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Control of human cytomegalovirus gene expression by differential histone modifications during lytic and latent infection of a monocytic cell line☆

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Abstract

Non-differentiated THP-1 cells can be infected by human cytomegalovirus (HCMV) Towne strain, which persists in these cells in a non-active (latent) form without undergoing a productive cycle. The same cells become permissive for HCMV lytic infection after induction of cell differentiation by treatment with 12-*O*-tetradecanoylphorbol-13-acetate. We used this cellular model to study the possible role of histone modifications in the control of HCMV latency. Using chromatin immunoprecipitation with antibodies against histone H3 acetylated or dimethylated in position K9, we demonstrated that in lytically infected cells the HCMV enhancer was associated with heavy acetylated but not dimethylated H3. In the case of latent infection, the HCMV enhancer was associated with neither acetylated nor dimethylated H3. HCMV genes encoding DNA polymerase (early), pp65 (early–late) and pp150 (late) proteins were associated preferentially with acetylated H3 in lytically infected cells and with dimethylated H3 in latently infected cells. These data strongly suggest that K9 methylation of H3 is involved in HCMV gene repression, while association of the above genes with acetylated histones is likely to be necessary for active transcription. It can be postulated that the same histone modifications are used to mark active and repressed genes in both cellular and viral chromatin.

Keywords: Cytomegalovirus latency; Gene repression; Histone methylation; THP-1 monocytic cell line

1. Introduction

Human cytomegalovirus (HCMV) is a ubiquitous human pathogen. Although infection with this virus is asymptomatic in otherwise healthy individuals, it may cause serious diseases particularly in immunocompromised persons (Escuissato et al., 2005; Griffiths and Walter, 2005; Magro et al., 2005; Rowshani et al., 2005; Schleiss and McVoy, 2004). This happens when HCMV, persisting lifelong in a latent condition in normal individuals, switches to a productive cycle. The mechanisms which make some cells permissive for lytic infection and others non-permissive are largely unknown. In infected cells (irrespective of the type of infection), the viral DNA is packed into nucleosomes and, perhaps, in higher order chromatin structures (Chen et al., 1997; Murphy et al., 2002; Reeves et al., 2005; St Jeor et al., 1982).

Recent studies have demonstrated that histone modifications play a crucial role in the regulation of cellular gene transcription and also in facilitating the transcription of templates packed in nucleosomes ("transcription through nucleosomes") (Imhof, 2003; Khan and Krishnamurthy, 2005; Margueron et al., 2005; Vermaak et al., 2003). Furthermore, it becomes increasingly

Abbreviations: aa, amino acid(s); Ab, antibody(ies); bp, base pair(s); BSA, bovine serum albumin; cDNA, DNA complementary to RNA; DNase, deoxyribonuclease; dNTPs, deoxyribonucleoside triphosphate; DTT, dithiothreitol; EtdBr, ethidium bromide; FBS, foetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HCMV, human cytomegalovirus; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IE, Immediate–Early; kb, kilobase(s); kDa, kilodalton(s); mAb, monoclonal Ab; moi, multiplicity of infection; pfu, plaque forming unit(s); Pipes, 1,4-piperazinediethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; Sm, streptomycin; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; U, unit(s).

☆ This article is dedicated to the memory of Christophe Mérieux.

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evident that targeted modifications of histone tails at specific positions constitute signals for the formation of either active or repressed chromatin structures. Acetylation of histone H3 lysine 9 (K9) triggers a chain of molecular events resulting in the formation of an active chromatin domain. In contrast, methylation of H3 at the same position results in silencing of a chromatin domain (Dillon and Festenstein, 2002; Richards and Elgin, 2002). Thus, modifications of histone H3 at K9 position represent a molecular switch which allows to choose between active and repressed chromatin configurations. Some recent publications suggest that specific epigenetic markers, such as histone modifications, play an important role in determining the type of HCMV infection. It has been reported that treatment of cells that are non-permissive for HCMV lytic infection with inhibitors of histone deacetylases converts these cells into permissive ones (Murphy et al., 2002). It was also reported that the HCMV enhancer is preferentially associated with acetylated histone H4 in lytic infection (Reeves et al., 2005). In order to further investigate the role of histone modifications in the regulation of HCMV gene expression, we studied here the patterns of H3-K9 modifications of HCMV DNA-associated histones in cultured cells undergoing lytic or latent infection. As a model system, we used non-differentiated THP-1 cells that do not support lytic infection by HCMV unless they are differentiated by pretreatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Lee et al., 1999; Turtinen and Seufzer, 1994; Weinshenker and Rice, 1988). Using this cellular system and HCMV Towne strain to infect the cells, we demonstrated that in non-differentiated cells, all HCMV genes tested are associated with H3 preferentially dimethylated at K9 position. In contrast, in the course of lytic infection of differentiated THP-1 cells, the same genes were preferentially associated with H3 acetylated at K9. These data suggest that the cellular system of histone modifications is also used to regulate the expression of the majority of HCMV genes, specifically suppressing it in non-permissive cells.

