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The Host-Cell Architectural Protein HMG I(Y) Modulates Binding of Herpes Simplex Virus Type 1 ICP4 to Its Cognate Promoter

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The productive infection cycle of herpes simplex virus is controlled in part by the action of ICP4, an immediate-early gene product that acts as both an activator and repressor of transcription. ICP4 is autoregulatory, and IE-3, the gene that encodes it, contains a high-affinity binding site for the protein at its cap site. Previously, we had demonstrated that this site could be occupied by proteins found in nuclear extracts from uninfected cells. A HeLa cell cDNA expression library was screened with a DNA probe containing the IE-3 gene cap site, and clones expressing the architectural chromatin proteins HMG I and HMG Y were identified by this technique. HMG I is shown to augment binding of ICP4 to its cognate site in *in vitro* assays and to enhance the activity of this protein in short-term transient expression assays.

INTRODUCTION

Expression of herpes simplex virus type 1 (HSV-1) genes during the course of a lytic infection proceeds in a temporally ordered cascade (Honess and Roizman, 1974). The virus genes are classified in three major kinetic classes, immediate-early (IE), early (E), and late (L), based on their temporal pattern of expression during infection (Honess and Roizman, 1974, 1975). The IE genes, which are the first to be expressed, encode four regulators of gene expression [infected-cell proteins (ICPs) 4, 27, 0, and 22), and a protein, ICP47, that facilitates immune evasion by blocking the presentation of viral antigens (Hill *et al.,* 1995; York *et al.,* 1994). Regulation of virus gene expression by the IE proteins takes place at both the transcriptional and posttranscriptional levels. Transcriptional regulation is orchestrated by ICP4, the major transcriptional regulator of HSV-1. ICP4, which is essential for virus replication (DeLuca *et al.,* 1985; Dixon and Schaffer, 1980; Knipe *et al.,* 1978; Preston, 1979; Watson and Clements, 1978, 1980), is a sequence-specific DNA-binding protein (DiDonato *et al.,* 1991; Faber and Wilcox, 1986; Michael and Roizman, 1989; Michael *et al.,* 1988) that can act as a repressor of IE gene expression and as an activator of E and L genes (DeLuca *et al.,* 1985; Gelman and Silverstein, 1985; Lium *et al.,* 1996; O'Hare and Hayward, 1985a; Roberts *et al.,* 1988; Watson and Clements, 1980). The IE proteins ICP0 and ICP22, although not essential for completion of an HSV-1 lytic cycle, appear to participate in transcriptional regulation as well. ICP0, a promiscuous transactivator of viral and cellular promoters (Everett, 1984; Gelman and Silverstein, 1985; O'Hare and Hayward, 1985a, 1985b; Quinlan and Knipe, 1985), acts at the level of transcription (Jordan and Schaffer, 1997). ICP22 might also affect transcription of HSV-1 genes by altering the phosphorylation state of RNA polymerase II (Rice *et al.,* 1995).

Post-transcriptional regulation of gene expression is affected both negatively and positively by the essential protein ICP27 (Everett, 1986; Hardwicke *et al.,* 1989; McCarthy *et al.,* 1989; Rice and Knipe, 1990; Sacks *et al.,* 1985; Sekulovich *et al.,* 1988; Su and Knipe, 1989). The inhibitory effects of ICP27 result from inhibition of pre-mRNA splicing (Hardy and Sandri-Goldin, 1994; Phelan *et al.,* 1993; Sandri-Goldin and Mendoza, 1992), whereas its positive effects have been linked to its ability to promote 3′ end maturation of certain RNA transcripts (McGregor *et al.,* 1996; McLauchlan *et al.,* 1989, 1992; Sandri-Goldin and Mendoza, 1992) and possibly aid their nucleocytoplasmic transport (Sandri-Goldin, 1998, Soliman, 1997). Recent evidence also suggests that ICP27 may act at the level of transcription (Panagiotidis *et al.,* 1997; Samaniego *et al.,* 1995).

Although ICP4 appears to be the master switch in the coordinated temporal expression of HSV-1 genes (for a review, see Roizman and Sears, 1996), its exact molecular mechanism of action is largely unknown. Several lines of evidence suggest that it is involved in a large number of interactions with both viral and host proteins. ICP4 has been found to interact with the TATA binding protein (TBP), the basal transcription factor TFIIB, and the TBP-associated protein 250 (TAF250) (Carrozza and DeLuca, 1996; Gu and DeLuca, 1994; Gu *et al.,* 1995; Smith *et al.,* 1993). Therefore, it is possible that ICP4 regulates transcription by affecting transcription preini-

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tiation complex assembly. ICP4 also interacts with the HSV-1 regulatory proteins ICP0 and ICP27 (Panagiotidis *et al.,* 1997), and these interactions appear to affect the intracellular compartmentalization of these proteins (Mullen *et al.,* 1995; Zhu *et al.,* 1994; Zhu and Schaffer, 1995). This imposition of compartmental constraints by the virus regulatory proteins on each other is likely to have an impact on regulation of virus gene expression. Moreover, these interactions could alter the biochemical activities of the proteins involved. Indeed, the post-translational modification state of ICP4 is affected by ICP27 (McMahan and Schaffer, 1990; Rice and Knipe, 1988; Su and Knipe, 1989; Xia *et al.,* 1996). Although the molecular basis for this phenomenon is not known, it is likely to involve physical interactions between ICP27 and hypomodified forms of ICP4 (Panagiotidis *et al.,* 1997). This interaction may affect transcription because less modified forms of ICP4 have different DNA-binding specificities (Michael *et al.,* 1988). In fact, the ability of ICP4 to bind a regulatory region within the thymidine kinase (TK) gene depends on the presence of ICP27 (Panagiotidis *et al.,* 1997).

The ability of ICP4 to interact with DNA, and thus regulate gene expression, can also be affected by components of the cell milieu that alter DNA structure. We have shown that polyamines (biological cations, which bind to and neutralize the anionic phosphate charges of DNA (Tabor and Tabor, 1984), enhance the DNA-binding activity of ICP4 at physiological concentrations (Panagiotidis *et al.,* 1995). A recent report by Carrozza and DeLuca (1998) revealed that the high-mobility group (HMG) protein HMG 1 augments ICP4 activation of *in vitro* transcription. Therefore, it appears that the intracellular environment of the host can influence the recognition of regulatory DNA sequences by ICP4 and affect its ability to regulate HSV-1 gene transcription. In this report, we provide evidence that the architectural chromatin protein HMG I(Y) binds to DNA sequences within the HSV-1 IE-3 gene promoter/regulatory region. This interaction appears to have regulatory significance because it affects both the transcriptional activity of this gene regulatory region and the binding of ICP4 to these same DNA sequences.

RESULTS

Screening for host-cell proteins interacting with DNA sequences recognized by ICP4

Factors affecting the binding of ICP4 to DNA have the potential to regulate virus gene expression. Physiological concentrations of the polyamines spermidine and spermine can enhance the binding of ICP4 to a high-affinity site positioned at the cap site of the IE-3 gene (Panagiotidis *et al.,* 1995). This same site is readily occupied by proteins prepared from uninfected cell nuclear extracts (Papavassiliou and Silverstein, 1990b). The fact that polyamines also enhanced the binding of a host-cell protein or proteins to the same DNA (Panagiotidis *et al.,* 1995) raised the possibility that the binding of ICP4 might also be affected by host-cell proteins binding in the vicinity of its DNA-binding site. To test this hypothesis, and to identify the factors involved, a HeLa cell cDNA expression library, in phage λ gt11, was screened in the presence of 1 mM spermidine with a radiolabeled DNA probe containing six concatamerized copies of the IE-3 gene cap region (−17/+32). Phages expressing proteins that bind specifically to the IE-3 cap were verified by repeating the screening procedure three times. The cDNAs contained in these phages were amplified by PCR and cloned. DNA sequencing of three positive clones revealed two encoding for the chromatin architectural protein HMG I and one encoding for its isoform HMG Y (Eckner and Birnstiel, 1989; Johnson *et al.,* 1988, 1989). These proteins are encoded by the same gene, and their mRNAs are generated by alternative splicing (Johnson *et al.,* 1989). HMG I, the larger of the two, is composed of 107 amino acids, whereas HMG Y lacks an 11-amino-acid sequence of unknown functional significance. These proteins are commonly referred to as HMG I(Y) because no functional differences have been found so far between these two isoforms.

