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The major late promoter and bipartite leader sequence of fowl adenovirus*

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Summary. The region of the fowl adenovirus serotype 10 (FAV-10) genome containing the major late promoter (MLP) and leader sequences was determined and appropriate genomic fragments were cloned and sequenced. A TATA box was identified and the location of the putative transcription start site was determined. By using synthetic primers from the transcription start site in conjunction with oligonucleotides from the coding regions of the penton base and hexon genes, cDNA was produced from late mRNA isolated from cell cultures infected with FAV-10 at 24 h post-infection. The resulting cDNA was cloned and sequenced and the leader sequences thus identified. It was found that the FAV-10 MLP utilized only two leader sequences (a bipartite leader). By comparison with human adenoviruses (HAVs) it appeared that the second leader in HAVs was absent from the FAV-10. The second leader sequences of FAV-10 was larger than either the second or third leaders of HAVs, but was 29 baseparirs shorter than the combined size of the leader sequences 2 and 3 from HAV-2. To confirm the transcription start site and leader sequences, single stranded cDNA was produced from mRNA using the primers from within the coding sequence for the penton base or hexon. A tail of dGTP's was added and cDNA synthesis was completed using an oligonucleotide from within the hexon or penton base coding sequence and a second poly-dCTP oligonucleotide. Sequencing of the resultant G-tailed DNA confirmed the location of the transcription start site as an adenosine residue 24 basepairs upstream from the 3-prime (3′) end of the TATA box. Sequencing 5′ of the TATA box failed to

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reveal any sequence similarity with the human adenovirus upstream stimulatory factor (USF). Various plasmids were constructed which placed the determined sequences of the MLP, leader, and the region upstream of the TATA box linked to the co-acetyl acid transferase (CAT) gene. These expression plasmids in transient expression assays of CAT activity in primary chicken kidney cell culture with or without FAV-10 co-infection were determined. These experiments showed that the cassette containing sequences 5′ of the TATA box expressed CAT to a much greater level than cassettes not containing this upstream region and that the presence of virus significantly increased the activity of the promoter following the onset of viral DNA replication. Without the 5′ region, cassettes failed to express above background levels. These results suggest that the basic structure of the fowl adenovirus MLP is similar to that of the human adenovirus although it utilizes a bipartite rather than a tripartite leader sequence.

Introduction

Adenoviruses are found in a diverse range of animals and with a few exceptions, the genome organization of those examined to date appears to be well conserved. Genes are transcribed from 11 major regions, 6 of which are expressed early in the viral replication cycle (E1a, E1b, E2a, E2b, E3 and E4) and 5 being transcribed late (L1 to L5). All the early regions utilize their own promoters with the exception of the two E2 regions, which use a single promoter to express both regions [3]. All the late regions are transcribed from a single promoter called the major later promoter (MLP). The human adenovirus MLP is active at both early and late stages of an adenovirus infection [16]. At early stages of infection the MLP shows similar activity to the early gene promoters, whereas at late stages of infection the rate of transcription from the MLP is greatly increased [12, 16, 23]. Efficient constitutive transcription in HAV from the MLP depends on a series of well defined promoter elements [9, 27, 37] comprising a correctly positioned TATA box, an upstream element located between -67 to -49 (relative to the MLP transcriptional start site), and a CAAT box located between -80 to -76 [27]. It has been reported that the rate of transcription from the MLP, at the onset of DNA replication, can increase as much as 20- to 50-fold [16, 18, 21, 31]. The significantly increased rate of transcription from the MLP after DNA replication, requires virus-induced trans-acting and sequence specific binding proteins [8, 12, 15, 21, 31]. Some of these proteins interact with sequences downstream from the transcription start site, and fall within the first intron of the MLP mRNA [8, 12, 15, 16, 18, 24]. The two main sequence elements are known as downstream element (DE) 1 and downstream element 2 (DE2) and are located between $+85$ to $+98$ and $+100$ to $+120$, respectively [23]. Comparisons made between nucleotide sequence of a variety of adenovirus serotypes demonstrate conservation of the DEI and DE2 elements, which can be divided into 3 district adenovirus groups. These are serotypes 2, 4 and 5 (non-oncogenic), serotype 7 (weekly oncogenic) and serotype 12 (highly oncogenic) [12].

