Elongin B-Mediated Epigenetic Alteration of Viral Chromatin Correlates with Efficient Human Cytomegalovirus Gene Expression and Replication

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ABSTRACT **Elongins B and C are members of complexes that increase the efficiency of transcriptional elongation by RNA polymerase II (RNAPII) and enhance the monoubiquitination of histone H2B, an epigenetic mark of actively transcribed genes. Here we show that, in addition to its role in facilitating transcription of the cellular genome, elongin B also enhances gene expression from the double-stranded DNA genome of human cytomegalovirus (HCMV), a pathogenic herpesvirus. Reducing the level of elongin B by small interfering RNA- or short hairpin RNA-mediated knockdown decreased viral mRNA expression, viral protein accumulation, viral DNA replication, and infectious virion production. Chromatin immunoprecipitation analysis indicated viral genome occupancy of the elongating form of RNAPII, and monoubiquitinated histone H2B was reduced in elongin B-deficient cells. These data suggest that, in addition to the previously documented epigenetic regulation of transcriptional initiation, HCMV also subverts cellular elongin B-mediated epigenetic mechanisms for enhancing transcriptional elongation to enhance viral gene expression and virus replication.**

IMPORTANCE **The genetic and epigenetic control of transcription initiation at both cellular and viral promoters is well documented. Recently, the epigenetic modification of histone H2B monoubiquitination throughout the bodies of cellular genes has been shown to enhance the elongation of RNA polymerase II-initiated transcripts. Mechanisms that might control the elongation of viral transcripts are less well studied. Here we show that, as with cellular genes, elongin B-mediated monoubiquitination of histone H2B also facilitates the transcriptional elongation of human cytomegalovirus genes. This and perhaps other epigenetic markings of actively transcribed regions may help in identifying viral genes expressed during *in vitro* latency or during natural infections of humans. Furthermore, this work identifies a novel, tractable model system to further study the regulation of transcriptional elongation in living cells.**

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The elongin BC complex is a heterodimer composed of the 118-amino-acid (18-kDa) elongin B and 112-amino-acid (15-kDa) elongin C proteins (1). These two proteins, which are found almost exclusively in this binary complex, were identified as positive regulators of elongin A, an RNA polymerase II (RNAPII) elongation factor (1). The trimeric elongin ABC complex (termed SIII) stimulates the rate of elongation by RNAPII *in vitro* by decreasing transient pauses, likely through promoting proper alignment of the 3′ end of the nascent RNA with the enzyme active site, thus preventing polymerase backtracking and stalling (2).

The elongin BC complex also facilitates the clearance of persistently stalled polymerases that result, for example, during transcriptional elongation on damaged templates (3, 4). Phosphorylation of serine 5 in the C-terminal domain (CTD) of Rpb1, the largest subunit of RNAPII, and monoubiquitination by the yeast Rsp5 ubiquitin ligase (human homologue NEDD4) appear to be required for elongin ABC-mediated polyubiquitination of arrested polymerases (3, 4). The subsequent proteasomal degradation of the stalled and polyubiquitinated RNAPII clears the template, enabling subsequent transcription (3, 4).

Furthermore, elongin C participates in a ubiquitin ligase complex with the SWI/SNF-A component BAF250b to mediate the monoubiquitination of histone H2B at lysine 120 (5). Monoubiquitinated H2B (UbH2B) is found associated with the transcribed regions of highly expressed human genes (6–9). This modification is dynamic in nature and thought to facilitate the passage of RNA polymerases through the nucleosomes they encounter during the process of transcriptional elongation (10–12). The prevailing model posits that UbH2B increases the association of histone chaperones with actively transcribed chromatin, allowing more efficient disassembly of nucleosomes in front of RNAPII, as well as their efficient reassembly behind the transcribing enzyme. Thus, elongin proteins facilitate transcriptional elongation by multiple mechanisms.

In addition to enhancing transcription elongation, elongins B and C are found in complexes with a cullin family protein (Cul2 or Cul5), the ring finger protein Rbx1, and a substrate-targeting

component that polyubiquitinate targeted substrates, leading to their eventual proteasomal degradation. Such substrates include p53 (13), HIF-1α (14), Rb (15), and APOBEC3G (16). Because the elongin BC proteins associate with sequence-specific motifs found within the substrate-binding proteins, these factors are often called BC-box proteins (17–20). Multiple cellular (e.g., elongin A, BAF250b, and the von Hippel-Lindau tumor suppressor) and viral (e.g., human immunodeficiency virus Vif, adenovirus E4orf6, and human papillomavirus E7) BC-box proteins, as well as their substrates, have been identified (3, 5, 13–16, 20, 21).

The majority of DNA viruses, including human cytomegalovirus (HCMV), depend on cellular transcriptional machinery to transcribe functional viral mRNAs (22). Therefore, viruses such as HCMV provide tractable models to understand transcriptional regulation. HCMV is the prototypic member of the *Betaherpesvirus* family (22). It is a ubiquitous human pathogen that causes severe morbidity and mortality, especially in people with an immature or compromised immune system. HCMV is the leading viral cause of birth defects (23, 24) and is associated with age-related immunosenescence and proliferative diseases, including atherosclerosis, restenosis, and certain cancers (25–29).

