Biochemical and Biophysical Research Communications 391 (2010) 322–328

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Protein arginine methyltransferase 1 regulates herpes simplex virus replication through ICP27 RGG-box methylation

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ARTICLE INFO

Article history:
Received 6 November 2009
Available online 12 November 2009

Keywords:
PRMT1
ICP27
HSV-1
Viral replication
Protein arginine methylation

ABSTRACT

Protein arginine methylation is involved in viral infection and replication through the modulation of diverse cellular processes including RNA metabolism, cytokine signaling, and subcellular localization. It has been suggested previously that the protein arginine methylation of the RGG-box of ICP27 is required for herpes simplex virus type-1 (HSV-1) viral replication and gene expression *in vivo*. However, a cellular mediator for this process has not yet been identified. In our current study, we show that the protein arginine methyltransferase 1 (PRMT1) is a cellular mediator of the arginine methylation of ICP27 RGG-box. We generated arginine substitution mutants in this domain and examined which arginine residues are required for methylation by PRMT1. R138, R148 and R150 were found to be the major sites of this methylation but additional arginine residues serving as minor methylation sites are still required to sustain the fully methylated form of ICP27 RGG. We also demonstrate that the nuclear foci-like structure formation, SRPK interactions, and RNA-binding activity of ICP27 are modulated by the arginine methylation of the ICP27 RGG-box. Furthermore, HSV-1 replication is inhibited by hypomethylation of this domain resulting from the use of general PRMT inhibitors or arginine mutations. Our data thus suggest that the PRMT1 plays a key role as a cellular regulator of HSV-1 replication through ICP27 RGG-box methylation.

Protein arginine methylation, a common post-translational modification, is involved in the modulation of diverse intracellular events that lead to cellular proliferation, activation, differentiation and apoptosis [1,2]. The methylation of arginines is basically catalyzed via *S*-adenosyl-L-methionine (SAM) as the methyl donor and a family of enzymes known as the protein arginine methyltransferases (PRMTs) [1]. In humans, 11 PRMTs (PRMT1–11) have so far been identified and PRMT1 is the most extensively characterized of these enzymes in manifold cellular processes including RNA processing, transcription, cytokine signaling, and cell fate [1–3].

Protein arginine methylation may be very important for viral infection and replication through the modulation of viral replicative proteins [4–7]. In our previous studies, we also reported that the arginine methylation of NS3, a viral replicative protein, of Hepatitis C virus can be modulated by PRMT1 or 5 [8,9]. However, the mechanism by which the host–cell arginine methylation system is utilized by viruses has not been well documented thus far in studies of viral replication. Hence, it is of interest to determine whether viral replication is directly modulated by arginine methylation events catalyzed by the PRMTs.

HSV-1 ICP27 is an immediate-early protein with multifunctional regulatory roles that are essential for viral replication, transcription and translation [10]. It contains arginine-rich RGG-box, nuclear localization signal (NLS), nuclear export sequence (NES) and KH domains and shuttles between the nucleus and cytoplasm [10]. Interestingly, Mears and Rice have previously suggested the possibility that protein arginine methylation participates in HSV-1 replication [11]. Significantly, the RGG-box is the exclusive target motif in ICP27 for arginine methylation and is required for efficient HSV-1 replication [11,12]. However, it is still unclear how this domain is methylated or what the biological significance of this modification is *in vivo*.

In our current study, we have investigated some of these issues using HSV-1 as our model system. We focused on the functional role of the arginine methylation of ICP27 RGG-box by PRMT1 during HSV-1 replication. Moreover, we analyzed the effects of

Abbreviations: PRMT, protein arginine methyltransferase; ICP27, infected cell protein 27; HSV-1, herpes simplex virus type-1.

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doi:10.1016/j.bbrc.2009.11.057

these methylation events upon the regulation of viral replication through the modulation of its subcellular localization, SRPK1 interaction, RNA-binding and cellular signaling. Our results implicate the PRMT1 as a crucial cellular regulator of HSV-1 replication through ICP27 RGG-box methylation.