The MLP produces a long primary transcript that is differentially spliced to yield five 3′ co-terminal families of mRNAs [31]. All of these mRNAs have been shown to share a common 5′ untranslated region which, in HAVs, is approximately 200 nucleotides in length and is spliced onto each late mRNA [2, 5]. This 200-nucleotide sequence has been termed the tripartite leader (TPL) sequence, which is encoded by 3 distinct and spatially separated sequences in the HAV genome [7]. The TPL is required for preferential translation of viral mRNAs produced at late stages of the infection cycle [19]. It has been demonstrated that, at least for HAV, the TPL enhances translation of mRNAs at late but not early times post infection [4, 19]. Deletion analysis performed by Kaufman [13] on HAV indicated that all 3 leader sequences of the TPL are required for the translation enhancement effect. However, the action of the TPL has been shown not to be solely due to a structural or sequence effect that alters the intrinsic efficiency with which a mRNA is translated since, if the intrinsic efficiency of translation were altered, mRNAs that carry the sequences would be more efficiently translated under all conditions, and this is clearly not the case [19]. The TPL exerts its effect within HAV infected cells only late in the infection cycle. Therefore, there is a change in the intracellular environment following the onset of HAV DNA replication that enables the TPL sequences to enhance viral translation.

Fowl adenoviruses (FAVs) have been demonstrated to be the causative agent for many diseases in poultry including egg drop syndrome, aplastic anemia, infectious inclusion body hepatitis and various respiratory diseases [10, 39]. It is only recently that the molecular biology of FAVs has been examined in any detail. It is known that the FAV genome is some 30% larger (approximately an extra 10 kb) than the HAV genome [38] and that the virus is composed of at least 11 to 14 structural proteins [17]. DNA cross-hybridization studies between HAV and FAV demonstrated limited homology [1]. Complete sequencing of the chick embryo lethal orphan (CELO) virus [6] revealed that large sections of the genome are unique to avian adenovirus. Further studies on FAV-10 showed that many of the functional and structural genes were located in positions similar to that of HAV. Despite an apparent lack of relatedness at the DNA level, it was shown that the amino acid compositions of several FAV and HAV proteins are similar [32, 34–36]. The FAV genome like HAV, also possesses an inverted terminal repeat at each end of the genome [1, 33] and was shown to produce low molecular weight virus associated RNAs [14] even though these differ from HAV in their genomic location and primary sequence.

The adenovirus MLP/TPL has a major effect on transcription and mRNA translation within the cell at late times post adenovirus infection. To date only HAV MLP/TPL regions have been studied in detail. In this paper, we describe the location and characterization of the MLP and leader sequences of an avian adenovirus (FAV-10) and discuss the structural differences and similarities with HAV MLP/TLP.

Materials and methods

Virus culture and purification

A strain of FAV serotype 10 isolated and plaque purified in this laboratory and designated CFA20 was used for all experiments in this report. It was cultured in primary chicken kidney (CK) cells in Eagle's Modified Essential Medium, 5% CO_2 at 37 °C for 24 to 48 h. Cells and supernatants were transferred into plastic centrifuge tubes and were frozen and thawed at least twice before being centrifuged at 3 000 rpm in a benchtop centrifuge (Jouan, France). Supernatants were concentrated in an Amicon stir cell (Amicon, USA) with a PM 10 filter under a pressure of approximately 60 psi. The concentrated virus suspension was then centrifuged through a 30 to 60% sucrose gradient at 28 000 rpm in an SW28 rotor for 2 h. Virus bands were removed and the virus pelleted at 28 000 rpm for 1.5 h. The virus was resuspended in 10 mM Tris/lmM EDTA buffer, pH 8.0 (10:1 TE) and stored at −20 °C until use.

