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Regulation of Cellular Genes Transduced by Herpes Simplex Virus

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Previous studies demonstrated that the rabbit β-globin gene is transcribed from its own promoter and regulated as a herpes simplex virus (HSV) early gene following insertion into the early HSV thymidine kinase gene in the intact viral genome (J. R. Smiley, C. Smibert, and R. D. Everett, J. Virol. 61:2368–2377, 1987). We report here that the β-globin promoter remained under early control after insertion into the late HSV gene encoding glycoprotein C. On the basis of these findings, we concluded that the β-globin promoter is functionally equivalent to an HSV early-control region. We found that a transduced human α-globin gene was also regulated as an early HSV gene, while two linked *Alu* elements mimicked the behavior of HSV late genes. These results demonstrate that certain aspects of HSV temporal regulation can be duplicated by cellular elements and provide strong support for the hypothesis that the regulation of HSV gene expression can occur through mechanisms that do not rely on recognition of virus-specific temporal control signals.

The 70 herpes simplex virus (HSV) genes are transcribed by RNA polymerase II in a regulatory cascade driven by viral products (33). Five immediate-early (IE) genes are expressed first (1, 6, 43, 51, 68), and four of the IE polypeptides play crucial roles in activating transcription of the remaining early (E) and late (L) genes (8, 9, 16, 18, 24, 47, 49, 50, 53–55, 61, 66; reviewed in reference 19). E genes are maximally expressed before the onset of viral DNA replication, while two subclasses of L genes require DNA replication for high-level expression. Promoter transplant experiments have shown that the temporal regulation of individual HSV genes during infection is dictated mainly by sequences present in their respective promoter regions (31, 48, 57), and nuclear run-on transcription assays suggest that this control occurs largely at the transcriptional level (25, 69).

The detailed mechanisms of action of the HSV IE proteins remain unknown. Although the IE polypeptide ICP4 binds directly to specific sequences present in HSV and some heterologous DNAs (2, 20, 21, 38, 39, 45, 46), the role of sequence-specific DNA binding in the transactivation mediated by this polypeptide remains unclear; for example, an ICP4-binding site located in the upstream region of the glycoprotein D gene (2, 20) does not appear to be required for transactivation of this gene by ICP4 (14, 15). Extensive studies of several HSV E and L promoters have indicated that many of the *cis*-acting regulatory sequences required for activation by IE proteins and temporal regulation during infection correspond to the binding sites of cellular transcription factors (7, 12, 13, 15, 31, 32, 35, 56). These results suggest that the temporal control of HSV E and L genes relies at least in part on changes in the activity of cellular transcription factors that recognize distinctive constellations of binding sites in E and L promoters.

An independent line of evidence supporting this view comes from studies of the regulation of cellular promoters by HSV products. The rabbit β-globin gene is activated by HSV IE polypeptides when it is newly introduced into fibroblasts by transfection (15–17) or as part of an infecting HSV genome (60). In the latter case, β-globin is regulated as an HSV E gene following insertion into the E thymidine kinase gene (*tk*). A straightforward interpretation of these results is that the β-globin control region is functionally equivalent to a bona fide HSV E promoter. An alternative explanation is that cellular genes resident in the viral genome are regulated by HSV-specific temporal control signals present in the flanking viral DNA sequences. According to this hypothesis, β-globin is controlled as an E gene following insertion into the *tk* locus because it falls under the influence of putative HSV-specific E-regulatory signals that govern *tk* gene expression.

The hypothesis that the β-globin promoter is equivalent to an HSV E-control region predicts that its regulation does not depend on the temporal class of the viral gene into which it is inserted. We tested this prediction by inserting the β-globin gene into the HSV L gene encoding glycoprotein C (gC) and found that the β-globin promoter remained under E control in this situation. From these results, we concluded that the temporal regulation of β-globin expression by HSV products depends on features of the β-globin promoter rather than on the nature of the flanking viral sequences.

The finding that the β-globin promoter was regulated as an HSV E promoter in a context-independent fashion prompted us to study the control of several additional cellular genes following incorporation into the HSV genome. We found that the human α-globin gene was also regulated as an HSV E gene, while two linked *Alu* elements closely mimicked the behavior of HSV L genes. These results demonstrate that a variety of cellular promoters are able to function efficiently in the context of the HSV genome and lend further support to the hypothesis that temporal regulation during HSV infection can occur through mechanisms that do not involve recognition of virus-specific signals.

MATERIALS AND METHODS

Viruses and cells. HSV type 1 (HSV-1) strain KOS PAA'5 (27) was used throughout this study. Virus stocks were propagated and titers were determined on monolayers of Vero cells. Expression of virally transduced cellular genes was monitored following infection of Vero or BHK21 cells with a multiplicity of 10 PFU per cell. When indicated, cycloheximide (100 μg/ml) or aphidicolin (10 μg/ml) was added 30 min prior to infection and maintained continuously.

