

The core histone-binding region of the murine cytomegalovirus 89K immediate early protein

Konrad Münch, Brigitte Bühler, Martin Messerle and Ulrich H. Koszinowski*

Department of Virology, Institute for Microbiology, University of Ulm, 7900 Ulm, Germany

The gene regulatory immediate early protein, pp89, of murine cytomegalovirus interacts with both DNA-associated and isolated histones *in vitro*. We characterized the histone-binding region of pp89 and its cellular localization during cell division to examine the possible interaction between pp89 and chromatin. pp89 expressed constitutively in cell line BALB/c 3T3 IE1 does not interact with condensed chromatin. As observed in infected cells, pp89 is localized within the nucleus of cells during interphase but spreads throughout the cell plasma following degradation of the

nuclear membrane during early mitosis. In late telophase, pp89 is reorganized within the nucleus. Analysis of pp89 deletion mutants and of fragments generated by cleavage at pH 2.5 revealed that the regions responsible for association with histone are located between amino acids 71 and 415, and are not identical with the domain that shows homology to histone H2B or the highly acidic carboxy-terminal region. A potential gene-activating role of the high affinity of pp89 for isolated histones and the low affinity for DNA-associated histones is discussed.

Introduction

As with other herpesviruses, the expression of genes from the 235 kbp DNA genome of murine cytomegaloviruses (MCMV) is temporally controlled and regulated in a cascade fashion (Keil *et al.*, 1984). At least three classes of MCMV genes, immediate early (IE or α), early (E or β) and late (or γ), can be differentiated. Three major MCMV IE genes, IE1, IE2 and IE3, have been identified (Keil *et al.*, 1984, 1987*b*; Messerle *et al.*, 1991); genes IE1 and IE3 share the same promoter, and are separated from gene IE2 by a regulator region containing enhancer elements. Whereas gene IE2 has no counterpart among human cytomegalovirus (HCMV) IE genes (Messerle *et al.*, 1990), the structural organization of the HCMV IE1/IE2 gene complex is comparable with that of MCMV IE1/IE3 (Keil *et al.*, 1987*a*; Stinski *et al.*, 1983); IE1 products are encoded by exons 2, 3 and 4, and IE3 products by exons 2, 3 and 5. IE proteins are required for the trans-activation of E gene promoters, but no function in transcriptional regulation has been assigned to the IE2 product (Koszinowski *et al.*, 1986). Activation of heterologous, viral and cellular promoters by the MCMV IE1 protein, pp89, has been demonstrated by transfection of cells with IE1 gene (Koszinowski *et al.*, 1986; Schickedanz *et al.*, 1988). This is in contrast to the inability of the IE1 protein expressed in transfected cells to activate the MCMV early promoter E1 which requires the presence of IE3 gene products (Buehler *et al.*, 1990).

However, transient expression experiments have revealed that E gene activation by the IE3 gene products can be augmented by the IE1 protein (M. Messerle, unpublished results).

Thorough functional analysis of the homologous gene complex, IE1/IE2, in HCMV has revealed that IE1 products activate the IE1 gene promoter, whereas IE2 products have been found to be essential for the activation of E gene promoters (Sambucetti *et al.*, 1989; Malone *et al.*, 1990; Stenberg *et al.*, 1990).

To understand better the role of the MCMV IE1 protein pp89 in the activation of heterologous promoters, we have started to examine the properties of the IE1 protein predicted from amino acid sequence data. The amino acid sequence of the MCMV IE1 protein contains a region of homology to histone H2B and a highly acidic carboxy-terminal region. *In vitro* studies have revealed that pp89 is eluted from chromatin at low salt concentration, but binds strongly to isolated histones (Muench *et al.*, 1988). As the HCMV IE1 protein, which also contains an acidic carboxy-terminal region, recently has been shown to interact with metaphase chromosomes (LaFemina *et al.*, 1989), we investigated whether the high affinity of pp89 for histones observed *in vitro* correlates with an association of pp89 with chromatin *in vivo*. From the data presented we conclude that pp89 does not bind to condensed chromatin and that neither the region of homology to histone H2B nor the acidic region are essential for histone binding.

Methods

Virus and cell culture. MCMV (mouse salivary gland virus, strain Smith, ATCC VR-194) was used to infect BALB/c mouse embryonal fibroblasts (MEFs) as described (Muench *et al.*, 1988). Construction of vaccinia virus recombinants has been described by Volkmer *et al.* (1987). KD2SV cells were infected with recombinant vaccinia viruses at an m.o.i. of 20 and 12 h post-infection (p.i.) were labelled with 1 mCi/ml [³⁵S]methionine (Amersham) for 4 h.