Virus DNA preparation

Resuspended purified virus was made up to 400 μl in 10:1 TE and 10 μl 10% sodium dodecyl sulfate, 15 μl 10/mg/ml proteinase K and 40 μl 1M NaCl added. The reaction mixture was incubated at 37 °C for 1 h before being extracted once with 400 μl of 50:50:1 phenol:chloroform:isoamylalcohol. DNA was precipitated from the resulting aqueous phase with isopropanol and resuspended in sterile distilled deionized water (sdd. H_2O).

Genome characterization and mapping

Restriction enzyme maps of the virus were constructed using standard techniques. In general, enzyme digests were performed using the buffers and guidelines as provided by the supplier. Individual fragment hybridizations were performed on Southern blots of genomic digests run on 1% agarose electrophoresis gels transferred onto nitrocellulose. Hybridizations were performed overnight at 65 °C and blots washed after probing firstly with 2 × SSC (NaCl/sodium citrate/1% SDS) and then 0.5 × SSC buffer. The region believed to contain the MLP was identified from the resulting maps and appropriate fragments cloned into the plasmid vector pIC19R.

Preparation of plasmid DNA

DNA from plasmid clones containing the required sequence was prepared using a standard midi-plasmid preparation method [29]. DNA prepared in this way was resuspended in sdd H_2O and stored at −20 °C until used.

DNA sequencing

All sequencing was performed on dsDNA fragment clones using a modification of the chain termination method with Taq DNA (Promega, USA). DNA for sequencing was prepared by first precipitating with 0.8 M sodium chloride and an equal volume of 13% polyethylene glycol for 30 min on ice. The precipitates were centrifuged at 10,000 rpm for 10 min with the resultant pellet washed once with 70% ethanol. The pellets were resuspended in 20 μl of sdd H_2O and treated with 2 μl 2mM sodium hydroxide for 5 min at room temperature. The alkaline treatment was halted by addition of 3 μl 3M sodium acetate and the DNA precipitated with 2.5 volumes of ethanol. Labeled sequencing reactions were run on 6% acrylamide gels.

Identification of the splice leader sequences

In order to determine the structure and sequence of the leader sequence spliced to the 5′ end of the late mRNAs, CK cells were infected with FAV-10 and incubated for between 22 to 24 h. Total RNA was then purified from the infected cells using RNAzol solution (Bresatec, Australia) and the RNA precipitated in isopropanol before being stored at −70 °C or used immediately to isolate mRNA. Poly-A mRNA was isolated using the PolyATract system (Promega, USA) and again stored at −70 °C or used immediately. To produce cDNA of the appropriate transcripts, oligonucleotides were produced to the complementary strands of both the penton base and hexon genes. These oligonucleotides were used to make single stranded cDNA using reverse transcriptase (Boehringer Mannheim, Germany). To produce double stranded cDNA, a further oligonucleotide covering the putative cap site (24 bases downstream from the TATA box) of the major late transcript was also produced and this used in conjunction with the first oligonucleotides in Taq polymerase chain reaction (PCR) (Promega, USA). The resulting DNA was cloned and sequenced and the sequence compared to that of the genomic fragment clones in order to identify spliced DNA fragments.

To confirm the transcription start site, single stranded cDNA was prepared and a poly-dGTP tail added to the 5′ end using terminal polynucleotide transferase (Promega, USA). Double stranded cDNA was then produced using a poly-dCTP oligonucleotide in conjunction with the penton base or hexon oligonucleotide in PCR.

