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The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3' termini

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ABSTRACT

Our previous DNA sequence comparisons of 3' terminal portions from equivalent herpes simplex virus type 1 (HSV-1) and HSV-2 genes identified a conserved sequence (consensus YGTGTTYY; Y=pyrimidine) located approximately 30bp downstream from the AATAAA signal. We report here that this signal is located downstream from 67% of the mammalian mRNA 3' termini examined. Using constructions with the bacterial chloramphenicol acetyl transferase (CAT) gene linked to an HSV 'terminator' fragment, we show that deletions in the 'terminator' reduce CAT activities and the levels of CAT mRNA 3' termini. Specifically: (1) deletions of downstream sequences which extend up to the consensus YGTGTTYY signal reduce CAT levels to values 35% of those obtained with undeleted plasmids, (2) a deletion of a further 14bp, which removes the YGTGTTYY consensus but not the poly A site, reduces CAT activities to 1%-4%. The levels of CAT mRNA 3' termini reflect the reductions in CAT activities howevever, levels of mRNA 5' termini are unaffected by these deletions. The RNA produced in the absence of the YGTGTTYY signal is present in the cytoplasm although no CAT activity is detectable.

INTRODUCTION

Sequence comparison of genes transcribed by RNA polymerase II has identified a number of consensus sequences certain of which have been shown by functional assays to be important in the control of transcription. For example, the TATA homology, initially identified by comparison of 5' flanking regions (1), was shown to be required for transcription initiation (2). We have used HSV-1/HSV-2 DNA sequence comparison of equivalent genomic regions to identify discrete blocks of homology and have shown that these correspond to transcription regulatory signals which are located upstream from mRNA 5' termini (3, 4).

In contrast to 5' end formation, analysis of DNA sequences required for the formation of mRNA 3' termini has received less

attention. The highly conserved hexanucleotide sequence AATAAA (5) is required for polyadenylation of mRNAs transcribed by RNA polymerase II (6); a point mutation within this sequence abolishes the ability to form correctly terminated mRNA (7), and a α-thalassaemic defect is due to an altered AATAAA signal (8). However, it is clear that sequences apart from the AATAAA signal are required for the polyadenylation process since this sequence is present at sites other than at mRNA 3' termini.

Our HSV DNA sequence comparisons also identified a conserved sequence (consensus YGTGTTYY) located downstream from the poly A site at about 30 nucleotides from the AATAAA signal (3, 9, 10, 11) which is similar to a sequence noted by Taya et al (12). Our study of DNA sequences at the 3' termini of mammalian poly A^+ mRNAs reveals that the YGTGTTYY signal is present downstream from many genes. We also report, using plasmid constructions with the bacterial CAT gene, that the YGTGTTYY signal, together with other further downstream signals, is required for efficient formation of an mRNA 3' terminus.

MATERIALS AND METHODS

Growth of Cells and Calcium Phosphate Transfection

HeLa cells were grown as monolayers on 90mm Petri dishes in Dulbecco's modified Eagles medium supplemented with 2.5% foetal calf serum (Flow Laboratories) and 2.5% calf serum.

Subconfluent HeLa cells were transfected (13) with calcium phosphate precipitates containing 15µg of pTER plasmid, 10µg of the pseudorabies (PRV) plasmid, pPRVKpnh (14, 15), and 10µg of pβ(244+)β, a β-globin plasmid (16). In experiments where CAT activities alone were assayed, the β-globin plasmid was omitted. At 24h after addition of the calcium phosphate precipitates, the medium was removed and 15ml of fresh medium was added to each plate.

Cloning Procedures

The host bacterium was E.coli K12 strain JM 83 (17) and the cloning vector was pUC9 (18). Cloning procedures for the construction of the pTER plasmid series and for pBTER1 are given in Results, and the construction of plasmid pLW1 is described in Gaffney et al (19). The various deletions in plasmid pTER3 were

generated by linearisation with Bam HI followed by treatment with exonuclease III; blunt-ended DNA was produced using Mung Bean nuclease and then ligated to give the plasmid series pTER3Δ. Deletion end-points were determined by DNA sequencing (20).

Radiolabelling of DNA Fragments

DNA fragments were either 5'- or 3'-labelled as described by McLauchlan and Clements (21). In order to generate single-stranded DNA probes, the end-labelled fragments were denatured and electrophoresed on a 5% polyacrylamide gel at 4°C (22).

RNA Isolation and Structural Analysis of mRNAs

Cytoplasmic cellular fractions were prepared and RNA was isolated as described by Easton and Clements (23).

Structural analysis of mRNAs was performed using the nuclease S1 procedure (24, 25). Either 5'- or 3'-labelled DNA probes and 15-20µg of cytoplasmic RNA were mixed in a buffer containing 50% (v/v) formamide (deionised with Amberlite monobed resin MB-2), 400mM NaCl, 40mM PIPES pH6.8 and 1mM EDTA to a final volume of 30µl. This mixture was heated to 90°C for 3min and then incubated at 42°C for 16h. Prior to nuclease S1 treatment, the hybridisation mixtures were quenched in ice.

Nuclease S1 digestion was performed at 30°C for 2-3h in 300µl of 400mM NaCl, 40mM sodium acetate pH 4.5, 1mM $ZnSO_4$ with 5000 units of nuclease S1. Following phenol/chloroform extraction and ethanol precipitation, the nuclease S1-resistant hybrids were electrophoresed on a 6% denaturing polyacrylamide gel.