Materials and methods

Cells, viruses and materials. Vero, V27, CRT-MG, and 293T cells were maintained in DMEM supplemented with 10% FBS. The HSV-1 wild-type strain KOS1.1 was obtained from J. Ahn (CNU, Korea) and its d27–1 mutant virus was obtained from D. Knipe (HMS, MA). Adenosine-2,3-dialdehyde (AdOx), *S*-(5′-adenosyl)-L-homocysteine (AdoHcy), and Histone (type II AS) were purchased from Sigma–Aldrich. Antibodies specific for FLAG, Myc, and actin were from Sigma–Aldrich; for ICP27 and His (Santa Cruz Biotech); for JNK, p-JNK, p38, p-p38, ERK, p-ERK and PRMT1 (Cell Signaling).

Plasmids and site-directed mutagenesis. Bacterial expression plasmids, pGEX-27 (GST-ICP27), pGEX-d4–5 (GST-d4–5), and pGEX-RGG (GST-RGG) were obtained from S.A. Rice (UMMS, MN) and PRMT1 (His-PRMT1) and pGST-GAR (GST-GAR) have been described previously [8,9,13]. To construct mammalian expression plasmids, the corresponding coding regions of ICP27, Hsc70, and Sam68 were amplified by PCR and subcloned into pDsRed-N1 (Clontech), pcDNA3.1-myc-His (Invitrogen), or pCDH1-MCS1-EF1-puro (System Bioscience). Expression constructs for Flag-tagged human SRPK1 and SRPK2 were obtained from X.-D. Fu (UCSD, CA). Site-directed mutagenesis of arginine residues within the ICP27 RGG-box to alanine residues was performed using the Quickchange site-directed mutagenesis kit (Stratagene) with the mutated oligonucleotides (Table 1).

Protein preparations and methylation assays. Bacterially expressed His-PRMT1 and GST-fusion proteins were purified as described [9,11,13]. *In vitro* methylation assays were carried out as described [9] in which purified PRMTs were incubated in buffer for 2 h at 30 °C with substrate proteins of the indicated amounts in the Figures and 0.1 μCi of *S*-[*methyl*-^{14}C] adenosyl-L-methionine (^{14}C-SAM, Amersham Pharmacia Biotech) in PRMT buffer (25 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, and 1 mM PMSF). For an affinity bead-bound methylation assay, methylated products of partially purified ICP27 point mutant proteins by PRMT1 were analyzed as described [9]. For methylation assay of Vero cell extracts, cell extracts from 10^8 cells were prepared as described [9] and the PRMT assay was carried out with 100 μg of a soluble Vero cell extracts and 1 μg of GST or GST-RGG in the presence of 0.1 μCi of ^{14}C-SAM as described above.

Virus infection and plaque assay. Viral titers were determined by plaque assays using Vero or V27 cells as described [14,15]. To perform an indirect plaque assay, viral titers were determined as described [14,16]. Briefly, lentiviruses expressing WT ICP27-GFP or its mutants were infected into CRT-MG cells and the cells were selected with puromycin (0.2 μg/ml). The d27–1 mutant viruses were infected at an MOI of 1. After 24 h post-infection, viruses were prepared by three cycles of freeze–thawing at −80 °C. The quantities of infectious virus were determined by plaque assay using V27 cells. Mouse brains were sliced into 1-mm thick sections (0.12–0.13 g in weight) which were then infected with 10^6 pfu of WT HSV-1 for 1 h. Infected brain slices were washed, treated with 1 mM of AdOx or PBS, and cultured for 24 h. The viral titer was measured using Vero cells.