Construction of recombinant viral strains. Viral strains

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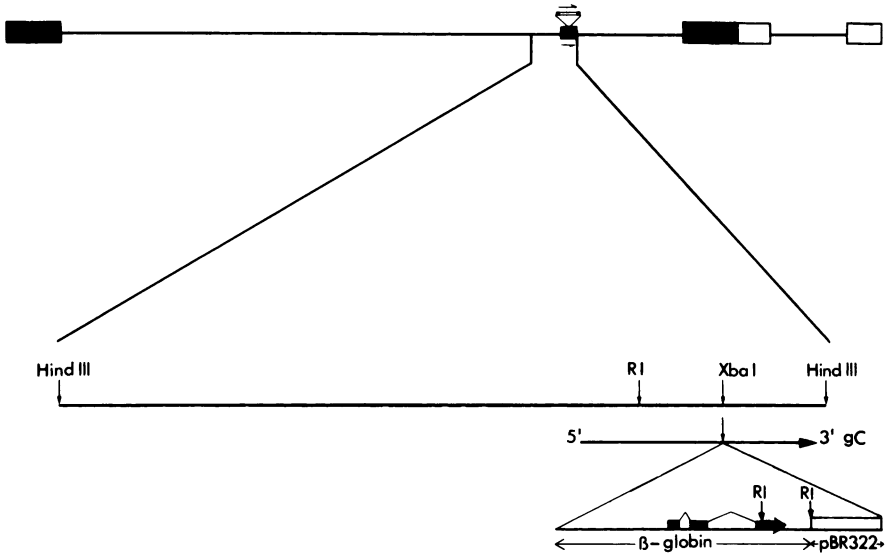

FIG. 1. Structure of strain gC-beta. A 3.7-kb *Xba*I fragment bearing the rabbit β-globin gene and 1,200 nt of 5′ flanking sequences was inserted into the *Xba*I site within gC-coding sequences present on p*Hin*dIII L. The resulting insertion mutation was transferred to the *gC* locus in the viral genome by in vivo recombination, as described in Materials and Methods.

bearing inserts of cellular sequences were derived by in vivo recombination following cotransfection of Vero cells with KOS PAA^{r}5 DNA and plasmids bearing the desired insertion mutation, as previously described (58–60).

Strain gC-beta, bearing the rabbit β-globin gene inserted into the HSV gene encoding glycoprotein C, was constructed by converting a previously described 3.7-kilobase (kb) globin-bearing *Sst*I fragment (60) into an *Xba*I fragment, then inserting this into the *Xba*I site within gC-coding sequences (22) present on p*Hin*dIII L (Fig. 1; p*Hin*dIII L was provided by E. K. Wagner). Globin-bearing viral clones were identifed by plaque hybridization (32), plaque purified, and then screened for the desired insertion by Southern blot hybridization.

Strain tk-alpha, bearing the human α_2 globin gene inserted into the viral gene encoding thymidine kinase (*tk*), was constructed by inserting a 4.3-kb α_2 globin *Sst*I fragment (provided by A. Bernstein) into the *Sst*I site located within the *tk* coding sequences present on pTK173 (65). *tk*-deficient viral recombinants were selected by plaque purification on Vero cells in the presence of 20 μM acycloguanosine.

Primer extension and S1 nuclease analysis. Cytoplasmic RNA was extracted by the method of Berk and Sharp (3). Primer extension and S1 nuclease analysis were performed exactly as previously described (60).

The following synthetic primers were purchased from the Central Facility of the Institute for Molecular Biology and Biotechnology, McMaster University: (i) gC, 5′-AAACG ACCTCCACACGGCCCACCGG-3′, predicted extension product of 79 nucleotides (nt) (22, 32); (ii) US11, 5′-GAT GCGTTGGGGGCGATTTCGGGCA-3′, predicted extension product of ca. 80 nt (36); (iii) glycoprotein D (gD), 5′-CCCCATACCGGAACGCACCACACAA-3′, predicted extension product of ca. 80 to 90 nt (67); (iv) α_2 globin, 5′-AGGCGGCCTTGACGTTGGTCTTGTC-3′, predicted extension product of 80 nt (41); (v) *Alu*I, 5′-TTAGTATAAC TGGGGTTTCTCCATA-3′, predicted extension product of 120 nt (11); and (vi) *Alu*II, 5′-TTAGTAGAGACGGGGTT TCTCCATG-3′, predicted extension product of 120 nt.