To confirm the results of the library screening, recombinant HMG I (rHMG I) carrying a hexahistidine tag fused to its amino terminus was expressed in bacteria, purified by nickel-binding affinity chromatography and assayed for its ability to bind to the IE-3 cap DNA. Protein–DNA-binding reactions were performed with increasing amounts of purified rHMG I (1–50 ng) and end-labeled IE-3 gene cap site probe, and the complexes were analyzed by electrophoretic mobility shift assay (EMSA). The results of this analysis confirmed that rHMG I forms stable complexes with DNA containing the IE-3 cap site (Fig. 1A, lanes 3–6).

HMG I(Y) enhances ICP4 binding to the IE-3 gene cap site

The aim of the present study was to identify proteins that potentially alter the DNA-binding activity of ICP4. Therefore, we next asked whether HMG I(Y) affects the DNA-binding activity of ICP4 to the IE-3 gene cap site probe using recombinant HMG I(Y). The addition of increasing amounts of purified rHMG I to reactions containing 10 ng of a purified GST-fusion protein with the ICP4 DNA-binding domain (amino acids 245–523) revealed that HMG I(Y) enhances the DNA-binding activity of ICP4. In the absence of rHMG I, GST–ICP4–DNA complexes were barely detectable by EMSA (Fig. 1B, lanes 2–5). However, the abundance of these complexes was greatly enhanced with the addition of increasing amounts of rHMG I to the DNA-binding reaction mixture (Fig. 1B, lanes 2–5). Furthermore, the abundance of GST–ICP4–DNA complexes in the presence of increasing amounts of rHMG I was higher than that observed when

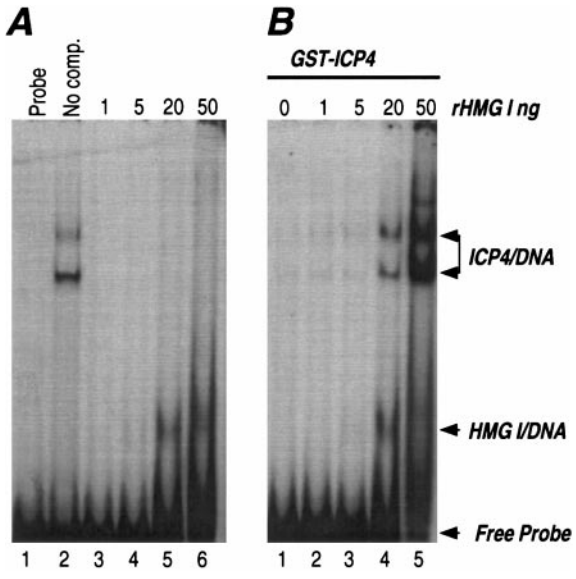

FIG. 1. Binding of rHMG I to the IE-3 cap site and its effects on the DNA-binding activity of a recombinant ICP4 protein. The ability of purified rHMG I to bind the IE-3 cap site, as well as its effect on the DNA-binding activity of a purified, bacterially expressed, fusion of the DNA-binding domain of ICP4 (amino acids 245–523) with GST (GST-ICP4), was assessed by EMSA. The DNA-binding reactions were performed as described in *Materials and Methods*, and the amounts of rHMG I added to each reaction mix are indicated above the figure. The amount of GST-ICP4 used was 10 ng (A, lanes 2–6; B, lanes 1–5). Sonicated salmon sperm DNA (0.5 μg/reaction) was used as nonspecific competitor except in A, lane 2 (No comp), where no competitor was added. The positions of the unbound IE-3 cap probe and the rHMGI–DNA and ICP4–DNA complexes are indicated.

the DNA-binding reaction was performed in the absence of nonspecific competitor DNA (Fig. 1A, lane 2). This eliminates the possibility that the enhancement of the DNA-binding activity of ICP4 by rHMG I results from titration of the nonspecific sonicated salmon sperm DNA competitor by rHMG I.

The effect of HMG I on the DNA-binding activity of full-length ICP4 was also measured using nuclear extracts from HSV-1-infected HeLa cells as the source of ICP4. The addition of increasing amounts of rHMG I resulted in an increase in the accumulation of an ICP4-DNA complex (Fig. 2, lanes 3–6). Supershift analysis using 58S monoclonal antibody (MAb) confirmed that ICP4 was present in the HMG I-enhanced protein–DNA complex (Fig. 2, lanes 7–10). The results of this analysis demonstrate that the effect of HMG I is not limited to the bacterially expressed, purified DNA-binding domain of ICP4 but that it can be reproduced with the full-length ICP4. Furthermore, because rHMG I does not enhance the formation of other IE-3 cap site–protein complexes (Fig. 2), we believe that the HMG I effect is specific only for the binding of ICP4 to the IE-3 cap site and not for other IE-3 cap site-binding proteins. Further evidence for the specificity of the HMG I effect comes from our observation that HMG I does not enhance the binding of ICP4 to the TK B region of the HSV-1 TK gene that is bound by ICP4 (Papavassiliou and Silverstein, 1990b), which lacks an HMG I site and is not bound by the protein (data not shown).

HMG I(Y) binds the minor groove of DNA at AT-rich regions (Solomon *et al.,* 1984; Thanos and Maniatis, 1992). To further demonstrate that the enhanced binding was specific, a poly(dA)/poly(dT) polynucleotide was used as a specific competitor and poly(dG)/poly(dC) was used as a nonspecific competitor in binding reactions. The resulting protein–DNA complexes were analyzed by EMSA. The addition of increasing amounts of either polynucleotide had little, if any, effect on the abundance ICP4–DNA complexes in the absence of exogenous rHMG I (Fig. 3, lanes 2–7). As expected, the addition of rHMG I to the binding reactions greatly increased the abundance of ICP4–DNA complexes (Fig. 3, compare lanes 2 and 8). However, only poly(dA)/poly(dT) competed for formation of both the rHMG I–DNA complexes and the HMG I-enhanced ICP4 complexes (Fig. 3, lanes 8–13). The decrease in the abundance of ICP4–DNA complexes paralleled that of HMG I-DNA complexes, as would be expected for an HMG I-specific effect (Fig. 3). Similar data are also obtained when distamycin, an oligopeptide antibiotic, is added to the binding reactions. Distamycin binds in the minor groove of DNA with similar specificity to HMG I(Y) (Bellorini *et al.,* 1995; Mote *et al.,* 1994). It also competes with HMG I(Y) for binding to DNA

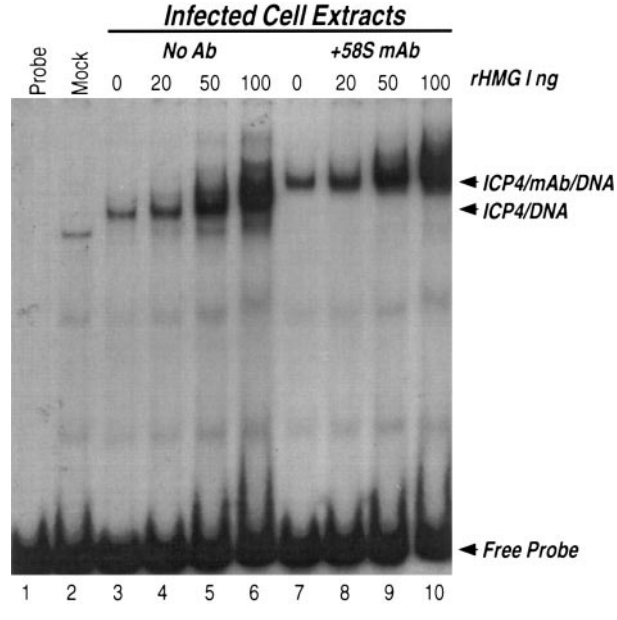

FIG. 2. Enhancement of ICP4 binding by rHMG I. Protein–DNA-binding reactions and EMSAs were performed as described in *Materials and Methods* using nuclear extracts from HSV-1-infected HeLa cells, except in lane 2, where an extract from mock-infected cells was used. Five micrograms of HeLa nuclear extract proteins and 2 μg of nonspecific competitor DNA were used in each binding experiment. The amounts of rHMG I added to each binding reaction are indicated above the figure. Supershift analysis was performed using 1 μg of purified 58S MAb per reaction (lanes 7–10). The positions of the unbound IE-3 cap probe and the ICP4–DNA and MAb–ICP4-DNA complexes are indicated.