Subcellular localization, immunoprecipitation (IP), and RNA binding assay. For subcellular localization, the RFP expression plasmids for ICP27 or its counterparts expressing GFP were transiently transfected into Vero cells (10^5 cells/ml) using Lipofectamine 2000 (Invitrogen). The cells were then observed with a confocal microscope (Zeiss). For IP, protein interactions between ICP27 and SRPKs were determined as described [9]. RNA binding assay was performed using poly(G)-agarose beads as described [11,13].

Results

The RGG-box of HSV-1 ICP27 is methylated by PRMT1

We examined whether PRMT1, the predominant PRMT in cells, can methylate the RGG-box of ICP27. We purified His-tagged-PRMT1 from *Escherichia coli* (Fig. 1A) and its activity was verified with previously known methyl acceptors such as histone or GST-GAR (Fig. 1B). The methylation of GST-ICP27 was found to be serially increased in the presence of His-PRMT1 in a dose-dependent manner (Fig. 1B). Next, we constructed ICP27-deletion mutants (Fig. 1C) and performed a PRMT assay with His-PRMT1. GST-ICP27 and GST-RGG, but neither GST-d4–5 nor GST, were found to be methylated by PRMT1 (Fig. 1D). Among relatively well known PRMTs (PRMT1–6), the methylation of GST-ICP27 and GST-RGG was predominantly mediated by PRMT1, but weak methylation of GST-RGG by PRMT3, 5 or 6 was evident (Supplementary Fig. 1). Furthermore, we determined that the ICP27 RGG-box is methylated in Vero cell extracts (Fig. 1E). These results thus indicate that the arginine residues in the ICP27 RGG-box are methylated by PRMT1.

The R138, R148 and R150 residues are a major sites of arginine methylation and fundamentally required for the efficient SRPK1 interaction and RNA binding of ICP27

We generated single point mutants of RGG-box by substitution of arginine (R) for alanine (A) using site-directed mutagenesis

Table 1
Primer sequences for site-directed mutagenesis.

Mutant	Primer sequence
R1A	5′-GCC CAG CCT GCC GCA GGC GGA CGC CGT-3′
R2A	5′-GCC CGC GGC GGA GCA CGT GGG CGT CGC-3′
R3A	5′-CGC GGC GGA CGC GCA GGG CGT CGC AGG-3′
R4A	5′-GGA CGC CGT GGG GCA CGC AGG GGT CGG-3′
R5A	5′-ACG CCG TGG GCG TGC AAG GGG TCG GGG TC-3′
R6A	5′-CGT GGG CGT CGC GCA GGT CGG GGG TCG C-3′
R7A	5′-GCG TCG CAG GGG TGC AGG TCG CGG TGG TC-3′
R8A	5′-CAG GGG GTC GGG GTG CAG GTG GTC CCG GGG G-3′
R17A	5′-GCC CAG CCT GCC GCA GGC GGA CGC CGT GGG CGT CGC AGG GGT GCA GGT CGC GGT GGT C-3′
R18A	5′-GCC CAG CCT GCC GCA GGC GGA CGC CGT GGG CGT CGC AGG GGT CGC GGT GCA GGT GGT C-3′
R78A	5′-GCC CAG CCT GCC CGC GGC GGA CGC CGT GGG CGT CGC AGG GGT GCA GGT GCA GGT GGT C-3′
R178A	5′-GCC CAG CCT GCC GCA GGC GGA CGC CGT GGG CGT CGC AGG GGT GCA GGT GCA GGT GGT C-3′
R12378A	5′-CAG CCT GCC GCA GGC GGA GCC GCT GGG CGT CGC AGG GGT GC-3′
RallA	5′-GCA GGC GGA GCC GCT GGG GCT GCC GCG GGT GCA GGT GCA GGT GGT-3′