CAT Assays

Cell extracts were prepared as described by Gorman et al (26). The levels of CAT activity were measured by incubating aliquots from cell extracts in a mixture containing 1mM acetyl CoA, 250mM TRIS pH7.8, and 0.125µCi ^{14}C chloramphenicol (68nmoles/µCi) at 37°C. Aliquots were removed at various times, extracted with 200µl of ethyl acetate and the organic phase was dried down. The ^{14}C chloramphenicol was resuspended in 30µl ethyl acetate and spotted onto 0.25mm silica gel thin layer chromatography plates. Ascending chromatography was performed in 95% chloroform: 5% methanol, after which the chromatograms were

autoradiographed. The percentage conversion to acetylated ^{14}C chloramphenicol was calculated by removing the spots from the chromatograms and counting in a scintillation counter. CAT activities were normalised by measuring the protein content of each extract (27) which allowed calculation of CAT specific activities.

RESULTS

To test the effect of removing sequences downstream from the poly A site, plasmid constructions were made using the CAT gene. An HSV-2 IE promoter fragment was inserted upstream from the CAT gene giving plasmid pLW1 (19), and a 'terminator' fragment from the same IE gene was inserted downstream to give plasmid pTER5.

The HSV-2 IE promoter was a 210bp SmaI/Sau3A fragment from IE gene-4/5 which comprised 91bp of 5' flanking sequence and 119bp of leader sequence (10). This promoter fragment contains all the sequences required for the initiation of transcription and the location of the mRNA 5' terminus is identical to that determined for the HSV-2 IE mRNA-4/5 synthesised in vivo (10, 19). Plasmid pLW1 has no ATG in the HSV-2 leader sequence; the first initiation codon in the mRNA is the CAT gene ATG.

The 'terminator' was a 100bp Sma I/Xba I fragment from the 3' terminus of HSV-2 IE gene-5. This fragment contains an AATAAA signal, and located 8 nucleotides downstream from the poly A site is the sequence TGTGTTGC which fits the YGTGTTYY consensus (10) except in one position. A comparison of the HSV-2 and HSV-1 DNA sequences from the 3' terminal portions of IE gene-5 (Fig. 1) reveals conservation of the AATAAA signal together with two blocks of homology downstream from the poly A site. The conserved block between positions 62-65 (Fig. 1) contains the sequence corresponding to the YGTGTTYY consensus.

Construction of 'terminator' plasmids

Fig. 2 details the construction of plasmids used for short-term expression assays. Briefly, the 'terminator' fragment was cloned into pUC9 to give plasmid pTER3. The 'terminator' was then inserted into the pLW1 promoter/CAT plasmid at a site just downstream from the CAT gene to give plasmid pTER5. In pTER5,

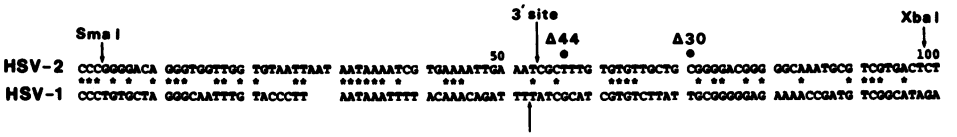


FIG. 1. DNA sequence comparison at the mRNA 3' termini of the HSV-2 and HSV-1 IE genes-5 (10). The asterisks denote nucleotides which are conserved. Locations of the 3' termini and the deletion end-points in pTER5 plasmids are indicated. The AATAAA signal and the sequences homologous to the YGTGTTYY motif are at positions 31-36 and 61-68 respectively.

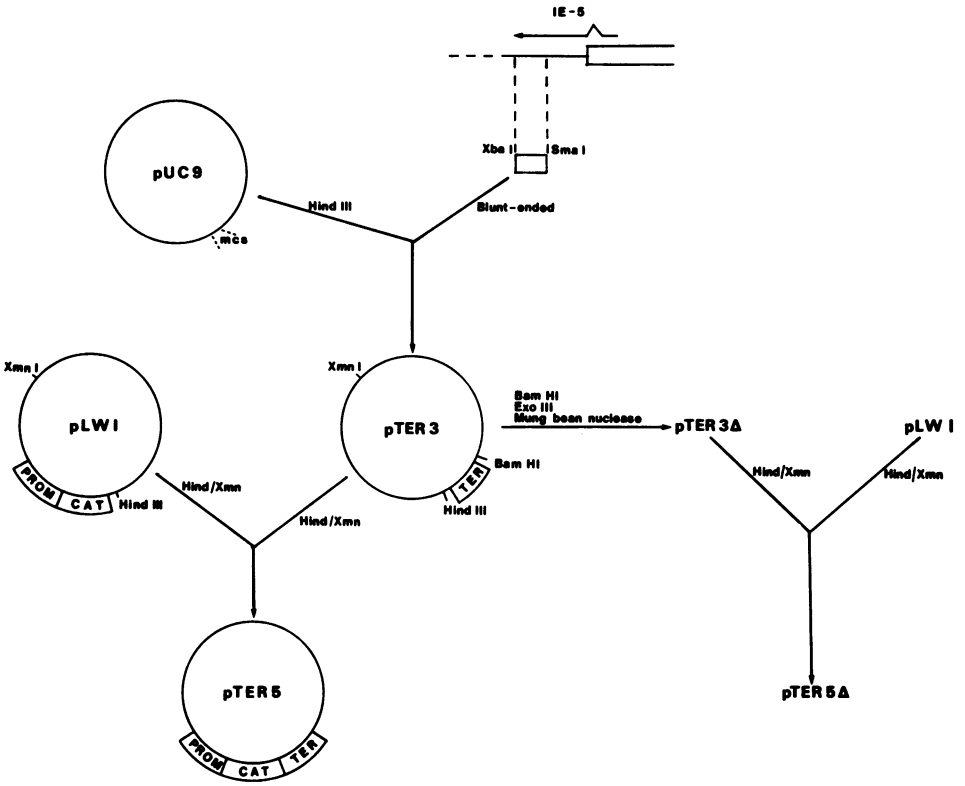


FIG. 2. Construction of pTER plasmids. The Sma I/Xba I fragment (Fig. 1) was derived from the HSV-2 plasmid pBam b' (10) and blunt-ended using Klenow fragment polymerase with all four dNTP's. The multiple cloning site of pUC9 is designated mcs.