RESULTS

Insertion of the rabbit β-globin gene into the HSV gC locus. Previous studies demonstrated that the intact rabbit β-globin gene is transcribed from its own promoter and regulated as an HSV E gene following insertion into the E *tk* gene in the viral genome (60). We wished to determine whether the β-globin promoter remained under E control when the globin gene was placed within the body of a true L HSV gene. We constructed a plasmid in which a 3.7-kb *Xba*I fragment bearing the rabbit β-globin gene and 1,200 nt of 5′ globin-flanking sequences was inserted into the *Xba*I site within the dispensable L gene encoding gC (22, 32), and then we transferred the resulting insertion mutation into the viral genome by in vivo recombination to produce strain gC-beta (Fig. 1). The introduction of β-globin sequences into the *gC* gene resulted in the replacement of a wild-type 2.2-kb *Hin*dIII-*Eco*RI *gC* fragment with the expected *gC*-globin fusion fragments of 3.3 and 1.9 kb (Fig. 2) and the acquisition of a 590-base-pair internal globin *Eco*RI fragment (data not shown).

E expression of rabbit β-globin in strain gC-beta. We first tested whether the insertion of β-globin sequences disrupted the regulation of transcripts initiated from the *gC* promoter in strain gC-beta by studying the effects of inhibiting viral DNA replication with aphidicolin. Cytoplasmic RNA extracted from Vero cells infected with gC-beta and the parental PAA^{r}5 strain was analyzed by primer extension by using a probe complementary to residues +54 to +79 relative to the gC mRNA cap site. Accumulation of correctly initiated gC RNAs was strongly inhibited by blocking viral DNA replication in both viral strains (Fig. 3). We therefore concluded that expression from the *gC* promoter remained highly dependent on DNA replication in gC-beta.

We studied the regulation of the inserted β-globin gene by S1 nuclease analysis of globin transcripts produced during lytic infection of Vero cells. The S1 probe was derived from a previously described gD-globin fusion (14) and allowed differentiation of globin RNAs initiated from the globin

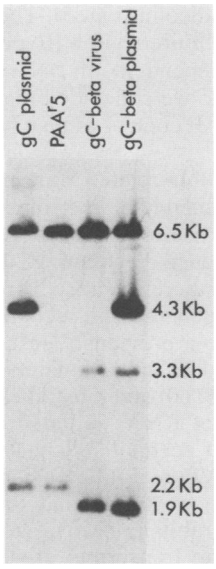

FIG. 2. Southern blot analysis of strain gC-beta DNA. The indicated DNAs were cleaved with a mixture of *Hin*dIII and *Eco*RI, transferred to nitrocellulose, and then probed with p*Hin*dIII L (lacking the β-globin insert). Insertion of globin sequences disrupted the 2.2-kb PAA^r5 *Hin*dIII-*Eco*RI fragment and led to the appearance of two gC-globin fusion fragments (3.3 and 1.9 kb).

promoter from those arising by readthrough from upstream sequences (Fig. 4D). Correctly initiated β-globin transcripts were detectable at 3 h postinfection and did not increase in abundance thereafter. These globin RNAs accumulated with

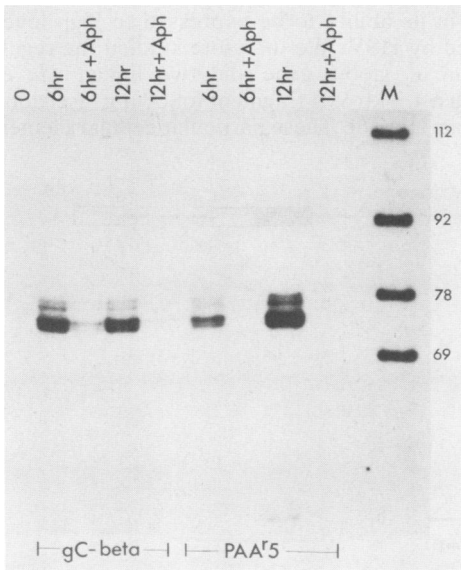

FIG. 3. Expression of gC RNAs in strain gC-beta. Vero cells were infected with 10 PFU of the indicated viral strain per cell. Cytoplasmic RNA (20 μg), harvested at the indicated times postinfection, was analyzed by primer extension with a 5′-labeled synthetic gC primer. Following treatment with reverse transcriptase, products were displayed on an 8% sequencing gel. Where indicated, 10 μg of aphidicolin (Aph) per ml was added 30 min prior to infection and maintained continuously. Markers (M) were 3′-labeled *Hpa*II fragments of pBR322 DNA. Marker fragment sizes (in nucleotides) are shown at the right.

