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Messenger RNAs from the E1 region of bovine papillomavirus type 1 detected in virus-infected bovine cells

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Abstract

Bovine papillomavirus type 1 DNA replicated to a high copy number in virus-infected bovine fibroblasts. Infected bovine cells were therefore used as a source of RNA for Northern blotting analysis to search for viral transcripts hybridizing to the E1 gene region, implicated in viral DNA replication. Cytoplasmic polyadenylated RNA preparations contained at least five different E1-region transcripts, ranging from 1200 to approximately 4500 nucleotides in length. All of these species contained sequence information from the 5'-end of the E1 open reading frame, but only the largest species included sequences from its central portion. The latter transcript is a candidate mRNA for a stimulatory replication factor (R) previously mapped by genetic experiments (1).

Introduction

Warts have been shown to be transmitted by a virus in many animal species (2). The wart (papilloma-) viruses are non-enveloped DNA-containing viruses which replicate in the nucleus of infected cells. They differ from other papova viruses (e.g. SV40) in the arrangement of the viral "early" and "late" genes in the same transcriptional orientation (3,4). A notorious feature of papillomaviruses is their limited host and tissue range: virus replication is confined to the keratinocytes within a wart, and is linked to the terminal differentiation programme of these cells. This has undoubtedly hindered the development of a wart virus culture system (5).

The bovine papillomavirus (BPV-1) has been widely used for studies on papillomavirus molecular genetics. This virus differs from most other wart viruses in its ability to transform dermal fibroblasts as well as epidermal keratinocytes, giving rise to fibropapillomas in cattle (6). Moreover, such bovine fibro-papillomaviruses (BPV-1 and BPV-2) can transform *in vitro*-cultured fibroblastic cells from cattle and from certain species of rodent (7-9). Many copies of the viral genome persist in the nucleus of such transformed cells as extrachromosomal circular plasmids (10-12): a feature which has stimulated the development of BPV-1-based eukaryotic expression vectors (13).

Sequence analysis of BPV-1 (14) revealed an organization of open

reading frames (ORFs) similar to that of a human papillomavirus (4) and later confirmed for many more wart viruses (15,16). Systematic dissection and mutational analysis of the BPV-1 genome has defined genetic elements and protein domains involved in cellular transformation, the control of plasmid replication, and BPV-1 transcriptional activation (17-28). The integrity of the E1 open reading frame has been reported to be necessary for maintaining BPV-1 as a plasmid (21). The conservation of this ORF between papillomaviruses leads us to infer that it is expressed during some stage of the virus life cycle, and recent genetic data have indicated that it encodes two functions with opposing influences on viral DNA replication (1). However, mapping of BPV-1 transcripts in transformed mouse C127 cells has failed to identify mRNAs covering the E1 ORF (22,29,30) (see Fig.1). Neither have E1 mRNAs been identified for the cottontail rabbit papillomavirus nor for human papillomavirus type 1

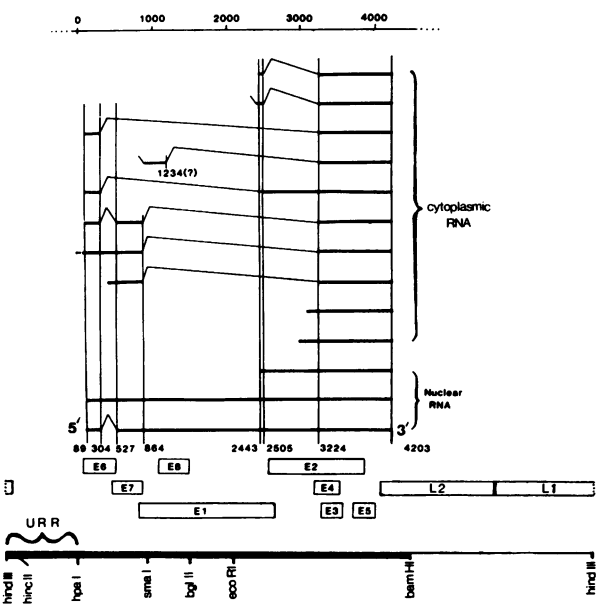

Figure 1. Organization and transcription of the BPV-1 genome.

