analysed by S1-mapping as described in Materials and Methods. +1 β-GLOB and +1 Ad2MLP indicate the major start sites of transcription of the reference rabbit β-globin gene and from the Ad2MLP, respectively.

Figure 5. Insertions between the SV40 enhancer and the Ad2MLP TATA-box. The pML16Δ series of recombinants were transfected into HeLa cells. Cytoplasmic RNA was isolated and analysed by S1-mapping as described in Materials and Methods. +1 β-GLOB and +1 Ad2MLP indicate the major start sites of transcription of the reference rabbit β-globin gene and from the Ad2MLP, respectively.

Figure 6. Stereospecific relationships between promoter elements in the heterologous recombinant pML15. S1-nuclease resistant products of Figures 2, 3, 4 and 5 were quantitated by scanning densitometry. The values for transcription from the Ad2MLP were standardized to those from the β -globin internal reference gene and plotted as the percentage of wild-type transcription from pML15 or pML16 as appropriate, against the length of the DNA insert. (A) Insertions between the SV40 enhancer and the Ad2MLP upstream element (UE): pML15 series, (B) insertions between the Ad2MLP upstream element and the TATA-box: pML16 series, (C) insertions between the SV40 enhancer and a mutated UE, and (D) insertions between the SV40 enhancer and the Ad2MLP TATA-box pML16 δ series. Each point is the mean value of at least two independent observations using two independent plasmid preparations. On average the data for a single point has a standard deviation of $\pm 10\%$. When sequence effects were observed between A and B series of insertions, the outer limits of the values for A and B are indicated.

with pML16 δ .4 using pML16 δ .27A/B, and little 'cyclic' response is evident. The apparent 'Dip' in transcription for pML16 δ .11A/B is not statistically significant. No DNA sequence effects were observed for the inserts when comparing the A series of constructs with the B series.

The slow decrease in enhancer activity with distance away from the TATA-box is in agreement with the well documented ability of the SV40 enhancer to function at a distance from homologous and heterologous promoters (26, 27). The lack of any obvious periodicity of transcription with increasing multiples of half DNA turns, especially with the insertions containing single nucleotide increments from 4 to 9bp., indicates that stereospecific interactions between enhancer-binding factors and the TATA-box binding factor are either very weak or very strong. As discussed below the apparently contradictory results obtained by either mutating or deleting the Ad2MLP-UE within our heterologous constructions can be explained by 'saturation' of the promoter.

DISCUSSION

Insertion of DNA sequences between promoter elements has frequently shown that the efficient function of many of these elements requires the preservation of their alignment on the DNA double helix (for example, the effects of spacing mutations between: λ -repressor binding to two λ operator sites (17, 20), certain araCBAD promoter elements in *E. coli* (14,15), the A and C-boxes in *X. laevis* 5S DNA which bind TFIIC and TFIIA,

respectively (18) and, the SV40 enhancer and the 21bp repeats (19).

The favoured interpretation for the effects of spacing mutations on the transcription efficiency is that in the wild-type situation two DNA- binding factors or complexes directly or indirectly interact and that their efficient action requires this interaction. Insertion of a half DNA turn between the two interacting entities disrupts their alignment, thereby inhibiting their interaction. Insertion of a complete DNA turn restores the alignment of the binding sites, allowing the factors to interact by 'looping out' of the intervening sequences. The simplest interpretation of our results, using a heterologous promoter is that a particular stereospecific alignment is required between the SV40 72 bp repeat enhancer and a downstream Ad2MLP element, for optimal transcription from the Ad2MLP TATA-box.