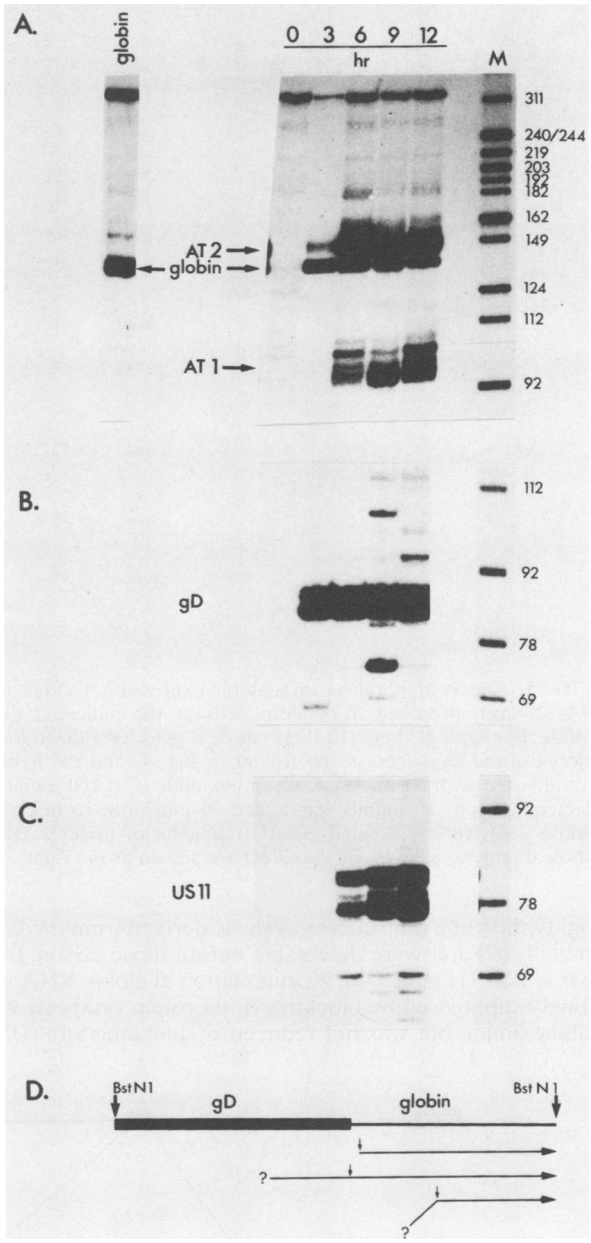

FIG. 4. Time course of β-globin RNA expression during infection with gC-beta. Cytoplasmic RNA (20 μg), prepared from Vero cells infected with gC-beta (10 PFU per cell), was hybridized to the 5′-labeled probe diagrammed in panel D to detect globin transcripts. Following treatment with S1 nuclease, digestion products were displayed on an 8% sequencing gel. These RNA samples were also analyzed for gD and US11 transcripts by primer extension with 5′-labeled synthetic 25-mers. (A) Time course of β-globin RNA accumulation. AT1 and AT2, Aberrant transcripts (described in the text). A portion of the globin probe was also hybridized to 1 ng of purified rabbit globin mRNA (alpha plus beta) to provide a marker for the position of correctly initiated globin RNAs. Markers (M) for panels A to C were 3′-labeled *Hpa*II fragments of pBR322 DNA. Marker fragment sizes (in nucleotides) are shown at the right. (B) Time course of gD RNA accumulation. (C) Time course of US11 RNA accumulation. (D) Probe fragment used in panel A. The fragment was derived from a gD-globin fusion (14) and allows transcripts initiated from the globin promoter to be distinguished from those arising by splicing (AT1) and readthrough (AT2) from upstream sequences.

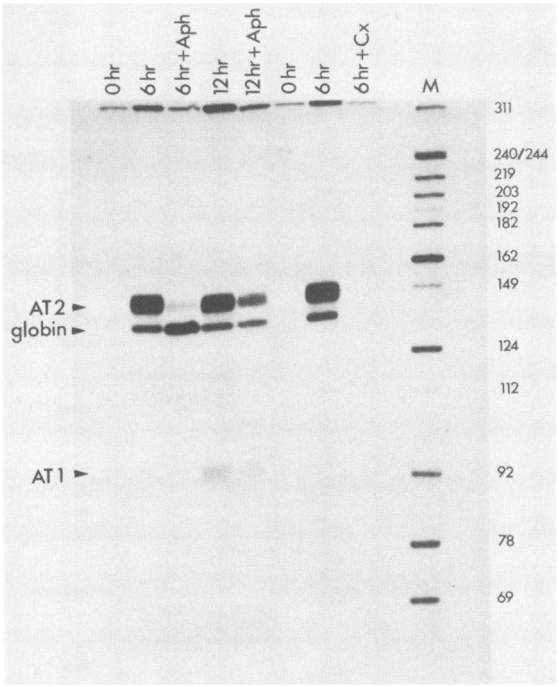

FIG. 5. Effects of inhibitors on β-globin expression. Cytoplasmic RNA (20 μg), prepared from Vero cells at the indicated times postinfection with gC-beta (10 PFU per cell), was hybridized to the β-globin probe described in the legend to Fig. 4, and the hybrids were digested with S1 nuclease. Cycloheximide (Cx; 100 μg/ml) or aphidicolin (Aph; 10 μg/ml) was added 30 min prior to infection. Markers (M) were 3'-labeled *Hpa*II fragments of pBR322 DNA. Marker fragment sizes (in nucleotides) are shown at the right.

roughly the same time course as those derived from the E gD gene (14, 67) and were detectable before those arising from the true L US11 gene (36). Accumulation of globin RNA was strongly suppressed by blocking viral protein synthesis with cycloheximide but was not reduced by blocking viral DNA replication with aphidicolin (Fig. 5). These data suggest that globin expression required viral IE polypeptide synthesis and was largely independent of DNA replication. On the basis of these results, we concluded that the rabbit β-globin gene remained under E control when it was embedded within a viral L gene.