HCMV gene expression is temporally regulated (22) and initiates with the immediate-early (IE) genes from the major IE promoter (MIEP). The activity of the MIEP is controlled in part by an intrinsic cellular antiviral defense that silences the promoter, mediated in part by the cellular transcriptional corepressor Daxx (30, 31). Upon productive, lytic HCMV infection, this defense is neutralized by the viral pp71 protein. A component of the tegument layer of infectious virions (32), pp71, is delivered directly to cells upon viral entry and migrates to the nucleus, where it induces the proteasomal degradation of Daxx to facilitate viral IE gene expression (30). These newly expressed viral IE proteins trigger the subsequent events of the productive infectious cycle, such as early gene expression, viral DNA replication, late gene expression, and the release of infectious progeny virions (22).

With a desire to address how additional cellular proteins might regulate HCMV gene expression, we investigated a potential role for the elongin BC complex during HCMV infection. We found that depletion of elongin B reduced viral mRNA levels, viral protein expression, and viral DNA replication, resulting in reduced progeny virus production. Negative effects on viral protein, DNA, and progeny virion levels are likely secondary to the decreases in viral mRNA levels that we observed. Elongin B depletion did not restrict the ability of pp71 to mediate the degradation of Daxx but did decrease the viral genome association of UbH2B and the elongating form of RNAPII. Our data indicate that elongin B function is required for efficient HCMV transcript accumulation and productive replication and implicate efficient transcript elongation as a significant determinant of successful HCMV infection.

RESULTS

Elongin B is required for efficient HCMV replication. To determine if elongin B or elongin C is required for HCMV replication, we generated, by retroviral transduction and drug selection, cells that should constitutively express short hairpin RNAs (shRNAs) designed to knock down the levels of either protein. While the levels of elongin B or the control protein Skp1, an elongin C homolog, were substantially reduced in the relevant drug-selected cell populations (~90% reduction), those of elongin C were not (Fig. 1A). That elongin C levels are reduced in shEloB cells is not surprising because elongin B is known to stabilize elongin C by forming a heterodimer (1). Because of our inability to generate stable elongin C knockdown cells, we focused our subsequent experimentation on elongin B.

HCMV appeared to enter parental telomerase-immortalized human foreskin fibroblasts (Tert-HFs; control) and shEloB and shSkp1 cells with equal efficiency, as evidenced by the equivalent levels of tegument-delivered pp71 found in cell lysates at 4 h postinfection (hpi) (Fig. 1B). Newly expressed viral proteins of the immediate-early (IE1), early (UL44 and pp71), and late (pp28) temporal classes accumulated to roughly equivalent levels in control and shSkp1 cells but to reduced levels in shEloB cells. Off-target effects of the specific targeting sequence are unlikely to be responsible for the decreased viral gene expression observed in cells constitutively expressing the shRNA to elongin B because transient transfection of Tert-HF cells with either of two small interfering RNAs (siRNAs), but not a scrambled siRNA, also resulted in decreased viral gene expression upon HCMV infection (Fig. 1C).

Other assays used to measure efficient lytic replication also indicated that viral replication was reduced in shEloB cells. Viral DNA synthesis at 72 hpi was decreased over 5-fold in shEloB cells compared to that in control cells, even though viral DNA levels at 6 hpi, a measure of viral entry, were equivalent (Fig. 1D). Similarly, both growth curve (Fig. 1E) and endpoint (Fig. 1F) analyses showed that infectious progeny virion release from shEloB cells was approximately 100-fold lower than that from control or shSkp1 cells. Taken together, these results demonstrate that elongin B is required for efficient HCMV replication.

Elongin B is not required for pp71-mediated Daxx degradation but is required for viral transcript accumulation during HCMV infection. Elongin B negatively regulates the stability of p53, HIF-1α, Rb, APOBEC3G (20, 21), and likely other proteins and positively regulates the process of transcription by facilitating transcript elongation as described above (1, 3, 4). We wanted to determine whether effects on protein degradation and/or transcription correlate with the decreased replication of HCMV in cells with reduced elongin B.

Both elongin B (20, 21) and pp71 (30, 33, 34) participate in reactions that lead to the proteasomal degradation of targeted substrates. One pp71 substrate, Daxx, represses viral IE gene expression prior to its proteasomal degradation induced by pp71 (31, 35). Viruses that lack pp71 (36) or express a mutant protein unable to bind (37, 38) or degrade (30, 39) Daxx show reduced viral gene expression and grow to lower titers, similar to wild-type virus in shEloB cells (Fig. 1). To determine if the growth defect in shEloB cells could possibly result from an inability of pp71 to degrade Daxx, we asked if elongin B is required for pp71-mediated Daxx degradation. Neither the transient knockdown of either elongin B or elongin C (Fig. 2A) nor the constitutive knockdown of elongin B (Fig. 2B) impaired the ability of tegument-delivered pp71 to induce Daxx degradation upon HCMV infection. These experiments suggest that neither elongin B nor elongin C is required for pp71-mediated Daxx degradation and that elongin B participates in a different step required for efficient HCMV replication.