Comparing transcription efficiency of pML15, which contain both the SV40 enhancer and the Ad2MLP wild type sequence, and pML16X which contain the wildtype SV40 enhancer and the double mutation in the UE (known to inhibit the factor binding in an in vitro assay (8) we observe the same rate of in vivo transcription. Furthermore, in both cases insertion of half a helix turn diminishes transcription efficiency whereas a 10 bp insertion partially restores the wild type transcription. This apparently contradictory result can be explained by 'promoter saturation'. If the factor(s) responsible for SV40 enhancer action in our heterologous constructions is likely to be working near to maximum capacity, a small decrease in UEF binding capacity of a mutated Ad2MLP-UE would maintain near maximal transcription. When the Ad2MLP-2-UE is deleted there is zero binding of the UEF resulting in a reduction in transcription. Alternatively, the destabilisation caused by the Ad2MLP-UE mutations [sufficient to inhibit UEF-binding in an in vitro assay using a construction without the SV40 enhancer (8)] is circumvented by the enhancer factors binding the UEF. It has been shown that TATA box factors are stabilized by upstream factors such as ATF (activating transcription factor ; ref. 12) or by GAL4 (13). The potential role of the UEF, even when its UEF target site is mutated, should not be neglected. Indeed, when the Ad2MLP-UE is completely deleted there is zero binding of the UEF and hence a reduction in transcription to around 20% (compare the transcription of pML16 and pML16.84, Fig. 5).

Interactions between factors binding to the Ad2MLP-UE and the TATA-box have previously been suggested by DNase I 'footprinting' (3) in which binding of the TATA-box factor to the Ad2MLP stabilised binding of the UEF. This cooperative binding of the UEF is most probably via protein-protein interactions, yet a periodic stereospecific relationship was not observed between the upstream element and the TATA-box. For the SV40 early promoter, insertions between the 21 bp repeats and the TATA-box revealed a slight dependence upon the stereoalignment of these elements (19). The difference between the SV40 and our system could lie in the nature of the TATA-box element and/or the Ad2MLP has a good consensus TATA-box whereas the SV40 early promoter does not have a consensus TATA sequence.

Surprisingly, insertion of half of a DNA turn between the Ad2MLP-UE and the TATA-box significantly increased transcription from the Ad2MLP. It is not clear that this apparently non-optimal wild-type spacing between the Ad2MLP-UE and the TATA-box, in this heterologous system, reflects stereospecificity since insertion of 15 bp DNA did not increase transcription relative to a 10 bp insert.

In agreement with our insertions between an upstream element and a TATA-box, the upstream Gal4 binding site shows no strict stereochemical alignment when placed 30 to

70bp upstream to the TATA-box (28). However, it has also been reported that moving the γ Fibrinogen (γ Fbg) UEF-like factor binding site closer to the TATA-box by 2, 5 and 14bp. did not have any effect on in vitro transcription from the γ Fbg promoter (29), whereas we observed an increase in transcription from the Ad2MLP when four or five base pairs of DNA were inserted between the Ad2MLP-UE and the TATA-box. The apparent discrepancy with the result of Chodosh et al. (29) may well be a result of the nature of the γ Fbg promoter itself, whose affinity for the UEF is much less than for the Ad2MLP and which contains other uncharacterised factor binding sites; furthermore a SV40 enhancer element is not present in their recombinants.

A characteristic of enhancer sequences is that they function at a distance from the promoter start sites (27). Alternatively, upstream elements such as the SV40 early promoter 21 bp repeats lose activity rapidly with distance from the start sites (19). The Ad2MLP-UE functions at 66% the efficiency of the wild-type Ad2MLP, when placed 21 bp from the TATA-box and probably works well at substantially longer distances. This confirms that the proximal upstream element such as the 21 bp of the SV40 promoter and the Ad2MLP-UE exert interaction only when positioned at a short distance from the general transcription machinery. The slow decrease in Ad2MLP-UE activity with distance could be explained by tight UEF binding to the upstream sequence, strong interaction with the TATA-box factor and a concomitant 'looping out' of the intervening sequence.

It is interesting that the SV40 enhancer is capable of a stereospecific relationship with an upstream element from another source (i.e. the Ad2MLP-UE) as well as the SV40 21 bp repeats. This suggests that a factor(s) that binds to the enhancer may interact with both Sp1 and the UEF in a similar manner. Therefore Sp1, the UEF and other upstream factors may either share a common structural feature responsible for interacting with a SV40 enhancer factor(s), or Sp1 and the UEF may interact with different enhancer-binding factors.