The viral genome is depicted linearized at its single HindIII site (position 6958). The 69% HindIII-BamHI transforming segment of the viral genome is indicated by the thick solid line, and the position of various restiction endonuclease sites pertinent to this study are shown. The open boxes represent the translational early (E) and late (L) open reading frames (ORFs). Above, are shown the BPV-1 cytoplasmic and nuclear transcripts previously identified in BPV-1 transformed mouse C127 cells (30). Transcription in BPV-1 is unidirectional. The position of the 5' ends of the mRNAs indicate three separate promoter regions within the 69% segment which have previously been mapped (29,30,41,42) . The upstream regulatory region (URR) contains elements involved in the control of virus transcription and replication (see text for references).

(31-33) suggesting that they can represent but only a small fraction of the papillomavirus transcription products.

The present communication reports the discovery of a set of mRNAs from the E1 gene region of BPV-1 which have not previously been described. Furthermore, we have carried out this study using primary cells derived from the natural animal host for this virus. Similarities and differences in the interaction of BPV-1 with bovine cells and the widely used murine C127 cells are outlined.

Materials and Methods

Cells and cell culture.

Fibroblasts derived from foetal bovine conjunctivae explanted in culture dishes were used for virus infection experiments between their fourth and eighth passage *in vitro*. Murine C127 cells were obtained from Dr. Peter Howley (National Institutes of Health, Bethesda, Maryland, U.S.A.), and were grown under the same conditions as the bovine cells.

Virus purification and typing.

Bovine papilloma virions were isolated from pooled head and neck fibropapillomas of individual cattle, and were purified as described for elk warts (34). Virus preparations were typed as BPV-1 by restriction endonuclease analysis of their DNA component. Infection of cell monolayers was carried out at 37deg.C for three hours with 0.25 ml of medium containing approx. one million focus forming units of BPV-1. Virus activity was assayed as described previously (9).

Recombinant viral plasmids.

As specific molecular hybridization probes, the entire BPV-1 genome cloned in pBR322 via the HindIII site (35) was used, as well as the following BPV-1 restriction endonuclease fragments derived from it and subcloned in the pSP6 vector system: the E1-region SmaI-EcoRI fragment (nucleotides 945-2113); the late region BamHI-HindIII fragment (nucleotides 4450-6958); and the upstream regulatory region (URR) HincII-HpaI fragment (nucleotides 7142-7945).

Isolation and analysis of cellular DNA and RNA.

Total cellular DNA was purified by phenol extraction of virus-infected cells (36). Restriction endonuclease analysis, agarose gel electrophoresis and Southern blotting and hybridization protocols were carried out using standard techniques (37). Hybridization and washing of filters was performed using stringent conditions (36). Specific hybridization probes labelled to a specific activity of greater than fifty million c.p.m. per microgram of DNA were prepared by random priming using reagents obtained from Amersham International, Amersham, U.K..

Total cellular RNA was isolated from tissue culture cells lysed in 6M guanidine hydrochloride as described previously (36). Cytoplasmic RNA was prepared as described elsewhere (38) and nuclear RNA was obtained by lysis of pelleted nuclei with guanidine hydrochloride using the same procedure as for total cellular RNA. Induction of BPV-1 transcription was carried out by the addition of cycloheximide to a final concentration of 25ug per ml in the tissue culture medium, followed by a 3-4hr incubation at 37deg.C. Polyadenylated RNA was selected by oligo dT cellulose chromatography. Analysis of denatured RNA samples was performed in 1.0% or 1.2% agarose gels containing formaldehyde (38). Prior to blotting, the RNA gels were treated sucessively with: 50mM NaOH, 10mM NaCl (45 min., r.t.); 0.1M Tris-HCl pH 7.5 (45 min., r.t.) ; and finally, 3M NaCl, 0.3M Na citrate (1hr., r.t.). Blotting of the RNA gels and Northern hybridization analysis was performed using the same conditions as for the DNA blots. For autoradiography, Southern and Northern blots were exposed to Kodak XAR film at -70 deg.C using intensifier screens for 1-14 days. RNA bands visualized by autoradiography were sized approximately by comparison of their mobilities with the 18S and 28S rRNAs. Transcript sizes given in the text include an estimated error margin of +/-100 nucleotides (nt) for transcripts smaller than 2500nt, and +/-250nt for transcripts larger than 2500 nt.