Construction of expression cassettes

The required fragments were produced using PCR with suitable restriction enzyme sites included onto the ends of the oligonucleotides used in the PCR reactions. The fragments were ligated to form the required sequence and inserted into an appropriate plasmid vector. The SV40 poly-A recognition site (AATAAA) was used as the 3′ untranslated region of the expression cassettes.

Transient expression of cassettes

The eukaryotic cell transfection reagent DOTAP (Boehringer Mannheim, Germany) was mixed with the appropriate DNA (5 μg DNA/50 cm^2 culture dish) the used to transfect primary CK cells using the method described by the manufacturer. For each time point, duplicate tissue culture dishes were used and then pooled for testing of CAT activity. CK cells were transfected and re-incubated for 24 h, then infected with FAV-10 at multiplicity of infection (MOI) of 10 plaque forming units (pfu) per cell. After adsorption to the cells for 1 h, cells were then incubated for a further 0, 2 and 18 h (where all cells were visibly infected but not lysed). At least two separate transfections were carried out and assayed for CAT activity. CAT assays were performed as described by the manufacturer (Promega, USA) for use in a liquid scintillation counter. All normalized CAT activities were scaled to the normalized values obtained for the negative control (p207) set at a relative unit of 1.0.

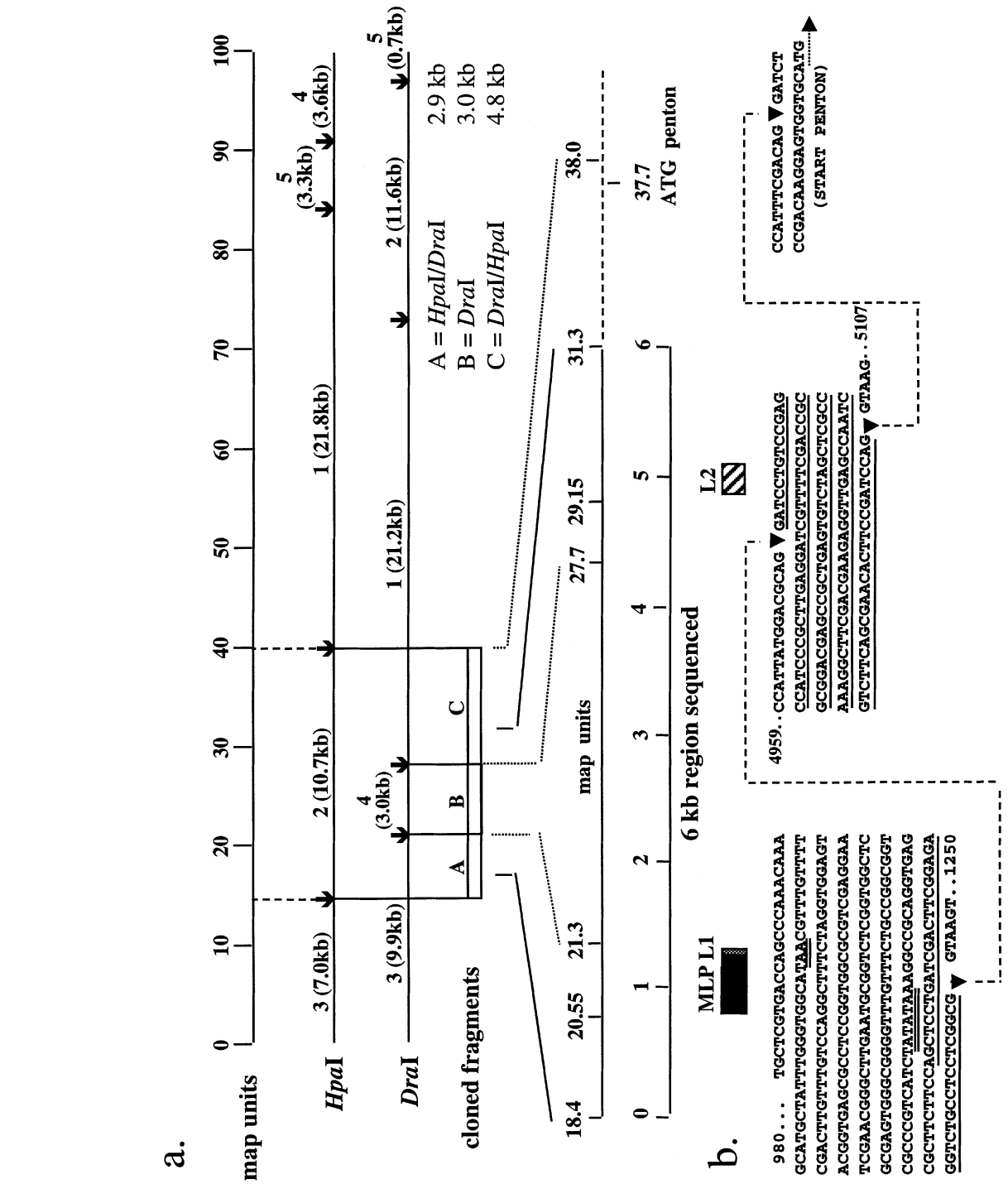
Results and discussion

Location of the MLP on the FAV-10 genome

A region of 10.4 kilobase pairs (kb) from 15.1 to 38.1 map units of the FAV-10 genome was cloned as three fragments. These fragments were selected based on the location of the MLP and TPL in the HAV-2 genome [14]. The fragments cloned were a 2.8 kb *Hpa*I/*Dra*I fragment (15.1 to 21.3 map units), a 3.0 kb *Dra*I fragment (21.3 to 27.8 map units) and a 4.8 kb *Dra*I/*Hpa* I fragment (27.8 to 38.1