(French *et al.,* 1996; Ghersa *et al.,* 1997). We verified this result, using the IE-3 cap site probe, and found that distamycin also competed with the HMG I-enhanced binding of ICP4 to DNA, whereas it did not affect the binding of ICP4 in the absence of exogenously added HMG I (data not shown).

Identification of the HMG I(Y) binding sites within the IE-3 promoter/regulatory region

DNase I protection footprint analysis was used to identify DNA sites within the IE-3 regulatory region that are bound by HMG I(Y). This analysis revealed that rHMG I protected, albeit weakly, two DNA sequences (from −2 to −4 and from +9 to +12, relative to the transcription start site) within the IE-3 cap site (Fig. 4). The weak protection by rHMG I is not unexpected because the IE-3 gene cap site bears little resemblance to sequences typically recognized by HMG I(Y). The addition of more rHMG I (at 120 and 200 ng) to the reactions resulted in DNase I hypersensitivity at two positions (+6 and −8) within the IE-3 cap site (Fig. 4). This may indicate that rHMG I binding to the IE-3 cap alters DNA structure. This putative alteration may facilitate the increased binding of ICP4 to this sequence.

The DNase I footprint analysis revealed that rHMG I binds to the IE-3 cap site and to two other sequences within the IE-3 promoter. One of these sites (from −26 to −21) corresponds to the TATA box. The strongest rHMG I-dependent protection within the IE-3 probe was observed further upstream (−106 to −98) at the DNA region overlapping the TAATGARAT motif. The binding of

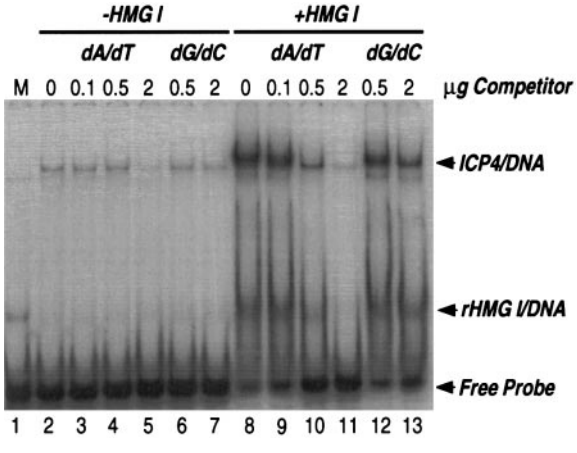

FIG. 3. Specificity of rHMG I enhancement of ICP4 binding. DNA-binding reactions with nuclear extracts from HSV-1-infected (7 h) HeLa cells and EMSAs were performed as described in *Materials and Methods*. Reactions were performed in the absence (−HMG I, lanes 1–7) or presence (+HMG I, lanes 8–13) of 100 ng of rHMG I. The amounts of poly(dA)/poly(dT) and poly(dG)/poly(dC) competitor polynucleotides are shown above the figure. No competitor was added in the reactions analyzed in lanes 2 and 8. The reaction in lane 1 (M) was performed using a nuclear extract from mock-infected HeLa cells. Arrows indicate the positions of the free IE-3 probe and its complexes with rHMG I or ICP4.

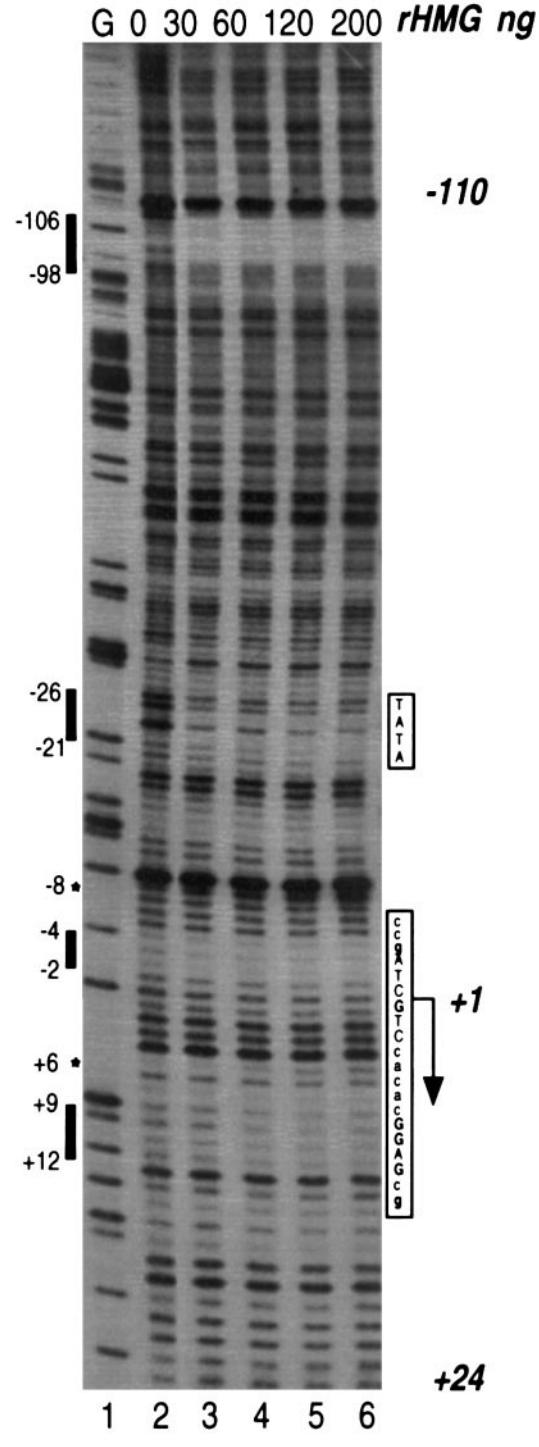

FIG. 4. DNase I footprinting analysis of HMG I binding sites within the IE-3 gene promoter/regulatory region. The reactions were performed as described in *Materials and Methods* with the indicated amounts of rHMG I added to each reaction. The black boxes at the left identify regions protected from DNase I action in the presence of rHMG I. The asterisks identify sites that become hypersensitive to DNase I action in the presence of rHMG I. The boxed sequences contain the TATA box and a high-affinity ICP4 recognition site. The arrow at +1 identifies the IE-3 gene transcription initiation site.

HMG I(Y) to several sites within the IE-3 gene promoter/regulatory region is consistent with the multivalent binding of this protein to several other promoters where it has

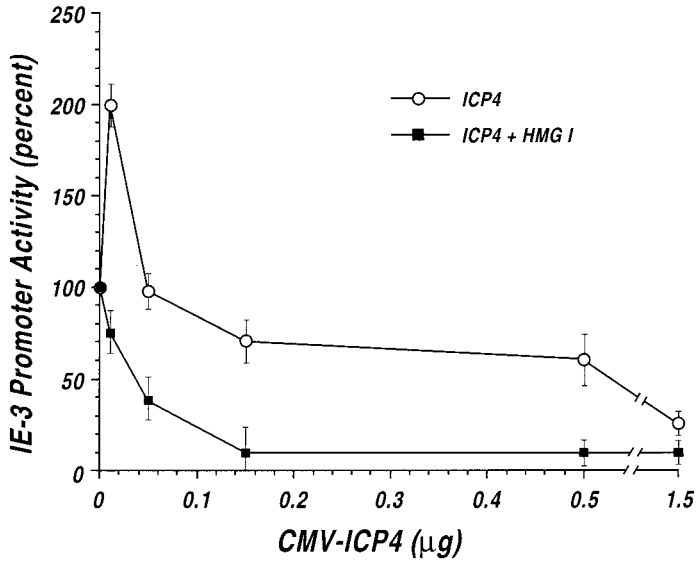

FIG. 5. Cotransformation of plasmids expressing rHMG I and ICP4. Vero cells were transfected with 1 μg of the reporter plasmid pCPC-4P-LUC and the indicated amounts of pCPC-CMV4. These transfections were performed in the absence (○) or presence (□) of 1 μg of pCPC-I_E. Cells were harvested at 36 h postinfection, and luciferase activities were determined as described in *Materials and Methods*. The results are expressed as a percentage of the luciferase activity value (relative light units) obtained from cells transfected with the reporter construct in the absence of any effector plasmids.

been found to alter DNA architecture (Carey, 1998; Maher and Nathans, 1996; Thanos and Maniatis, 1995).