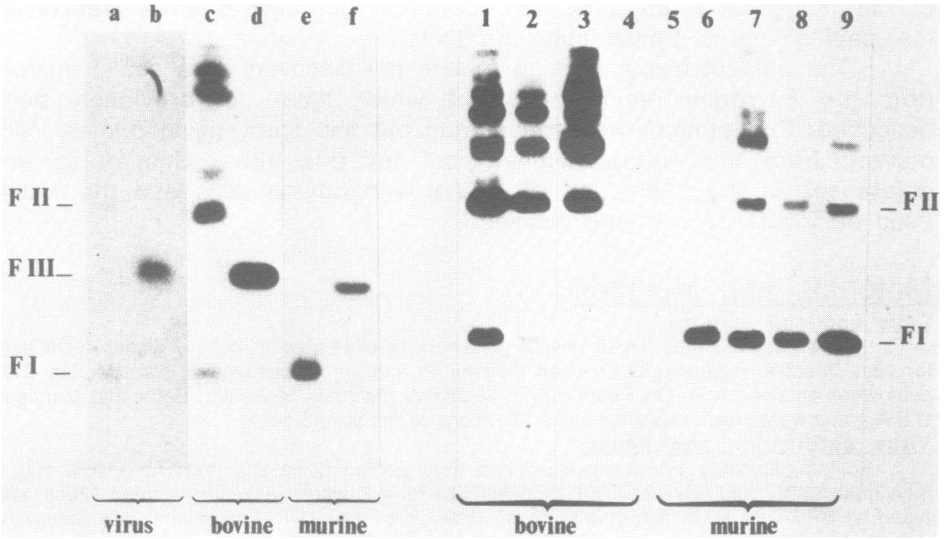

Figure 2. Southern blotting analysis of BPV-1 DNA in virus-infected bovine and murine cell cultures. Five ug of cellular DNA treated with the endonucleases indicated below, were electrophoresed in 0.8% (left panel) or 1.0% (right panel) agarose gels. Following blotting onto nitrocellulose, filters were hybridized to (32)P-labelled cloned viral DNA (whole genome). Lanes a and b: cellular DNA from uninfected bovine fibroblasts containing 100pg of BPV-1 virion DNA as marker (these lanes were overexposed to show the bands). Lanes c and d: BPV-1 infected bovine fibroblasts (third passage, three weeks following infection) cleaved with the endonucleases SstI (no sites in BPV-1) and EcoRI (one site in BPV-1) respectively. Lanes e and f: BPV-1 infected C127 cells (third passage, three weeks following infection) cleaved with SstI and EcoRI respectively. The DNA samples in the autoradiograph shown at the right (lanes 1-9) were all treated with the endonuclease SstI. An identical pattern of bands was obtained with non-digested cell DNA (data not shown) however they were less distinct due to the high viscosity of the DNA samples. Lanes 1 and 2: low and high passage virus-infected bovine fibroblasts (three and ten weeks following infection). Lane 3: a BPV-1 transformed bovine fibroblast subclone. Lanes 4 and 5: uninfected bovine fibroblasts and murine C127 cells, respectively. Lanes 6 and 7: low and high passage virus infected C127 cells (three and ten weeks following infection). Lanes 8 and 9: BPV-1 transformed C127 subclones. The position of the input virion DNA components (FI,FII and FIII) are indicated at the left side of the figure.

Results

BPV-1 infected bovine fibroblasts contain a high level of replicating viral DNA.

During a study of the replication kinetics of BPV-1 in bovine conjunctival fibroblasts and mouse C127 cells, we noticed that the bovine cultures contained a higher level of BPV-1 DNA. A further infection experiment confirmed this observation, and moreover, demonstrated that the majority of the viral DNA in the infected bovine cells had undergone replication, since it became established as concatemeric and/or

oligomeric complexes of the complete viral genome, distinguishable from the low molecular weight input virion components by Southern hybridization analysis (Fig. 2, compare lanes a and c). This was not the case in the C127 culture infected in parallel, where the monomeric viral forms predominated (Fig. 2, lane e).