map units) (Fig. 1a). Using these cloned fragments, a region of 6 kb of continuous sequence from both DNA strands was determined (GenBank submission, accession number AF007577). From this sequence the putative MLP TATA at 1174 to 1181 nucleotides was identified. Overall, sequence identity between FAV-10 and HAV-2 from the TATA (at 1 174 nucleotides) to the 3′ end of the sequence (at 6 078 nucleotides) was only 47%. While short regions with higher levels of identity were found at intervals throughout this FAV-10 sequence, these were found to concide with reported conserved regions of the adenovirus DNA polymerase gene [28]. The DNA polymerase gene forms part of the E2 transcription unit and is encoded by the complementing strand to that of the MLP and leader sequences [28]. When each of the 3 HAV-2 leader sequences were individually compared to this region of the FAV-10 genome, no significantly homologous regions were found that were not accounted for by sequence requirements for the DNA polymerase gene (data not shown). From the sequence data, it was possible to locate a putative transcription start site 24 bp downstream of the 3′A of the TATA with a surrounding sequence of CTTCTTCCAGCTCCT which conforms to the consensus sequence of 5′PyPyCA/GPyPyPyPyPy3′ [37]. However, based on sequence comparison with HAV, it was not possible to locate any downstream elements (DE) located in intron 1 or an upstream stimulatory factor (USF). Both of these features were previously reported to be involved with late gene transcription from the MLP in HAV [8, 12, 16, 20, 23, 24, 26, 30], although a reverse CAAT box was located 148 bp 5′ to the TATA box (Fig. 1b).