Two additional globin-related transcripts, AT1 and AT2 (Fig. 4 and 5), accumulated at later times postinfection. One of these, AT2, gave rise to an S1 signal mapping to the site of sequence divergence between gC-beta DNA and the probe used and must therefore arise by readthrough from the upstream globin sequences. The second (AT1) generated an S1 signal mapping to a previously described cryptic splice-acceptor site located at approximately +46 in the globin-coding sequences (26, 60) and most likely originates through alternative splicing of RNAs initiated at one or more upstream promoters. It seems likely that some of the RNAs that give rise to the AT1 and AT2 S1 signals initiate at the gC promoter. However, we found that the AT1 and AT2 S1 signals were considerably less sensitive to inhibition of viral DNA replication than transcripts driven from the gC promoter (compare Fig. 3 and 5). We therefore suspect that transcripts initiated at one or more additional promoters, perhaps located upstream of the gC promoter or within the 5' flanking globin sequences, also contributed to the AT1 and AT2 S1 signals.

Insertion of the human α_2 globin gene and two *Alu* elements into the *tk* locus. Tackney et al. (62) reported that the intact Chinese hamster adenine phosphoribosyltransferase (*aprt*) gene was not detectably transcribed following incorporation into the HSV genome and interpreted these results as indicating that HSV regulators distinguish between cellular and viral promoters. Because of the contrasting behavior of the rabbit β-globin and hamster *aprt* genes, we wished to learn whether the β-globin gene was unique among cellular elements in its ability to be expressed to high levels when transduced by HSV. We therefore studied the regulation of the human α_2 globin gene and two linked *Alu* elements present on a 4.3-kb *Sst*I fragment following insertion into the viral *tk* gene (Fig. 6). These particular cellular elements were

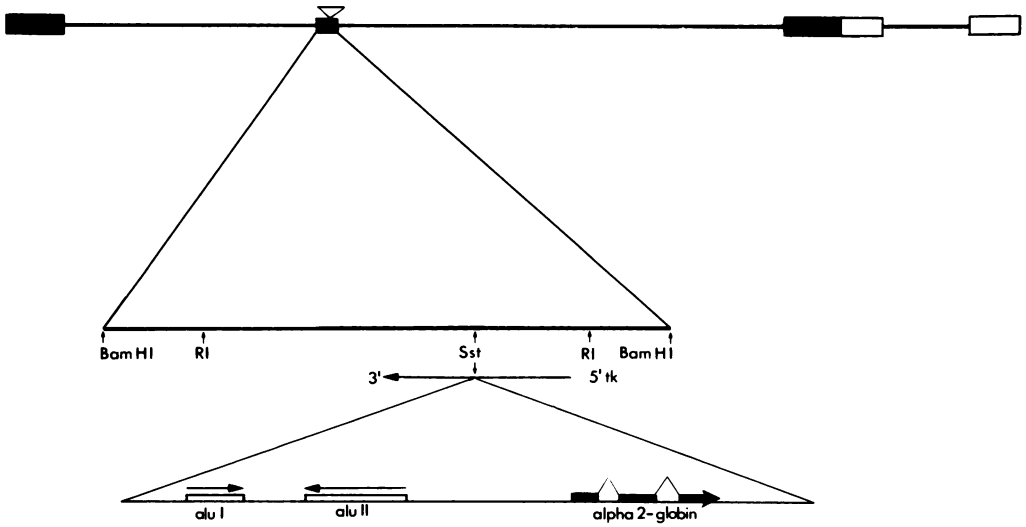

FIG. 6. Structure of strain tk-alpha. A 4.3-kb *Sst*I fragment of human DNA bearing the α_2-globin gene and two *Alu* repetitive elements was cloned into the *Sst*I site within *tk* coding sequences, and the resulting tk-deficient insertion mutation was transferred into the *tk* locus in the viral genome by recombination in vivo. The intron-exon arrangement of the α-globin gene and the transcriptional polarities of the *Alu* elements are indicated. RI, *Eco*RI.