There was no gross variation in the proportion of cells transformed in the two types of culture : both the bovine and the murine cultures responded rapidly to infection with greater than fifty percent of the cells becoming morphologically transformed within one week. In this experiment the bovine culture harboured a four-to-five fold greater level of BPV-1 DNA than the C127 culture at the third passage level after infection, as judged by comparison of the intensity of the hybridization signals obtained using an endonuclease which linearized the viral genome (Fig. 2, compare lanes d and f). Further passaging led to a slight decrease in the viral DNA content in the bovine culture (Fig. 2, lanes 1 and 2) whereas the C127 culture retained a consistently lower level of viral DNA both following passaging and in transformed subclones derived from the virus-infected culture (Fig. 2, lanes 6-9). A transformed bovine fibroblast subclone contained a still higher virus DNA content than the uncloned bovine culture (Fig. 2, lane 3), further indicating a greater potential for BPV-1 DNA replication in the bovine cells.

Transcription of the BPV-1 genome in bovine fibroblasts.

To analyze BPV-1 transcription, RNA was prepared from virus-infected bovine cultures, or control virus-infected C127 cells, and used in Northern blotting experiments. An initial analysis of the overall viral transcriptional activity was carried out using the whole virus genome as a hybridization probe. The polyadenylated BPV-1 transcripts present within total cellular RNA preparations from virus-infected bovine and murine cells are shown in Fig. 3 (lanes 1 and 2, respectively). Although the overall number and size distribution of the major transcripts was similar in the bovine and murine cells, there were differences in the relative abundance of the various RNA species. For example, in the bovine cells the shorter transcripts were more abundant, whereas in the C127 cells the largest (approx. 4500nt) species appeared to predominate, as indicated by a comparatively stronger hybridization signal.

Further variations were noted in the pattern of transcripts within the high molecular weight range. This region of the blot is shown with greater resolution in lanes 3 and 4 of Fig. 3. At least three components (A, B and C) were present in the infected bovine cells, with approximate sizes of 5500nt (component A), 4500nt (component B) and 4000nt (component C). The murine cells contained no detectable component A, except following treatment with cycloheximide : an inhibitor of protein translation which has been shown to induce BPV-1 transcription via an unknown mechanism (39).

The structure of nuclear component A was investigated further, since

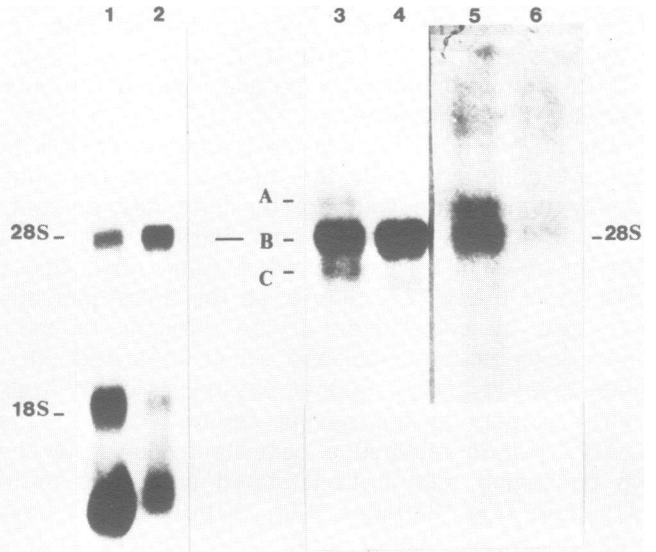

Figure 3. Analysis of BPV-1 transcripts in virus-infected bovine fibroblasts and murine C127 cells. All lanes contained 2.5 ug of polyA-enriched RNA isolated from whole cells. The positions of the 28S and 18S murine rRNAs (4710 and 1869nt respectively) are indicated at the sides of the figure. The analysis was performed in 1.0% (lanes 1,2) and 1.2% (lanes 3-6) agarose gels. Lanes 1 and 3 : virus infected bovine cells, whole genome probe. Lanes 2 and 4 : virus infected murine cells, whole genome probe. Lanes 5 and 6: virus infected bovine and murine cells respectively, late region probe. Lanes 5 and 6 were exposed to X-ray film five times longer than lanes 3 and 4. A = component A; B = component B; C = component C.