We next compared HCMV mRNA levels in control and shEloB cells to determine if decreased levels of elongin B affect viral transcript levels. We found that messages encoding the major immediate-early proteins (Fig. 2C) and overlapping messages

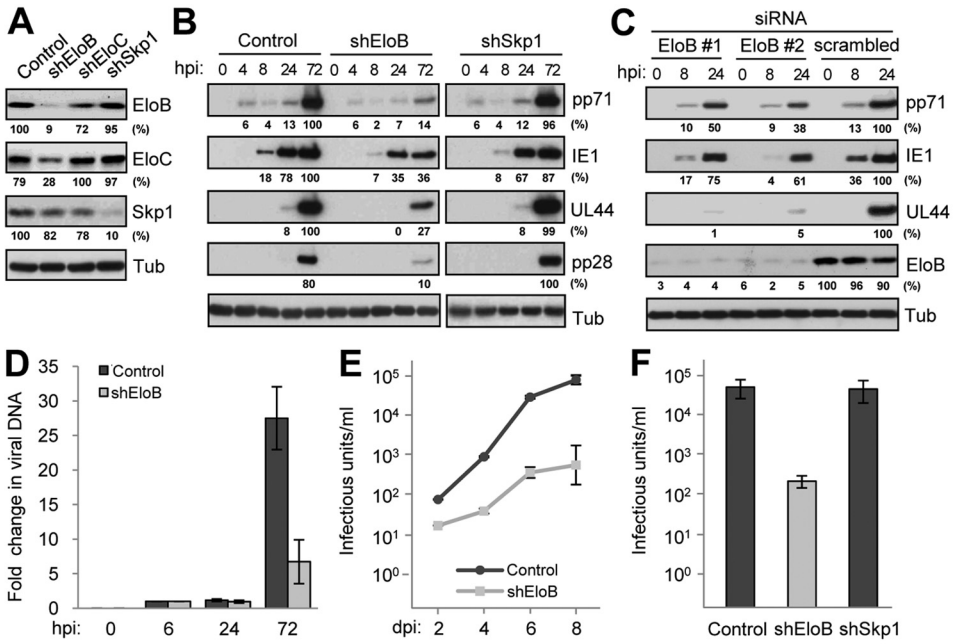

FIG 1 Elongin B is required for efficient HCMV replication. (A) Western blot analysis of whole-cell lysates from Tert-HFs (control) or derivatives selected to express shRNAs to elongin B (EloB), elongin C (EloC), or Skp1. In all Western blot assays, tubulin (Tub) served as a loading control. Band intensities were quantified using the Scion image program, normalized relative to the quantity of their respective tubulin bands, and expressed as percentages of the strongest value. (B) Lysates from cells infected with HCMV were harvested at the indicated times (hours) postinfection and analyzed by Western blot assay with the indicated antibodies. (C) Lysates from HFs transfected with distinct siRNAs directed at EloB (no. 1 or 2) or with a scrambled siRNA for 48 h and then infected with HCMV were harvested at the indicated times and analyzed by Western blot assay. (D) Viral DNA accumulation was measured by qPCR with a primer-probe set corresponding to the major IE gene using total DNA isolated from HCMV-infected control or shEloB cells at the indicated times postinfection. Values were normalized to cellular β-actin levels and are expressed as *n*-fold changes versus IE levels measured in control cells at 6 hpi. Error bars represent the standard error of the mean. (E) Viral growth curves after infection with HCMV in control or shEloB cells were determined by plaque assay of culture supernatants. Error bars represent the standard error of the mean. (F) Viral titers achieved at 7 days in supernatants from HCMV-infected cells were determined by plaque assay. Data from triplicate experiments with standard errors are shown.

(40) encoding the early proteins pp65 and pp71 (Fig. 2D) accumulated in shEloB cells to levels approximately 10-fold lower than those in control cells. Therefore, elongin B is required for efficient HCMV transcript accumulation. Furthermore, it is likely that the observed defects in viral protein accumulation, viral DNA replication, and infectious virion release in shEloB cells are secondary effects resulting from defects in viral transcription.

Less monoubiquitinated histone H2B and elongating RNA polymerases are found associated with an HCMV gene in cells with reduced levels of elongin B. The elongin BC complex mediates the polyubiquitination of RNAPII, leading to its proteasomal degradation (3, 4), as well as the monoubiquitination of histone H2B, without affecting its stability (5). Either of these activities has the potential to modulate transcriptional elongation. To determine if altering levels of RNAPII or UbH2B correlate with decreased HCMV transcript accumulation in shEloB cells infected with HCMV, we analyzed the steady-state levels of these proteins in infected cell lysates. We used two different antibodies to monitor RNAPII, one (H14) that detects the serine 5-phosphorylated CTD initiating form and one (H5) that detects the serine 2-phosphorylated CTD elongating form (41, 42). In experiments where HCMV protein accumulation was clearly lower in shEloB cells than in control cells, no significant difference in either form of RNAPII was detected (Fig. 3A). Thus, global effects on RNAPII stability do not correlate with the reduced viral gene expression observed upon HCMV infection of shEloB cells.