Protein extraction. Whole cell extracts were prepared as described previously (Muench *et al.*, 1988).

pp89 was isolated and cleaved at pH 2.5 as follows. Infected and radiolabelled MEFs (10⁶) were suspended in 1 ml 3% (w/v) SDS and subjected to SDS gel electrophoresis, after which the gel was exposed to Kodak XAR film to identify the pp89 band. The region of the gel containing proteins of *M*_r 85K to 95K was excised and homogenized, and 250 mg of this material was suspended in 1 ml cleavage buffer (0.1% w/v SDS, 200 mM-glycine, 0.025 mM-Tris-HCl, adjusted to pH 2.5 with TCA) and incubated for 20 min at 95 °C. This treatment cleaves pp89 quantitatively and elutes the resulting fragments from the gel homogenate. After centrifugation at 3000 *g* for 30 min, the supernatant was desalted against binding buffer (20 mM-Tris-HCl pH 7.5, 50 mM-NaCl, 1 mM-EDTA, 10% v/v glycerol) on Sepharose G25 columns. To elute uncleaved pp89, the gel homogenate was incubated in 0.1% (w/v) SDS, 200 mM-glycine, 0.025 mM-Tris-HCl at 4 °C for 16 h and desalted against binding buffer.

To extract histones, mouse livers were passed through a stainless steel mesh and the homogenate was washed three times with 50 ml PBS; nuclei were isolated from the pellet as described (Del Val *et al.*, 1989). Proteins soluble in 0.5 M-NaCl were extracted by shaking the nuclei three times for 30 min in 3 ml extraction buffer (20 mM-Tris-HCl pH 7.5, 0.5 M-NaCl, 10% v/v glycerol). Subsequently, histones were extracted by shaking the pellet in extraction buffer containing 2 M-NaCl for 30 min. Cell debris was removed by centrifugation at 100000 *g* for 30 min and, for the removal of DNA, the supernatant was incubated for 30 min with 500 mg hydroxylapatite and cleared by centrifugation at 100000 *g* for 30 min.

Histone-binding assays

(i) **Chromatography on histone-Sepharose.** Chromatography of cell extracts and pp89 fragments on histone-Sepharose was carried out as described previously (Muench *et al.*, 1988). Core histones H3, H2B, H2A and H4 were from Boehringer Mannheim.

(ii) **Blot hybridization.** Histones were separated by SDS gel electrophoresis and transferred to nitrocellulose. Free binding sites were blocked by incubation in PBS containing 1% bovine serum albumin. [³⁵S]Methionine-labelled pp89 (10⁵ c.p.m.) eluted from an SDS gel and 10⁵ c.p.m. ³²P-labelled viral DNA were diluted in 5 ml binding buffer and incubated with the nitrocellulose for 14 h at 4 °C. The filters were washed three times for 10 min in binding buffer, dried and exposed to Kodak XAR film.

Immunoprecipitation and immunofluorescence. Immunoprecipitation, gel electrophoresis and indirect immunofluorescence were carried out as described previously (Buehler *et al.*, 1990; Muench *et al.*, 1988). Analysis of the cellular distribution of pp89 during mitosis was carried out on BALB/c 3T3 IE1 cells grown to 50% confluence.

Transfection and selection of transfectants. BALB/c 3T3 cells were transfected with plasmids pIE100 (Koszinowski *et al.*, 1986), containing the IE1 gene, and pAG60, providing the kanamycin-neomycin resistance gene. Cell lines were selected as described previously (Buehler *et al.*, 1990).

Antisera and monoclonal antibodies (MAbs). For the identification of pp89, antibodies from several sources were used. MAb 6/15/1 was

produced as described (Keil *et al.*, 1985); antisera against synthetic peptides composed of amino acids 34 to 53, 87 to 104 and 271 to 295 of pp89 were raised in rabbits (Del Val *et al.*, 1989); an antiserum was raised in mice against a bacterial TrpE fusion protein containing amino acids 532 to 595 of pp89, generated by fusing the relevant MCMV IE1 gene sequence to the *Sma*I site of the pATH 2 shuttle vector (Dieckmann & Tzagloff, 1985; Spindler *et al.*, 1984); serum from a mouse recovering from MCMV infection was collected (Reddehase *et al.*, 1984).

Results

Intracellular localization of pp89 during mitosis

During infection of MEFs, and following transfection or injection of the IE1 gene into LTK⁻ or NIH 3T3 cells, the IE1 gene product pp89 accumulates within the nucleus (Del Val *et al.*, 1989; Koszinowski *et al.*, 1986; Schickedanz *et al.*, 1988). *In vitro*, pp89 associates strongly with histones, presumably mediated by a region that shows homology to histone H2B or by the acidic carboxy-terminal region (Muench *et al.*, 1988). LaFeina *et al.* (1989) recently reported that the interaction of the HCMV IE1 protein with metaphase chromosomes depends on the presence of exon 4 sequences, which also make up an acidic region.

To determine whether pp89 also associates with cellular chromatin, BALB/c 3T3 cells, permissive for MCMV infection, were stably transfected with the IE1 gene and the intracellular distribution of the polypeptide was followed during cell division (Fig. 1). Using indirect immunofluorescence, pp89 was detected in the nucleus in interphase, but was dispersed throughout the cell plasma during metaphase (arrow in *b*), whereas the chromatin was condensed (arrow in *a*) but clearly was not associated with the pp89-specific immunofluorescence. This distribution of pp89 was the same during the subsequent anaphase (arrow in *d*) and in early telophase (not shown). During late telophase, in which the nuclear membrane is reformed, pp89-specific immunofluorescence was found in the cytoplasm and also at a higher concentration within the nuclei (arrow in *f*), indicating that pp89 again accumulates within the nucleus at this stage of cell division. The same results were obtained using a series of pp89-specific MAbs and antisera (data not shown); 200 cells with typical metaphase, anaphase and telophase patterns, respectively, were analysed for each MAb and antiserum. The amount of pp89 expressed in individual cells varied greatly, but no deviation from the distribution patterns of pp89 described above could be detected.

