Functional Roles of the Human Cytomegalovirus Essential IE86 Protein

M. F. Stinski(✉), D. T. Petrik

Contents

Abstract The IE86 protein of human cytomegalovirus (HCMV) is unique among viral and cellular proteins because it negatively autoregulates its own expression, activates the viral early and late promoters, and both activates and inhibits cellular promoters. It promotes cell cycle progression from Go/G1 to G1/S and arrests cell cycle progression at the G1/S interface or at G2/M. The IE86 protein is essential because it creates a cellular environment favorable for viral replication. The multiple functions of the IE86 protein during the replication of HCMV are reviewed.

Introduction

Human cytomegalovirus (HCMV) infection in utero is the leading infectious cause of birth defects that cause developmental disabilities. Infection of immunocompromised individuals can result in retinitis, pneumonitis, hepatitis, and gastroenteritis. There is no vaccine for HCMV, and the available antiviral therapies are fraught with

M.F. Stinski
Department of Microbiology, Interdisciplinary Program in Molecular Biology,
University of Iowa, Iowa City,
IA 52242, USA
mark-stinski@uiowa.edu

T.E. Shenk and M.F. Stinski (eds.), *Human Cytomegalovirus.*
Current Topics in Microbiology and Immunology 325.

limited efficacy and high rates of adverse affects. Investigations of the essential proteins for HCMV replication may lead to novel antiviral strategies. This chapter will focus on the immediate early two gene (IE2) of HCMV, which encodes the essential multifunctional IE protein designated IE86. The IE2 gene is in a region of the viral DNA referred to as the major immediate early (MIE) gene locus. Transcription from the MIE gene locus does not require de novo viral protein synthesis, and MIE viral transcripts are detected in the presence of an inhibitor of protein synthesis. The MIE gene locus consists of an enhancer-containing promoter upstream of the IE1 (UL123) and IE2 (UL122) genes. The transcriptional binding sites and functions of the MIE enhancer have been recently reviewed (Stinski 1999; Meier and Stinski 2006). The functions of the IE1 gene product are reviewed (see the chapter by G. Maul, this volume). This chapter will review our current understanding of the functions of the IE2 gene product.

The MIE enhancer-containing promoter generates a primary transcript that undergoes differential splicing and polyadenylation to produce multiple mRNA species (Fig. 1). The IE1 and IE2 mRNAs contain the first three exons in common, but IE1 contains exon 4, while IE2 contains exon 5 (Fig. 1). Translation initiates in exon 2; consequently the IE1 and IE2 gene products have the first 85 amino acids in common (Stenberg et al. 1984). Minor isoforms are produced from the IE1 and IE2 genes, as diagramed in Fig. 1. There is also a late promoter in exon 5 that is activated after viral DNA synthesis. Less is known about the functions of the isomers

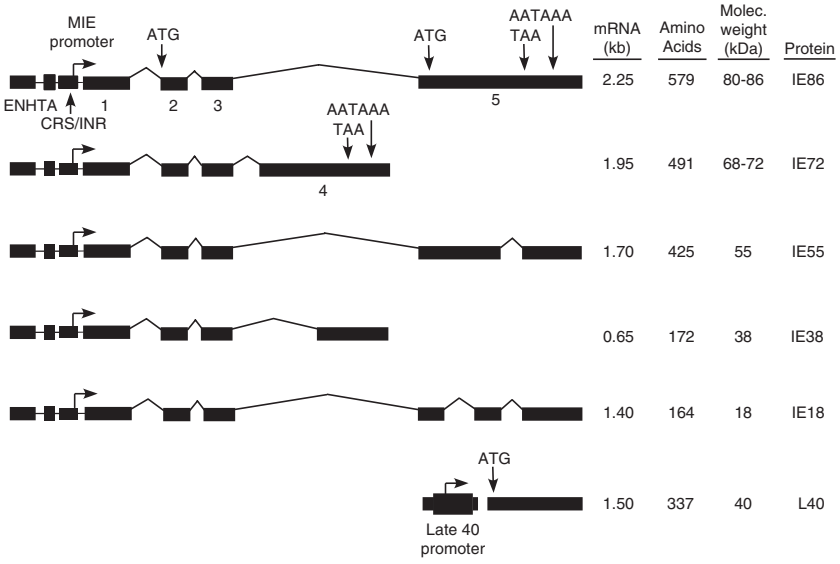

Fig. 1 The mRNAs and viral proteins encoded by the IE1 and IE2 genes of HCMV. Abbreviations: *ENH* enhancer, *TA* TATA box, *crs cis* repression sequence, *INR* initiator, *MIE* major immediate early, *ATG* start codon, *TAA* stop codon, *AATAAA* polyadenylation signal, *kb* kilobases, *kDa* kilodaltons, *IE* immediate early, *L* late

since they are lower in abundance and more difficult to study independently. Even though the isomers are dispensable, they are required for efficient early and late viral gene expression (White et al. 2007). Exon 5 encodes the unique amino acid sequence for the major IE2 gene product designated IE86 (Fig. 1). Recombinant viruses deleted in exon 5 cannot express early or late viral genes; consequently viral replication does not occur (Angulo et al. 2000; Marchini et al. 2001). Therefore, the IE86 protein is essential for viral replication. Both IE72 and IE86 are key regulatory proteins in the switch from latent to lytic infections. However, the molecular mechanisms that trigger and sustain CMV reactivation are related to cellular differentiation and are largely unknown.

After the viral DNA enters the nucleus, subsets of viral genomes are found at nuclear structures referred to as nuclear domain 10 (ND10) or promyelocytic leukemia protein oncogenic domains (PODs). IE72 and IE86 proteins localize at and adjacent to the ND10s, respectively (Ishov et al. 1997). Although IE86 does not directly affect ND10s, it has many other functions, which occur in the nucleus. The functions of the IE86 protein are negative autoregulation of the viral MIE promoter, transactivation of viral and cellular promoters, inactivation of cellular cytokine and chemokine promoters, and control of cell cycle progression. While the IE86 protein is necessary for viral DNA synthesis, initiation of DNA synthesis from oriLyt by an IE86/UL84 protein complex can occur with UL84 alone when a transactivator function in oriLyt is replaced with a constitutive promoter such as SV40 (Xu et al. 2004). The effect of UL84 on the IE86 and lytic viral DNA replication are reviewed (see the chapter by G.S. Pari, this volume). More importantly, IE86 prepares the cell for viral DNA synthesis by activating the expression of early viral genes and cellular genes. The IE86 protein of HCMV is unique among viral and cellular regulatory proteins because it both negatively and positively regulates viral and cellular promoters, and it promotes and arrests cell cycle progression. A better understanding of this essential viral protein may spawn novel strategies for preventing HCMV-induced disease.

Mapping the Functional Domains of the IE86 Protein

The functional domains of the IE86 protein have been studied by in vitro protein-protein binding assays, transient transfection assays, and the construction and isolation of recombinant viruses with either amino acid deletions or substitutions. The use of bacterial artificial chromosomes (BACs) containing the HCMV genome has been useful in confirming and extending the results of these early assays. These studies have indicated that the IE86 protein is a homodimer with critical functional domains located primarily toward the carboxyl end of the viral protein. Structural analysis of the IE86 protein by X-ray crystallography has not been done to date. In addition to tertiary structure of the IE86 protein, posttranslational modifications such as sumoylation and phosphorylation affect the biological activity of the viral protein. The IE86 protein of 579 amino acids is sumoylated at lysine residues 175

and 180 to give an apparent molecular weight of 105 kDa (Hofmann et al. 2000; Ahn et al. 2001). Mutation of lysine residues 175 and 180 negatively affects the efficiency of early viral promoter activation, but sumoylation is not required for viral growth (Lee and Ahn 2004). The IE86 protein is phosphorylated in vitro by the extracellular regulated kinase (ERK) and presumably affected in vivo by both the mitogen activated protein kinase (MAPK) and ERK signal transduction pathways in the infected cell (Harel and Alwine 1998; Heider et al. 2002). The IE86 protein is phosphorylated at multiple serine and threonine residues (Fig. 2a). Mutation of the threonine residues at positions 27 and 233 or the serine residues at positions 144 and 234 positively affects the efficiency of early viral promoter activation (Harel and Alwine 1998). Deletion of the serine residues between 258