its estimated size of 5500nt exceeded the total length of the early gene region (approximately 4200nt). When tested with a probe specific for the virus upstream regulatory region (URR; see Fig.1), we noted hybridization to the 5500nt component A species in cycloheximide-treated bovine (Fig. 4, lane 11) and murine cells (Fig.4, lane 12, overexposed to show the bands). It hybridized also to a probe from the E1 ORF (Fig 4, lane 10), and furthermore, weak hybridization was observed using the late region probe (Fig.3, lane 5). These observations suggested that component A transcripts originated from a promoter upstream of the previously mapped RNA initiation site at nucleotide 89 (see Fig.1), and probably included the entire early region as well as possibly a portion of the late region. Component A was confined to the nuclear fraction in BPV-1 infected bovine cells (Fig.4, lane 7) indicating that it represented a nuclear precursor RNA. It is likely that it was transcribed from the initiation site at position 7185, recently reported to be active in bovine fibropapillomas,

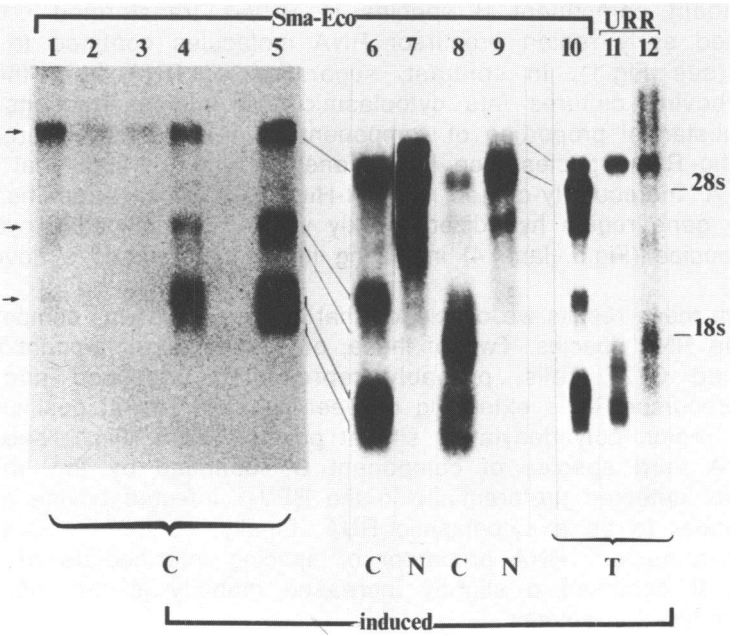

Figure 4. Northern blotting analysis of cycloheximide-inducible E1-region BPV-1 transcripts. Polyadenylated RNA (2.5ug per lane, with the exception of lane 4 which contained 1.0ug) prepared from cytoplasmic (C) or nuclear (N) fractions, or from whole cells (T), were electrophoresed in denaturing 1.2% (left panel) or 1.0% (middle and right panels) agarose gels. Following blotting onto nitrocellulose, the filters were hybridized with the E1 region Sma-Eco probe or the upstream regulatory region (URR) probe, as indicated along the top of the figure.With the exception of lanes 1-3, all preparations were from cycloheximide treated cultures (induced). Lanes1-3: cytoplasmic RNA from bovine fibroblast cultures infected with three separate BPV-1 preparations. Lanes 4,5: cytoplasmic RNA (1.0 and 2.5ug respectively) from BPV-1 infected bovine fibroblasts (induced). Lanes 6,7: cytoplasmic (lane6) and nuclear (lane7) RNA from virus infected bovine fibroblasts (induced). Lanes 8,9: cytoplasmic (lane 8) and nuclear (lane 9) preparations from infected C127 cells (induced). Lanes 10,11: whole cell polyadenylated RNA from infected bovine cells (induced). Lane 12: whole cell polyadenylated RNA from infected C127 cells (induced : autoradiograph exposed three times longer than lanes 10 and 11). The arrows at the left side of the figure indicate the positions of the E1 transcripts in non-induced bovine cells.

and induced by cycloheximide treatment of BPV-1 transformed C127 cells (41).