We next examined the steady-state levels of total histone H2B and monoubiquitinated histone H2B. The overall level of histone H2B did not vary substantially in any of the samples analyzed (Fig. 3B). However, in uninfected cells, the levels of UbH2B were substantially lower in shEloB cells than in control or shSkp1 cells (Fig. 3B). Interestingly, HCMV infection appeared to induce an increase in the level of UbH2B at 4 hpi, most notably in shEloB cells. As H2B ubiquitin ligases associate with the complexes that activate transcription (7, 8) and as HCMV induces a burst of cellular gene expression early after infection (43), this is perhaps not surprising. This increase in UbH2B, especially in shEloB cells, is likely mediated by RNF20 or another H2B ubiquitin ligase (44–46). Importantly, lower levels of UbH2B clearly correlated with reduced HCMV gene expression in cells with reduced levels of elongin B.

UbH2B is an epigenetic mark for sites of active transcription (7–9, 11). Therefore, we determined, with a chromatin immunoprecipitation (ChIP) assay, whether or not the levels of DNA-associated UbH2B (normalized to total H2B occupancy) varied between the viral and cellular genomes in the presence of normal or reduced levels of elongin B. For the cellular genome, we analyzed UbH2B association with a single locus in the body of the actin gene. For the HCMV genome, we analyzed four loci within the UL47 and UL48 genes (Fig. 4A), the two longest open reading frames (ORFs) present in the virus, at 2,944 and 6,722 bp, respectively (47). As most HCMV genes are unspliced, rather short, and

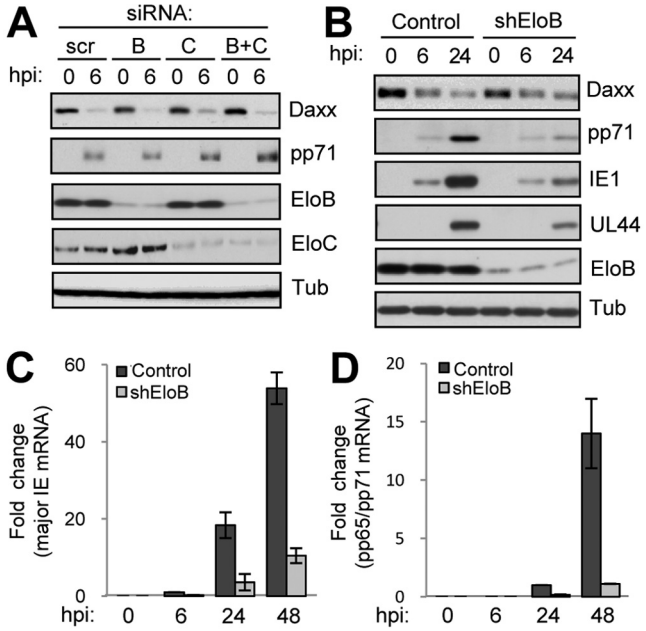

FIG 2 Elongin B is not required for pp71-mediated degradation of Daxx but is required for viral transcript accumulation during HCMV infection. (A) HFs were transfected with scrambled siRNA (columns scr) or siRNAs directed at elongin B (columns B), elongin C (columns C), or elongins B and C (columns B+C) for 48 h. Cells were then infected with HCMV. Lysates were harvested at the indicated times postinfection and analyzed by Western blot assay with the indicated antibodies. (B) Lysates from HCMV-infected cell populations were harvested at the indicated times postinfection and analyzed by Western blot assay. (C and D) Control or shEloB cells were infected with HCMV, and total RNA was harvested at the indicated times. Expression of major IE or pp65/pp71 RNA (C and D, respectively) was determined by reverse transcription-qPCR. Samples were normalized to cellular β-actin RNA levels, and data are expressed as *n*-fold changes compared to the IE level measured in control cells at 6 hpi (C) or pp65/pp71 levels measured in control cells at 24 hpi (D). Mean values from three independent experiments are shown. Tub, tubulin.