The pp89 polypeptides synthesized in BALB/c 3T3 IE1 cells migrated similarly to pp89 from MCMV-infected cells in SDS-PAGE, and their proteolytic processing, recognition by a series of MAbs and affinity

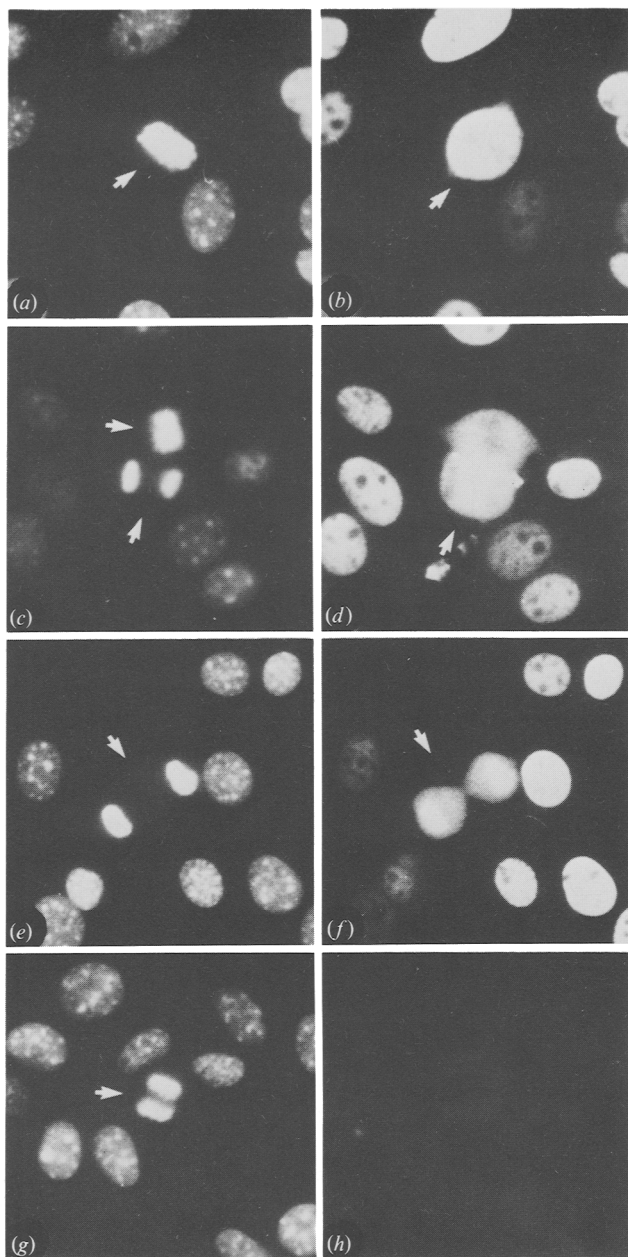


Fig. 1. Cellular location of pp89 during mitosis. BALB/c 3T3 IE1 cells (a to f) expressing pp89 and non-transfected 3T3 cells (g and h) were used for indirect immunofluorescence with MAb 6/15/1 (b, d, f and h). To stain DNA, Hoechst dye 33258 was used (a, c, e and g). Cells showing characteristic distribution of chromatin during different mitotic stages are marked by arrows.

for histones also was similar (data not shown). This argued against the selection of a spontaneous pp89 mutant with peculiar properties. In addition, several transfectants expressing the IE1 and IE3 genes showed the same cellular distribution of pp89 during cell division (data not shown). The non-transfected control cell line, 3T3, was stained for detection of chromatin (g) and pp89 (h), and did not react with antibodies to pp89. These data

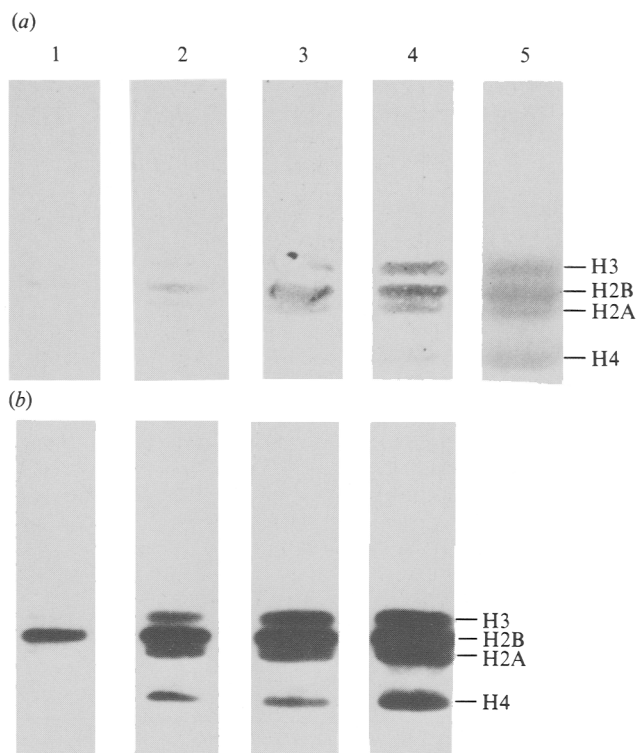


Fig. 2. Recognition of different histone species by denatured pp89. SDS gel electrophoresis was used to separate 2 μ g, 5 μ g, 10 μ g and 20 μ g of murine histones (lanes 1 to 4), which were transferred to nitrocellulose and incubated with [35 S]methionine-labelled pp89 (a) or 32 P-labelled DNA (b). Proteins binding DNA or pp89 were detected by autoradiography. Coomassie blue-stained histones are shown in lane 5.