Fig. 1. **a** *Hpa*I and *Dra* I restriction enzyme maps of the FAV-10 genome. The vertical arrows indicate the enzyme cleavage sites. The vertical dashed lines indicate the restriction sites used for cloning fragments A (*Hpa* I/*Dra* I, 2.9 kb), B (*Dra* I, 3.0 kb) and C (*Dra* I/*Hpa* I, 4.8 kb). The open box indicates the region sequenced. **b** The 6 kb sequenced from map units 18.4 to 31.3 is shown. The position of the major late promoter and first leader sequence are indicated by the solid box and dotted box, respectively. The hatched box indicates the position of the second leader. Below each box is the sequence of the MLP, including the upstream region and first leader and second leader, respectively. The nucleotide positions shown refer to the sequence lodged in GenBank accession number AF007 577. The leader sequences are single underlined. Features of the MLP such as the inverted CAAT and TATA box are double underlined. The MLP and leader sequences were used to construct expression cassettes which contained either the entire 5′ MLP sequence and both leaders (5′ MLP-LS) or the MLP sequence from the TATA box and both leader sequences (MLP-LS). The splice donor sites at the end of the first and second leader sequences (CG▼GTAAGT and CAG▼GTAAGT, respectively), compare well with the consensus sequence C/AAG▼GTA/GAGT [24]. The splice acceptor site between the intron and the second leader (CCATTATGGACGCAG▼G) also compared favorable with the consensus sequence $(C/T)_n$NT/CAG▼G [24]. cDNA cloning of late message RNA using hexon or penton oligonucleotides established that there were only two leader sequences. The splice acceptor site for the FAV-10 penton is indicated by the solid triangle. The dashed line indicates the joining of the leader sequences from the end of the first leader sequence to the second leader, and subsequently from the second leader to the penton


a.
map units
0
10
20
30
40
50
60
70
80
90
100
HpaI
3 (7.0kb)
2 (10.7kb)
1 (21.8kb)
DraI
3 (9.9kb)
4 (3.0kb)
1 (21.2kb)
2 (11.6kb)
5 (3.3kb)
4 (3.6kb)
5 (0.7kb)
A
B
C
cloned fragments
A = HpaI/DraI 2.9 kb
B = DraI 3.0 kb
C = DraI/HpaI 4.8 kb
18.4
20.55
21.3
map units
27.7
29.15
31.3
38.0
0
1
2
3
4
5
6
37.7
ATG penton
6 kb region sequenced
MLP L1
L2
b.
980... TGCTCGTGACCAGCCCAAACAAA
GCATGCTATTTGGGTGGCATAACGTTTGTTTT
CGACTTGTTTGTCCAGGCTTTCTAGGTGGAGT
ACGGTGAGCGCCTCCGGTGGCGCGTCGAGGAA
TCGAACGGGCTTGAATGCGGTCTCGGTGGCTC
GCGAGTGGGCGGGGTTTGTTTCTGCCGGCGGT
CGCCCGTCATCTATATAAAGGCCGCAGGTGAG
CGCTTCTTCCAGCTCCTGATCGACTTCGGAGA
GGTCTGCCTCCTCGGCG ▼ GTAAGT..1250
4959..CCATTATGGACGCAG ▼ GATCCTGTCCGAG
CCATCCCGCTTGAGGATCGTTTTCGACCGC
GCGGACGAGCCGCTGAGTGTCTAGCTCGCC
AAAGGCTTCGACGAAGAGGTTGAGCCAATC
GTCTTCAGCGAACACTTCCGATCCAG ▼ GTAAG..5107
CCATTTCGACAG ▼ GATCT
CCGACAAGGAGTGGTGCATG
(START PENTON)

Identification of the FAV-10 leader sequences

An oligonucleotide was synthesized corresponding to the transcription start site of the MLP, two additional oligonucleotides were synthesized, one complementary to and within the penton base coding region and the other complementary to and within the hexon coding region. The three oligonucleotides were used to make cDNA from late stages of the FAV-10 infection cycle. The resulting fragments were cloned and sequenced. Both the hexon and penton base cDNAs resulted in a leader sequences of identical length and sequences. Interestingly, the FAV-10 leader sequence was only 168 nucleotides is length, some 35 nucleotides shorter than the HAV-2 TPL [2, 5]. Comparison with the FAV-10 genomic DNA sequence revealed that the leader sequence consisted of only two parts separated by 3 726 nucleotides on the FAV-10 genome (Fig. 1b).