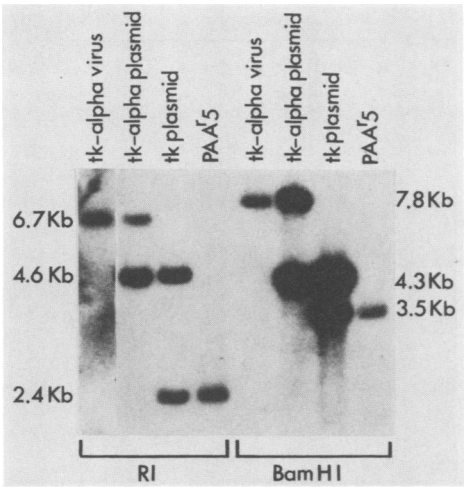

FIG. 7. Southern blot analysis of tk-alpha DNA. The indicated DNAs were cleaved with *Eco*RI (RI) or *Bam*HI, transferred to nitrocellulose, and then probed with a *tk* plasmid. Insertion of the α-globin fragment increased the size of the PAA'5 *Eco*RI and *Bam*HI *tk* fragments by 4.3 kb.

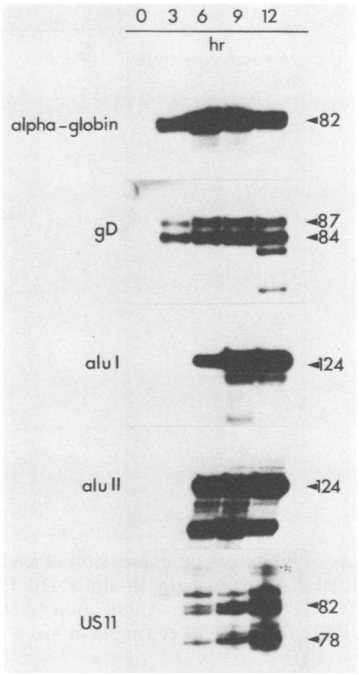

FIG. 8. Time course of α-globin and *Alu* expression during infection with tk-alpha. Cytoplasmic RNA (20 μg), extracted from BHK21 cells at the indicated times postinfection, was analyzed by primer extension with 5'-labeled synthetic 25-mers designed to detect α-globin, *Alu*I, *Alu*II, gD, and US11 RNAs. The sizes (in nucleotides) of the major extension products (estimated relative to *Hpa*II fragments of pBR322 DNA) are indicated.

chosen for two reasons. First, the α- and β-globin promoter regions have little primary nucleotide sequence homology, and these two genes are regulated very differently in transfection assays (5, 34, 63). Thus, if the α-globin gene was also activated by HSV products, the result would reduce the likelihood that this control results from recognition of "virus-specific" signals accidentally present in globin DNA. Second, *Alu* elements are transcribed by RNA polymerase III in vitro (10, 11; reviewed in reference 52), and we wished to learn whether certain cellular *pol*III-transcribed genes can also be expressed to high levels following transduction by HSV.

Insertion of the 4.3-kb α-globin fragment into the *tk* gene of strain tk-alpha resulted in loss of the wild-type 3.5-kb *Bam*HI and 2.4-kb *Eco*RI *tk* fragments and acquisition of the predicted 7.8-kb *Bam*HI and 6.7-kb *Eco*RI fragments bearing the globin insert (Fig. 7).

E expression of the α-globin gene. We studied the regulation of the inserted α-globin and *Alu* transcription units by primer extension analysis of cytoplasmic RNAs produced during lytic infection of Syrian hamster BHK21 cells with tk-alpha. BHK21 cells were chosen instead of Vero cells to reduce the risk of cross-hybridization between primers designed to detect transcripts arising from the virally transduced human genes and the closely related endogenous primate sequences present in Vero cells. Control experiments demonstrated that RNA prepared from PAA'5-infected BHK21 cells did not react with the α-globin and *Alu* primers (data not shown).

Correctly initiated α-globin transcripts accumulated with an early time course: transcripts were detected at 3 h postinfection, reached maximal levels by 6 h, and remained relatively constant in abundance thereafter (Fig. 8). In addition, α-globin expression was blocked by inhibiting protein synthesis with cycloheximide but was not greatly affected by suppressing viral DNA replication with aphidicolin (Fig. 9). Similar results were obtained during infection of Vero cells with strain tk-alpha (data not shown).

L expression of *Alu* elements. The two *Alu* elements transduced by strain tk-alpha differ in size: *Alu*I is a standard human monomer, while *Alu*II is a dimer composed of two fused *Alu* elements. In addition, *Alu*I and *Alu*II differ significantly in their primary sequences, necessitating the use of separate primers to detect their respective transcripts. The primers were complementary to residues 95 to 120 of the *Alu* elements and were designed to prevent cross-hybridization to the related 7SL RNA (64).

Both *Alu* elements gave rise to abundant cytoplasmic transcripts initiated at the first residue of the *Alu* repeat, i.e., the initiation site of RNA polymerase III in vitro (Fig. 8 and 9) (11). *Alu* transcripts were first detected 6 h postinfection, and the levels of *Alu* RNA increased at later times. Accumulation of *Alu* transcripts was completely suppressed by blocking DNA replication with aphidicolin and by inhibiting protein synthesis with cycloheximide (Fig. 9). In these respects, the *Alu* elements were regulated in a fashion that closely mimics the behavior of HSV true L genes.