TABLE 1 CAT activites obtained with various plasmid constructions are shown for 4 separate transfection experiments.

	1		2	3	4	poly A site		YGTGTTYY
pTER5	100	(4.01)	100	100	100	+		+
pTER5 Δ 30	35	(1.41)	34	32	39	+		+
pTER5 Δ 44	3	(0.12)	4	1	1	+		-
pTER6	1.5	(0.02)	2	0.6	1		inverted	
pLW1	0.4	(0.01)	U	U	U	-		-
pBTER1	110	(4.45)				+	β-globin	+

Activities are expressed as a percentage of the pTER5 value; U indicates undetectable CAT activity. The figures in brackets from experiment 1 are typical CAT values expressed as nmoles converted chloramphenicol/ ug protein/ h. Plasmids pTER Δ 30 and pTER5 Δ 44 contain deletions in the HSV 'terminator' fragment; pTER6 has an 'inverted terminator' ; pLW1 has no 'terminator' fragment; PBTER1 contains a 1250bp rabbit β-globin fragment. The presence (+) or absence (-) of the poly A site and YGTGTTYY signal in each plasmid is indicated.

the 'terminator' is orientated with the AATAAA signal on the mRNA sense strand.

A series of deletions was made in plasmid pTER3 from a BamHI site located in the multiple cloning site downstream from the 'terminator'. These deletions removed sequences from the 3' portion of the 'terminator' and extended for different lengths through the YGTGTTYY homology towards the AATAAA sequence to give the plasmid series pTER3Δ. These 'terminator' deletions were then inserted into the pLW1 promoter/CAT plasmid to give the expression plasmid series pTER5 Δ (Fig. 2).

Removal of 'terminator' sequences reduces CAT activity

Plasmid pLW1 lacks the pTER5 'terminator' and Table 1 shows that Hela cells transfected with pLW1 produced little detectable CAT activity whereas high levels of activity were obtained with pTER5. The 'terminator' fragment therefore was required for detectable CAT gene expression. To determine the effect on CAT activity of an alternative 'terminator', a 1250bp Bam HI/Xho I fragment of the rabbit β-globin gene containing all the IVS 2 sequences and some 600bp of flanking DNA (28) was inserted into the Hind III site of pLW1. Plasmid pBTER1 contained this rabbit β-globin fragment with the AATAAA signal on the mRNA sense strand. Table 1 shows that CAT activity obtained with pBTER1 was

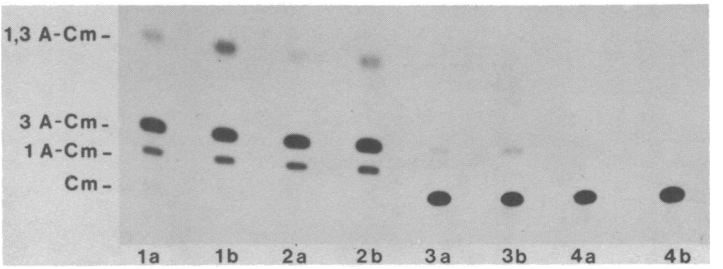


FIG. 3. CAT activities produced by the pTER plasmids as determined by thin layer chromatography. Aliquots were removed from each reaction after (a) 20min and (b) 40min. The positions of the acetylated and non-acetylated forms of ^{14}C chloramphenicol are indicated. Lanes 1a, 1b pTER5; lanes 2a, 2b pTER5△30; lanes 3a, 3b pTER5△44; lanes 4a, 4b pTER6.

comparable to that obtained with pTER5.

The CAT levels obtained with the pTER5△ series were compared with pTER5 CAT activity. On this basis, two deleted plasmids with reduced CAT activities were selected, and the deletion end-points were determined by DNA sequencing. These two plasmids pTER5△30 and pTER5△44 had 30bp and 44bp respectively deleted from the 3' portion of the 'terminator'. Locations of the deletion end-points are shown in Fig. 1; pTER5△30 has a deletion extending to 3bp from the YGTGTTYY signal while in pTER5△44 this signal is removed but the poly A site is retained.

Fig. 3 shows an autoradiograph of the CAT assays obtained with the pTER5 plasmid series and the relative CAT activities from a number of separate experiments are listed in Table 1. The CAT levels produced by pTER5△30 and pTER5△44 were low and transactivation by co-transfection with the PRV plasmid, pPRVKpnh, was required to boost activities to levels which could be accurately estimated. Plasmid pPRVKpnh expresses the IE regulatory protein (29) which increases transcription from other plasmid-borne eukaryotic promoters and from certain cellular promoters (15, 30, 31).