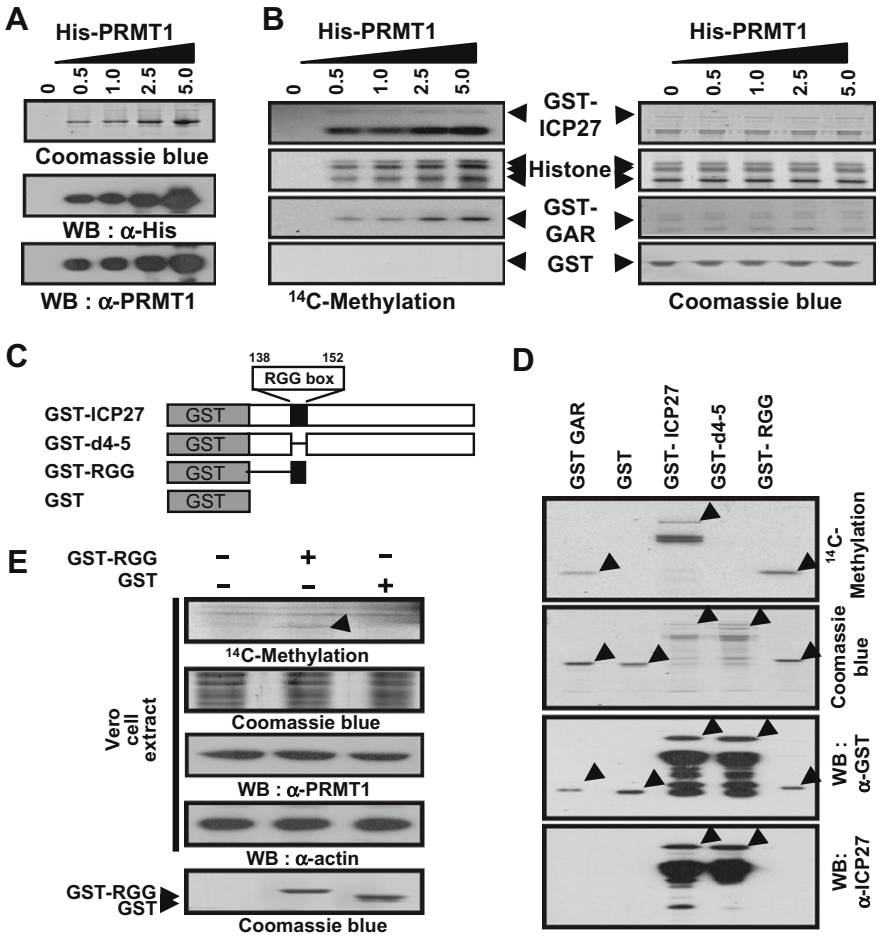

Fig. 1. The RGG-box of ICP27 is methylated by PRMT1 *in vitro*. (A) Purification of recombinant PRMT1. Purified 6×His-tagged-PRMT1 (His-PRMT1) was visualized by coomassie blue staining and immunoblotting. WB, western blotting. (B) ICP27 is methylated by PRMT1 *in vitro*. A purified GST-glycine–arginine-rich domain (GST-GAR) and histone were used as a positive controls and GST was used as a negative control. The methylated products were detected by fluorography (left panels, ^{14}C-methylation). The purified substrate proteins were stained with coomassie blue (right panels) and the arrowheads indicate the position of the full-length substrate proteins. (C) Schematic illustration of ICP27-deletion mutants shown as GST-tagged forms. The black box represents the RGG-box domain and the number of residues is indicated. (D) The RGG-box domain is required for ICP27 methylation *in vitro*. The arrowheads indicate the position of the full-length mutant proteins. (E) The RGG-box of ICP27 is methylated by Vero cell extracts. The arrowhead indicates the position of methylated GST-RGG.

(Fig. 2A) and carried out an affinity bead-bound methylation assay to test for methylated arginine residues in RGG-box by PRMT1. The methylation of the triple mutant, R178A, was significantly reduced by more than 90%, whereas the arginine methylation of double mutants was slightly reduced (20–60%) compared with WT ICP27 (Fig. 2B). Interestingly, we could not detect any methylated forms of the R12378A and RallA mutants (Fig. 2B). These results indicate that the R138, R148 and R150 residues are the major sites in the ICP27 RGG-box for arginine methylation by PRMT1, although additional arginine residues appear to be required as minor methylation sites to sustain fully the methylated form of ICP27 RGG-box.