2. Materials and methods

2.1. Cell culture

THP-1 monocytic cell line (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna) was maintained in suspension in RPMI 1640 medium, supplemented with 1% L-glutamine, 1% sodium pyruvate, 50 μM β-mercaptoethanol, 10% foetal bovine serum (FBS) and antibiotics (10,000 U/ml penicillin and 10,000 μg/ml Sm). For differentiation experiments, THP-1 cells were seeded at a final density of 6×10^5 cells/ml in 12 mm-round cover-slips, or at 1×10^6 cells/ml in 6-well plates. Cell differentiation was induced by adding of 80 nM 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Sigma-Aldrich) to the medium. Differentiated (adherent) THP-1 cells were used for infection experiments 72 h after induction. MRC5 human embryo lung fibroblasts (ATCC) were grown in Earle's modified Minimum Essential Medium, supplemented with 1% L-glutamine, 1% non-essential aa, 1% sodium pyruvate, 10% FBS and antibiotics (10,000 U/ml penicillin and 10,000 μg/ml Sm). Cell culture media and supplements were from Invitrogen.

2.2. Viral infection

Human cytomegalovirus (HCMV) Towne strain (ATCC VR-977) was propagated in MRC5 cells; after the planned infections, virus-enriched culture supernatants were centrifuged at low speed, then stored at −80 °C. Viral yield was determined as previously described (Arcangeletti et al., 2003). Infection experiments were carried out on non-differentiated THP-1 cells first by centrifuging them at low speed for 10 min, then gently resuspending the cellular pellet in viral-enriched culture medium at a moi of 1 pfu/cell. THP-1 cells were then transferred to a 6-well plate, centrifuged for 45 min at 700 ×*g* and finally incubated for 1 h and 15 min at 37 °C. After the absorption period, the cells were gently washed twice with culture medium, then incubated for the planned times.

2.3. Antibodies

A mAb (clone E13, Argene-Biosoft) against HCMV immediate–early (IE) major products (72 and 86 kDa proteins) was used (1:30 dilution).

Rhodamine isothiocyanate-conjugated goat anti-mouse serum (EuroClone) was used as the secondary Ab (1:100 dilution). The rabbit polyclonal Ab against histone H3 acetylated at position K9 (H3 acetyl-K9; Ab 4441) and histone H3 dimethylated at position K9 (H3 dimethyl-K9; Ab 7312) were from Abcam. The mouse serum (M5905) was from Sigma-Aldrich.

2.4. Indirect immunofluorescence

After the planned infections differentiated THP-1 cells grown on cover-slips were rinsed with cytoskeleton buffer (CSKB) [10 mM Pipes (pH 6.9), 100 mM NaCl, 1.5 mM $MgCl_2$, 300 mM sucrose], whereas non-differentiated cells were cytospinned (2×10^5 cells/spot) and then air dried. The cells were simultaneously permeabilized and fixed in CSKB containing 0.5% Triton X-100 and 1% paraformaldehyde at room temperature for 20 min (Arcangeletti et al., 1997), washed three times with phosphate buffer saline (PBS) [7 mM Na_2HPO_4, 1.5 mM KH_2PO_4 (pH 7.4), 137 mM NaCl, 2.7 mM KCl], then saturated with 1% BSA (pH 8.0) in PBS for 10 min. The cells were incubated in a humid chamber for 1 h at 37 °C with the anti-IE Ab diluted in 0.2% BSA. After three washes in PBS, the secondary Ab was applied for 45 min as above. The cells were then washed twice in PBS, mounted with an anti-fading medium (Dakocytomation) and observed under a fluorescence microscope (Zeiss-Axiophot). Chemicals were from Sigma-Aldrich.

2.5. DNA extraction from cell nuclei and PCR amplification

Both the non-differentiated and differentiated THP-1 cells (1×10^6) infected with HCMV Towne strain (moi = 1) for 30 h, were resuspended in 1 ml of lysis buffer [5 mM $MgCl_2$, 10 mM Hepes, 320 mM sucrose, 1% Triton X-100] and left on ice for 10 min. After incubation, the cells were centrifuged at 2000 ×*g* for 10 min at 4 °C, then the nuclear pellet was gently washed