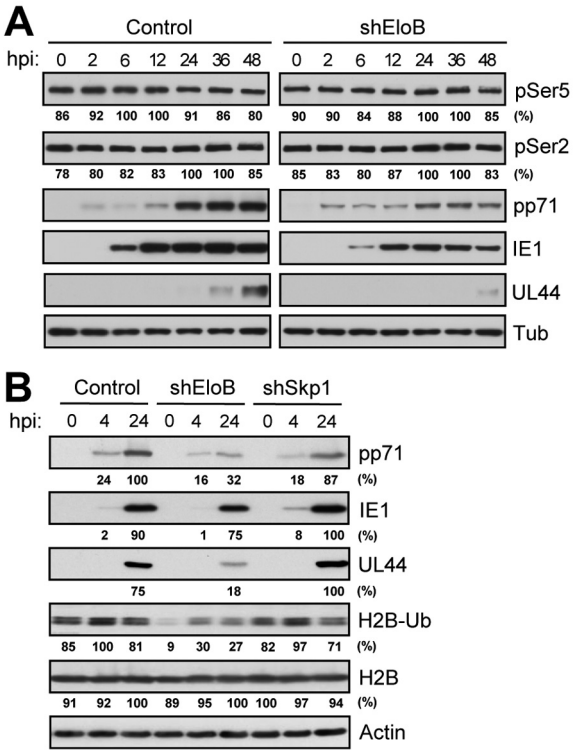

FIG 3 Elongin B does not change global RNAPII stability but does affect the overall levels of histone H2B monoubiquitination. (A and B) Lysates from HCMV-infected cell populations were harvested at the indicated times postinfection and analyzed by Western blot assay with the indicated antibodies. Actin served as a loading control in panel B. Band intensities were quantified using the Scion image program, normalized relative to the quantity of their respective tubulin (Tub) or actin bands, and expressed as percentages of the strongest value.

similar in size to the DNA fragments generated during a ChIP protocol, distinguishing proteins associated with promoter regions from those associated with the bodies of HCMV genes is challenging. However, utilizing these long ORFs provides the best opportunity to distinguish between these different gene positions with this assay. Note that splicing does not appear to significantly alter the rate of transcriptional elongation, at least at the loci tested (48).

Transcription initiates just upstream of the initiating codons of both UL47 and UL48 and terminates subsequent to a polyadenylation site just beyond the terminating codon of UL48 (47). Thus, the transcript encoding UL47 also passes through the UL48 gene. Primers were designed to analyze the 5′ region of UL47 (L0) or the 5′ (L1), central (L2), and 3′ (L3) regions of UL48 (Fig. 4A). We observed UbH2B association with both the cellular and viral genomes in both control and shEloB cells (Fig. 4B). The levels of UbH2B found on the cellular actin gene were, on average, 30% lower in shEloB cells than in control cells, although the observed difference was not statistically significant. In contrast, the average levels of UbH2B associated with different regions of the HCMV genome were substantially lower (L0, 47%; L1, 51%; L2, 69%; L3, 64%) in shEloB cells than in control cells (Fig. 4B). Importantly, these observed differences between the two cell populations were statistically significant. These data indicate that lower UbH2B occupancy on the viral genome correlates with decreased viral gene expression during HCMV infection of cells with decreased levels of elongin B.

Finally, we sought to uncover a potential mechanism by which elongin B and UbH2B might enhance HCMV gene expression. As elongin B has long been associated with transcriptional elongation (1), we focused on this aspect of gene expression. Elongation efficiency *in vivo* can be measured by examining the occupancy of differently modified forms of RNAPII at different loci in an actively transcribed gene (2, 49). Therefore, we used a ChIP assay to analyze nonphosphorylated, initiating (serine 5-phosphorylated), or elongating (serine 2-phosphorylated) RNAPII at the 5′, central, and 3′ regions of the viral UL47 and UL48 genes (Fig. 4C). Note that RNAPII occupying the genomic region surveyed by primer pair L1 will represent elongating polymerases that started at UL47 and initiating polymerases that started at UL48 (Fig. 4A).

The 8WG16 antibody recognizes the nonphosphorylated form, as well as the serine 5-phosphorylated (initiating) form, of RNAPII. ChIP analysis using 8WG16 or H14 (initiating) detected indistinguishable levels of RNAPII at all locations throughout UL47 and UL48 in control and shEloB cells (Fig. 4C). However, UL47 and UL48 occupancy by the serine 2-phosphorylated elongating form of RNAPII was significantly lower in shEloB cells than in control cells (Fig. 4C). The magnitude of the difference between