suggest that pp89 does not associate with cellular chromatin during mitosis; it differs from the HCMV IE1 protein in this respect. To determine the reasons for this unexpected result, we analysed the properties of pp89 which mediate its interaction with histones.

Non-selective binding of pp89 to histone species

pp89 and its post-translational modification product, pp76, bind to isolated histones *in vitro* with an affinity that is resistant to dissociation at an ionic strength of 2 M-NaCl (Muench *et al.*, 1988). The region spanning amino acids 96 to 153 shows striking homology to a sequence between amino acids 27 and 87 of histone H2B (Keil *et al.*, 1987a). This region of histone H2B is involved in its interaction with histone H2A (Isenberg, 1979; Kedes, 1979).

To determine whether pp89 also binds preferentially to histone H2A, 35 S-labelled pp89 was eluted from SDS gels after electrophoresis of cell extracts containing a large amount of pp89 (see Fig. 5a) and incubated with nitrocellulose-bound histones (Fig. 2a, lanes 1 to 4); all four core histone species were recognized by pp89.

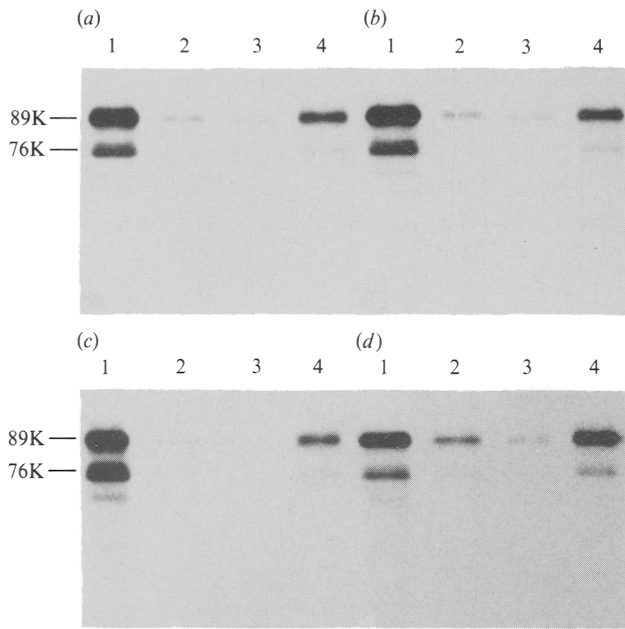


Fig. 3. Recognition of different histone species by native pp89. Extracts of cells labelled with [³⁵S]methionine during the immediate early phase of infection were applied to columns containing histones H3, H2A, H2B and H4, respectively (*a* to *d*). Unbound proteins were eluted with 0.5 M-NaCl (lanes 1); histone-bound proteins were eluted subsequently with 1 M- or 2 M-NaCl (lanes 2 and 3), or 4 M-guanidine hydrochloride (lanes 4). Eluted proteins were immunoprecipitated and subjected to gel electrophoresis and fluorography.

Histone-binding proteins from mock-infected cells with gel migration properties similar to pp89 were not detectable (data not shown). Binding of pp89 to histone H2B appeared to be most efficient. To determine whether this was due to a higher affinity for histone H2B or to a larger amount of histone H2B, the same protocol was carried out using ³²P-labelled DNA as probe (Fig. 2*b*, lanes 1 to 4); again, all four core histone species were labelled and histone H2B showed the strongest reaction, indicating that this histone represented the most abundant species and arguing against selective binding to histone H2B. Coomassie blue staining of histones also revealed that histone H2B was the most abundant species in this preparation (lane 5).

To examine whether denaturation of pp89 during isolation influenced the selectivity of histone binding, extracts from infected cells were chromatographed on Sepharose columns containing purified histones H3, H2A, H2B or H4 (Fig. 3); again, all four core histone species were recognized. The amount of histone-bound pp89 varied to some extent, but all histone species were bound with an affinity that was not affected by 2 M-NaCl. Thus, there was no indication that pp89 has preferential affinity for any histone species.