HMG I(Y) potentiates the repressive effect of ICP4 on the IE-3 promoter

ICP4 binding to IE-3 promoter sequences results in repression of gene expression. Thus if *in vivo* HMG I(Y) enhances the binding of ICP4 to the IE-3 promoter, as it does *in vitro*, then it should enhance repression by ICP4. To test this hypothesis, the ability of ICP4 to repress the IE-3 promoter was measured in transfection experiments in the presence or absence of coexpressed HMG I. Vero cells were transfected with an IE-3 promoter-luciferase reporter construct and increasing amounts (10 ng to 1.5 μg) of a plasmid expressing ICP4 under the control of a cytomegalovirus (CMV) promoter, in the presence or absence of 1 μg of a CMV promoter-driven HMG I construct. As expected, ICP4 activated the IE-3 promoter at low ratios of ICP4 to IE-3 promoter-luciferase construct but repressed it when cotransfected at higher ratios (Fig. 5). However, the repressive effect of ICP4 on the IE-3 promoter was significantly enhanced when HMG I was coexpressed (Fig. 5). Specifically, the amount of input CMV-ICP4 DNA required to effect 50% repression was reduced from 750 ng, in the absence of coexpressed HMG I, to 40 ng in its presence. Similar results were also obtained with a construct expressing HMG Y (data not shown).

It was possible that the enhanced expression was caused by HMG I-mediated activation of the CMV promoter that was driving the expression of ICP4. If this were the case, then overproduction of ICP4 should generate similar results. Therefore, we determined the effect of cotransfecting a plasmid expressing HMG I with a CMV promoter-luciferase reporter construct on the activity of the CMV promoter. HMG I had only a very small effect (<5%) on the activity of the CMV promoter (data not shown). Thus the HMG I(Y)-mediated enhancement of the DNA-binding activity of ICP4 can take place not only *in vitro* but also within the intracellular milieu.

Effect of HMG I(Y) on the activity of the IE-3 promoter

HMG I(Y) is neither a transcriptional activator nor repressor (Carey, 1998; Thanos and Maniatis, 1992, and references therein). However, in its function as an architectural protein (Bustin and Reeves, 1996), it can alter the conformation of DNA-regulatory regions affecting the binding of transcriptional activators and repressors. The identification of three HMG I(Y) binding sites in the IE-3 gene promoter/regulatory region suggests that HMG I(Y) may alter the architecture of this promoter and affect its basal activity. To test this hypothesis, Vero cells were transfected with the IE-3 promoter-luciferase reporter constructs (1 μg) and increasing amounts (0.1 μg-2 μg) of plasmids expressing HMG I or antisense HMG I(Y) RNA, each of which was under the control of a CMV promoter. HMG I expression increased the activity of the IE-3 promoter by more than twofold, whereas expression of the antisense HMG I(Y) RNA resulted in a modest reduction (~30%) of the IE-3 promoter activity (Fig. 6). It is noteworthy that expression of either HMG I(Y), or the antisense HMG I(Y) RNA has no effect on the activity of the minimal IE-3 promoter (−108 to +32) reporter con-

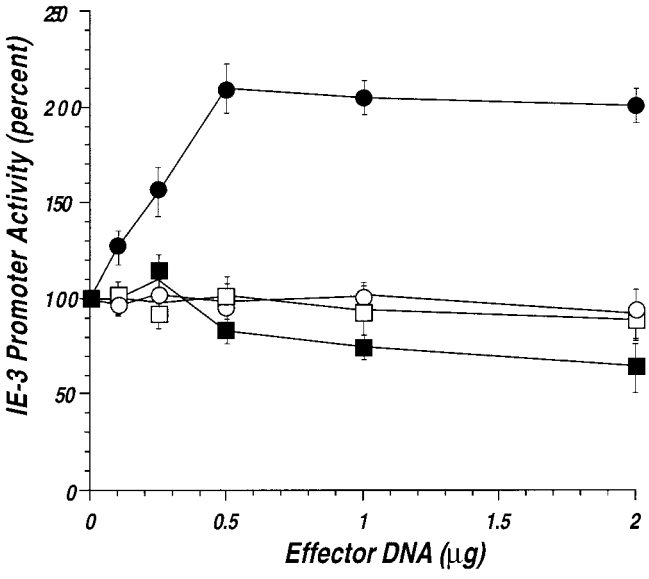

FIG. 6. Effect of HMG I on the activity of the IE-3 gene promoter. Vero cells were transfected with 1 μg of pCPC-4P-LUC (● and ■) or pCPC-4P$_1$-LUC (○ and □) and the indicated amounts of pCPC-I$_E$ (HMG I, ● and ○) or the HMG I(Y) antisense plasmid pα-HMG I (■ and □). Cells were harvested at 36 h postinfection, and luciferase activities were determined as described in *Materials and Methods*. The results are expressed as described in Fig. 5.

struct pCPC-4P$_1$-LUC (Fig. 6). This minimal promoter contains two of the three HMG I(Y) binding sites (at the IE-3 cap site and at the TATA box). However, it lacks the third site that overlaps with the TAATGARAT motif and other binding sites for transcription factors that interact further upstream of the minimal IE-3 promoter. Despite the absence of this upstream HMG I binding site, there is no diminution of the HMG I effect on ICP4-mediated repression. Indeed, the addition of HMG I enhanced the repressive effect of ICP4 even when plasmid pCPC-4P-$_1$-LUC was used as the reporter (data not shown). These results suggest that HMG I(Y) positively affects the activity of the IE-3 promoter. Increased reporter activity requires the upstream sequences that are bound by transcription factors that recognize DNA sequences within this regulatory region, such as Sp1 and Oct-1. In addition, HMG I, by changing the architecture of the promoter, might facilitate contacts between transcription factors and the transcription preinitiation complex.

DISCUSSION

The highly ordered temporal expression of HSV-1 genes is primarily effected by ICP4, the major virus regulatory protein (for a review, see Roizman and Sears, 1996). Although the exact molecular mechanism or mechanisms of the action of ICP4 are largely unknown, it has been shown to act as a transcriptional repressor of IE genes and an activator of E and L genes. DNA binding plays an important role in the regulatory activities of ICP4 (Shepard *et al.,* 1989). Therefore, factors that affect the DNA-binding activity of ICP4 have the potential to modulate HSV-1 gene expression.

We previously demonstrated that a high-affinity binding site for ICP4, located at the transcriptional start site of the IE-3 gene, was bound by host proteins extracted from uninfected cells (Panagiotidis *et al.,* 1991; 1995). This present study was initiated to identify host proteins that occupy sites in the HSV-1 chromosome that are subsequently bound by ICP4 after infection. The ICP4 DNA-binding site used in this analysis represents one of the most studied sites in the virus chromosome. Moreover, the biological consequences of ICP4 binding can be readily assessed, (i.e., ICP4 binding leads to IE-3 gene repression) (DeLuca *et al.,* 1985; Gelman and Silverstein, 1985; O'Hare and Hayward, 1985a; Roberts *et al.,* 1988). Analysis of a HeLa cDNA expression library with a DNA probe containing the high-affinity binding site for ICP4 revealed that the chromatin architectural proteins HMG I and HMG Y bound to the probe. Binding of HMG I(Y) to the IE-3 cap site was further verified by *in vitro* experiments with purified recombinant HMG I (Fig. 1).