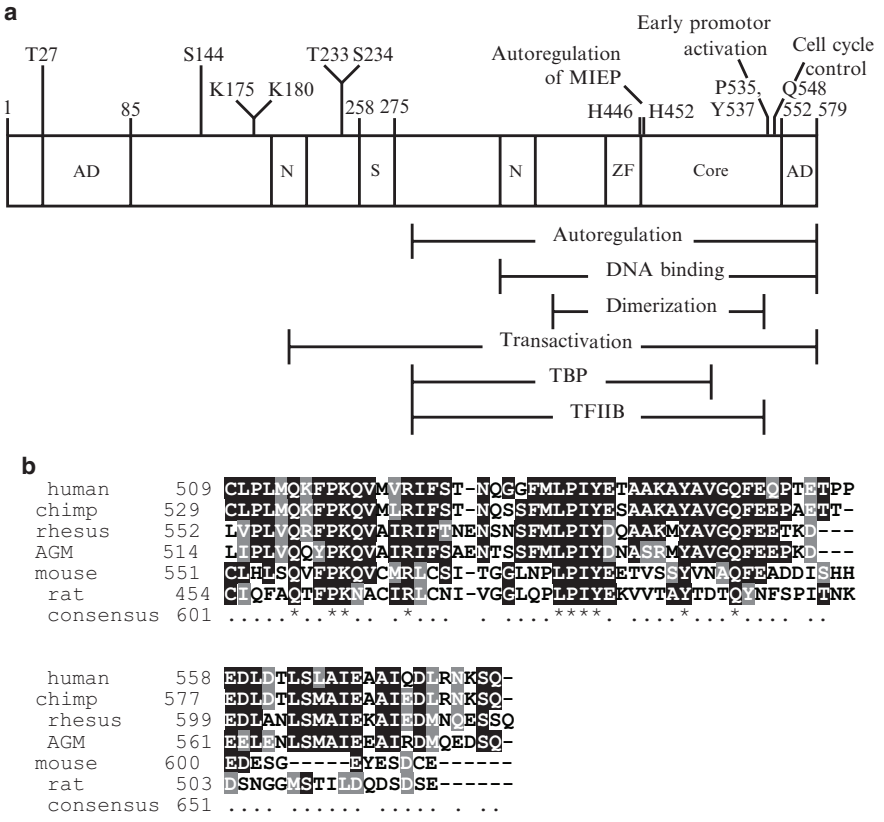

Fig. 2 Functional domains of the HCMV IE86 protein. **a** Abbreviations: *AD* activation domain, *S* serine domain, *K* lysine, *T* threonine, *H* histidine, *P* proline, *Y* tyrosine, *Q* glutamine, *N* nuclear localization signal, *ZF* putative zinc finger. **b** Amino acid sequence alignment of primate and nonprimate IE86 protein homologs. Identical amino acid residues are shaded in black and similar residues in gray. A *star* indicates a consensus sequence, and a *dot* a conserved sequence. A *hyphen* designates a gap in the sequence. MultAlin was used for the alignment of the IE86 residues for Towne strain (AAR31449), chimpanzee (NP612745), rhesus (AAB00488), African green monkey (AAB16881), mouse (AAA74505), and rat (AAB92266)

and 275 positively or negatively affects viral growth depending on the location of the residues (Barrasa et al. 2005) . Mutation of residues between 271 and 275 accelerates viral growth and mutation of residues between 258 and 264 or 266 and 269 delays viral growth (Table 1).

The IE86 protein has two nuclear localization signals (N) that can independently target the viral protein to the nucleus (Fig. 2a) (Pizzorno et al. 1991). In the nucleus, the viral protein affects viral promoters through two transcriptional activation domains, one amino and the other carboxyl (Fig. 2a) (Malone et al. 1990; Pizzorno et al. 1991; Yeung et al. 1993; Stenberg 1996). For transcriptional regulatory activity, the IE86 protein functions as a homodimer and dimerizes through the region broadly designated in Fig. 2a (Macias et al. 1996). Within this region, there is a putative zinc finger between amino acids 428 and 452, which may be part of a double zinc finger motif between amino acids 428 and 480 ($CX_5CX_{11}HX_5HXDXCX_{13}HXH$) (Fig. 2a). Double zinc finger motifs are important for interactions with other viral or cellular proteins such as transcription factors that either activate or repress transcription (Bachy et al. 2002; Moreno et al. 2003).

Figure 2a summarizes the regions of posttranslational modification and the broadly mapped functional domains of the IE86 protein. Multiple amino acid deletions were made to determine the functional domains of the IE86 protein and these mutations are summarized in Table 1. Large deletions resulted in nonreplicating genomes and confirmed that the IE86 protein is essential for HCMV replication. Smaller deletions affected the efficiency of either early or late viral gene expression. Frequently more than one viral protein function was affected by these deletions. Mutations that affected DNA binding and negative autoregulation produced high levels of the IE86 protein, which also affected cell viability. Mutations that failed to interact with TBP or TFIIB affected activation of early viral promoters. Mutations that affected phosphorylation of the viral protein affected the rate of viral growth. Delayed viral growth was associated with reduced expression of viral tegument proteins pp65 and pp28 (Sanchez et al. 2002). Many of the mutations made it difficult to assign a particular function to a specific region of the IE86 protein. The region between amino acids 450 and 552 was defined as a core domain because the IE86 regulatory functions of negative autoregulation of the MIE promoter, early promoter transactivation, and cell cycle arrest were all affected by any deletion within this region (Asmar et al. 2004).

Figure 2b shows regions of conserved amino acids in the carboxyl end of the viral protein between primate and nonprimate CMV homologs of the IE86 protein. The carboxy terminus is more conserved than the amino terminus. There are conserved stretches of amino acids suggesting critical structural and functional domains within the protein. Petrik et al. made rationally designed amino acid substitutions in the core region based on sequence conservation (Petrik et al. 2006, 2007); these results are also summarized in Table 1. These mutations separated the transactivation domain from the cell cycle arrest domain and the autoregulation domain from the transactivation domain (Fig. 2a). However, mutations in the putative zinc finger motif affected all functions of the

Table 1 Effect of amino acid deletions or substitutions on the functions of the IE86 protein

Effect on function	Amino acids	Assay	Reference
Dimerization	388–542	DNA binding	Ahn et al. 1998
	463–513		Macias et al. 1996
			Macias and Stinski 1993
Still dimerizes	551–579		Waheed et al. 1998
DNA binding	388–542		Ahn et al. 1998
	346–579		Chiou et al. 1993
	542–579		Jupp et al. 1993b
			Macias and Stinski 1993
Still binds DNA	551–579		Macias et al. 1996
Autoregulation of MIEP	427–435	Reporter	Chiou et al. 1993
	505–511		Hermiston et al. 1990
	H446/H452		Macias and Stinski 1993
			Macias et al. 1996
		Virus	Petrik et al. 2007
Interaction with TBP	290–504	Protein	Caswell et al. 1993
	266–269	Binding	Hagemeier et al. 1992a
	271–275		Jupp et al. 1993a
Interaction with TFIIB	290–542		Caswell et al. 1993
Interaction with CREB	290–410		Lang et al. 1995
Transactivation of promoters	1–98	Reporter	Malone et al. 1990
	25–85		Pizzorno et al. 1991
	169–194		Stenberg et al. 1989
	175–180		Stenberg 1996
	195–579		Yeung et al. 1993
	501–511		
	544–579		
Early gene expression	258–275	Virus	Heider et al. 2002
	266–275		
	E550, E554, E558, D559, D561, E568, D573	Reporter	Yeung et al. 1993
	P535,Y537	Virus	Petrik et al. 2007
Late gene expression	136–290	Virus	Sanchez et al. 2002
	356–359		White et al. 2004
No viral growth	501–511		White et al. 2004
Delayed viral growth	258–275		Barrasa et al. 2005
	266–275		
Accelerated viral growth	271–275	Virus	Barrasa et al. 2005
Interaction with Rb	290–390	Protein Binding	Sommer et al. 1994
Interaction with p53	325–448		Zhang et al. 2006
Interaction with mdm2	326–449		Zhang et al. 2006
Cell cycle arrest	260–279	Reporter	Wiebusch and Hagemeier 1999
	451–579		
	Q548	Virus	Petrik et al. 2006

IE86 protein because this region is necessary for dimerization of the IE86 protein in vivo. The various functions of the IE86 protein and their role in the replication of HCMV are reviewed.