Deletion of 30bp from the 3' portion of the 'terminator' fragment reduced CAT levels to approximately 35% of the pTER5 value while removal of a further 14bp markedly reduced activity to a level of 1%-4%; pTER5△44 produced CAT activities only just above the background levels obtained with plasmids pLW1 and pTER6

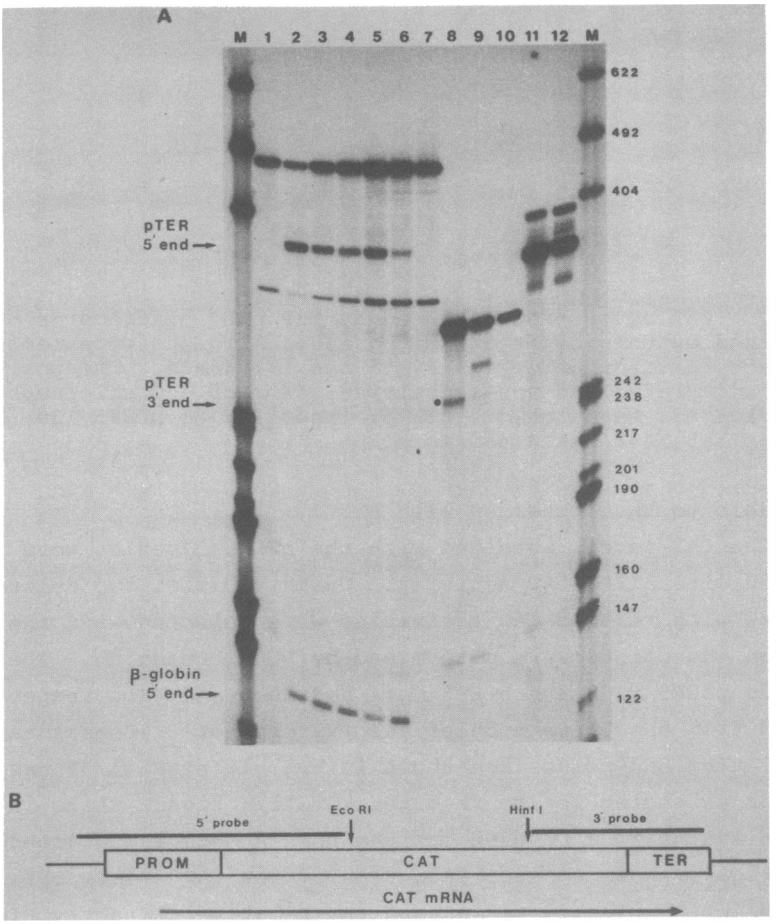


FIG. 4. (A) The abundance of mRNA 5' and 3' termini produced by the pTER plasmids. The DNA probes used in lanes 1-7 were (1) an Eco RI/Sma I fragment 5'-labelled at the Eco RI site to determine the pTER CAT mRNA 5' termini and (2) a 5'-labelled Bst NI fragment to detect the β-globin mRNA 5' termini. The single-stranded DNA probes used to detect the pTER CAT mRNA 3' termini were a Hinf I fragment from pTER5 3'-labelled at the Hinf I site in the CAT gene coding sequences (lanes 8-10) and a Hinf I/Bgl I fragment from pTER5Δ44 again 3'-labelled at the Hinf I site (lanes 11 and 12). RNA samples shown; lanes 1, 7, 10 and 12, mock-infected RNA; lanes 2 and 8, pTER5 RNA; lanes 3 and 9, pTER5 Δ30 RNA; lanes 4 and 11, pTER5Δ44 RNA; lane 5, pTER6 RNA; lane 6, pLW1 RNA. Molecular weight markers (M) were pAT 153 DNA cleaved with Hpa II.

(B) Approximate locations for the 5' and 3' termini of pTER CAT mRNA are shown. Restriction endonuclease cleavage sites within the CAT coding sequences which were used for making end-labelled DNA probes (see Fig. 4A) are indicated.

(Table 1). Plasmid pLW1 has no 'terminator' fragment and pTER6 has the 'terminator' inserted in the opposite orientation to that of pTER5.

Levels of mRNA 3' and 5' termini produced by the pTER5 plasmid series

Cytoplasmic RNA isolated from cells transfected with the pTER5 plasmid series was hybridised to strand-separated DNA probes which were 3'-labelled at the Hinf I site within the CAT coding sequences (Fig. 4B). RNAs produced by pTER5 and pTER5 △ 30 were hybridised to a probe produced from pTER5 DNA. As the deletion end-point of pTER5 △ 44 is only 4bp from the poly A site (Fig. 1), hybrids produced by RNA transcribed across the poly A site could not be resolved from correctly terminated RNAs, therefore RNA from pTER5 △ 44 was hybridised to a probe from pTER5 △ 44 DNA. The DNA probes were end-labelled to similar specific activities. After treatment with nuclease S1, the protected DNA fragments were electrophoresed on 6% denaturing polyacrylamide gels (Fig. 4A).

RNA produced from pTER5 protected a fragment of 247 nucleotides (Fig. 4A, track 8) and this positioned the 3' end at nucleotide 54 (Fig. 1) which is the same location as the mRNA 3' terminus for IE mRNA-5 from HSV-2 infected cells (10). The 3' end of mRNA produced by pTER5 △ 30 was located at the same position as that obtained with pTER5 but the amount of protected DNA fragment was reduced to approximately 30% of the pTER5 value (Fig. 4A, track 9). No discrete 3' end was detected with pTER5 △44 RNA (Fig. 4A, track 11). Hence, the levels of mRNA 3' termini obtained with the pTER5 series reflected the CAT activities obtained with these plasmids.

Plasmids pTER5 and pTER5△30 have identical sequences up to 18bp beyond the poly A site then the DNA sequences are dissimilar due to the terminator deletion (30bp) in plasmid pTER5△30. Therefore, the band below the fully protected probe (Fig. 4A, track 9) represents hybridisation of the pTER5 DNA probe to pTER5 △30 RNA transcribed through the poly A site. Hence the size of this band reflects the sequence homology between pTER5△30 RNA and the pTER5 DNA probe.

To assess the validity of the comparative 3' terminal data,

plasmid, pβ(244+)β, containing the β-globin promoter and the polyoma virus enhancer (16), was co-transfected with the various pTER5 plasmids. A strand-separated DNA probe, 5'-labelled at a Bst NI site approximately 130bp from the β-globin mRNA 5' terminus was used to estimate β-globin mRNA levels (32). As Fig. 4A tracks 2-6 indicate, the β-globin mRNA levels were similar in all the RNA samples.