Thus, the FAV-10 has a bipartite leader (BPL) sequences which differs significantly from all other adenoviruses studied to date which have a TPL. The FAV-10 first leader sequence was 39 bp in length from nucleotide 1 207 to 1 245 and the second leader sequence was 129 bp from 4 975 to 5 103 nucleotides. While the length of the first leader was found to be similar in size to the first leader of HAV-2 (40 nucleotides) the sequence identity across the region was very low. This indicated the role of the leader is probably not related to its sequence. The remaining FAV-10 leader sequence cloned showed identity with only one other region in the genomic sequence, namely a 129 bp region forming the second leader sequence of FAV-10 but in a similar genomic position as the third leader sequence of HAV-2 [27]. This identical bipartite leader sequence was found for cDNA produced from both the hexon and penton base.

In order to confirm the translation start site and the leader sequence, mRNA was isolated from late stages of the FAV-10 replication cycle and cDNA synthesized. Following first strand synthesis a poly-dGTP tail was added to the 3′ end of the cDNA and PCR performed using a poly dCTP oligonucleotide (15 dCTP residues) and the internal hexon or penton base oligonucleotides. Cloned cDNA from the PCR was sequenced and confirmed the adenosine residue 24 bases downstream of the TATA to be the transcription initiation site as well as confirming the sequence and bipartite structure of the FAV-10 MLP leader sequence. Recently, the complete genomic sequence of FAV-1 (CELO) has been reported [6]. While the authors did not locate the MLP or leader sequences, comparison with the FAV-10 MLP/BPL sequence revealed some homologies, especially the second leader of FAV-10 (data not shown).

Identification of the splice donor and acceptor sites

The splice sites from the FAV-10 leader sequences were compared with the consensus splice recognition sequences for acceptor and donor splice sites. The consensus sequence for acceptor sites is (C/T)n NT/CAG▼G [25] with the splicing occurring between the two G residues as indicated by the (▼). It was found that the acceptor splice site, between the intron and leader sequence 2, with a sequence of CCATTATGGACGCAG▼G (Fig. 1b) compared favorably with the

consensus sequence. The donor splice sites for the two leader sequences were then compared with the donor splice site consensus sequence of C/AAG▼GTA/GAGT [25]. Both of the FAV-10 leaders conformed closely to the consensus sequence, leader 1 CG▼GTAAGT and leader 2 CAG▼GTAAG (Fig. 1b). It is most likely therefore, that the process of splicing that takes place in the FAV MLP closely resembles the mechanisms of HAV splicing.

Construction of expression cassettes

To test the functionality of the FAV-10 MLP/BPL, four expression cassettes were constructed in plasmid vectors. The first contained sequence form the TATA to the donor splice site of the first leader sequence incorporated at a *Bam*HI site (MLP/LS) (Fig. 1b). The second construct contained 200 bases 5′ of the TATA (5′ MLP/LS) to include an inverted CAAT box 148 bp upstream of the TATA (Fig. 1b) and a potential upstream stimulatory factor (USF) even though a FAV-10 USF could not be detected by sequence comparison with HAV-2. A third cassette was constructed that contained the human cytomegalovirus (CMV) major immediately early (IE) gene (HCMVIE P/E). A fourth cassette was constructed that contained the CAT gene and no promoter element (p207). All cassettes contained an SV40 poly A sequence at the 3′ end of the CAT gene.