Autoregulation of the MIE Promoter

Negative autoregulation of the MIE promoter by the IE86 protein is almost certainly important to the replication of the virus because recombinant viruses that fail to autoregulate can not be isolated (H. Isomura and M.F. Stinski, unpublished data). The IE86 protein mutated at histidine residues 446 and 452 fails to negatively autoregulate the MIE promoter in in vitro transcription reactions (Macias and Stinski 1993), transient transfection assays (Macias et al. 1996), and recombinant BACs (Petrik et al. 2007). The MIE promoter region of HCMV has a *cis*-acting element that serves as a binding site for the IE86 protein or the late L40 protein (see Fig. 1). Point mutations in histidine residues 446 and 452 abolish DNA binding (Macias and Stinski 1993; Macias et al. 1996). Dimerization of the IE86 protein is required for viral DNA binding (Table 1) (Macias et al. 1996; Waheed et al. 1998). The viral DNA element is located immediately upstream of the transcription start site for IE1/IE2 RNA and is referred to as the *cis*-repression sequence (crs). The crs consists of a pair of dinucleotide CG separated by an A/T-rich region of 10 nucleotides. The CG dinucleotides are critical and the spacing is important for binding by the IE86 protein (Waheed et al. 1998). The crs functions between the TATA box and the transcription start site in either orientation. However, it does not function downstream of the transcription initiation site (+1) (Pizzorno and Hayward 1990; Cherrington et al. 1991; Liu et al. 1991). The IE86 protein binds to the minor groove between −14 and −1 without inhibiting the binding of TBP to the upstream TATA box (Jupp et al. 1993b; Lang and Stamminger 1994).

The first step in negative autoregulation of the MIE promoter by IE86 is blockage of RNA polymerase II occupancy at the transcription start site (Wu et al. 1993; Lee et al. 1996). There is also an initiator-like element between +1 and +7, and cellular protein binding to this element is also affected by the IE86 protein, which in turn negatively affects transcription from the MIE promoter (Macias et al. 1996). The ie3 gene product of murine CMV also negatively autoregulates its MIE promoter (Messerle et al. 1992). Repression of the HCMV MIE promoter is detectable approximately between 6 and 8 h after high multiplicity of infection when the IE86 protein reaches levels to compete for binding to the transcription initiation region (Stamminger et al. 1991; Meier and Stinski 1997).

The second stage of negative autoregulation by the IE86 protein, occurring 24 h postinfection, requires histone deacetylase (HDAC1), which causes deacetylation of histones at the MIE promoter (Reeves et al. 2006). Deacetylated histones are targets for methylation by histone methyltransferases, G9a and Suvar(3-9)H1. Since mutation of the crs abrogates the association of repressive chromatin to the

MIE promoter at late times, it was proposed that IE86 mediated the changes in the chromatin structure (Reeves et al. 2006). During latency, the HCMV MIE promoter is also associated with repressive chromatin (Reeves et al. 2005). The chromatin associated with the MIE promoter during latency and reactivation are reviewed (see the chapter by M. Reeves and J. Sinclair, this volume).

Transcription from Viral and Cellular Promoters

Viral Promoters

A strong heterochromatin structure forms quickly on the HCMV genome in nonpermissive, undifferentiated cells (Reeves et al. 2005; Ioudinkova et al. 2006; Yee et al. 2007). The transcriptional repressors may cause a closed viral chromatin structure (see the chapter by M. Reeves and J. Sinclair, this volume). The MIE promoter is repressed by heterochromatin, and consequently there is no expression of the MIE proteins. The IE86 protein is a master regulator of HCMV transcription required for early viral gene expression. The IE72 protein augments the activity of the IE86 protein by inhibiting histone deacetylase activity (Tang and Maul 2003; Nevels et al. 2004). Early viral gene expression from the viral genome in a latently infected cell, such as HCMV-infected undifferentiated THP-1 cells or murine CMV-infected mice, requires the expression of both of the MIE proteins (Kurz et al. 1999; Kurz and Reddehase 1999; Yee et al. 2007). Nevertheless, this is not sufficient to reactivate virus replication without cellular differentiation. Cellular factors induced or reduced by cellular differentiation appear to be critical for viral DNA replication and production of infectious virus (Murphy et al. 2002; Reeves et al. 2005). Therefore, sporadic expression of the MIE genes may occur, but it is not always sufficient to signal viral reactivation and replication.

The IE86 protein transactivates early viral promoters by interacting with cellular basal transcription machinery and requires a TATA box-containing promoter to transactivate downstream transcription (Lukac et al. 1994). Figure 2a shows the broad regions of the IE86 protein that interact with the basal transcription machinery and Table 1 summarizes the number of mutations in this region. Truncated forms of the IE86 protein interact in vitro with TBP, TFIIB, and TAFII130/TAF4 and the IE86 protein rescues defective TAFII250 (Caswell et al. 1993; Lukac et al. 1994, 1997). Other cellular transcription factors and chromatin remodeling proteins also interact and contribute to the activity of the IE86 protein such as CREB, SP1, Tef-1, Egr-1, p300/CBP, and P/CAF (Lukac et al. 1994; Sommer et al. 1994; Lang et al. 1995; Scully et al. 1995; Schwartz et al. 1996; Yoo et al. 1996; Bryant et al. 2000). Chromatin immunoprecipitation (ChIP) assays demonstrated that TBP is associated with HCMV early promoters, but activation of the viral promoter requires the presence of functional IE86 protein (Petrik et al. 2007). IE86 protein attracts histone acetylases, which are key to activating the early viral promoters (Bryant et al. 2000).

More acetylated chromatin was found on early viral promoters in nonpermissive undifferentiated THP-1 cells when both the IE72 and IE86 proteins were expressed in *trans* prior to infection (Yee et al. 2007).

As shown in Fig. 2a, there is a core domain between amino acids 450 and 552 and when mutated all functions of the IE86 protein are affected (Asmar et al. 2004). The longest stretch of conserved amino acids within the core domain (LPIYE) is shown in Fig. 2b. Mutation of the proline (P) and tyrosine (Y) residues within this conserved stretch of amino acids affected transactivation of early viral promoters without affecting autoregulation of the MIE promoter (Petrik et al. 2007). Mutation of P535 and Y537 to alanines results in a nonreplicating BAC (Table 1). The mutant IE86 protein is found associated with the MIE promoter in ChIP assays, but fails to transactivate early viral genes and is not found associated with viral early promoters in a ChIP assay (Petrik et al. 2007). Therefore, recruitment to the MIE promoter and early viral promoters by the IE86 protein occur through independent mechanisms. The LPIYE conserved sequence may be important for protein-protein interactions. This conserved sequence in the IE86 protein is also found in other cellular proteins that regulate events on a DNA template such as histone methyltransferase, DNA topisomerase III, and CHROM2. If the LPIYE sequence is mutated in the critical proline and tyrosine residues, there is no activation of the viral early promoters. In contrast, a mutation in the noncritical leucine (L) residue has little to no effect (Petrik et al. 2007). An activation domain of IE86 is carboxyl to the core domain, and it is also necessary to activate transcription of a viral early promoter (Pizzorno et al. 1991; Yeung et al. 1993). This region is rich in acidic amino acids. Mutation of the glutamic and aspartic acids between residues 550 and 573 to valines inactivates the activation domain (Yeung et al. 1993).