Levels of mRNA 5' termini were estimated using a strand-separated DNA probe 5'-labelled at an EcoRI site within the CAT coding sequences of pTER5 (Fig. 4B). Using this probe, a protected DNA fragment of 369 nucleotides was obtained with RNA produced by all the pTER5 series plasmids and by pLW1 (Fig. 4A, tracks 2-6). A similar amount of labelled probe was protected with the different RNA samples. Therefore, unlike the situation with the 3'-labelled probe, the levels of protected 5' probe obtained with pTER5 △ 30 and pTER5 △ 44 were similar to that obtained with pTER5 although the CAT activities were lower than the pTER5 values. This suggests that cytoplasmic RNA with a normal 5' terminus but an incorrect 3' terminus does not produce functional protein.

DISCUSSION

Our results have demonstrated that removal of sequences downstream from the poly A site of HSV-2 IE gene-5 reduces the level of correctly processed mRNA 3' termini, and this effect is reflected in lower levels of CAT activity. Deletion plasmid pTER5 △ 30 gave reduced mRNA 3' termini and the CAT levels were 35% of the values obtained with pTER5 which contains all the 'terminator' sequences. Moreover, the removal of a further 14bp, which includes the YGTGTTYY signal but not the poly A site, markedly reduced CAT activity to 1%-4% of the pTER5 value, and no correct 3' termini were detected.

When the DNA sequences at the 3' terminal region of HSV-2 IE gene-5 were compared with those of HSV-1 IE gene-5, a portion of the 14bp segment required for efficient 3' end formation was conserved (10). Furthermore, a similar downstream sequence was conserved in other equivalent HSV-1 and HSV-2 genes at approximately the same position relative to the AATAAA signal (3,

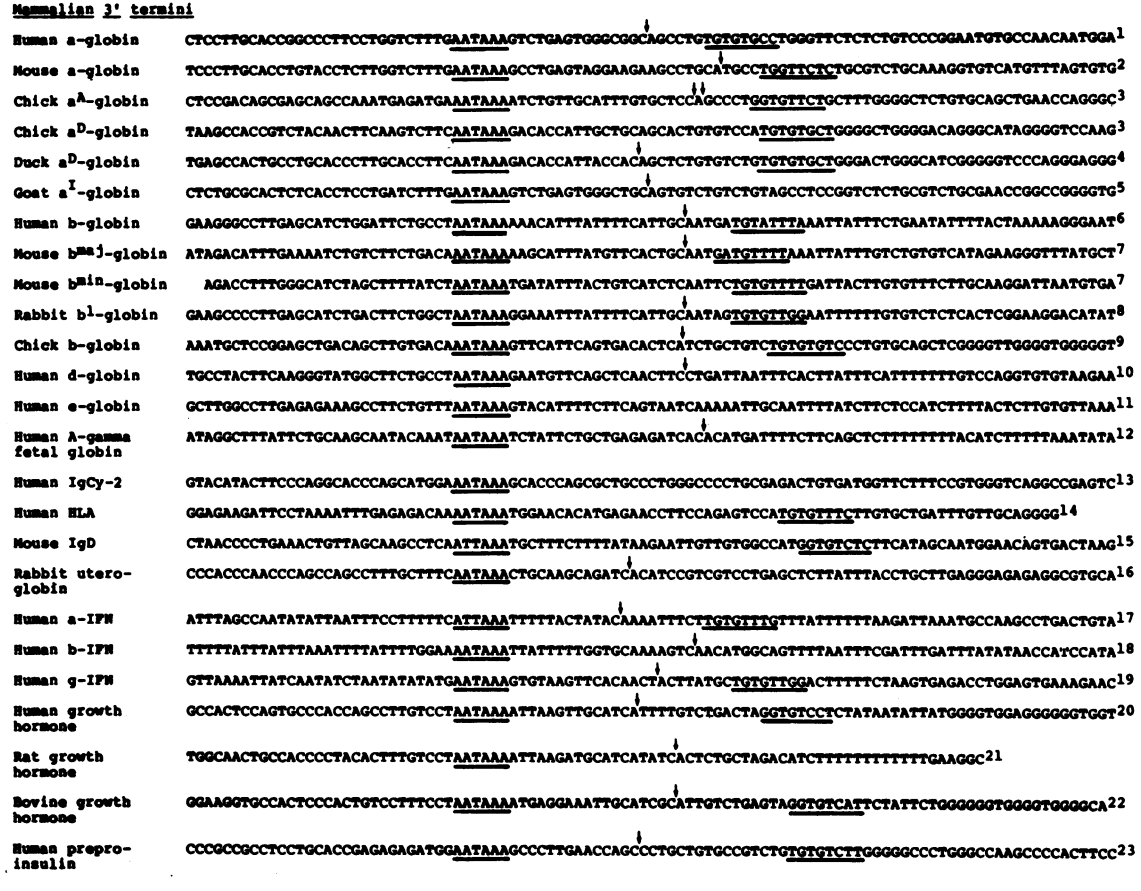
9). The 3' terminal DNA sequences from a number of mammalian and eukaryotic viral genes which specify poly A^+ mRNAs were examined (Fig. 5) and sequences similar to those conserved downstream from the poly A sites of HSV genes were identified in 67% of the genes examined. From a study of 3' terminal sequences from 100 genes, we have derived the consensus sequence YGTGTTYY, and the preferred location for this sequence is 24-30 nucleotides downstream from the AATAAA signal. We conclude from our analysis that this conserved element is likely to play an important role in the formation of mRNA 3' termini for a wide variety of mammalian genes.