Component B was more abundant in the murine cells (Fig.3, lane 2 compare with lane 1). Yet, when a probe specific for the virus late gene region was tested, we reproducibly noted weak but clearly positive hybridization to component B transcripts preferentially in the bovine cells (Fig.3, lane 5, compare with lane 6: these lanes were overexposed to show the bands). On the basis of RNA fractionation experiments performed previously (30), and confirmed in the present study, it was concluded that

the abundant component B species in BPV-1 transformed C127 cells represented early region precursor RNA molecules confined to the cell nucleus (see Fig.1). In contrast, separation of RNA from the BPV-1 infected bovine cultures into cytoplasmic and nuclear fractions showed that a substantial proportion of component B in these cells represented a cytoplasmic RNA species (see Fig.4, lanes 6 and 7 ; additional data not shown). A molecularly-cloned BamHI-HindIII fragment specific for the virus late gene region hybridized weakly yet reproducibly to a cytoplasmic 4500nt species (Fig.5, lane 4) indicating that it represented a novel BPV-1 transcript.

From these results we concluded that component B was composed of at least three RNA species. Two of these, present in larger amount in BPV-1 transformed C127 cells, probably represented unspliced and spliced nuclear precursor RNAs extending between the cap site at position 89 and the early region polyadenylation site at position 4205 (Fig.1; see (30) for details). A third species of component B, identified by the late region probe, was detected preferentially in the BPV-1 infected bovine cells, and would appear to be a cytoplasmic RNA. Finally, component C seems to represent a nuclear RNA precursor or splicing intermediate of unknown structure. It exhibited a slightly increased mobility in the murine cells and was reduced in amount.

Identification of mRNAs from the E1 gene region of BPV-1.

In view of the high level of viral DNA replication observed in the bovine fibroblast cultures, we were encouraged to search for mRNAs which might be involved in this process. To obtain a hybridization probe specific for the E1 region, a SmaI-EcoRI fragment (nucleotides 945-2113; see Fig.1) was subcloned into a bacterial plasmid vector.

Cytoplasmic polyadenylated RNA preparations from BPV-1 infected bovine fibroblast cultures contained three size classes of transcripts which hybridized to the Sma-Eco probe (Fig.4, lanes 1-3, arrowed). Cycloheximide pretreatment of the infected bovine cells led to a several fold induction of the E1 region transcripts (Fig.4, lanes 4 and 5) thus enabling a more detailed characterization to be made. Thus it was found that the three size classes contained at least five RNA species with estimated lengths of 1200, 1400, 2000, 2200, and 4500nt.

Cellular fractionation analysis indicated that all of these five species represented *bona fide* cytoplasmic RNA molecules, since there was little cross-contamination of the cytoplasmic and nuclear RNA preparations (Fig.4, lanes 6 and 7 repectively). These results supported the notion that component B included a cytoplasmic E1 mRNA species which was present in considerably higher amounts in BPV-1 infected bovine cells than in virus infected C127 cells.

Molecules with the mobility of component A (5500nt) also hybridized to the E1 region probe, and as discussed above, these were confined to the nuclear fraction (Fig.4, lane 7). Additional hybridization was observed to