RNA polymerase III terminates transcription immediately following a run of four or more T residues in the nontemplate strand (4, 11, 40). As an indirect test of whether the *Alu* transcripts arising from the virally transduced elements were transcribed by polymerase III, we mapped the 3' end of the *Alu*I transcript by S1 nuclease protection analysis. Using a 3'-labeled *Ava*I-*Nco*I probe fragment labeled at an *Ava*I site ca. 100 nt upstream of the 3' end of the element, we detected a protected fragment of ca. 162 nt (Fig. 10). This result maps the 3' end of the *Alu*I transcript within a run of 6 T residues (Fig. 10C), which corresponds to the first run of four or more T residues in the downstream flanking human sequences. Thus, the position of the 3' end of this *Alu* transcript provides indirect evidence that it is transcribed by RNA polymerase III.

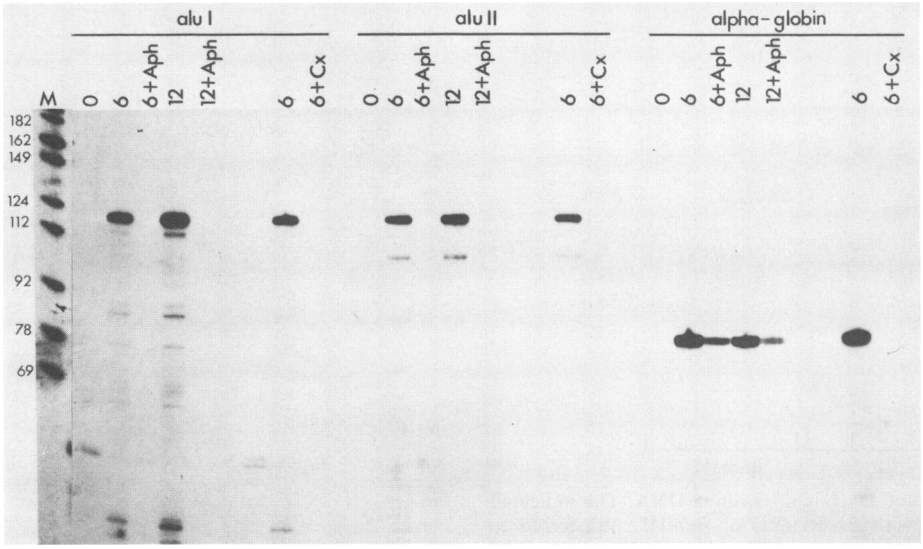

FIG. 9. Effects of inhibitors on expression of α-globin and *Alu* RNAs. Cytoplasmic RNA (20 μg), prepared at the indicated times (hours) postinfection of BHK21 cells with tk-alpha (10 PFU per cell), was analyzed for α-globin and *Alu* transcripts by primer extension. Cycloheximide (Cx; 100 μg/ml) or Aphidicolin (Aph; 10 μg/ml) was added 30 min prior to infection. Markers (M) were 3′-labeled *Hpa*II fragments of pBR322 DNA. Marker fragment sizes (in nucleotides) are shown at the left.

DISCUSSION

Previous studies demonstrated that the rabbit beta-globin gene was transcribed from its own promoter and regulated as an HSV E gene following insertion into the E *tk* gene in the intact viral genome (60). The results presented in this paper show that the globin promoter remained under E control when it was inserted into the body of the true L viral gene encoding gC. Thus, in this instance, the regulation of a cellular gene residing in the HSV genome was not dictated by the temporal class of the viral gene into which it was inserted. These data strongly suggest that the β-globin control region provides the functional equivalent of an HSV E promoter and demonstrate that E regulation can occur through mechanisms that are not restricted to viral promoters. Consistent with this view, we found that the highly diverged human α_2 globin gene was also expressed under E control in a viral recombinant. This latter finding reduces the likelihood that the regulation of globin genes by HSV products relies on recognition of virus-specific temporal control sequences accidentally present in the upstream regions or transcribed bodies of these genes. Rather, it seems much more likely that this control results at least in part from virus-induced modifications that facilitate the interaction of one or more cellular factors with the globin control regions. Interpreted in this way, our data support the hypothesis that HSV regulators alter the activity of cellular transcription factors (44) in a fashion similar to that of the adenovirus E1a proteins (reviewed in reference 37).

It is intriguing that the expression of an HSV-transduced α-globin gene required viral polypeptide synthesis. In contrast to β-globin genes, the human α-globin gene is efficiently expressed following transfection into a variety of cell types (5, 34, 63). Thus, one might have anticipated that the α-globin gene would be directly expressed upon infection in the absence of viral regulators. Indeed, Hearing and Shenk (28) found that this gene was expressed in the absence of IE polypeptides during infection with an E1a-deficient adenovirus recombinant. One interpretation of our data is that the α-globin gene is somehow prevented from interacting with the cellular transcriptional apparatus when it is placed in the HSV genome and that one or more viral products are required to overcome this negative control. If this idea is correct, then it seems possible that similar mechanisms contribute to the severe restriction of viral E and L gene expression that is observed in the absence of HSV IE polypeptides.