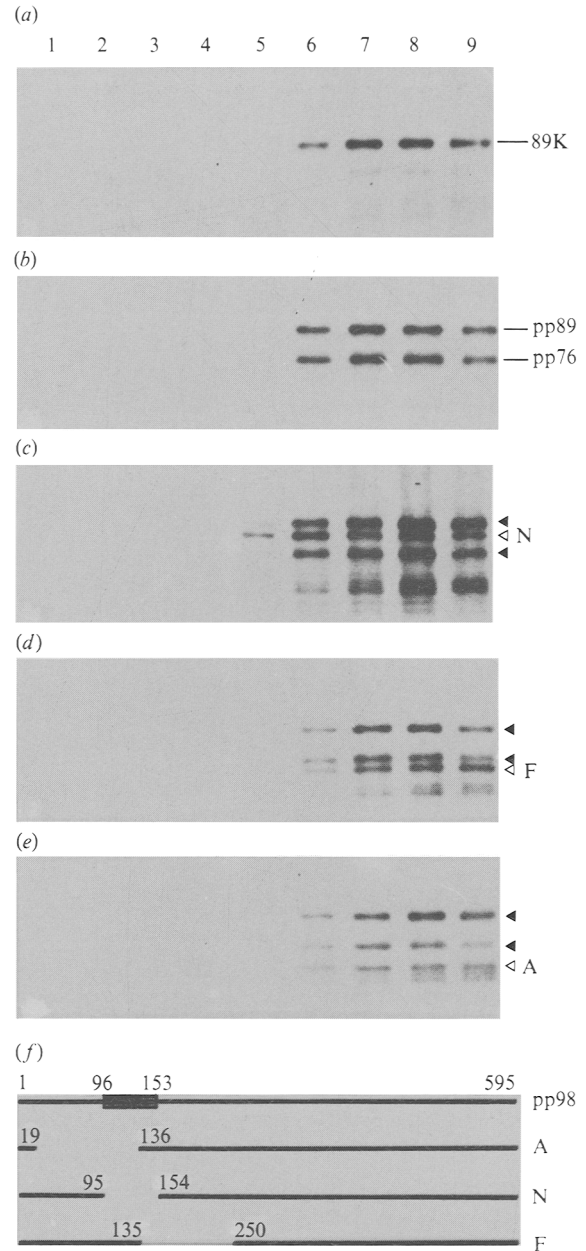


Fig. 4. Analysis of the histone affinity of mutant pp89 polypeptides. [³⁵S]Methionine-labelled extracts of cells infected with recombinant vaccinia viruses expressing wild-type pp89 or deletion mutants were applied to histone-Sepharose columns. Unbound proteins were eluted with binding buffer (lanes 1); bound proteins were eluted stepwise with binding buffer containing 2 M-NaCl (lanes 2) and subsequently with 1, 1.5, 2, 2.5, 3, 3.5 and 4 M-guanidine hydrochloride in 0.25, 0.375, 0.5, 0.625, 0.75, 0.875 and 1 M-ammonia, respectively (lanes 3 to 9). Eluted proteins were immunoprecipitated and subjected to gel electrophoresis and fluorography. (*a*) Extracts of cells infected with vaccinia virus expressing wild-type pp89. (*b*) Extracts of MEFs infected with MCMV under IE conditions. (*c*, *d* and *e*) A mixture of MCMV IE extracts and extracts of cells infected with vaccinia virus expressing deletion mutants N, F or A, respectively. (*f*) Physical map of the pp89 polypeptides expressed by recombinant vaccinia viruses. Amino acid positions of deleted sequences of mutants A, N and F are indicated in rows 2 to 4. The hatched box marks the region that shows homology to histone H2B.

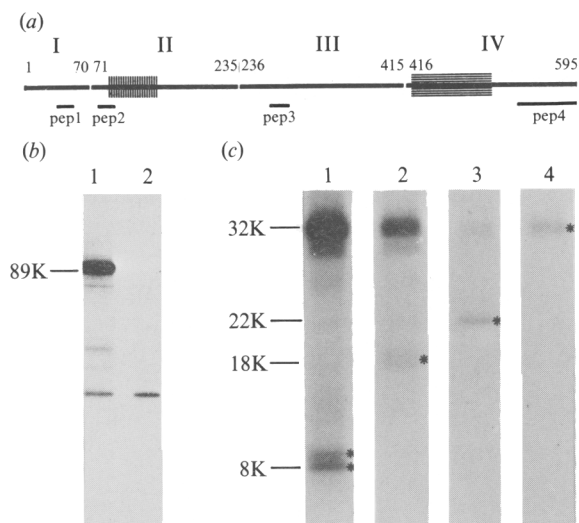


Fig. 5. (a) Physical map of sites in pp89 cleaved at pH 2.5. Aspartic acid-proline bonds that are cleaved at pH 2.5 are found at amino acid positions 70 to 71, 235 to 236 and 415 to 416. Peptides (pep1 to pep4) used to elicit antibodies against different epitopes of pp89 are shown as bars. The region that shows homology with histone H2B is marked as a vertically hatched box and the acidic region is marked as a horizontally hatched box. (b) Enhanced expression of pp89. MEFs were either infected (lane 1) or mock-infected (lane 2) in the presence of cycloheximide, replaced 5 h p.i. with actinomycin D. Cells were disrupted by sample buffer 11 h p.i., and subjected to gel electrophoresis and autoradiography. (c) Immunological characterization of pp89 fragments produced at pH 2.5. pp89 was cleaved at pH 2.5 and the resulting fragments were precipitated with sera raised against peptides 1 to 4 (lanes 1 to 4).