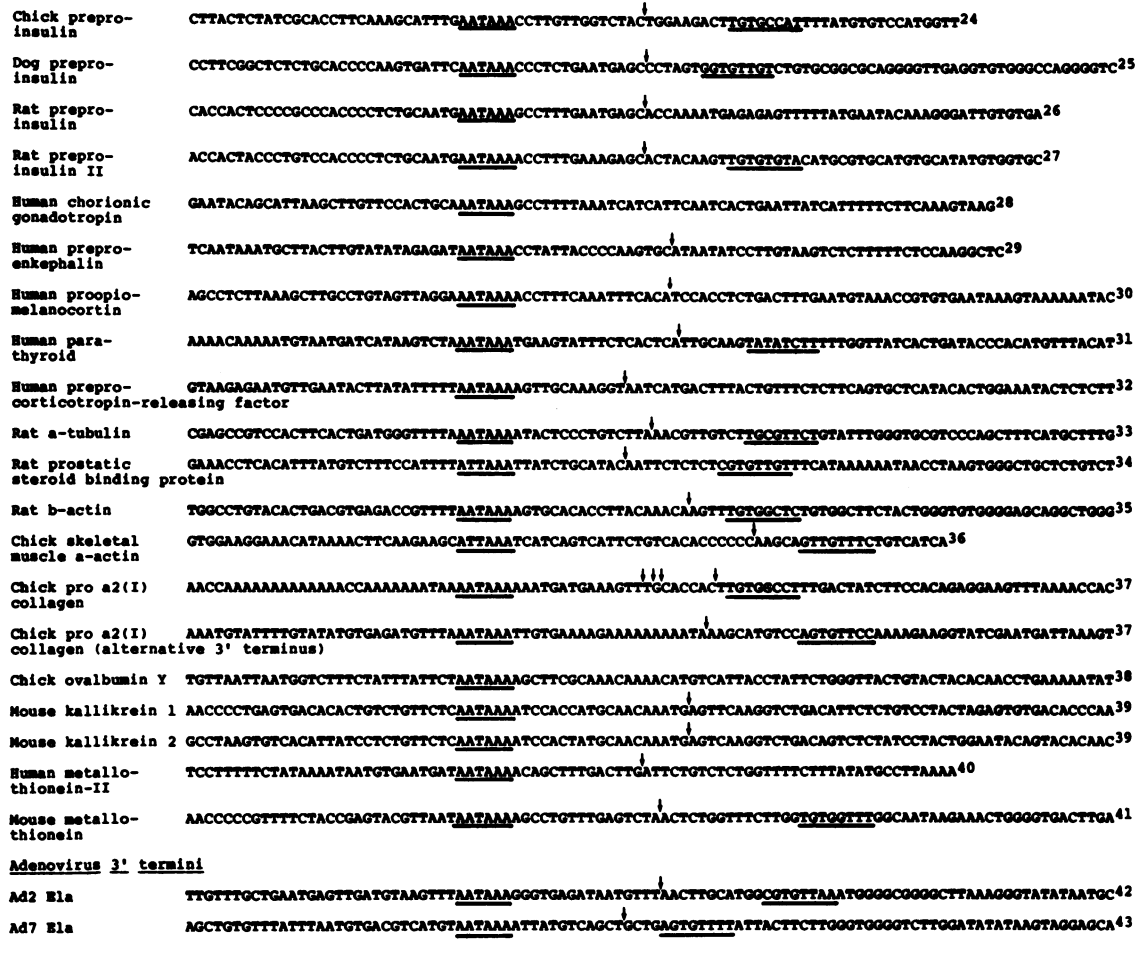
Nevertheless, it appears that other sequences further downstream are required for efficient polyadenylation. McDevitt et al (33) have shown that sequences between 20 and 35 nucleotides downstream from the poly A site (ie downstream from YGTGTTYY) are required for the production of functional adenovirus 2 (Ad2) E2a mRNA however correctly terminated mRNA was produced at detectable levels. Our data also shows that removal of sequences downstream from the YGTGTTYY signal reduces the level of 3' end formation but comparison of the HSV-2 and Ad2 sequences in this region revealed no significant homology. A deletion which removes the poly A site of Ad2 E2a mRNA and the sequence AGTGTCTC (this sequence is 26 nucleotides from the AATAAA signal and fits our consensus) resulted in no detectable mRNA. Similarly, a 52bp deletion which removes the poly A site together with sequences containing TGTGTTGG also abolishes rabbit β-globin 3' end formation (34).