HMG proteins are a heterogeneous group of small, acid-soluble, non-histone chromatin proteins (Bustin and Reeves, 1996). They are classified in three general families: the HMG1/2, the HMG 14/17, and the HMG I(Y) families (Bustin and Reeves, 1996, and references therein). It was initially thought that HMGs represent structural components of the eucaryotic chromatin with limited gene-regulatory potential. They are now known to be involved in a wide variety of cellular functions involving DNA, such as transcription, replication, and recombination (Bustin and Reeves, 1996; Carey, 1998; Falvo *et al.,* 1995; Fashena *et al.,* 1992; Ogawa *et al.,* 1995; Thanos and Maniatis, 1992, 1995). HMGs exert their effects by modifying chromatin architecture and/or by functionally interacting with regulatory and structural protein components of the chromatin (Bustin and Reeves, 1996; Carey, 1998; Falvo *et al.,* 1995; Fashena *et al.,* 1992; Ogawa *et al.,* 1995; Thanos and Maniatis, 1992, 1995). HMG I(Y) binds to the minor groove of AT-rich DNA regions through three short basic amino acid repeats called the AT-hooks (Reeves and Nissen, 1990). Although not a transcriptional activator *per se*, HMG I(Y) influences the activity of several cellular and viral promoters (for a review, see Carey, 1998). It produces its effects in multiple ways by: facilitating transcription factor binding to DNA, stabilizing higher-order multiprotein–DNA transcription-enhancing (enhanceosome) complexes, or competing with histone-mediated repression (Carey, 1998).

In this study, we demonstrate that HMG I(Y) induces the *in vitro* binding of both full-length ICP4 and a GST-fusion with the ICP4 DNA-binding domain to the IE-3 cap site DNA probe (Figs. 1 and 2). HMG I(Y) is known to exert its effects not only by altering the DNA structure but also by physically interacting with several transcription factors (Lehming *et al.,* 1994; Thanos and Maniatis, 1992;

Wood *et al.,* 1995; Zhou and Chada, 1998). Therefore, its effects on ICP4 could be a result of HMG I(Y)-induced DNA conformational change or changes and/or protein–protein interactions between HMG I(Y) and ICP4. However, despite repeated efforts using both immunoprecipitation and protein capture techniques, no evidence was found for protein–protein interactions between HMG I(Y) and ICP4. In contrast, binding of HMG I(Y) to the IE-3 cap DNA results in DNase I hypersensitivity of two bases in the vicinity of HMG I(Y) binding sites (Fig. 4). This hypersensitivity is indicative of HMG I(Y)-induced DNA conformational changes that could result in the enhancement of ICP4 binding in the presence of HMG I(Y).

ICP4 binding to the IE-3 gene cap site results in repression (Roberts *et al.,* 1988). The enhanced binding of ICP4 in the presence of HMG I *in vitro* suggested to us that ICP4 binding in the presence of HMG I(Y) should potentiate the repressive effect of ICP4 *in vivo*. This hypothesis was tested in transfection experiments, and the HMG I and HMG Y isoforms were found to be equally effective in enhancing the repressing activity of ICP4 on the IE-3 promoter. These results indicate that HMG I(Y) has the potential to modulate the activity of ICP4 and, consequently, virus gene expression.

HMG I(Y), unlike other HMG-box containing proteins that induce sharp bends in the DNA structure, has only a relative modest effect on DNA conformation (Carey, 1998; Falvo *et al.,* 1995). However, it frequently binds to multiple sites within target promoters, and this multivalent binding could produce extensive changes in chromatin architecture that might be essential for its effects on the binding of transcription factors. HMG I(Y) binds to at least three sites within the IE-3 gene promoter-regulatory sequences, as shown by DNase I footprint analysis (Fig. 4). Interestingly, these sites overlap with sequences that are important in the regulation of transcription from the IE-3 gene. These sequences include the TATA box, the TAATGARAT element, and the ICP4 binding site. Protection of these DNA elements suggests that the role of HMG I(Y) in the regulation of IE-3 gene expression is not limited to its effects on ICP4. Indeed, in transfection experiments, HMG I(Y) enhances the basal activity of the IE-3 promoter in the absence of viral factors (Fig. 6). A further role for HMG I(Y) in regulating transcription from this promoter is suggested by its failure to activate a minimal IE-3 promoter lacking the HMG I(Y) binding site that overlaps with the TAATGARAT sequence. This result suggests that multivalent HMG I(Y) binding to the IE-3 promoter/regulatory sequences is essential for its function. Alternatively, HMG I(Y) might recruit transcription factors that bind to upstream sequences that are missing from the minimal IE-3 promoter and/or it might facilitate the effects of upstream binding factors on the transcription preinitiation complex. To discriminate among these possibilities or to identify alternatives will require further experimentation.

The results presented here indicate that architectural protein components of the host cell chromatin may modulate HSV-1 gene expression by affecting the binding of host and virus-encoded transcription regulatory proteins. We do not at present know whether HMG I(Y) affects other classes of HSV-1 promoters. The recent observation (French *et al.,* 1996) that HMG I(Y) binds to and modulates the activity of another HSV-1 promoter, the latency-active promoter 2 (LAP-2), points toward a more general role for HMG I(Y) in the regulation of virus gene expression. This study provides further evidence that architectural proteins can play a significant role in the regulation of HSV-1 gene expression.

MATERIALS AND METHODS

Cells and virus

Vero cells were grown and maintained in DMEM (GIBCO BRL, Grand Island, NY) containing 5% bovine calf serum (Hyclone Laboratories Inc., Logan, UT). The media were supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO BRL), unless otherwise noted. HeLa cells were propagated in monolayers as previously described (Papavassiliou and Silverstein, 1990a). HSV-1 strain 17 was used in this study.

Screening of the HeLa cDNA expression library with a DNA probe

The HeLa cDNA library, was constructed in the λ gt11 *Sfi–Not* vector (Promega, Madison, WI) by Jeh-Ping Liu and Scott Zeitlin (Columbia University). A ^{32}P end-labeled 450-bp *Hin*dIII–*Eco*RI restriction fragment from plasmid pCPC-1B (see below) was used to screen ~750,000 plaques from the expression library for clones that produced protein that was bound by the probe. This DNA fragment contained six randomly concatamerized copies of the −17/+32 sequence from the HSV-1 IE-3 gene. The screening of the cDNA expression library was performed as previously described with some modifications (Singh *et al.,* 1988). Filters were blocked with Blotto overnight at 4°C, with gentle agitation. The incubation was performed in 10 mM Tris–HCl, pH 7.5, 50 mM NaCl, 5% (v/v) glycerol, 1 mM EDTA, 4 mM dithiothreitol, and 0.2 mg/ml BSA, and 0.5 mM spermidine and salmon sperm DNA (100 μg/ml) was used as the nonspecific competitor. Positive plaques were isolated, verified by rescreening, and purified three times. The cDNA inserts were amplified by PCR using λ gt11 primers (Promega), and the amplimers were cloned into the TA cloning vector pCRII (InVitrogen, San Diego, Ca.). The DNA sequence of each clone was determined using an ABI 377 automated sequencer (Perkin–Elmer, Foster City, CA) by the Columbia Cancer Center sequencing facility.