The region of homology to histone H2B is dispensable for histone binding

In the absence of selective affinity for histone H2A, it was important to determine whether the region of homology to histone H2B did contribute to the binding of pp89 to histones. Therefore, internal deletion mutants of the IE1 gene, which partially or completely lack the sequence encoding the region of homology to H2B, were constructed (Fig. 4f) and expressed in recombinant vaccinia virus.

To determine the histone affinity of the resulting polypeptides, extracts of infected cells were applied to histone-Sepharose columns (Fig. 4). Proteins without binding activity were eluted with binding buffer (lanes 1) and bound proteins were eluted stepwise with binding buffer containing 2 M-NaCl, and subsequently with increasing concentrations of guanidine hydrochloride and ammonia (lanes 2 to 9). About 70% of the labelled protein was eluted from the column by 0.1 M-NaCl and a further 25% by 2 M-NaCl. Immunoprecipitates of these eluates did not contain IE proteins (lanes 1 and 2). Wild-type pp89, expressed by recombinant vaccinia virus, bound avidly to histones and was eluted by 2.5 and 4 M-guanidine hydrochloride (Fig. 4a, lanes 6 to 9). This

elution profile is identical to that of pp89 and pp76 expressed by MCMV (Fig. 4b). In MCMV-infected cells, a large amount of pp89 is proteolytically cleaved to produce pp76 (Fig. 4b), whereas in vaccinia virus-infected cells pp89 seems to be more stable (Fig. 4a). As the faint bands below the 89K band in Fig. 4(a) were also seen in precipitates of non-infected 3T3 cells (data not shown), they were considered to represent proteins derived from 3T3 cells, which can vary to some extent (compare the bands below those indicated in Fig. 4a, c, d and e). To compare directly the affinity of pp89 deletion mutants for histone with that of the wild-type protein, extracts of MCMV-infected cells were mixed with extracts of cells infected with vaccinia virus recombinants prior to chromatography.

pp89 deletion mutants A and F exhibited an elution profile comparable to that of wild-type pp89. Only pp89 deletion mutant N, which completely lacks the region of sequence homology to histone H2B, showed a slightly different elution profile (Fig. 4c); histone-pp89 deletion mutant N complexes were less stable and started to dissociate with 2 M-guanidine hydrochloride. As this change in histone binding is very subtle, it was concluded that, in addition to the region of histone H2B homology, other regions of pp89 must contribute to its affinity for histones.

Affinity of pp89 fragments for histones

The pp89 amino acid sequence includes a highly acidic domain, amino acids 424 to 532, which contains 42 acidic amino acids and only one basic amino acid.

To test the histone-binding capability of this acidic region, pp89 was isolated and fragmented by pH 2.5 cleavage; aspartic acid-proline bonds are cleaved under acidic conditions (Jauregui & Marti, 1975; Landon, 1977) owing to the more basic nature of the proline nitrogen and an enhanced α - β isomerization reaction for aspartic acid residues linked to proline (Piszkiwicz *et al.*, 1970). The 595 amino acid sequence of pp89 contains three aspartic acid-proline bonds at positions 70 to 71, 235 to 236 and 415 to 416 (Fig. 5). Complete pH 2.5 cleavage of pp89 should yield four fragments of calculated M_r of 7.8K, 18.8K, 19.4K and 20.9K. The latter fragment should contain the acidic domain.

Enrichment of pp89 for cleavage at pH 2.5 was achieved by enhanced expression of MCMV IE proteins (Keil *et al.*, 1985) and the excision of 89K polypeptide bands from gels after SDS-PAGE. Following MCMV infection in the presence of cycloheximide, which was later replaced by actinomycin D, the major IE protein, pp89, represented the prominent radiolabelled protein (Fig. 5a, lane 1), containing about 20-fold the precipitable counts of the corresponding band from non-infected