The interaction between the RGG-box of ICP27 and SRPK1 modifies SRPK1 activity resulting in hypophosphorylation of SR proteins, thereby impairing their ability to function in spliceosome assembly [17]. Therefore, we examined which of arginine residues in this domain are essential for this binding. In IP, the triple mutant R178A and multiple point mutants R12378A or RallA cannot bind SRPK1 and SRPK2 (Fig. 2C and Supplementary Fig. 2A). Consistently, we could not detect any SRPK1 shuttling to nucleus in conjunction with R178A, R12378A or RallA as seen for WT ICP27 and double mutants (Supplementary Fig. 2B). Our findings indicate that the R138, R148 and R150 residues within the RGG-box are required for the interaction of ICP27 with the SRPK1.

Next, we postulated that the modification of arginine residues in ICP27 RGG-box by PRMT1 could modify the RNA-binding affinity of this protein. To test this possibility, we performed a poly(G)-agarose bead binding assay with cell extracts expressing WT ICP27 or its mutants as described previously [21,22]. Single and double point mutants did not show appreciable differences in this assay (Fig. 2D and Supplementary Fig. 3). However, the RNA-binding activity of the triple mutant R178A was dramatically reduced by 50% compared with WT ICP27 (Fig. 2D and Supplementary Fig. 3). Furthermore, the RNA-binding activity of the R12378A mutant was decreased to almost basal levels, whilst the RallA mutant was completely deficient in this binding (Fig. 2D and Supplementary Fig. 3). We thus concluded that the R138, R148 and R150 residues of the RGG-box primarily contribute to RNA binding by ICP27, although additional arginine residues appear to be required for full activity in this regard.

The nuclear foci-like structure of ICP27 is induced by the inhibition of RGG-box methylation

We examined whether the nuclear transition of ICP27 is affected by RGG-box methylation. Large foci-like nuclear structures were observed for d4–5 mutant but these were few in number,