E2F Promoters

After HCMV infection, gene microarray assays demonstrated a fourfold or greater increase of at least 124 cellular mRNAs (Zhu et al. 1998). While the IE86 protein can activate cellular promoters through the same mechanisms for viral promoters discussed above (Hagemeier et al. 1992b), it can also activate cellular promoters by inhibiting a repressor of transcription. The expression of genes by the transcription factor E2F is repressed by the cellular tumor suppressor protein pRb. IE86 binds to pRb in vitro and IE86 protein-Rb complexes can be immunoprecipitated from HCMV-infected cells (Hagemeier et al. 1994; Sommer et al. 1994; Fortunato et al. 1997). In the HCMV-infected cell, Rb is converted from the hypophosphorylated to hyperphosphorylated form. An analysis of gene expression using microarrays of cellular genes indicated that HCMV or the IE86 protein strongly activated E2F response genes (Song and Stinski 2002). These are the cellular genes that regulate the enzymes for DNA precursor synthesis, the initiation factors of cellular DNA synthesis, and the movement of the cell cycle. They are expressed prior to the S phase and they include the following: DNA precursor enzymes such as

ribonucleotide reductase, thymidylate synthetase, and dihydrofolate reductase, DNA initiation factors such as MCM3 and MCM7, the enzyme DNA polymerase alpha, and the cell cycle control proteins such as cyclin E, cdk-2, and E2F (Salvant et al. 1998; Gribaudo et al. 2000; Song and Stinski 2002). Since HCMV infects terminally differentiated cells of the host that are in the Go phase of the cell cycle, the virus requires a mechanism to overcome cellular quiescence. The IE86 protein-along with the IE72 protein and the viral tegument protein pp71-induce cell cycle progression by inactivating the cellular Rb family of repressor proteins.

NFκ-B Promoters

After HCMV infection, gene microarray assays also demonstrated a fourfold or greater decrease of at least 134 cellular mRNAs (Zhu et al. 1998). Repression of some cellular genes would favor viral replication. In cells infected with an IE2-86 mutant virus lacking amino acids 136-290 (referred to as delta SX) (Sanchez et al. 2002), HMGA2 expression is not significantly reduced, suggesting that the IE86 protein is involved in the regulation of the HMGA2 expression (see the chapter by V. Sanchez and D.H. Spector, this volume). Repression of select cytokine and chemokine expression would favor survival of the virus-infected cell. HCMV infection attenuates interleukin (IL)-1 beta and tumor necrosis factor (TNF)-alpha proinflammatory signaling (Jarvis et al. 2006; Montag et al. 2006). The IE86 protein inhibits the induction of interferon (IFN)-beta, TNF-alpha, RANTES, MIG, MCP-2, and IL-6 (Taylor and Bresnahan 2005, 2006). The effect of IE86 is downstream of the inhibitor of kappa B (IκB) kinase. While IE86 directly effects the binding of NFκ-B to its cognate site on the DNA by an unknown mechanism (Taylor and Bresnahan 2006), IE86 also blocks the activity of NFκ-B when the cellular transcription factor is brought to the promoter via a GAL4 DNA-binding domain (Gealy et al. 2007). Therefore, the inhibition of NFκ-B by IE86 is also subsequent to NFκ-B binding. The regions required to inhibit NFκ-B activity are amino terminal of the IE86 carboxyl activation domain (Gealy et al. 2007). The exact region of the IE86 protein involved in blocking NFκ-B activity requires further investigation.

Cell Cycle Progression

CMVs typically infect terminally differentiated cells in the Go/G1 phase of the cell cycle. The pool of dideoxynucleotide triphosphates and biosynthetic enzymes to make precursors for DNA synthesis are low in these cells. Since CMVs do not encode many of the biosynthetic enzymes for DNA precursor synthesis, the virus must have a mechanism to overcome G1 arrest. The effect of HCMV infection on the cell cycle is reviewed (see the chapter by V. Sanchez and D.H. Spector, this volume). As discussed above, the IE86 protein binds to pRb and activates E2F

responsive promoters. Cyclin E/cdk1 expression and activity are increased by HCMV or by the IE86 protein, which causes a feed-forward loop allowing amplification of signals that promote cell cycle progression from the G1 to S phase (Jault et al. 1995; Salvant et al. 1998; McElroy et al. 2000; Sinclair et al. 2000). In a normal cell, p53 induces cell cycle arrest, possibly to allow for DNA repair (Kastan et al. 1992; Kuerbitz et al. 1992) or apoptosis to eliminate cells with damaged genomes (Kuerbitz et al. 1992; Levine 1997; Prives and Hall 1999; Vogelstein et al. 2000). In a $p53^{+/+}$ cell infected with a high multiplicity of HCMV, most of the cells are blocked at G_1/S (Jault et al. 1995; Bresnahan et al. 1996; Lu and Shenk 1996; Morin et al. 1996; Dittmer and Mocarski 1997; Wiebusch and Hagemeier 1999; Casavant et al. 2006). In a p53 mutant cell infected with HCMV, viral replication is delayed and infectious virus production is decreased compared to $p53^{+/+}$ cells (Murphy et al. 2000; Casavant et al. 2006). Wild type p53 expressed in *trans* restores replication efficiency. Although p53 is not an essential cellular protein for HCMV replication, a functional p53 contributes significantly to the progression of infection.

In a $p53^{+/+}$ cell, the IE86 protein binds to p53 and the level of p53 increases in the nucleus (Speir et al. 1994; Bonin and McDougall 1997; Fortunato and Spector 1998). p53 is stabilized by the IE86 protein in an atazia telangiectasis mutated (ATM) kinase-positive cell but not in a ATM kinase-negative cell (Song and Stinski 2004). ATM kinase phosphorylates p53 at serine residue 15, which stabilizes the cellular protein by preventing Mdm2 ubiquitination (Song and Stinski 2004). Mdm2 is a p53-specific E3 ubiquitin ligase that promotes the degradation of p53 (Haupt et al. 1997; Honda et al. 1997; Fang et al. 2000). Mdm2 forms an autoregulatory feedback loop with p53 and allows p53 to control its own level and activity by inducing the expression of Mdm2 (Fang et al. 2000). The IE86 protein interrupts this cellular regulatory control by binding to Mdm2 and facilitating its degradation (Zhang et al. 2006). Therefore, the IE86 protein decreases the level of Mdm2, which increases the level of p53 in the HCMV-infected cell. In addition, phosphorylation at serine residues 15 and 20 of p53 hampers the Mdm2-p53 interaction, which prevents ubiquitination and degradation (Shieh et al. 1997; Dumaz et al. 2001; Louria-Hayon et al. 2003).

There is also a correlation between high levels of IE86 and increased levels of p21 (Shen et al. 2004; Song and Stinski 2004). The early increase in p53 and p21 in the HCMV-infected cell may be related to an early intrinsic cellular defense to virus replication (Garcia et al. 1997). Both IE72 and IE86 induce p21 in a p53-dependent manner (Song and Stinski 2004; Castillo et al. 2005). The p21 promoter is activated by p53, but how IE72 or IE86 proteins increase p21 is currently not understood. The IE86/p53 complex can still bind to the p53 cognate site on DNA (Tsai et al. 1996), but it is uncertain whether IE86 or p53 activates the p21 promoter. Alternatively, p21 could be activated prior to sequestration of p53 in the nucleus with IE86. p21 can inhibit cdk2 and block the activity of cyclin E, arresting the cell cycle progression, but this does not occur in the HCMV-infected cell. It has been reported that IE86 can bind p21 and thereby prevent p21 repression of cyclinE-dependent kinase activity (Sinclair et al. 2000). However, the cyclin E/cdk2 levels increase in both the HCMV-infected

cell and the IE86 protein expressing cell and consequently, the virus primes the cell for DNA synthesis (Song and Stinski 2002). In contrast, cyclin A decreases because IE86 protein induces degradation of Mdm2, and Mdm2 normally induces cyclin A expression (Zhang et al. 2006).