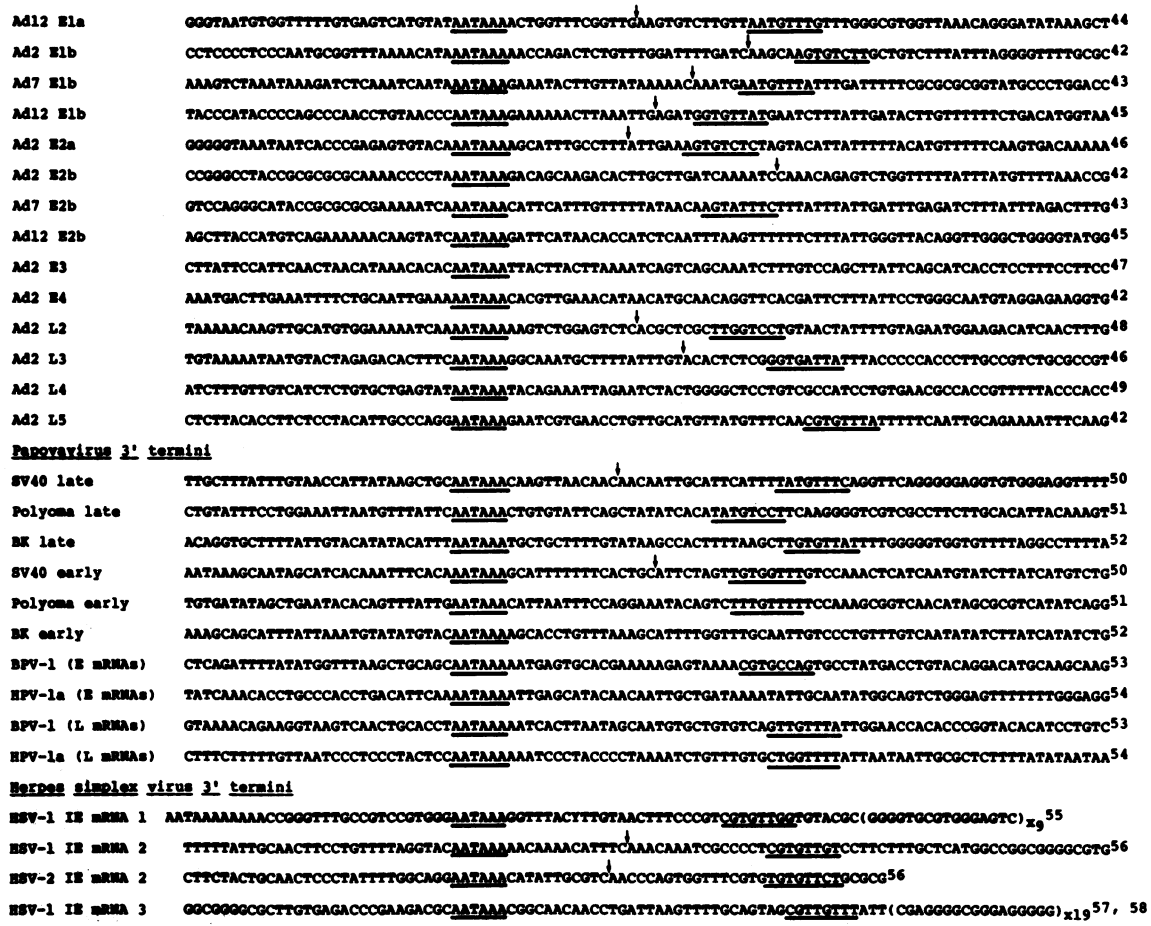
Indirect but compelling evidence for the importance of sequences downstream from the poly A site may be derived from an analysis of gene duplications and from the locations of small tandem repeat sequences which are present downstream from the 3' termini of HSV mRNAs. The human α 1 and α 2 globin genes have arisen from a gene duplication event and have areas of high homology (35). Sequence conservation at the 3' portions of α 1 and α 2 genes is markedly reduced beyond 15bp downstream from the poly A site, and it has been proposed that this decrease in homology delimits those sequences involved in gene duplication. We consider it significant that the sequence TGTGTGCC, which fits

COMPARISON OF MAMMALIAN AND EUKARYOTIC VIRAL mRNA 3' TERMINI

Mammalian 3' termini




Chick prepro-insulin
Dog prepro-insulin
Rat prepro-insulin
Rat prepro-insulin II
Human chorionic gonadotropin
Human prepro-enkephalin
Human proopio-melanocortin
Human para-thyroid
Human prepro-corticotropin-releasing factor
Rat a-tubulin
Rat prostatic steroid binding protein
Rat b-actin
Chick skeletal muscle a-actin
Chick pro a2(I) collagen
Chick pro a2(I) collagen (alternative 3' terminus)
Chick ovalbumin Y
Mouse kallikrein 1
Mouse kallikrein 2
Human metallo-thionein-II
Mouse metallo-thionein
Adenovirus 3' termini
Ad2 Ela
Ad7 Ela


Ad12 E1a
Ad2 E1b
Ad7 E1b
Ad12 E1b
Ad2 E2a
Ad2 E2b
Ad7 E2b
Ad12 E2b
Ad2 E3
Ad2 E4
Ad2 L2
Ad2 L3
Ad2 L4
Ad2 L5
Papovavirus 3' termini
SV40 late
Polyoma late
BK late
SV40 early
Polyoma early
BK early
BPV-1 (E mRNAs)
HPV-1a (E mRNAs)
BPV-1 (L mRNAs)
HPV-1a (L mRNAs)
Herpes simplex virus 3' termini
HSV-1 IE mRNA 1
HSV-1 IE mRNA 2
HSV-2 IE mRNA 2
HSV-1 IE mRNA 3