Transient expression experiments were performed by transfecting plasmid DNA into primary CK cells. The cells were incubated for 24 h at which time they were either infected with adenovirus or had the media changed and re-incubated. CAT assays were performed at various times post-infection with FAV-10 (Fig. 2). Three transfection experiments were performed for each time point with or without FAV-10 coinfection. The results shown in Fig. 2 demonstrates that in the absence of FAV-10 very little or no CAT activity can be detected from the two FAV-10 MLP constructs, whereas the HCMV IE promoter produces CAT activity significantly above background. At early stages, following coinfection with FAV-10 the presence of virus has no discernable effect on CAT activity (2 hpi). At a time after FAV-10 DNA replication has commenced (18 hpi) there was a significant increase in the level of CAT activity from the 5′ MLP/LS construct. Interestingly, the same effect was not observed with the smaller version (MLP/LS) promoter construct. An increase in the HCMV IE promoter is also evident but this may be due to an accumulation over time. These results showed that the 5′MLP/BPL was active and upregulated following the onset of FAV-10 DNA replication. While this is similar to that found with the HAV MLP/TPL [11–12, 13, 19, 20] the mechanism of up regulation of the FAV-10 MLP/BPL must be due in part to transacting factors produced following DNA replication and as yet unidentified sequences that function in a similar manner to the USF of HAV. Since the intron between the two leader sequences was removed in the construction of the expression cassettes, it is not yet known whether there are sequences that have a similar function to the DEs of HAV, or in FAV-10 consist of totally different sequences.

By using a combination of DNA sequencing and transient expression assays we have identified a region of the FAV genome required for FAV MLP activity.

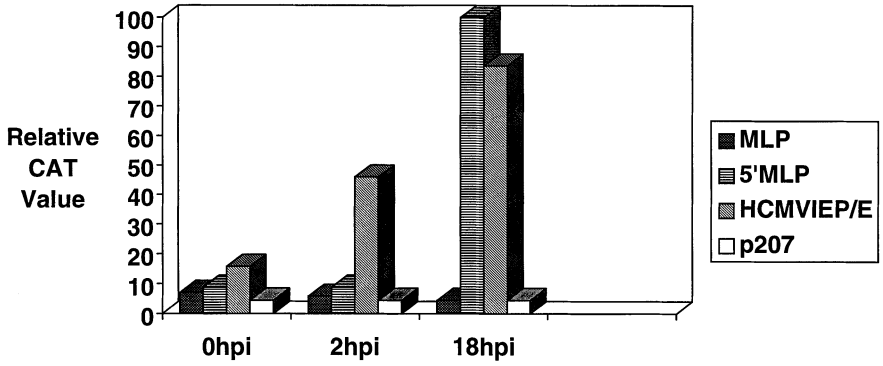


Fig. 2. The four expression cassettes were transfected into CK cells and CAT expression assayed 24 h followed by no FAV-10 infection (0 hpi) or infection with FAV-10 for 2 h (2 hpi) or 18 h (18 hpi). Bars represent the average of at least two separate transfection assays. Variation was less than 10% between experiments. All bars are expressed in relative CAT values with the highest given the arbitrary value of 100 and all other expressed as relative to that value

We have also demonstrated that the FAV MLP is typical of many mammalian cell promoters in that it possesses two essential elements located upstream of the transcription start site. One is the highly conserved TATA box located between -3 to -25 (with respect to the transcription start site) and the other is a less well conserved region between the TATA box and -226, but as yet unidentified. Why FAV has a bipartite as opposed to a tripartite leader sequence is not known, but may reflect some type of evolutionary progression. The lack of sequence homology, difference in length and a bipartite structure all raise questions as to how the leader sequences actually operate and exert their effect on late gene translation. Our results confirm the conclusions of Logan and Shenk [19] that the action of the leader sequence is not solely due to a structural or sequence effects resulting in an increase in translation. Clearly if this were the case, one would expect greater conservation of sequence between these two adenoviruses and that translation would be enhanced equally at all times, not just late times post infection. As in the HAV infected cell, at late times post infection of CK cell by FAV, something different allows the leader sequence to enhance translation. The finding that fowl adenoviruses utilize a bipartite leader sequence is significant and will contribute to a better understanding of the molecular biology of FAVs and potentially the use of the FAV MLP-LS for the expression of antigens from important viral disease of poultry in recombinant fowl adenoviruses.

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