The IE86 protein upregulates p21 in a $p53^{+/+}$ cell and not in a $p53^{-/-}$ cell (Song and Stinski 2004). However, p21 is upregulated in a p53-null cell by expressing p53 and IE86 in *trans* (Song and Stinski 2004). In the HCMV-infected cell, the increase in p21 is transient and then p21 levels decrease (Chen et al. 2001). The decrease in p21 may be related to the senescent state of a HCMV-infected cells since senescence causes a decrease in p21 (Stein et al. 1999; Chen et al. 2004). Senescence also occurs in an IE86-expressing HF cell (Noris et al. 2002).

HCMV inhibits cell cycle progression in a $p53^{+/+}$ cell to utilize the cellular DNA precursors for its own DNA synthesis. In a $p53^{+/+}$ cell, IE86 protein activates a quasi G1/S program for cellular gene expression, inhibits cellular DNA synthesis, and consequently co-opts valuable enzymes and macromolecular precursors for viral DNA replication. The inhibition of cellular DNA synthesis appears to be dependent on high multiplicity of infection and on a functional p53 because inhibition does not occur in a p53-null cell unless wild type p53 is introduced in *trans*. Therefore the IE86 protein initially stimulates the cell and then blocks cell cycle progression. The IE86 protein may induce p53 in the permissive HF cell by deregulating E2F activity, activating ATM kinase activity, degrading Mdm2 and consequently stabilizing p53.

The viral IE86 protein and the cellular p53 protein play major roles in control of the cell cycle during HCMV infection. The IE86 protein promotes cell cycle progression in the G_o/G_1 phase. In the presence of the cellular p53 protein, the IE86 protein stops the cell cycle before the S phase. If cells progress into the S phase, then the IE86 protein stops cell cycle progression at the G2/M phase even in the absence of cellular p53 protein (Song and Stinski 2004). The IE86 protein is necessary for cell cycle progression because at least four times more serum-starved p53 mutant glioblastoma U373 or 293T cells are induced into the S phase by wild type IE86 protein compared to a mutant IE86 protein (Murphy et al. 2000). While cell division occurs in the presence of the mutant IE86 protein, it does not occur in the presence of the wild type IE86 protein. In the p53-null Saos-2 cell, wild type IE86 protein blocks cell cycle progression at the G_2/M phase, which correlates with an aberrant increase in cyclin B and cdk1 levels (Song and Stinski 2004). Cells in the S and G_2/M phases suppress transcription of the HCMV MIE genes by an unknown mechanism. When the cell cycle re-enters G_o/G_1, transcription of the MIE genes resumes (Fortunato et al. 2002).

This is important for the replication of the virus because a single amino acid substitution of IE86 at residue 548 from a glutamine to an arginine (Q548R) (Fig. 2b) inactivates the ability of IE86 to arrest the cell cycle at either G1/S or G2/M (Petrik et al. 2006). Under these conditions, the recombinant virus replicates viral DNA slowly, fails to inhibit cellular DNA synthesis or cellular division, and exhibits smaller, nondistinct plaques. The Q548R recombinant virus negatively autoregulates the MIE promoter, activates early viral promoters and cellular

E2F-responsive promoters, but fails to regulate cell cycle progression (Petrik et al. 2006). The virus replicates slowly because viral DNA synthesis is in competition with cellular DNA synthesis. Therefore, arrest of cell cycle progression by the IE86 protein and prevention of cellular DNA synthesis are critical steps for efficient HCMV replication.

The mutation of glutamine at residue 548 to arginine in the Q548R recombinant virus is unique because the IE86 protein fails to regulate the cell cycle at both the G1/S and G2/M phases (Petrik et al. 2006). In stable cell lines, the Q548R mutant IE86 protein complexes with p53 and the cells continue to divide (Bonin and McDougall 1997; Murphy et al. 2000). While the Q548R mutant IE86 protein stimulates cellular E2F gene expression and unscheduled entry into the S phase, it cannot transform cells. The mutant viral protein may not be able to overcome contact inhibition or other cellular processes necessary for cellular transformation. It is likely that the Q548R mutant IE86 protein fails to bind and degrade Mdm2, allowing both cellular DNA synthesis and normal progression into G2/M. The introduction of a positively charged arginine at residue 548 may inhibit IE86 protein interaction with a cellular protein. In contrast, substitution of residue 548 with a neutral alanine residue had little to no effect (Petrik et al. 2006). In addition, the Q548R mutant viral protein may also fail to degrade the anaphase promoting complex/cyclosome (APC/C), which is an E3 ubiquitin ligase that targets regulators of cell division for degradation by the proteasome (Peters 2006). APC/C causes lower levels of cyclin B/cdk1, and cyclin B degradation is necessary to release the cell cycle from G_2/M back into G_1 (Peters 2006). HCMV infection inactivates APC/C and the IE86 protein may be the cause of this inactivation (Wiebusch et al. 2005).

The UL69 gene product is also reported to arrest the cell cycle when overexpressed from a viral vector (Hayashi et al. 2000). Our results with the Q548R recombinant virus indicate that the cell cycle is not arrested when UL69 is present. These results may suggest that both UL69 and a functional IE86 protein are required to efficiently arrest the cell cycle during viral infection. The IE86 protein of HCMV is different from DNA tumor virus early regulatory proteins because it also stops cycle progression at the G1/S interface in $p53^{+/+}$ cells or at G_2/M in p53-null cells. Nevertheless, apoptosis is prevented in the HCMV-infected cell by the viral IE proteins expressed from the UL36, UL37X1, and UL38 genes (see the chapter by A.L. McCormick, this volume). In addition, the IE86 protein itself is reported to inhibit apoptosis under special circumstances (Zhu et al. 1995).

Perspectives

The IE86 protein of HCMV is an essential viral protein that does the following during viral replication:

1. It negatively autoregulates the MIE enhancer-containing promoter, which controls the expression of IE72 and IE86 proteins.

2. It transactivates transcription from viral early and late promoters.
3. It inactivates the cellular Rb repressor, which activates E2F responsive promoters to move the cell cycle from the G_o/G_1 to the G_1/S interface.
4. It prevents expression of cellular cytokines and chemokines expression with antiviral properties.
5. It controls both the G_1/S and G_2/M transition points in the cell cycle.

The regions of the viral protein involved in these functions were broadly mapped, and consequently more than one function was usually affected by the mutation. A core region was identified between amino acid residues 450 and 552 in which mutations in this region affected all identified functions of the IE86 protein. Site-specific mutations in this region demonstrated that the functions of the IE86 protein can be separated and more clearly defined. There are other functional domains and conserved regions within the IE86 protein that need to be defined. The crystal structure of the IE86 protein should be demonstrated to better understand the functional domains. The tertiary structure of the IE86 protein appears critical for function. For example, the histidine residues at 446 and 452 are critical and possibly associated with a double zinc finger domain that determines a three-dimensional structure for the interaction with a variety of cellular and viral proteins. Mutation of the histidine residues at 446 and 452 affects all IE86 protein functions in the context of the viral genome. The proline and tyrosine residues 535 and 537, respectively, which are a part of a conserved five-amino acid stretch in the core domain, affect transcription of early viral promoters. The acidic amino acids between residues 550 and 573 affect early viral promoter activation. The conserved glutamine residue 548 affects control of cell cycle progression. These critical amino acids of the IE86 protein are potential targets for new and unique antivirals. Since the current antivirals for the treatment of HCMV infection are of limited efficacy with high frequencies of adverse effects, it is important to understand the tertiary structure of the carboxyl domain of the IE86 protein to develop new treatments for HCMV infection.

Acknowledgements We thank members of the Stinski lab and Jeffery Meier for critical review of this manuscript. Our work was supported by grant AI-13562 from the National Institutes of Health.