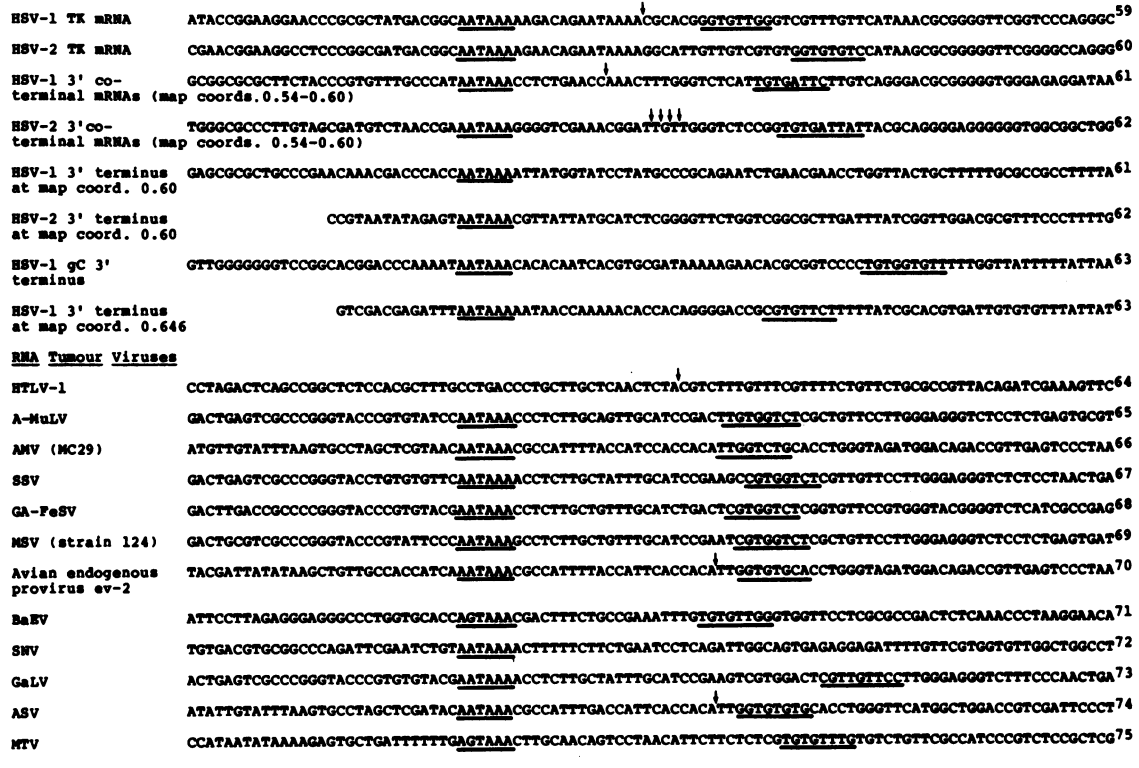


FIG. 5. Compilation of DNA sequences at the 3' termini of mammalian and eukaryotic viral mRNAs. Sequences are aligned using the AATAAA signals which are underlined. Locations of poly A sites are indicated by arrows and the sequences which resemble the YGTGTTYY consensus are underlined. The references numbered at the right of each sequence are listed in the Compilation References section.

TABLE 2 Derivation of the YGTGTTYY consensus from sequences located 24bp to 38bp downstream from the AATAAA signal.

A	9	6	2	2	3	0	8	9
G	13	57	6	66	10	7	8	13
C	12	1	0	0	2	20	15	15
T	36	6	62	2	55	43	39	33
	Y	G	T	G	T	T	Y	Y

This represents an analysis of those 70 sequences listed in Fig. 5 which contain the signal; numbers indicate the frequency of occurrence for each nucleotide.

our consensus, is contained within this 15bp stretch and suggest that retention of this sequence reflects the ability to correctly process α 1 and α 2 mRNAs. In HSV, small tandem repeats occur downstream from the 3' termini of at least three mRNAs and these repetitive sequences are believed to have no specific function (11). The HSV reiterations begin a few nucleotides downstream from sequences which fit our consensus and hence could mark the boundary of regions important in transcription.

Approximately 33% of the 3' terminal DNA sequences examined do not contain a YGTGTTYY sequence motif. Hence, absence of this sequence may be overcome by other downstream signals, and we note that many of these 3' terminal regions contain T-rich residues. The downstream YGTGTTYY sequence could therefore be analagous to the transcription regulatory signals present upstream of the TATA box, eg the GC-rich segments present in many but not all promoters. Removal of GC-rich sequences reduces the level of initiation (32, 36, 37, 38), however, as these sequences are not present in the upstream regions of every promoter presumably they are not obligatory, or other sequences mimic their function.

While the function of sequences downstream from 3' termini is unclear it is likely that they are involved in the cleavage/processing of nuclear RNAs which extend beyond the poly A site. Cleavage/processing of primary transcripts is thought to be the mechanism whereby 3' termini are generated for both poly A^+ mRNAs (39), and histone messages (40, 41, 42). Histone genes do not contain AATAAA signals and a palindromic sequence frequently is present close to mRNA 3' termini (43, 44). Point mutations within this palindromic sequence abolish the ability to

form a correctly terminated mRNA, and sequences at least 24 nucleotides downstream from the 3' terminus are required for efficient processing (45). We have not observed any sequences similar to our consensus downstream from histone mRNA 3' termini. This is not surprising since, although the mechanism for generating 3' termini in poly A^+ and histone mRNAs may be similar, the processing events could be different and therefore alternative signals may be used.

We have no evidence yet as to whether the YGTGTTYY signal either in DNA or in pre-mRNA is involved in mRNA termination or processing. Its presence downstream from most HSV genes analysed so far may indicate a role in termination as the different transcription units are closely located on the virus genome (46). By contrast, genes in mammalian cells are more scattered so that transcription beyond the poly A site may less frequently disturb transcription of an adjacent gene. Examples of alternative processing of mRNA 3' termini include the pro $\alpha 2$(I) collagen (47), β^2-microglobulin (48) and mouse α amylase (49) genes, and there are situations where alternative poly A sites are used with differing efficiencies. Our analysis of these situations indicates that the YGTGTTYY signal is not associated with 3' end selection.

Deletion of the YGTGTTYY signal reduced 3' end formation but had no detectable effect on 5' end initiation; the RNA synthesised runs through into the pUC vector sequences and does not appear to be polyadenylated (data not shown). However, these extended RNAs are transported to the cell cytoplasm but, as reflected in CAT levels, are not translated. The inability to translate these RNAs may imply that their mode of transport to the cytoplasm differs from that of poly A^+ mRNAs thus suggesting a coupling of mRNA polyadenylation, transport and translation.

The lack of transcript polyadenylation is not surprising as *in vitro* only pre-mRNAs that contain a 3' end located at or just downstream from the *in vivo* poly A site are efficiently polyadenylated (50). *In vitro* studies with pre-mRNA suggest that the specificity of polyadenylation is linked to RNA synthesis and indicate that the poly A polymerase interacts with a specific signal located at the 3' end of pre-mRNA (51).

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