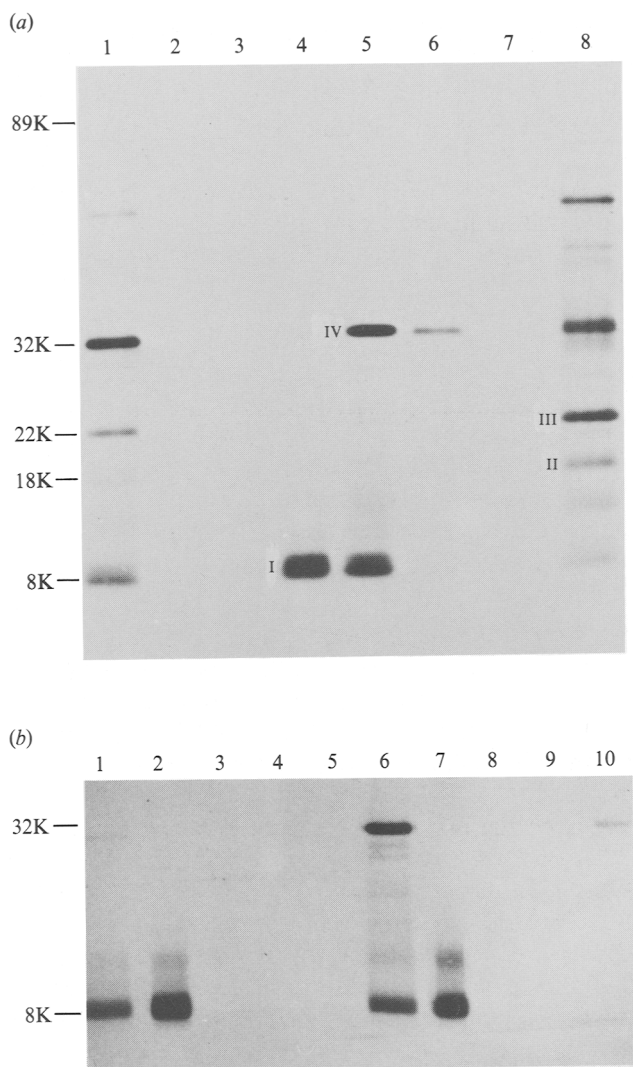


Fig. 6. Histone-Sepharose chromatography of pp89 fragments. (a) Affinity of pp89 fragments for histone. pp89 fragments generated by cleavage at pH 2.5 (lane 1) were applied to a histone-Sepharose column and unbound fragments were eluted with binding buffer (lane 2). Bound proteins were consecutively eluted with binding buffer containing 0.2, 0.4, 0.6, 1 and 2 M-NaCl, respectively (lanes 3 to 7), and subsequently with 4 M-guanidine hydrochloride, 1 M-ammonia (lane 8). (b) Identification of pp89 peptides eluted from the histone-Sepharose column using 0.4 and 0.6 M-NaCl, respectively. Eluates obtained using 0.4 M-NaCl (lane 1) and 0.6 M-NaCl (lane 6) were split into four aliquots, and proteins were precipitated with sera raised against peptides 1 to 4 (lanes 2 to 5 and 7 to 10).

cells. Acidic cleavage of pp89 eluted from gels resulted in near complete digestion and yielded fragments of apparent M_r 8K, 9K, 18K, 22K and 32K (Fig. 6a, lane 1).

The difference between calculated and apparent M_r was not surprising because the apparent M_r of pp89 is about 1.3-fold greater than predicted (Keil *et al.*, 1987a).

Owing to their aberrant migration pattern, these fragments could not be identified easily as the expected fragments, especially because fragments resulting from partial cleavage had to be taken into consideration. Therefore, peptides corresponding to regions of the four expected fragments were synthesized and used to raise antisera. The activities of some antisera were weak, but sufficient to identify the observed polypeptides (Fig. 5b).

Fragment I, of 8K to 9K, II, of 18K, III, of 22K and IV, of 32K, were recognized by peptide antisera pep1 to pep4, respectively (lanes 1 to 4), and therefore were identified as the expected fragments. A second, 32K peptide recognized by both pep1 and pep2 antisera (lanes 1 and 2) was identified as the product spanning amino acids 1 to 235, produced when cleavage of the aspartic acid-proline bond at positions 70 to 71 does not occur. The reason for the cleavage at position 70 to 71 resulting in two amino-terminal fragments migrating at 8K and 9K remained unclear.

Chromatography on histone-Sepharose revealed the histone affinity of the individual pH 2.5 fragments (Fig. 6a). In contrast to authentic pp89, the fragments of 8K and 9K, and one of the two 32K fragments were eluted by 0.4 M-, 0.6 M- and 1 M-NaCl, respectively (Fig. 6a, lanes 4, 5 and 6); with 2 M-NaCl, no further fragments were eluted (lane 7). The complexes of histones with fragments II and III remained stable with up to 2 M-NaCl, and were only dissociated by guanidine hydrochloride (lane 8). Within this fraction, several other pp89 products were found which represented partially cleaved products consisting of sequences from fragment II, fragment III or both.

To identify polypeptides with low affinity for histones, immunoprecipitation of the 0.4 M- and 0.6 M-NaCl eluates with all four antipeptide sera was carried out (Fig. 6b). As expected, pep1 identified the 8K polypeptide as fragment I (lane 2). The 0.6 M-NaCl eluate contained an 8K and a 32K polypeptide; pep1 precipitated the 8K polypeptide but not the 32K polypeptide (lane 7). Since pep1 strongly precipitates the incomplete cleavage product consisting of fragments I and II that migrates at 32K (Fig. 5b, lane 1), the 32K protein eluting at low salt concentration must represent a different part of pp89. Positive identification of this polypeptide was achieved by precipitation with pep4 (lane 10), which identified highly acidic fragment IV as a polypeptide with low affinity for histones.

Cleavage of pp89 at pH 2.5 yields incomplete cleavage products in addition to the four expected fragments and, therefore, it was concluded that the 32K polypeptide in the guanidine hydrochloride eluate represents fragments I and II. Together these results demonstrate that the domains represented by fragments II and III mediate the histone-binding property of pp89.

Discussion

In a previous study we observed that the MCMV IE1 protein, pp89, dissociates from DNA-bound histones in 0.3 M- to 0.6 M-NaCl, whereas that bound to Sepharose-coupled histones is resistant to 2 M-NaCl (Muench *et al.*, 1988). In this study we have tested the interaction between pp89 and histones *in vivo*, and located the domains of the protein that mediate this effect. We found that (i) pp89 is localized within the nucleoplasm of interphase cells, but clearly is excluded from the condensed chromatin during mitosis, and (ii) the domain that mediates the high affinity binding between pp89 and histones is encoded by exon 4 of the IE1 gene, but is not identical with the domain which shows homology to histone H2B, and the highly acidic carboxy-terminal region is not involved.