References

Ahn J-H, Chiou C-J, Hayward GS (1998) Evaluation and mapping of the DNA binding and oligomerization domains of the IE2 regulatory protein of human cytomegalovirus using yeast one and two hybrid interaction assays. Gene 210:25-36

Ahn J-H, Xu Y, Jang W-J, Matunis MJ, Hayward GS (2001) Evaluation of interactions of human cytomegalovirus immediate-early IE2 regulatory protein with small ubiquitin-like modifiers and their conjugation enzyme Ubc9. J Virol 75:3859-3872

Angulo A, Ghazal P, Messerle M (2000) The major immediate-early gene ie3 of mouse cytomegalovirus is essential for viral growth. J Virol 74:11129-11136

Asmar J, Wiebusch L, Truss M, Hagemeier C (2004) The putative zinc-finger of the human cytomegalovirus IE286-kilodalton protein is dispensable for DNA-binding and autorepression thereby demarcating a concise core domain in the C-terminus of the protein. J Virol 78:11853-11864

Bachy I, Failli V, Retaux S (2002) A LIM-homeodomain code for development and evolution of forebrain connectivity. Neuroreport 13:A23-A27

Barrasa MI, Harel NY, Alwine JC (2005) The phosphorylation status of the serine-rich region of the human cytomegalovirus 86-kilodalton major immediate-early protein IE2/IEP86 affects temporal viral gene expression. J Virol 79:1428-1437

Bonin LR, McDougall JK (1997) Human cytomegalovirus IE2 86-kilodalton protein binds p53 but does not abrogate G1 checkpoint function. J Virol 71:5861-5870

Bresnahan WA, Boldogh I, Thompson EA, Albrecht T (1996) Human cytomegalovirus inhibits cellular DNA synthesis and arrests productively infected cells in late G1. Virology 224:150-160

Bryant LA, Mixon P, Davidson M, Bannister AJ, Kouzarides T, Sinclair JH (2000) The human cytomegalovirus 86-kilodalton major immediate-early protein interacts physically and functionally with histone acetyltransferase P/CAF. J Virol 74:7230-7237

Casavant NC, Luo MH, Rosenke K, Winegardner T, Zurawska A, Fortunato EA (2006) Potential role for p53 in the permissive life cycle of human cytomegalovirus. J Virol 80:8390-8401

Castillo JP, Frame FM, Rogoff HA, Pickering MT, Yurochko AD, Kowalik TF (2005) Human cytomegalovirus IE1-72 activates ataxia telangiectasia mutated kinase and a p53/p21-mediated growth arrest response. J Virol 79:11467-11475

Caswell R, Hagemeier C, Chiou C-J, Hayward G, Kouzarides T, Sinclair J (1993) The human cytomegalovirus 86K immediate early (IE2) protein requires the basic region of the TATA-box binding protein (TBP) for binding, and interacts with TBP and transcription factor TFIIB via regions of IE2 required for transcriptional regulation. J Gen Virol 74:2691-2698

Chen J-H, Stoeber K, Kingsbury S, Ozanne SE, Williams GH, Hales CN (2004) Loss of proliferative capacity and induction of senescence in oxidatively stressed human fibroblasts. J Biol Chem 279:49439-49446

Chen Z, Knutson E, Kurosky A, Albrecht T (2001) Degradation of p21cip1 in cells productively infected with human cytomegalovirus. J Virol 75:3613-3625

Cherrington JM, Khoury EL, Mocarski ES (1991) Human cytomegalovirus IE2 negatively regulates a gene expression via a short target sequence near the transcription start site. J Virol 65:887-896

Chiou C-J, Zong J, Waheed I, Hayward GS (1993) Identification and mapping of dimerization and DNA-binding domains in the C terminus of the IE2 regulatory protein of human cytomegalovirus. J Virol 67:6201-6214

Dittmer D, Mocarski ES (1997) Human cytomegalovirus infection inhibits G1/S transition. J Virol 71:1629-1634

Dumaz N, Milne DM, Jardine LJ, Meek DW (2001) Critical roles for the serine 20, but not the serine 15, phosphorylation site and for the polyproline domain in regulating p53 turnover. Biochem J 359:459-464

Fang S, Jensen JP, Ludwig RL, Vousden KH, Weissman AM (2000) Mdm2 is a RNIG finger-dependent ubiquitin protein ligase for itself and p53. J Biol Chem 275:8945-8951

Fortunato EA, Sommer MH, Yoder K, Spector DH (1997) Identification of domains within the human cytomegalovirus major immediate-early 86-kilodalton protein and the retinoblastoma protein required for physical and functional interaction with each other. J Virol 71:8176-8185

Fortunato E, Sanchez V, Yen JY, Spector DH (2002) Infection of cells with human cytomegalovirus during S phase results in a blockade to immediate-early gene expression that can be overcome by inhibition of the proteasome. J Virol 76:5369-5379

Fortunato EA, Spector DH (1998) p53 and RPA are sequestered in viral replication centers in the nuclei of cells infected with human cytomegalovirus. J Virol 72:2033-2039

Garcia JF, Piris MA, Lloret E, Orradre JL, Murillo PG, Martinez JC (1997) p53 expression in CMV-infected cells: association with the alternative expression of the p53 transactivated genes p21/WAF1 and MDM2. Histopathology 30:120-125

Gealy C, Humphreys C, Stinski MF, Caswell R (2007) An activation-defective mutant of the HCMV IEp86 protein inhibits NF-kappa B-mediated stimulation of the human IL-6 promoter. J Gen Virol 88:2435-2440

Gribaudo F, Riera L, Lembo D, De Andrea M, Gariglio M, Rudge TL, Johnson LF, Landolfo S (2000) Murine cytomegalovirus stimulates cellular thymidylate synthase gene expression in quiescent cells and requires the enzyme for replication. J Virol 74:4979-4987

Hagemeier C, Walker S, Caswell R, Kouzarides T, Sinclair J (1992a) The human cytomegalovirus 80-kilodalton but not the 72-kilodalton immediate-early protein transactivates heterologous promoters in a TATA box-dependent mechanism and interacts directly with TFIID. J Virol 66:4452-4456

Hagemeier C, Walker SM, Sissons PJ, Sinclair JH (1992b) The 72K IE1 and 80K IE2 proteins of human cytomegalovirus independently trans-activate the c-fos, c-myc and hsp70 promoters via basal promoter elements. J Gen Virol 73:2385-2393

Hagemeier C, Caswell R, Hayhurst G, Sinclair J, Kouzarides T (1994) Functional interaction between the HCMV IE2 transactivator and the retinoblastoma protein. EMBO J 13:2897-2903

Harel NY, Alwine JC (1998) Phosphorylation of the human cytomegalovirus 86-kilodalton immediate-early protein IE2. J Virol 72:5481-5492

Haupt Y, Maya R, Kazaz A, Oren M (1997) Mdm2 promotes the rapid degradation of p53. Nature 387:296-299

Hayashi ML, Blankenship C, Shenk T (2000) Human cytomegalovirus UL69 protein is required for efficient accumulation of infected cells in the G1 phase of the cell cycle. Proc Natl Acad Sci USA 97:2692-2696

Heider JA, Bresnahan WA, Shenk TE (2002) Construction of a rationally designed human cytomegalovirus variant encoding a temperature-sensitive immediate-early 2 protein. Proc Natl Acad Sci USA 99:3141-3146

Hermiston TW, Malone CL, Stinski MF (1990) Human cytomegalovirus immediate-early two protein region involved in negative regulation of the major immediate-early promoter. J Virol 64:3532-3536

Hofmann H, Floss S, Stamminger T (2000) Covalent modification of the transactivator protein IE2-p86 of human cytomegalovirus by conjugation to the ubiquitin-homologous proteins SUMO-1 and hSMT3b. J Virol 74:2510-2524

Honda R, Tanaka H, Yasuda H (1997) Oncoprotein MDM 2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett 420:25-27

Ioudinkova E, Arcangeletti MC, Rynditch A, De Conto F, Motta F, Covan S, Pinardi F, Razin SV, Chezzi C (2006) Control of human cytomegalovirus gene expression by differential histone modifications during lytic and latent infection of a monocytic cell line. Gene 384:120-128