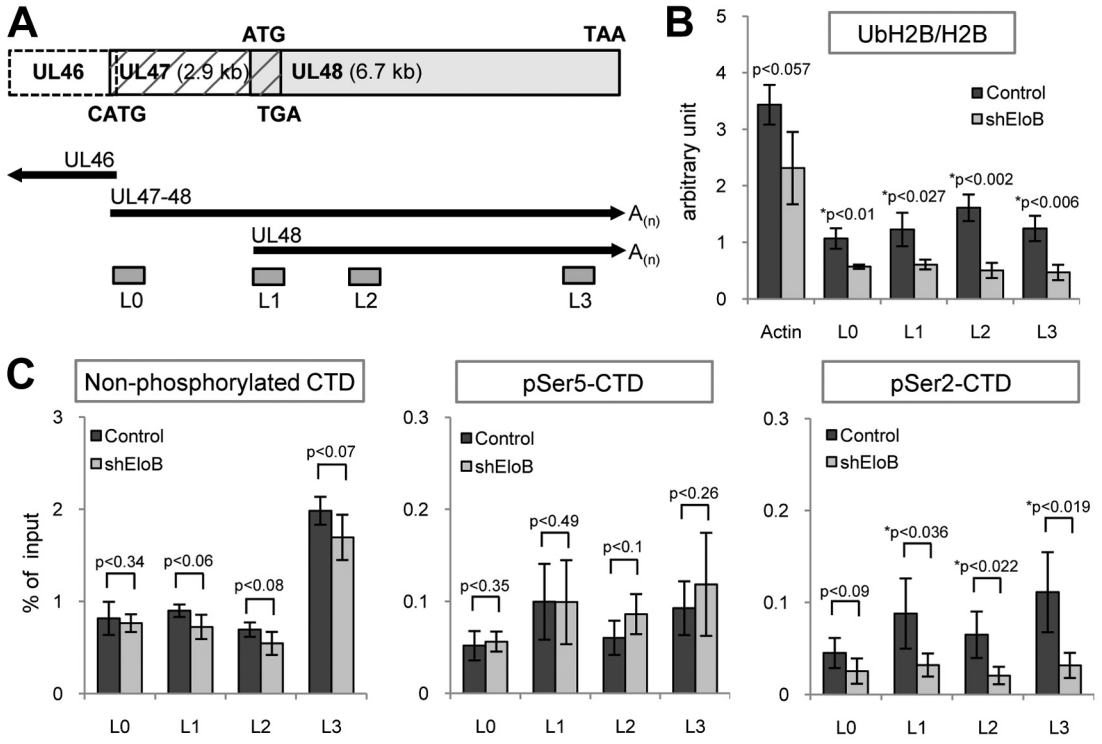

FIG 4 Less monoubiquitinated histone H2B and elongating RNAPIIs are associated with an HCMV gene in elongin B-depleted cells. (A) Schematic representation of the HCMV UL46 to UL48 genes. The dotted line, stripes, and the shading represent UL46, UL47, and UL48, respectively. Primer-probe pairs for the ChIP-qPCR analysis are indicated (L0 to L3 [L, locator]). (B and C) Control or shEloB cells were infected with HCMV at an MOI of 0.3. After 60 h, cells were subjected to ChIP with the indicated antibodies. Precipitated DNA was subjected to qPCR analysis with the primer-probe pairs described in panel A. All ChIP data represent the mean and standard error of the mean from three independent experiments. An asterisk indicates a statistically significant difference. (B) For each primer-probe pair, ChIP values were normalized to the corresponding total H2B ChIP and are represented as the ratio of UbH2B to H2B compared to the value obtained with the L0 primer-probe pair observed in control cells. (C) Each ChIP value is represented as a percentage of the respective input DNA.

the cell types was greatest near the shared polyadenylation site at the 3′ end of UL48. These data indicate that elongin B affects the occupancy of the elongating form of RNAPII on the viral UL47 and UL48 genes and imply that elongin B may facilitate efficient HCMV infection by promoting the elongation phase during the transcription of viral genes (Fig. 5).

DISCUSSION

Histone modifications that modulate transcript initiation affect both cellular and viral gene expression. For HCMV, histone H4, as well as H3K9 and H3K14 acetylation and H3K9 dimethylation correlate with transcriptionally active or silent viral genes, respec-

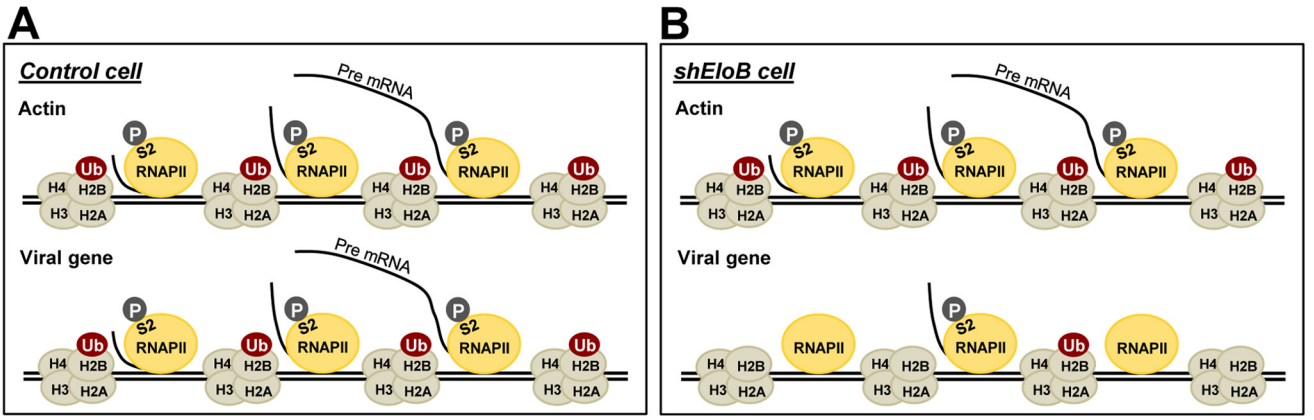

FIG 5 Proposed model of the mechanism of action of UbH2B in cellular and viral gene transcriptional elongation. (A) Ubiquitin ligase elongin BC complex induces monoubiquitination of histone H2B on genes, which enhances the elongation rate of RNAPII to efficiently transcribe genes. (B) Elongin B depletion results in inefficient monoubiquitination of histone H2B at the viral genome but not the cellular actin gene. Decreased levels of monoubiquitinated histone H2B results in less elongating RNAPII, which leads to decreased accumulation of viral transcripts.

tively (50, 51). Here we add H2B monoubiquitination, mediated in part by elongin B, to the list of epigenetic mechanisms that control HCMV gene expression and propose that efficient elongation is an additional step in the transcription cycle that is critical for productive HCMV infection.