Plasmid constructions

Plasmid pCPC-1A, containing the −17/+32 region of the IE-3 gene, was constructed by subcloning an end-

filled 49 bp *Ava*I–*Bam*HI fragment from plasmid pIGA103 (Gelman and Silverstein, 1987) in the *Hin*cII site of pUC19. Plasmid pCPC-1B, containing six tandem −17/+32 IE-3 gene regions, was constructed by subcloning a self-ligated 68-bp *Ava*I (filled) restriction fragment from pCPC-1A into the *Hin*cII site of pUC19. IE-3 promoter-luciferase reporter constructs pCPC-4P-LUC (−323/+32) and pCPC-4P_1-LUC (−108/+32) were constructed as follows. Plasmid pCPC-4P-LUC was generated by cloning a *Bam*HI fragment containing the luciferase coding sequences from plasmid p19LUC (van Zonneveld *et al.,* 1988) into the *Bam*HI site of pIGA103 (Gelman and Silverstein, 1987). Plasmid pCPC-4P_1-LUC was constructed in two steps. First, a *Bam*HI and *Eco*RI fragment from plasmid pIGA103, containing the −108/+32 sequences from the IE-3 promoter, was cloned in vector pIC-20H (cut with *Bam*HI–*Eco*R I) to yield pCPC-4P_1. Plasmid pCPC-4P_1-LUC was generated by cloning the *Bam*HI fragment, containing the luciferase coding sequences, from plasmid p19LUC into the *Bam*HI site of pCPC-4P_1. Plasmids pCPC-I and pCPC-Y were constructed by ligating the PCR-amplified HMG I and HMG Y cDNAs with pCRII (InVitrogen). Plasmids pCPC-I_E and pCPC-Y_E, which express HMG I or HMG Y, respectively, under the control of the CMV promoter, were generated by subcloning the *Eco*RI (filled)–*Not*I fragments from plasmids pCPC-I and pCPC-Y in pcDNAI.neo (InVitrogen) that had been digested with *Eco*RV–*Not*I. Plasmid panti-HMG I(Y), expressing an antisense HMG I(Y) RNA (Thanos and Maniatis, 1992), was a gift from Dr. T. Maniatis, as was the bacterial expression construct pET15b-HMG I (see below). Bacterial plasmids pCPC-X4(2–5) expressing the ICP4 DNA-binding domain (amino acids 245–523) and pCPC-X4(5–6) expressing amino acids 573–686 of ICP4, as amino-terminal fusions with GST, were previously described (Panagiotidis *et al.,* 1997).

Protein expression and purification

The GST-fusion proteins were expressed in bacteria and purified as previously described (Panagiotidis *et al.,* 1997; Smith and Johnson, 1988). Recombinant HMG I (rHMG I) protein, carrying a hexahistidine tag fused at its amino terminus, was expressed in *Escherichia coli* BL21(DE3) transformed with plasmid pET15b-HMG I (Thanos and Maniatis, 1992). Purification of rHMG I was performed by affinity chromatography on a nickel-NTA agarose column (Qiagen), as previously described (Thanos and Maniatis, 1992). After purification, proteins were dialyzed overnight at 4°C against 200 volumes of 10 mM HEPES-KOH, pH 7.9, 50 mM NaCl, 1 mM EDTA, 4 mM dithiothreitol, and 20% (v/v) glycerol, with two changes.

Protein concentrations were determined by the Bradford method (Bradford, 1976), and protein purity was determined by SDS–PAGE (Laemmli, 1970). Proteins were stored in aliquots at −80°C.

Transfections and luciferase assays

Transfections were performed using the calcium phosphate precipitation method (Wigler *et al.,* 1979). Briefly, Vero cells were seeded at a density of 5×10^5 cells/60-mm plate the day before transfection. The culture media were replaced 5 h before transfection. Transfections were performed with calcium phosphate, using 15 μg of total plasmid DNA/plate, and the transfection mixtures were left on the cells overnight. The next day, the cells were refed with fresh media after three washes of the cell monolayers with PBS. The cells were harvested at 48 h posttransfection, and luciferase activities were determined as described previously (Brasier *et al.,* 1989), using a Berthold Lumat LB9501 luminometer (Wallac Inc., Gaithersburg, MD). Luciferase activities were determined from triplicate transfections in at least two independent experiments.

Nuclear extract preparation and EMSAs

Nuclear extracts were prepared from uninfected and HSV-1 infected HeLa cells at 7 h postinfection (Dignam *et al.,* 1983), with some modifications (Papavassiliou and Silverstein, 1990a). EMSAs were performed by incubating either 4 μg of nuclear or total cell extracts or the indicated amounts of the purified proteins with ^{32}P end-labeled restriction fragments from the IE-3 gene (−17/+32), which contains a high-affinity ICP4 binding site, followed by native PAGE (Papavassiliou and Silverstein, 1990a). The DNA-binding reactions were incubated for 20 min at room temperature in 10 mM Tris–HCl, pH 7.5, 50 mM NaCl, 5% (v/v) glycerol, 1 mM EDTA, 4 mM dithiothreitol, and 0.2 mg/ml BSA in a final volume of 20 μl. Sonicated salmon sperm DNA or poly(dG)/poly(dC) (Pharmacia) were included as nonspecific DNA competitors. Supershift analyses were performed by adding 1 μg of purified anti-ICP4 mouse MAb 58S (Showalter, Zweig, and Hampar, 1981) to each binding reaction after 20 min of incubation, and the reaction mix was incubated for an additional 30 min. The 58S MAb was equilibrated in binding buffer by dialysis. After separation by native PAGE in 0.25× TBE, 4% gels, the DNA–protein complexes were visualized by autoradiography.

DNase I footprint analysis

The DNA probe used in the footprinting experiments was labeled on one strand by end-filling with the Klenow fragment of DNA polymerase I. The probe was a *Hin*dIII–*Nco*I fragment from plasmid pIGA103 (Gelman and Silverstein, 1987), and it was labeled at the *Hin*dIII site. DNase I protection experiments were performed as previously described (Panagiotidis *et al.,* 1995), and the products of the digestion were analyzed on 6% sequencing gels.

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REFERENCES

Bellorini, M., Moncollin, V., D'Incalci, M., Mongelli, N., and Mantovani, R. (1995). Distamycin A and tallimustine inhibit TBP binding and basal *in vitro* transcription. *Nucleic Acids Res.* **23,** 1657–1663.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Brasier, A. R., Tate, J. E., and Habener, J. F. (1989). Optimized use of the firefly luciferase assay as a reporter gene in mammalian cell lines. *BioTechniques* **7,** 1116–1122.

Bustin, M., and Reeves, R. (1996). High-mobility-group chromosomal proteins: Architectural components that facilitate chromatin function. *Prog. Nucleic Acids Res. Mol. Biol.* **54,** 35–100.

Carey, M. (1998). The enhanceosome and transcriptional synergy. *Cell* **92,** 5–8.

Carrozza, M. J., and DeLuca, N. A. (1996). Interaction of the viral activator protein ICP4 with TFIID through TAF250. *Mol. Cell. Biol.* **16,** 3805–3093.

Carrozza, M. J., and DeLuca, N. A. (1998). The high mobility group protein 1 is a coactivator of herpes simplex virus ICP4 in vitro. *J. Virol.* **76,** 6752–6757.

DeLuca, N. A., McCarthy, A. M., and Schaffer, P. A. (1985). Isolation and characterization of deletion mutants of herpes simplex virus type 1 in the gene encoding immediate early regulatory protein ICP4. *J. Virol.* **56,** 558–570.

DiDonato, J. A., Spitzner, J. R., and Muller, M. T. (1991). A predictive model for DNA recognition by the herpes simplex virus protein ICP4. *J. Mol. Biol.* **219,** 451–470.

Dignam, J. D., Lebowitz, R. M., and Roeder, R. G. (1983). Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11,** 1475–1489.

Dixon, R. F., and Schaffer, P. A. (1980). Fine structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate early protein VP175. *J. Virol.* **36,** 189–203.

Eckner, R., and Birnstiel, M. L. (1989). Cloning of cDNAs coding for human HMG I and HMG Y proteins: Both are capable of binding to the octamer sequence motif. *Nucleic Acids Res.* **17,** 5947–5959.

Everett, R. D. (1984). Trans activation of transcription by herpes virus products: Requirement for two HSV-1 immediate-early polypeptides for maximum activity. *EMBO J.* **3,** 3135–3141.

Everett, R. D. (1986). The products of herpes simplex virus type 1 (HSV-1) immediate early genes 1, 2, and 3 can activate HSV-1 gene expression in trans. *J. Gen. Virol.* **68,** 2507–2513.

Everett, R. D., Elliott, M., Hope, G., and Orr, A. (1991). Purification of the DNA binding domain of herpes simplex virus type 1 immediate-early protein Vmw175 as a homodimer and extensive mutagenesis of its recognition site. *Nucleic Acids Res.* **19,** 4901–4908.

Faber, S. W., and Wilcox, K. W. (1986). Association of the herpes simplex virus regulatory protein ICP4 with specific nucleotide sequences in DNA. *Nucleic Acids Res.* **14,** 6067–6083.