Ishov AM, Stenberg RM, Maul GG (1997) Human cytomegalovirus immediate early interaction with host nuclear structures: definition of an immediate transcript environment. J Cell Biol 138:5-16

Jarvis MA, Borton JA, Keech AM, Wong J, Britt WJ, Magun BE, Nelson JA (2006) Human cytomegalovirus attenuates interleukin-1beta and tumor necrosis factor alpha proinflammatory signaling by inhibition of NF-kappaB activation. J Virol 80:5588-5598

Jault FM, Jault J-M, Ruchti F, Fortunato EA, Clark C, Corbeil J, Richman D, Spector DH (1995) Cytomegalovirus infection induces high levels of cyclins, phosphorylated RB, and p53, leading to cell cycle arrest. J Virol 69:6697-6704

Jupp R, Flores O, Nelson JA, Ghazal P (1993a) The DNA-binding subunit of human transcription factor IID can interact with the TATA box as a multimer. J Biol Chem 268:16105-16108

Jupp R, Hoffmann S, Depto A, Stenberg RM, Ghazal P, Nelson J (1993b) Direct interaction of the human cytomegalvirus IE86 protein with the cis repression signal does not preclude TBP from binding to the TATA box. J Virol 67:5595-5604

Kastan MB, Zhan Q, el-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJ Jr (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71:587-597

Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB (1992) Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 89:7491-7495

Kurz SK, Reddehase MJ (1999) Patchwork pattern of transcriptional reactivation in the lungs indicates sequential checkpoints in the transition from murine cytomegalovirus latency to recurrence. J Virol 73:8612-8622

Kurz SK, Rapp M, Steffens H-P, Grzimek NKA, Schmalz S, Reddehase MJ (1999) Focal transcriptional activity of murine cytomegalovirus during latency in the lungs. J Virol 73:482-494

Lang D, Stamminger T (1994) Minor groove contacts are essential for an interaction of the human cytomegalovirus IE2 protein with its DNA target. Nucleic Acids Res 22:3331-3338

Lang D, Gebert S, Arlt H, Stamminger T (1995) Functional interaction between the human cytomegalovirus 86-kilodalton IE2 protein and the cellular transcription factor CREB. J Virol 69:6030-6037

Lee G, Wu J, Luu P, Ghazal P, Flores O (1996) Inhibition of the association of RNA polymerase II with the preinitiation complex by a viral transcriptional repressor. Proc Natl Acad Sci USA 93:2570-2575

Lee H, Ahn J (2004) The sumoylation of HCMV (Towne) IE2 is not required for viral growth in cultured human fibroblast. 29th International Herpesvirus Workshop, Reno, Nevada.

Levine AJ (1997) p53, the cellular gatekeeper for growth and division. Cell 88:323-331

Liu B, Hermiston TW, Stinski MF (1991) A cis-acting element in the major immediate early (IE) promoter of human cytomegalovirus is required for negative regulation by IE2. J Virol 65:897-903

Louria-Hayon I, Grossman T, Sionov RV, Alsheich O, Pandolfi PP, Haupt Y (2003) The promyelocytic leukemia protein protects p53 from Mdm2-mediated inhibition and degradation. J Biol Chem 278:33134-33141

Lu M, Shenk T (1996) Human cytomegalovirus infection inhibits cell cycle progression at multiple points including the transition from G1 to S. J Virol 70:8850-8857

Lukac DM, Manuppello JR, Alwine JC (1994) Transcriptional activation by the human cytomegalovirus immediate-early proteins: requirements for simple promoter structures and interactions with multiple components of the transcription complex. J Virol 68:5184-5193

Lukac DM, Harel NY, Tanese N, Alwine JC (1997) TAF-like functions of human cytomegalovirus immediate-early proteins. J Virol 71:7227-7239

Macias MP, Stinski MF (1993) An in vitro system for human cytomegalovirus immediate early 2 protein (IE2)-mediated site-dependent repression of transcription and direct binding of IE2 to the major immediate early promoter. Proc Natl Acad Sci USA 90:707-711

Macias MP, Huang L, Lashmit PE, Stinski MF (1996) Cellular and viral protein binding to a cytomegalovirus promoter transcription initiation site: effects on transcription. J Virol 70:3628-3635

Malone CL, Vesole DH, Stinski MF (1990) Transactivation of a human cytomegalovirus early promoter by gene products from the immediate-early gene IE2 and augmentation by IE1: mutational analysis of the viral proteins. J Virol 64:1498-1506

Marchini A, Liu H, Ahu H (2001) Human cytomegalovirus with IE-2 (UL122) deleted fails to express early lytic genes. J Virol 75:1870-1878

McElroy AK, Dwarakanath RS, Spector DH (2000) Dysregulation of cyclin E gene expression in human cytomegalovirus-infected cells requires viral early gene expression and is associated with changes in the Rb-related protein p130. J Virol 74:4192-4206

Meier JL, Stinski MF (1997) Effect of a modulator deletion on transcription of the human cytomegalovirus major immediate-early genes in infected undifferentiated and differentiated cells. J Virol 71:1246-1255

Meier JL, Stinski MF (2006) Major immediate-early enhancer and its gene products. In: Reddehase MJ (ed) Cytomegaloviruses molecular and immunology. Caister Academic Press, Norfolk, UK, pp 151-166

Messerle M, Buhler B, Keil GM, Koszinowski UH (1992) Structural organization, expression, and functional characterization of the murine cytomegalovirus immediate-early gene 3. J Virol 66:27-36

Montag C, Wagner J, Gruska I, Hagemeier C (2006) Human cytomegalovirus blocks tumor necrosis factor alpha- and interleukin-1beta-mediated NF-kappaB signaling. J Virol 80:11686-11698

Moreno N, Bachy I, Retaux S, Gonzalez A (2003) Pallial origin of mitral cells in the olfactory bulbs of *Xenopus*. Neuroreport 14:2355-2358

Morin J, Johann S, O'Hara B, Gluzman Y (1996) Exogenous thymidine is preferentially incorporated into human cytomegalovirus DNA in infected human fibroblast. J Virol 70:6402-6404

Murphy EA, Streblow DN, Nelson JA, Stinski MF (2000) The human cytomegalovirus IE86 protein can block cell cycle progression after inducing transition into the S-phase of permissive cells. J Virol 74:7108-7118

Murphy J, Fischle W, Verdin E, Sinclair J (2002) Control of cytomegalovirus lytic gene expression by histone acetylation. EMBO J 21:1112-1120

Nevels M, Brune W, Shenk T (2004) SUMOylation of the human cytomegalovirus 72-kilodalton IE1 protein facilitates expression of the 86-kilodalton IE2 protein and promotes viral replication. J Virol 78:7803-7812

Noris E, Zannetti C, Demurtas A, Sinclair J, De Andrea M, Gariglio M, Landolfo S (2002) Cell cycle arrest by human cytomegalovirus 86-kDa IE2 protein resembles premature senescence. J Virol 76:12135-12148

Peters JM (2006) The anaphase promoting complex/cyclosome: a machine designed to destroy. Nat Rev Mol Cell Biol 7:644-656

Petrik DT, Schmitt KP, Stinski MF (2006) Inhibition of cellular DNA synthesis by the human cytomegalovirus IE86 protein is necessary for efficient virus replication. J Virol 80:3872-3883

Petrik DT, Schmitt KP, Stinski MF (2007) The autoregulatory and transactivating functions of the human cytomegalovirus IE86 protein use independent mechanisms for promoter binding. J Virol 81:5807-5818

Pizzorno MC, Hayward GS (1990) The IE2 gene products of human cytomegalovirus specifically down-regulate expression from the major immediate-early promoter through a target located near the cap site. J Virol 64:6154-6165

Pizzorno MC, Mullen MA, Chang YN, Hayward GS (1991) The functionally active IE2 immediate-early regulatory protein of human cytomegalovirus is an 80-kilodalton polypeptide that contains two distinct activator domains and a duplicated nuclear localization signal. J Virol 65:3839-3852