In cells where the levels of elongin B have been reduced through RNA interference, the accumulation of viral mRNAs, proteins, DNA, and infectious progeny is reduced during HCMV infections (Fig. 1). The event most likely to be directly effected by elongin B deficiency is the transcriptional elongation of viral genes. We found reduced levels of HCMV genome-associated monoubiquitinated histone H2B, a known substrate of an elongin B-containing ubiquitin ligase (5), in cells with reduced levels of elongin B (Fig. 4B). Furthermore, we also detected reduced levels of elongating forms of RNAPII associated with the viral genome in these cells (Fig. 4C). From this, we conclude that the decrease in a specific epigenetic modification (UbH2B) of viral chromatin in the absence of sufficient elongin B correlates with, and perhaps causes, decreased HCMV transcription, leading in turn to reduced HCMV replication (Fig. 5).

Interestingly, substantial amounts of serine 5-phosphoarylated (initiating) polymerases were found throughout the body of the UL48 gene (Fig. 4C). Transcripts antisense to UL48 have been detected in HCMV-infected cells (52), and thus, promoter elements (where serine 5-phosphorylated RNAPII would be found) may be present within the UL48 gene on the opposite strand. Alternatively, this observation may be linked to splicing. The RNAPII CTD acts as a scaffold to assemble processing factors (48). Most HCMV genes (including UL48) produce mRNAs that are unspliced (53). Thus, the timing of serine 5 dephosphorylation may be subtly different between RNAPIIs transcribing (unspliced) viral genes and those transcribing (spliced) cellular genes.

Even though transcriptional elongation appears to be impaired in the absence of elongin B, stalled polymerases did not accumulate to high levels on the viral UL47 or UL48 gene (Fig. 4C). This agrees with our (Fig. 3A) and previous (42) observations that RNAPII levels are not reduced during HCMV infection. Irreversibly stalled polymerases are polyubiquitinated and degraded (54), for example, in herpes simplex virus type 1-infected cells (55, 56). This seems to imply that the elongation defect imposed by decreased levels of UbH2B, while significant, is not as severe as that imposed by other lesions, such as DNA damage. Alternatively, HCMV may have an as-yet-undescribed degradation-independent mechanism to clear stalled polymerases.

The overall level of UbH2B was increased upon HCMV infection, even in elongin B knockdown cells. As HCMV is known (22) to stimulate host cell gene expression (as well as to express numerous viral genes), and as multiple H2B ubiquitin ligases may exist (7), this is perhaps not surprising. Mechanistically unexplained is the more substantial decrease in UbH2B association with the viral UL47 and UL48 genes compared to the cellular actin gene (Fig. 4B). It remains to be determined how this might occur, whether it extends to additional viral and cellular genes, and whether or not similar results would be obtained in cells depleted in other potential H2B ubiquitin ligases.

Degradation of the transcriptional corepressor Daxx by the viral tegument protein pp71 stimulates viral gene expression (30). Although elongin B is known to serve as an adaptor for viral protein-assisted polyubiquitination and subsequent proteasomal degradation of cellular substrates (21), we found no indication that elongin B plays a substantial role in the pp71-mediated degradation of Daxx. This may not be surprising, as the undefined pathway through which pp71 targets its substrates for proteasomal degradation has been characterized as ubiquitin independent (33, 34).

Elongin B forms a complex with elongin C. While we were able to transiently knock down the levels of elongin C with a siRNA, we were unable to select for constitutive elongin C knockdown cells with a shRNA with the identical sequence. It is possible that low or absent elongin C protein levels are incompatible with human cell survival, as depletion of the ELC gene in *Caenorhabditis elegans* causes severe defects in chromosomal dynamics and cell proliferation (57). Thus, while we suspect that elongin C is required along with elongin B for efficient HCMV replication, we have not directly tested this hypothesis.

The epigenetic regulation of transcript initiation at promoters is well appreciated, but the observations that elongation also represents a significant control point during transcription that can be enhanced by the epigenetic modification of histone proteins (2, 49, 58) are not as well known. The data presented here suggest that the importance of the regulation of transcriptional elongation in cellular gene expression also extends to viral templates. The substantial effects of transcriptional elongation on HCMV gene expression reported here are somewhat surprising. The typical human gene is 30 times longer than the average HCMV gene (53, 59). Furthermore, the human genome displays, in general, a higher level of chromatinization than the HCMV genome (60). Thus, nucleosome traversal by elongating RNAPII over the entire length of a gene might be expected to represent a more significant impediment to cellular than to viral transcription. However, our results clearly show that elongin B, a protein historically implicated in transcriptional elongation, facilitates HCMV gene expression and productive infection. The viral capture of cellular transcriptional elongation mechanisms that we report here provides additional models with which to study them, as well as a potential therapeutic target for antivirals.