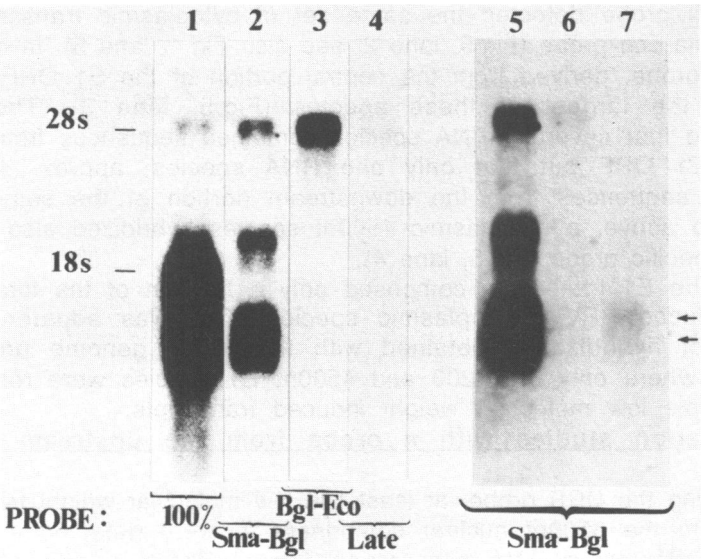

Figure 5. Northern blotting analysis of cytoplasmic BPV-1 E1-region transcripts using subgenomic virus probes. Lanes 1-5: cytoplasmic polyadenylated RNA (2.5ug per lane except for lane 3, which contained 5ug) from cycloheximide-treated infected bovine cells. Lanes 6,7: cytoplasmic polyadenylated RNA (2.5ug) from non-induced virus infected bovine and murine cells respectively. Additional control lanes (not shown) containing 20ug whole cell RNA from non-infected bovine fibroblasts did not reveal any positive hybridization.The probes used are indicated at the bottom of the figure : 100% = the whole virus genome; Sma-Bgl and Bgl-Eco = probes from the 5′ and central portions respectively of the E1 region; late = BamHI-HindIII probe specific for the late gene region. The arrows at the right side of the figure point to the low molecular weight BPV-1 transcripts in non-induced murine cells.

nuclear RNA in the size range 3000-4000nt, however discrete RNA species could not be resolved in this region of the blot.

Virus infected C127 cells, pretreated with cycloheximide (Fig.4, lanes 8 and 9) contained E1 region transcripts corresponding in size to the major E1 species present in the infected bovine cells. However, an additional abundant species of approx.1900nt was observed in the cytoplasmic fraction (Fig.4, lane 8). In contrast to the bovine cells, the abundant component B molecules in the murine cells were largely retained in the nucleus, as were molecules with the mobility of component C. Finally, the nuclear component A transcripts were detected in the murine cells with the E1-probe only after pretreatment with cycloheximide (Fig.4, lane 12).

Mapping of the E1 region-derived mRNAs.

To further characterize transcripts from the E1 region, the Sma-Eco probe was divided into 5' and 3' portions using the endonuclease BglII which cleaves within this sequence at nucleotide 1515 (see Fig.1). The 5'

(Sma-Bgl) probe detected the same set of cytoplasmic transcripts as the larger Sma-Eco probe (Fig.5, lane 2, see also Fig.4, lane 5). In contrast, the Bgl-Eco probe, derived from the central portion of the E1 ORF, hybridized only to the largest of these species (Fig.5, lane 3). These results suggested that several mRNA species contained sequences from the 5'end of the E1 ORF but that only one RNA species, approx. 4500nt-long, included sequences from the downstream portion of the same ORF. As described above, a cytoplasmic 4500nt species hybridized also to the late region specific probe (Fig.5, lane 4).

The E1 transcripts comprised only a fraction of the total cycloheximide-induced BPV-1 cytoplasmic species. This was apparent from the pattern of hybridization obtained with the whole genome probe (Fig.5, lane 1), where only the 2200 and 4500nt E1 species were resolved from the multiple low molecular weight induced transcripts.

Hybridization studies with a probe from the upstream regulatory region.

Using the URR probe, at least two low molecular weight transcripts, in addition to the 5500nt nuclear component A, were detected in the whole cell polyadenylated RNA preparation from infected bovine cells treated with cycloheximide (Fig.4, lane 11). Fractionation analysis (not shown) confirmed that these were cytoplasmic transcripts. These species were not detected in cytoplasmic preparations from virus-infected bovine cultures not treated with cycloheximide (data not shown). Although they exhibited a similar mobility to the short E1 region transcripts (lane 10), they did not correspond exactly in size. Therefore it did not seem likely that the E1-region transcripts represented spliced molecules derived from the component A nuclear, putative precursor, species. Nevertheless, the E1 mRNAs could still contain a short leader sequence from the URR region as our hybridization method would fail to detect exons below 50nt in length. The structure of the cycloheximide-induced cytoplasmic URR-derived species is currently under investigation.

A discrepancy was noted between the cycloheximide-induced infected bovine and C127 cells when tested with the URR probe (Fig.4, lanes 11 and 12, respectively). Neither of the shorter cycloheximide inducible URR-derived species in the bovine cells was detectable in the murine cells, and this supported our conclusion that the shortest E1 region transcripts, present both in bovine and in murine cells, probably did not contain a URR-derived leader sequence. Instead, the murine cells contained additional transcripts, one of which exhibited a mobility identical to that of the approx. 1900nt species detected with the Sma-Eco E1 probe (lane 8).

Ratio between different E1 mRNA classes in bovine and murine C127 cells, infected with BPV-1.