Falvo, J. V., Thanos, D., and Maniatis, T. (1995). Reversal of intrinsic DNA bends in the IFNβ gene enhancer by transcription factors and the architectural protein HMG I(Y). *Cell* **83,** 1101–1111.

Fashena, S. J., Reeves, R., and Ruddle, N. H. (1992). A poly(dA-dT) upstream activating sequence binds high-mobility group I protein and contributes to lymphotoxin (tumor necrosis factor) gene regulation. *Mol. Cell. Biol.* **12,** 894–903.

French, S. W., Schmidt, M. C., and Glorioso, J. C. (1996). Involvement of a high-mobility-group protein in the transcriptional activity of herpes simplex virus latency-active promoter 2. *Mol. Cell. Biol.* **16,** 5393–5399.

Gelman, I. H., and Silverstein, S. (1985). Identification of immediate early genes from herpes simplex virus that transactivate the virus thymidine kinase gene. *Proc. Natl. Acad. Sci. USA* **82,** 5265–5269.

Gelman, I. H., and Silverstein, S. (1987). Herpes simplex virus immediate-early promoters are responsive to virus and cell trans-acting factors. *J. Virol.* **61,** 2286–2296.

Ghersa, P., Whelan, J., Cambet, Y., DeLamarter, J. F., and Hooft van Huijsduijnen, R. (1997). Distamycin prolongs E-selectin expression by interacting with a specific NF-kappaB-HMG-I(Y) binding site in the promoter. *Nucleic Acids Res.* **25,** 339–346.

Gu, B., and DeLuca, N. (1994). Requirements for activation of the herpes simplex virus glycoprotein C promoter in vitro by the viral regulatory protein ICP4. *J. Virol* **68,** 7953–7965.

Gu, B., Kuddus, R., and DeLuca, N. (1995). Repression of activator-mediated transcription by herpes simplex virus via a mechanism involving interactions with the basal transcription factors TATA-binding protein and TFIIB. *Mol. Cell. Biol.* **15,** 3618–3626.

Hardwicke, M. A., Vaughan, P. J., Sekulovich, R. E., O'Conner, R., and Sandri-Goldin, R. M. (1989). The regions important for the activator and repressor functions of herpes simplex virus type 1α protein ICP27 map to the C-terminal half of the molecule. *J. Virol.* **63,** 4590–4602.

Hardy, W. R., and Sandri-Goldin, R. M. (1994). Herpes simplex virus inhibits host cell splicing, and regulatory protein ICP27 is required for this effect. *J. Virol.* **68,** 7790–7799.

Hill, A., Jugovic, P., York, I., Russ, G., Bennink, J., Yewdell, J., Ploegh, H., and Johnson, D. (1995). Herpes simplex virus turns off the TAP to evade host immunity. *Nature* **375,** 411–415.

Honess, R. W., and Roizman, B. (1974). Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.* **14,** 8–19.

Honess, R. W., and Roizman, B. (1975). Regulation of herpesvirus macro-molecular synthesis: Sequential transition of polypeptide synthesis requires functional viral polypeptides. *Proc. Natl. Acad. Sci. USA* **72,** 1276–1280.

Johnson, K. R., Lehn, D. A., Elton, T. S., Barr, P. J., and Reeves, R. (1988). Complete murine cDNA sequence, genomic structure, and tissue expression of the high mobility group protein HMG I(Y). *J. Biol. Chem.* **263,** 18338–18342.

Johnson, K. R., Lehn, D. A., and Reeves, R. (1989). Alternative processing of mRNAs encoding mammalian chromosomal high-mobility-group proteins HMG-I and HMG-Y. *Mol. Cell. Biol.* **9,** 2114–2123.

Jordan, R., and Schaffer, P. (1997). Activation of gene expression by herpes simplex virus type 1 ICP0 occurs at the level of mRNA synthesis. *J. Virol.* **71,** 6850–6862.

Knipe, D. M., Ruyechan, W. T., Roizman, B., and Halliburton, I. A. (1978). Molecular genetics of herpes simplex virus: Demonstration of regions of obligatory and nonobligatory identity within diploid regions of the genome by sequence replacement and insertion. *Proc. Natl. Acad. Sci. USA* **75,** 3896–3900.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685.

Lehming, N., Thanos, D., Brickman, J. M., Ma, J., Maniatis, T., and Ptashne, M. (1994). An HMG-like protein that can switch a transcriptional activator to a repressor. *Nature* **371,** 175–179.

Lium, E. K., Panagiotidis, C. A., Wen, X., and Silverstein, S. J. (1996). Repression of the α0 gene by ICP4 during a productive herpes simplex virus infection. *J. Virol.* **70,** 3488–3496.

Maher, J. F., and Nathans, D. (1996). Multivalent DNA-binding properties of the HMG-I proteins. *Proc. Natl. Acad. Sci. USA* **93,** 6716–6720.

McCarthy, A. M., McMahan, L., and Schaffer, P. A. (1989). Herpes simplex virus type 1 ICP27 deletion mutants exhibit altered patterns of transcription and are DNA deficient. *J. Virol.* **63,** 18–27.

McGregor, F., Phelan, A., Dunlop, J., and Clements, J. B. (1996). Regulation of herpes simplex virus poly(A) site usage and the action of

immediate-early protein IE63 in the early-late switch. *J. Virol.* **70,** 1931–1940.

McLauchlan, J., Phelan, A., Loney, C., Sandri-Goldin, R. M., and Clements, J. B. (1992). Herpes simplex virus IE63 acts at the posttranscriptional level to stimulate viral mRNA 3′ processing. *J. Virol.* **66,** 6939–6945.

McLauchlan, J., Simpson, S., and Clements, J. B. (1989). Herpes simplex virus induces a processing factor that stimulates poly(A) site usage. *Cell* **59,** 1093–1105.

McMahan, L., and Schaffer, P. A. (1990). Repressing and enhancing functions of the herpes simplex virus regulatory protein ICP27 map to C-terminal regions and are required to modulate viral gene expression very early in infection. *J. Virol.* **64,** 3471–3485.

Michael, N., and Roizman, B. (1989). Binding of the herpes simplex virus major regulatory protein to viral DNA. *Proc. Natl. Acad. Sci. USA* **86,** 9808–9812.

Michael, N., Spector, D., Mavromara-Nazos, P., Kristie, T. M., and Roizman, B. (1988). The DNA-binding properties of the major regulatory protein α4 of herpes simplex viruses. *Science* **239,** 1531–1534.

Mote, J., J., Ghanouni, P., and Reines, D. (1994). A DNA minor groove-binding ligand both potentiates and arrests transcription by RNA polymerase II. *J. Mol. Biol.* **236,** 725–737.

Mullen, M. A., Gerstberger, S., Ciufo, D. M., Mosca, J. D., and Hayward, G. S. (1995). Evaluation of colocalization interactions between the IE110, IE175, and IE63 transactivator proteins of herpes simplex virus within subcellular punctate structures. *J. Virol.* **69,** 476–491.

O'Hare, P., and Hayward, G. S. (1985a). Evidence for a direct role for both the 175000 and 110000 molecular-weight immediate early proteins of herpes simplex virus in the transactivation of delayed-early promoters. *J. Virol.* **53,** 751–760.

O'Hare, P., and Hayward, G. S. (1985b). Three trans-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. *J. Virol.* **56,** 723–733.

Ogawa, Y., Aizawa, S., Shirakawa, H., and Yoshida, M. (1995). Stimulation of transcription accompanying relaxation of chromatin structure in cells overexpressing high mobility group 1 protein. *J. Biol. Chem.* **270,** 9272–9280.

Panagiotidis, C. A., Artandi, S., Calame, K., and Silverstein, S. J. (1995). Polyamines alter sequence-specific DNA-protein interactions. *Nucleic Acids Res.* **23,** 1800–1809.

Panagiotidis, C. A., Lium, E. K., and Silverstein, S. J. (1997). Physical and functional interactions between herpes simplex virus immediate early proteins ICP4 and ICP27. *J. Virol.* **71,** 1547–1557.

Papavassiliou, A. G., and Silverstein, S. J. (1990a). Characterization of DNA-protein complex formation in nuclear extracts with a sequence from the herpes simplex virus thymidine kinase gene. *J. Biol. Chem.* **265,** 1648–1657.