MATERIALS AND METHODS

Cells, viruses, antibodies, and Western blot assays. HFs or Tert-HFs were maintained and infected with HCMV strain AD169 as previously described (61). In all experiments, cells were infected with strain AD169 at a multiplicity of infection (MOI) of 0.1, unless described otherwise. Viral titers in supernatants from growth assays were determined in triplicate by plaque assay. Antibodies to the following proteins were from commercial sources: Daxx (D7810; Sigma), tubulin (DM 1 A; Sigma), elongin B (sc-11447; Santa Cruz), elongin C (613102; Bio Legend), Skp1 (610530; BD Biosciences), UL44 (CA006; Virusys), histone H2B (17-10054; Millipore), UbH2B (17-650; Millipore), RNAPII 8WG16 (MMS-126R; Covance), H14 (MMS-134R; Covance), and H5 (MMS-129R; Covance). Antibodies against pp28 (CMV157), pp71 (2H10-9), and IE1 (1B12) have been previously described (30, 62). Secondary antibodies were from Chemicon. Cells were lysed in either 1% sodium dodecyl sulfate (SDS) lysis buffer (20 mM Tris [pH 8.0], 250 mM NaCl, 3 mM EDTA, 10% glycerol, 1% SDS, 0.5% NP-40, protease inhibitors) for H2B detection or radioimmunoprecipitation assay buffer as described previously (33). Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis, immobilized on Optitran membranes, and probed with the indicated antibodies as previously described (61).

qPCR analysis. Total RNA and DNA isolation and quantitative real-time PCR (qPCR) were performed as previously described (62). The oligonucleotides used for qPCR are listed in Table 1. qPCR was performed on an ABI 7900HT, and data were analyzed using the SDS 2.2.1 program.

TABLE 1 Sequences of primers and probes used for qPCR analysis and siRNA target sequences

Primer or siRNA	Target	Sequence[a]
Primers for qPCR analysis		
L0	UL47	Forward, 5′ ATGGCGAGGCGCACGGTA 3′
		Reverse, 5′ GCCGATCTCCAATTGGCT 3′
		Probe, FAM-GATAGAGCAGCTGCGGGCAC-TAMRA
L1	UL48	Forward, 5′ ATGAAAGTCACACAGGCCAGC 3′
		Reverse, 5′ GCAGCTTCTTTTGCAACTCGC 3′
		Probe, FAM-AATCAATGCGTCTGCAACGGCATCA-TAMRA
L2	UL48	Forward, 5′ CAACGTTTCGTAACCAAGCGA 3′
		Reverse, 5′ CTGCAGCGCTTTCAGAATTTC 3′
		Probe, FAM-ACT TTCAGCGTCTGACGCAGGTCAT-TAMRA
L3	UL48	Forward, 5′ CTGCTCCAGGACACGTGGAC 3′
		Reverse, 5′ GGTCATACAGCGGGAAGGTG 3′
		Probe, FAM-ACGGCTACGCGATTACCTGCGTTTC-TAMRA
IE1	IE1	Forward, 5′ CGACGTTCCTGCAGACTATG 3′
		Reverse, 5′ TCCTCGGTCACTTGTTCAAA 3′
		Probe, FAM-TGGGAGACCCGCTGTTTCCA-TAMRA
pp71	pp71	Forward, 5′ CGTTCATTTGGAACACCGAC 3′
		Reverse, 5′ CTCTTCCTCTTCTTCCTCCTCTTC 3′
		Probe, FAM-TCTCACACCCGGTAAACCGGACAAA-TAMRA
siRNAs		
EloB no. 1	Elongin B	5′ UGAACAAGCCGUGCAGUGA 3′
EloB no. 2	Elongin B	5′ AGCGGCUGUACAAGGAUGA 3′
EloC	Elongin C	5′ AAACCAAUGAGGUCAAUUU 3′

[a] FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

Results were normalized to those for β-actin (62). All qPCRs were performed in triplicate from at least three independent experiments.

ChIP-qPCR analysis. ChIP analysis with anti-H2B or anti-UbH2B was performed using 10^7 cells infected with HCMV (MOI = 0.3) for 60 h. Cells were fixed with 1% formaldehyde and lysed, and aliquots were stored as input controls. ChIP assays were performed as previously described (63), and immunoprecipitated DNA was analyzed by qPCR as described above. All ChIP-qPCRs were performed in triplicate from at least three independent experiments.

RNA interference. Synthetic siRNAs were purchased from Dharmacon. The sequences are listed in Table 1. HFs or Tert-HFs were transfected with siRNA (1 to 2 μg) by using the Nucleofector kit (VPI-1002; Amaxa Biosystems) and following the manufacturer's protocol. Transfected cells were cultured for 48 h and then infected with HCMV. Knockdown of endogenous elongin B, elongin C, or Skp1 was achieved using shRNAs stably expressed in Tert-HFs as previously described (39). Sequence 1 was used for the constitutive knockdown of elongin B.

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