In RNA preparations derived from virus-infected cells which had not been pretreated with cycloheximide, an altered ratio of cytoplasmic E1 transcripts was noted between the bovine and the murine systems. This

was apparent using the Sma-Bgl probe specific for the 5' end of the E1 region. Whereas in the C127 cells the low molecular weight species predominated (Fig.5, lane 7) the reverse was true for the bovine cells where the large transcript was most abundant (Fig.5, lane 6 ; compare also Fig.4, lanes 1-3). Cytoplasmic RNA from cycloheximide-treated infected bovine cells is shown for comparison in the adjacent lane (Fig.5, lane 5).

Discussion

The E1 region performs a fundamental role in the replication of papillomaviruses. The work of Lusky and Botchan and their colleagues (1,19-21,25) has indicated that its expression is required for BPV-1 plasmid replication in C127 cells, yet no mRNAs covering the E1 ORF have been detected for any papillomavirus. From experiments using BPV-1 DNA mutated *in vitro* and then transfected into C127 cells, two functions encoded by the E1 ORF which affect plasmid stability have been described (1). These consist of a modulator function, M, localized to the 5' end of the E1 ORF, which is proposed somehow to curb the activity of a positive replication factor, R, encoded by the remainder of E1.

In the present study we have shown that cytoplasmic candidate E1 mRNAs can be detected in *in vitro*-infected fibroblasts of the natural host for BPV-1. Earlier failures to detect any E1 mRNAs were undoubtedly due to their relative scarcity within virus-infected cells. Our preliminary characterization has revealed a set of five transcripts containing sequence information from the 5' end of the E1 region, and a single large transcript which includes also sequences from its central portion.

A likely possibility is that one of the 5' E1-region transcripts described herein encodes an M factor, and that the large cytoplasmic E1-species may be translated as an R factor. Our observation of an altered balance of putative M and R mRNAs between virus-infected bovine and murine cells could therefore be correlated with the increased replication potential of the virus plasmid in the bovine cells. It should be noted, however, that the 5'-E1 region transcripts are likely to include also coding information from the overlapping E8 ORF (see Fig. 1) for which no function has as yet been ascribed.

Particularly surprising was our observation of the presence of late region sequences within a putative messenger RNA. Previous studies have not described any such late region transcripts in BPV-1 transformed murine cells (29,30). Indeed, our data indicate that this RNA species was much reduced in amount in virus-infected murine C127 cultures. It remains to be established if this transcript is identical to the large E1 species. The differences in BPV-1 transcriptional patterns between bovine cells and the standard murine C127 cell system might reflect host-specific factors influencing promoter usage. In support of this, it has earlier been reported that a BPV-1 enhancer element stimulated linked

heterologous promoter activity to a higher level in bovine fibroblasts than in murine fibroblasts (40). Yet, to suggest merely a generalized reduction in BPV-1 promoter activity in murine cells is not sufficient to explain the presence of additional BPV-1 transcripts specifically in those cells, as we have observed following cycloheximide treatment. Therefore, it is further possible that alternative RNA processing pathways are used in these two systems. This possibility is currently being explored. The increased potential for BPV-1 plasmid replication in bovine cells could conceivably be exploited to obtain increased expression of foreign genes cloned into BPV-1 expression vectors.

We do not expect that BPV-1 infected bovine fibroblasts express any virus late (capsid) proteins since such cultures are unable to support lytic virus growth (12) and since virus late gene expression has been linked to the use of a specific viral promoter thought to be active only in differentiating keratinocytes (41). To substantiate this, we did not observe any late transcripts with the size of an L1 mRNA (approx. 2000nt (3,41)) which encodes the major capsid protein.

We have not yet determined the structure of the E1 mRNAs at the nucleotide level. However, in a previous study (30) (see also Fig.1), a splice donor site was mapped to position 1234. Hence, one or more of the shorter E1 mRNAs might contain an exon which ends at this position. Further structural analysis of the E1 region messenger RNAs is required in order to define their precise termini and splicing patterns as a prerequisite to studies on the regulation of their promoters. A reasonable proposition is that separate promoters control expression of the BPV-1 M and R products in view of the genetic data which has indicated that they have opposing properties (1).

Acknowledgements

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Note: During completion of this manuscript Stenlund et al.(43) reported the detection of BPV-1 E1 transcripts derived from the virus URR.

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