Expression of the *Alu* elements transduced by strain tk-alpha stringently required viral protein and DNA synthesis—in these respects mimicking the behavior of viral true L genes. It is not yet clear whether the requirement for protein synthesis reflects a direct effect of viral polypeptides on *Alu* expression: an equally plausible explanation is that *Alu* expression is driven entirely by viral DNA replication. One hypothesis to explain the requirement for viral DNA replication postulates that *Alu* promoters are very weak in vivo and that detectable expression therefore requires template amplification. Alternative explanations include (i) the generation of a transcriptionally permissive, altered DNA conformation during replication and (ii) a replication-dependent segregation of DNA molecules into specialized nuclear compartments containing the necessary transcription factors.

Epstein-Barr virus encodes small *pol*III-transcribed RNAs (42), but HSV is not known to bear *pol*III genes. While we have no direct evidence that the virally transduced *Alu* elements are transcribed by RNA polymerase III during infection, our observation that the *Alu* transcripts start at the predicted *pol*III initiation site and end at a classical *pol*III terminator provides indirect evidence that this is the case. Adenovirus and pseudorabies virus IE proteins can activate the transcription of certain *pol*III genes that have been newly introduced into cells (23, 30) by modifying the activity of the *pol*III transcription factor IIIC (29, 70). We are testing whether HSV IE products are also able to directly stimulate transcription by *pol*III.

Our results strongly suggest that some cellular *cis*-acting elements are able to specify control patterns that closely resemble those of HSV E and L genes. This in turn suggests that certain aspects of the temporal regulation of HSV genes

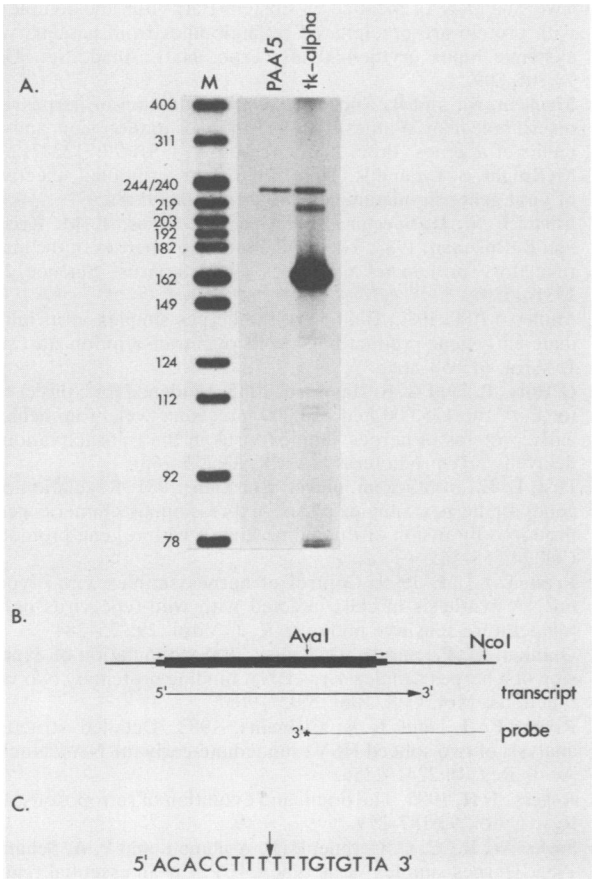

FIG. 10. Location of the 3′ end of the *Alu*I transcript. (A) Cytoplasmic RNA (20 μg) prepared from BHK21 cells 9 h postinfection with PAA'5 or tk-alpha (10 PFU per cell) was hybridized to the 3′-labeled probe diagrammed in panel B. After treatment with S1 nuclease, hybrids were displayed on an 8% sequencing gel. Markers (M) were 3′-labeled *Hpa*II fragments of pBR322 DNA. Marker fragment sizes (in nucleotides) are shown at the left. (B) Probe structure. The probe extends from an *Ava*I site in the *Alu*I element to an *Nco*I site in the 3′ flanking human sequences. The *Alu* element is represented as a thick closed bar, the 15-nt direct repeats of host sequences flanking the element are indicated by small arrows, and the structure of the *Alu*I transcript is diagrammed. (C) Nucleotide sequence at the 3′ end of the *Alu*I transcript. The sequence in the sense of the *Alu* transcript is presented. The arrow marks the approximate position of the 3′ end, as estimated from the data displayed in panel A.

arise through processes that do not involve recognition of HSV-specific *cis*-acting signals. It will be interesting to learn whether any of the IE polypeptides provide additional levels of control that are specifically targeted to HSV E and L genes.

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