Papavassiliou, A. G., and Silverstein, S. J. (1990b). Interaction of cell and virus proteins with DNA sequences encompassing the promoter/regulatory and leader regions of the herpes simplex virus thymidine kinase gene. *J. Biol. Chem.* **265,** 9402–9412.

Papavassiliou, A. G., Wilcox, K. W., and Silverstein, S. J. (1991). The interaction of ICP4 with cell/infected factors and its state of phosphorylation modulate differential recognition of leader sequences in herpes simplex virus DNA. *EMBO J.* **10,** 397–406.

Phelan, A., Carmo-Fonseca, M., McLauchlan, J., Lamond, A. I., and Clements, J. B. (1993). A herpes simplex virus type 1 immediate-early gene product, IE63, regulates small nuclear ribonucleoprotein distribution. *Proc. Natl. Acad. Sci. USA* **90,** 9056–9060.

Preston, C. M. (1979). Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant tsK. *J. Virol.* **29,** 275–284.

Quinlan, M. P., and Knipe, D. (1985). Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral factors. *Mol. Cell. Biol.* **5,** 957–963.

Reeves, R., and Nissen, M. S. (1990). The A-T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. *J. Biol. Chem.* **265,** 8573–8582.

Rice, S. A., and Knipe, D. M. (1988). Gene-specific *trans*-activation by the herpes simplex virus type 1 alpha protein ICP27. *J. Virol.* **62,** 3814–3823.

Rice, S. A., and Knipe, D. M. (1990). Genetic evidence for two distinct trans-activation functions of the herpes simplex virus α protein ICP27. *J. Virol.* **64,** 1704–1715.

Rice, S. A., Long, M. C., Lam, V., Schaffer, P. A., and Spencer, C. A. (1995). Herpes simplex virus immediate-early protein ICP22 is required for viral modification of host RNA polymerase II and establishment of the normal viral transcription program. *J. Virol.* **69,** 5550–5559.

Roberts, M. S., Boundy, A., O'Hare, P., Pizzorno, M. C., Ciufo, D. M., and Hayward, G. S. (1988). Direct correlation between a negative autoregulatory response element at the cap site of the herpes simplex virus type 1 IE175 (α4) promoter and a specific binding site for the IE175 (ICP4) protein. *J. Virol.* **62,** 4307–4320.

Roizman, B., and Sears, A. (1996). Herpes simplex viruses and their replication. *In* "Fields Virology," 3rd ed. (B. N. Fields, D. M. Knipe, and P. M. Howley, eds.), Vol. 2, pp. 2231–2295. Lippincott-Raven Publishers, Philadelphia.

Sacks, W. R., Greene, C. C., Aschman, D. P., and Schaffer, P. A. (1985). Herpes simplex virus type 1 ICP27 is an essential regulatory protein. *J. Virol.* **55,** 796–805.

Samaniego, L. A., Webb, A. L., and DeLuca, N. A. (1995). Functional interactions between herpes simplex virus immediate-early proteins during infection: Gene expression as a consequence of ICP27 and different domains of ICP4. *J. Virol.* **69,** 5705–5715.

Sandri-Goldin, R. M. (1998). ICP27 mediates HSV RNA export by shuttling through a leucine-rich nuclear export signal and binding viral intronless RNAs through an RGG motif. *Genes Dev.* **12,** 868–879.

Sandri-Goldin, R. M., and Mendoza, G. E. (1992). A herpesvirus regulatory protein appears to act post-transcriptionally by affecting mRNA processing. *Genes Dev.* **6,** 848–863.

Sekulovich, R. E., Leary, K., and Sandri-Goldin, R. M. (1988). The herpes simplex virus type 1 α protein ICP27 can act as a *trans*-repressor or a *trans*-activator in combination with ICP4 and ICP0. *J. Virol.* **62,** 4510–4522.

Shepard, A. A., Imbalzano, A. N., and DeLuca, N. A. (1989). Separation of primary structural components conferring autoregulation, transactivation, and DNA-binding properties to the herpes simplex virus transcriptional regulatory protein ICP4. *J. Virol.* **63,** 3714–3728.

Showalter, S. D., Zweig, M., and Hampar, B. (1981). Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP4. *Infect. Immunol.* **34,** 684–692.

Singh, H., LeBowitz, J. H., Baldwin, J., A. S., and Sharp, P. A. (1988). Molecular cloning of an enhancer binding protein: Isolation by screening of an expression library with a recognition site DNA. *Cell* **52,** 415–423.

Smith, C. A., Bates, P., Rivera-Gonzalez, R., Gu, B., and DeLuca, N. A. (1993). ICP4, the major transcriptional regulatory protein of herpes simplex virus type 1, forms a tripartite complex with TATA-binding protein and TFIIB. *J. Virol.* **67,** 4676–4687.

Smith, D. B., and Johnson, K. S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* **67,** 31–40.

Soliman, T., Sandri-Goldin, R., and Silverstein, S. (1997). Shuttling of the herpes simplex virus type 1 regulatory protein ICP27 between the nucleus and cytoplasm mediates the expression of late proteins. *J. Virol.* **71,** 9188–9197.

Solomon, M. J., Strauss, F., and Varshavsky, A. (1984). A mammalian high mobility group protein recognizes any stretch of six A-T base pairs in duplex DNA. *Proc. Natl. Acad. Sci. USA* **83,** 1276–1280.

Su, L., and Knipe, D. M. (1989). Herpes simplex virus α protein ICP27 can inhibit or augment viral gene transactivation. *Virology* **170,** 496–504.

Tabor, C. W., and Tabor, H. (1984). Polyamines. *Annu. Rev. Biochem.* **53,** 749–790.

Thanos, D., and Maniatis, T. (1992). The high mobility group protein HMG I(Y) is required for NF-κB-dependent virus induction of the human IFN-β gene. *Cell* **71,** 777–789.

Thanos, D., and Maniatis, T. (1995). Virus induction of human IFNβ gene expression requires the assembly of an enhanceosome. *Cell* **83,** 1091–1100.

van Zonneveld, A. J., Curriden, S. A., and Loskutoff, D. J. (1988). Type 1 plasminogen activator inhibitor gene: Functional analysis and glucocorticoid regulation of its promoter. *Proc. Natl. Acad. Sci. USA* **85,** 5525–5529.

Watson, R. J., and Clements, J. B. (1978). Characterization of transcription-deficient temperature-sensitive mutants of herpes simplex virus. *Virology* **91,** 364–379.

Watson, R. J., and Clements, J. B. (1980). A herpes simplex type 1 function continuously required for early and late virus RNA synthesis. *Nature* **285,** 329–330.

Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G., and Chasin, L. (1979). DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. *Proc. Natl. Acad. Sci. USA* **76,** 1373–1376.

Wood, L. D., Farmer, A. A., and Richmond, A. (1995). HMGI(Y) and Sp1 in addition to NF-kappa B regulate transcription of the MGSA/GRO alpha gene. *Nucleic Acids Res.* **23,** 4210–4219.

Xia, K., DeLuca, N. A., and Knipe, D. M. (1996). Analysis of phosphorylation sites of herpes simplex virus type 1 ICP4. *J. Virol.* **70,** 1061–1071.

York, I. A., Roop, C., Andrews, D. W., Riddell, S. R., Graham, F. L., and Johnson, D. C. (1994). A cytosolic herpes simplex virus protein inhibits antigen presentation to $CD8^+$ T lymphocytes. *Cell* **77,** 525–535.

Zhou, X., and Chada, K. (1998). HMGI family proteins: Architectural transcription factors in mammalian development and cancer. *Keio J. Med.* **47,** 73–77.

Zhu, Z., Cai, W., and Schaffer, P. A. (1994). Cooperativity among herpes simplex virus type 1 immediate-early regulatory proteins: ICP4 and ICP27 affect the intracellular localization of ICP0. *J. Virol.* **68,** 3027–3040.

Zhu, Z., and Schaffer, P. A. (1995). Intracellular localization of the herpes simplex virus type 1 major transcriptional regulatory protein, ICP4, is affected by ICP27. *J. Virol.* **69,** 49–59.