Prives C, Hall PA (1999) The p53 pathway. J Pathol 187:112-126

Reeves M, Murphy J, Greaves R, Fairley J, Brehm A, Sinclair J (2006) Autorepression of the human cytomegalovirus major immediate-early promoter/enhancer at late times of infection is mediated by the recruitment of chromatin remodeling enzymes by IE86. J Virol 80:9998-10009

Reeves MB, MacAry PA, Lehner PJ, Sissons JG, Sinclair JH (2005) Latency, chromatin remodeling, and reactivation of human cytomegalovirus in the dendritic cells of healthy carriers. Proc Natl Acad Sci USA 102:4140-4145

Salvant BS, Fortunato EA, Spector DH (1998) Cell cycle dysregulation by human cytomegalovirus: influence of the cell cycle phase at the time of infection and effects on cyclin transcription. J Virol 72:3729-3741

Sanchez V, Clark CL, Yen JY, Dwarakanath R, Spector DH (2002) Viable human cytomegalovirus recombinant virus with an internal deletion of the IE2 86 gene affects late stages of viral replication. J Virol 76:2973-2989

Schwartz R, Helmich B, Spector DH (1996) CREB and CREB-binding proteins play an important role in the IE2 86-kilodalton protein-mediated transactivation of the human cytomegalovirus 2.2-kilobase RNA promoter. J Virol 70:6955-6966

Scully AL, Sommer MH, Schwartz R, Spector DH (1995) The human cytomegalovirus IE2 86 kDa protein interacts with an early gene promoter via site-specific DNA binding and protein-protein associations. J Virol 69:6533-6540

Shen YH, Utama B, Wang J, Raveendran M, Senthil D, Waldman WJ, Belcher JD, Vercellotti G, Martin D, Metchelle BM, Wang XL (2004) Human cytomegalovirus causes endothelial injury through the ataxia telangiectasia mutant and p53 DNA damage signaling pathways. Circ Res 94:1-9

Shieh SY, Ikeda M, Taya Y, Prives C (1997) DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 91:325-334

Sinclair J, Baillie J, Bryant L, Caswell R (2000) Human cytomegalovirus mediates cell cycle progression through G(1) into early S phase in terminally differentiated cells. J Gen Virol 6:1553-1565

Sommer MH, Scully AL, Spector DH (1994) Transactivation by the human cytomegalovirus IE2 86-kilodalton protein requires a domain that binds to both the TATA box-binding protein and the retinoblastoma protein. J Virol 68:6223-6231

Song Y-J, Stinski MF (2002) Effect of the human cytomegalovirus IE86 protein on expression of E2F-responsive genes: A DNA microarray analysis. Proc Natl Acad of Sci USA 99:2836-2841

Song Y-J, Stinski MF (2004) Inhibition of cell division by the human cytomegalovirus IE86 protein: role of the p53 pathway or cdk1/cyclin B1. J Virol 79:2597-2603

Speir E, Modali R, Huang E-S, Leon MB, Shawl F, Finkel T, Epstein SE (1994) Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. Science 265:391-394

Stamminger T, Puchtler E, Fleckenstein B (1991) Discordant expression of the immediate-early 1 and 2 gene regions of human cytomegalovirus at early times after infection involves posttranscriptional processing events. J Virol 65:2273-2282

Stein GH, Drullinger LF, Soulard A, Dulic V (1999) Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. Mol Cell Biol 19:2109-2117

Stenberg RM (1996) The human cytomegalovirus major immediate-early gene. Intervirology 39:343-349

Stenberg RM, Thomsen DR, Stinski MF (1984) Structural analysis of the major immediate early gene of human cytomegalovirus. J Virol 49:190-199

Stenberg RM, Depto AS, Fortney J, Nelson J (1989) Regulated expression of early and late RNAs and proteins from the human cytomegalovirus immediate-early gene region. J Virol 63:2699-2708

Stinski MF (1999) Cytomegalovirus promoter for expression in mammalian cells. In: Ferandez JM, Hoeffler JP (eds) Gene expression systems: using nature for the art of expression. Academic Press, San Diego, pp 211-233

Tang Q, Maul GG (2003) Mouse cytomegalovirus immediate-early protein 1 binds with host cell repressors to relieve suppressive effects on viral transcription and replication during lytic infection. J Virol 77:1357-1367

Taylor RT, Bresnahan WA (2005) Human cytomegalovirus immediate-early 2 gene expression blocks virus-induced beta interferon production. J Virol 79:3873-3877

Taylor RT, Bresnahan WA (2006) Human cytomegalovirus immediate-early 2 protein IE86 blocks virus-induced chemokine expression. J Virol 80:920-928

Tsai HL, Kou GH, Chen SC, Wu CW, Lin YS (1996) Human cytomegalovirus immediate-early protein IE2 tethers a transcriptional repression domain to p53. J Biol Chem 271:3534-3540

Vogelstein B, Lane D, Levine AJ (2000) Surfing the p53 network. Nature 408:307-310

Waheed I, Chiou C, Ahn J, Hayward GS (1998) Binding of the human cytomegalovirus 80-kDa immediate-early protein (IE2) to minor groove A/T-rich sequences bounded by CG dinucleotides is regulated by protein oligomerization and phosphorylation. Virology 252:235-257

White EA, Clark CL, Sanchez V, Spector DH (2004) Small internal deletions in the human cytomeaglovirus IE2 gene result in nonviable recombinant viruses with differential defects in viral gene expression. J Virol 78:1817-1830

White EA, Del Rosario CJ, Sanders RL, Spector DH (2007) The IE2 60-kilodalton and 40-kilodalton proteins are dispensable for human cytomegalovirus replication but are required for efficient delayed early and late gene expression and production of infectious virus. J Virol 81:2573-2583

Wiebusch L, Hagemeier C (1999) Human cytomegalovirus 86-kilodalton IE2 protein blocks cell cycle progression in G(1). J Virol 73:9274-9283

Wiebusch L, Bach M, Uecker R, Hagemeier C (2005) Human cytomegalovirus inactivates the G(0)/G(1)-APC/C ubiquitin ligase by Cdh1 dissociation. Cell Cycle 4:1435-1439

Wu J, Jupp R, Stenberg RM, Nelson JA, Ghazal P (1993) Site-specific inhibition of RNA polymerase II preinitiation complex assembly by human cytomegalovirus IE86 protein. J Virol 67:7547-7555

Xu Y, Cei SA, Rodriguez-Huete A, Colletti KS, Pari GS (2004) Human cytomegalovirus DNA replication requires transcriptional activation via an IE2- and UL84-responsive bidirectional promoter element within oriLyt. J Virol 78:11664-11677

Yee L-F, Lin PL, Stinski MF (2007) Ectopic expression of HCMV IE72 and IE86 proteins is sufficient to induce early gene expression but not production of infectious virus in undifferentiated promonocytic THP-1 cells. Virology 363:174-188

Yeung KC, Stoltzfus CM, Stinski MF (1993) Mutations of the cytomegalovirus immediate-early 2 protein defines regions and amino acid motifs important in transactivation of transcription from the HIV-1 LTR promoter. Virology 195:786-792

Yoo YD, Chiou C-J, Choi KS, Yi Y, Michelson S, Kim S, Hayward GS, Kim S-J (1996) The IE2 regulatory protein of human cytomegalovirus induces expression of the human transforming growth factor beta 1 gene through an Egr-1 binding site. J Virol 70:7062-7070

Zhang Z, Evers DL, McCarville JF, Dantonel JC, Huong SM, Huang ES (2006) Evidence that the human cytomegalovirus IE2-86 protein binds mdm2 and facilitates mdm2 degradation. J Virol 80:3833-3843

Zhu H, Shen Y, Shenk T (1995) Human cytomegalovirus IE1 and IE2 proteins block apoptosis. J Virol 69:7960-7970

Zhu H, Cong JP, Mamtora G, Gingeras T, Shenk T (1998) Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays. Proc Natl Acad Sci USA 95:14470-14475