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REFERENCES

1. Wasylyk, B. (1988) *CRC Crit. Rev. in Biochem.* Vol. 23, pp77–120.
2. Davison, B.L., Egly, J.M., Mulvihill, E.R. and Chambon, P. (1983) *Nature* 301, 680–686.
3. Sawadogo, M. and Roeder, R.G. (1985) *Cell* 43, 165–175.
4. Moncollin, V., Miyamoto, N.G., Zheng, X.M. and Egly, J.M. (1986) *EMBO J.* 5, 2577–2584.
5. Gidoni, D., Dynan, W.S. and Tjian, R. (1984) *Nature* 312, 409–413.
6. Gidoni, D., Kadonaga, J.T., Barrera-Saldana, H., Takahashi, K., Chambon, P. and Tjian, R. (1985) *Science* 230, 511–517.
7. Briggs, M.R., Kadonaga, J.T., Bell, S.P. and Tjian, R. (1986) *Science* 234, 47–52.
8. Miyamoto, N.G., Moncollin, V., Egly, J.M. and Chambon, P. (1985) *EMBO J.* 4, 3563–3570.

9. Lennard, A.C. and Egly, J.M. (1987) *EMBO J.* 6, 3027–3034.
10. Chodosh, L.A., Carthew, R.W. and Sharp, P.A. (1986) *Mol. Cell. Biol.* 6, 4723–4733.
11. Wildeman, A.G., Zenke, M., Schatz, C., Wintzerith, M., Grundström, T., Matthes, H., Takahashi, K. and Chambon P. (1986) *Mol. Cell. Biol.* 6, 2098–2105.
12. Horikoshi, M., Hai, T., Lin, Y-S, Green, M.R. and Roeder, R.G. (1988) *Cell* 54, 1033–1042.
13. Horikoshi, M., Carey, M.F., Kakidrani, H., and Roeder, R.G. (1988) *Cell* 54, 665–669.
14. Dunn, T. M., Hahn, S., Ogden, S. and Schleif, R.F. (1984) *Proc. Natl. Acad. Sci. USA* 81, 5017–5020.
15. Martin, M., Huo, L. and Schleif, R.F. (1986) *Proc. Natl. Acad. Sci. USA* 83, 3654–3658.
16. Kramer, H., Niemoller, M., Amouyal, M., Revet, B., von Wilcken-Bergmann, B. and Muller-Hill, B. (1987) *EMBO J.* 6, 1481–1491.
17. Hochschild, A. and Ptashne, M. (1986) *Cell* 44, 681–687.
18. Pieler, T., Hamm, J. and Roeder, R.G. (1987) *Cell* 48, 91–100.
19. Takahashi, K., Vigneron, M., Matthes, H., Wildeman, A., Zenke, M. and Chambon, P. (1986) *Nature* 319, 121–126.
20. Griffith, J., Hochschild, A. and Ptashne, M. (1986) *Nature* 322, 750–752.
21. Loeken, M.R. and Brady, J. (1989) *J. Biol. Chem.* 264, 6572–6579.
22. Miyamoto, N.G., Moncollin, V., Egly, J.M. and Chambon, P. (1984) *Nucleic Acids Res.* 12, 8779–8799.
23. Matthes, H.W.D., Staub, A. and Chambon P. (1987) In Wu (ed.) *Methods in Enzymol.-Recombinant DNA*, Academic Press (N.Y.) Vol. 154, pp. 250–287.
24. Zenke, M., Grundström, T., Matthes, H., Wintzerith, M., Schatz, C., Wildeman, A. and Chambon, P. (1986) *EMBO J.* 5, 387–397.
25. Hen, R., Sassone-Corsi, P., Corden, J., Gaub, M.P. and Chambon, P. (1982) *Proc. Natl. Acad. Sci. USA* 79, 7132–7136.
26. Sassone-Corsi, P., Dougherty, J.P., Wasylyk, B. and Chambon, P. (1984) *Proc. Natl. Acad. Sci. USA* 81, 308–312.
27. Moreau, P., Hen, R., Wasylyk, B., Everett, R., Gaub, M.P. and Chambon, P. (1981) *Nucleic Acids Res.* 9, 6047–6068.
28. Ruden, D.M., Ma, J. and Ptashne, M. (1988) *Proc. Natl. Acad. Sci. USA* 85, 4262–4266.
29. Chodosh, L.A., Carthew, R.W., Morgan, J.G., Crabtree, G.R. and Sharp, P.A. (1988) *Science* 238, 684–688.

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