Recently, LaFemina *et al.* (1989) reported that the IE1 protein of HCMV expressed in BALB/c 3T3 cells associates with metaphase chromosomes. The intrinsic property which allows the HCMV IE1 protein to associate with condensed chromatin is unknown and its affinity for histones has not been tested. However, chromatin association requires amino acid sequences encoded by exon 4. We therefore retested BALB/c 3T3 cell transfectants that express the MCMV IE1 and IE3 genes, and which induce MCMV E gene transcription. Furthermore, BALB/c 3T3 cells were constructed which express only the IE1 gene. As in the transfected cell lines stably expressing the MCMV IE1 gene (Koszinowski *et al.*, 1986) which were resistant to MCMV infection, in the permissive BALB/c 3T3 cell line the distribution of pp89 is restricted to the nucleus of interphase cells and it does not associate with the condensed chromatin during mitosis.

This was at first glance a surprising finding because earlier experiments had revealed an interaction of pp89 with DNA-bound histones *in vitro* (Muench *et al.*, 1988) and the existence of a carboxy-terminal acidic region implied that pp89 interacts with chromatin. Acidic regions have been identified in several chromatin and chromosomal proteins, and are thought to play a role in nucleosome assembly and disassembly (Earnshaw, 1987). A function of such regions could be to unfold condensed chromatin by electrostatic capture of the basic domains of histones. A direct involvement of acidic sequences in histone binding has been demonstrated for the *Xenopus laevis* protein N1 (Kleinschmidt & Seiter, 1988). The acidic sequence was of particular interest because exons 4 of HCMV and simian CMV IE1 genes also encode similar acidic sequences, and these sequences have been implicated in chromatin binding (LaFemina *et al.*, 1989).

A second region of pp89 was of interest with regard to

histone and, probably, chromatin binding. This region, located between amino acids 96 and 153, shows homology to histone H2B and is unique for pp89 (Keil *et al.*, 1987*a*). However, because the deletion of this region does not affect the resistance of the tightly associated histone–pp89 complex to 2 M-NaCl or endow pp89 with a selective binding affinity for histone H2A, it was clearly of little importance for the histone-binding ability of pp89.

Analysis of the affinity of pp89 fragments for histone reveals that pp89 interacts with histones by domains located outside the region of homology with histone H2B between amino acids 154 and 415. A deletion mutant of pp89 containing amino acids 1 to 250 also binds histones with high affinity and this binding is resistant to 2 M-NaCl (data not shown), confirming that the acidic region consisting of amino acids 424 to 532 is not essential for the binding of pp89 to histone. We therefore assume that the binding of pp89 to DNA-bound histones does not involve the acidic region, which may then cause electrostatic repulsion between the DNA and the pp89–histone complex. This explanation is in good agreement with the low affinity of pp89 for DNA-bound histones and the high affinity for isolated histones observed *in vitro*.

Why do the two CMV IE proteins react differently? Although the structural organization of the MCMV and HCMV IE1 genes is fairly similar (Keil *et al.*, 1987*b*; Stinski *et al.*, 1983), the amino acid sequences of the gene products only show similarity in the acidic carboxy-terminal regions encoded by exons 4 (Keil *et al.*, 1987*a*), which could implicate different functional properties of the proteins. However, the difference in chromatin binding does not necessarily indicate a functional difference. The affinity of CMV IE proteins for histones could be important for the modulation of histone assembly by the histone–IE protein complex. The histone-binding capacity of the HCMV IE1 protein has been discussed (LaFemina *et al.*, 1989) but not yet tested. Our interpretation predicts that the affinity of the HCMV IE1 protein for histones is lower than that of MCMV pp89. This difference in histone and chromatin binding between the IE proteins of MCMV and HCMV could indicate that they use alternative methods to perform the same functions.

Two possible functions for the binding of pp89 to histone during productive MCMV infection and latency are indicated by these results. First, interaction of pp89 with DNA-bound histones could lead to the destruction of the nucleosomal structure and second, interaction of pp89 with free histones could affect DNA packaging into nucleosomal structures.

Nucleosome assembly represents a general mechanism for the repression of gene activity (Yaniv &

Cereghini, 1987), but whether CMV DNA is assembled into nucleosome structures or remains associated with virion proteins is not known. The DNA of another herpesvirus, Epstein-Barr virus, is folded into nucleosomes in latently but not lytically infected cells (Shaw *et al.*, 1978). If nucleosomal packaging of MCMV DNA does occur, factors are required that prevent promoter repression; herpesvirus IE proteins can have such functions. The IE protein of pseudorabies virus, a porcine herpesvirus, stimulates transcription by promoting the binding of TFIID to promoter sequences during chromatin reconstitution (Workman *et al.*, 1988).

We speculate that the histone-binding domains of pp89 support the establishment of stable transcription complexes during nucleosomal organization by direct interaction with histones. In addition, the highly acidic C-terminal sequence of pp89 is free to exert functions homologous to those of the highly acidic region of VP16 of herpes simplex virus, which has been found to be essential for IE gene activation (Triezenberg *et al.*, 1988; Stringer *et al.*, 1990).

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