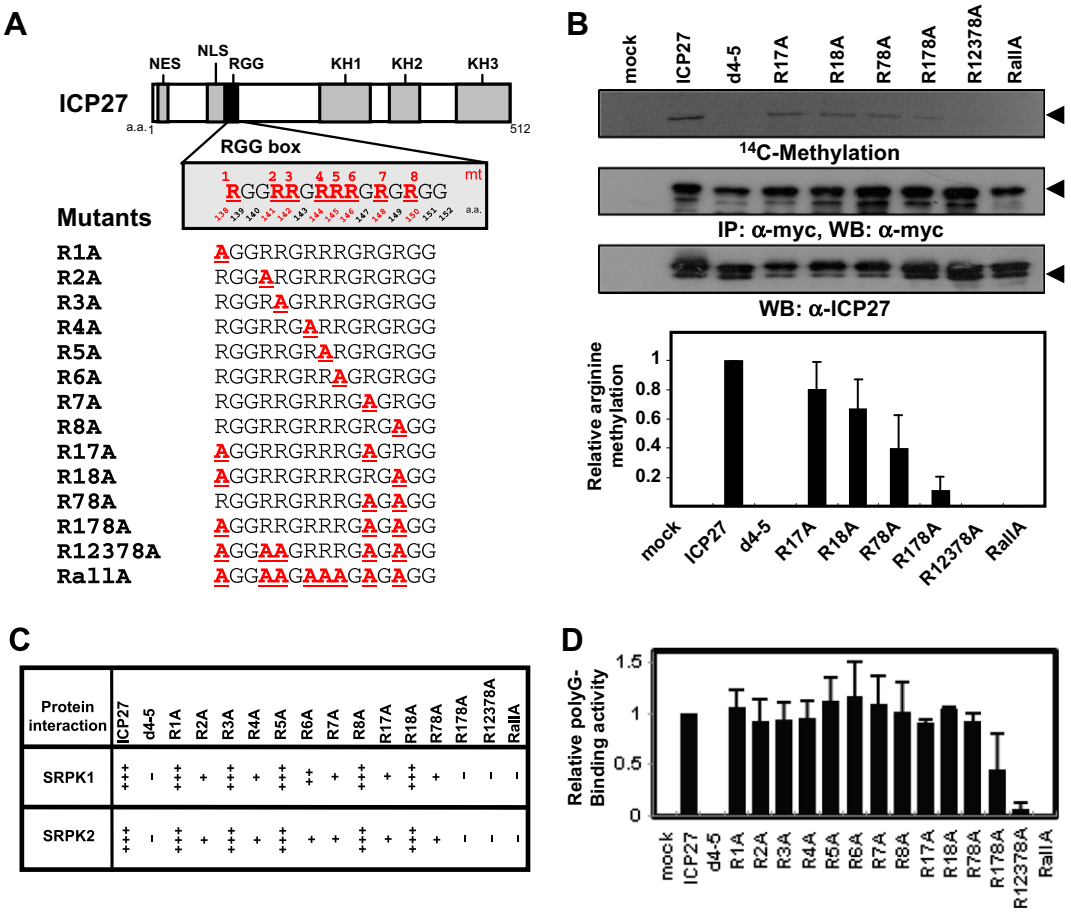

Fig. 2. The effects of arginine substitutions in the RGG-box. (A) Site-directed mutagenesis. Substitutions are indicated by the bold underlined. NES, nuclear export sequence; NLS, nuclear localization signal; RGG, RGG-box; KH, KH domain; a.a., position of amino acids; mt, the position of the R residue. (B) Affinity bead-bound methylation assay of point mutants. The arrowheads indicate the position of the Myc-tagged ICP27 proteins. The relative arginine methylation levels (bottom panel) were measured from triplicate experiments by densitometry using the Quantity One program (Bio-Rad). (C) Summary of the interaction between ICP27 mutants and the SRPKs. The relative intensities of the protein interactions were measured from the triplicate experiments. +++, strong interaction; ++, moderate interaction; +, weak interaction; -, no interaction. (D) Graphical depiction of the effects of the ICP27 RGG-box mutations on poly(G) RNA binding by ICP27. The relative poly(G) RNA binding was measured from triplicate experiments by densitometry using the Quantity One program (Bio-Rad).

whereas small foci-like structures in the nucleus were detected for WT ICP27 (Fig. 3A). The single point mutants showed nuclear localization patterns that were very similar to wild-type (Supplementary Fig. 4A). However, the large nuclear foci-like structures observed for d4–5 were also detected for mutants with two or more arginine substitutions (Fig. 3A). To identify whether these large foci are derived from the redistribution of ICP27 in the nucleus and not as a result of shuttling to the cytoplasm, we examined the subcellular location of d4–5, and also the ICP27 RGG-box double or triple mutants by cell fractionation assay. All of these mutants were detectable only in the nuclear fraction (Fig. 3B).

It has recently been reported that the interaction between ICP27 and Hsc70 is required for the formation of nuclear foci-like structures of Hsc70 during HSV-1 infection [18]. We therefore next examined whether these foci formed by ICP27 mutants colocalized with Hsc70. The colocalization of WT ICP27 and Hsc70 was detected in small nuclear foci-like structures (Fig. 3C). We obtained similar results for three or more arginine substitution mutants with Hsc70 in large nuclear foci-like structures (Fig. 3C). Next, we examined whether the large foci-like nuclear structures localize to the nucleoli. None of the ICP27 mutants tested in this experiment colocalized to any extent with B23/nucleophosmin, a standard marker of nucleoli [19] (Supplementary Fig. 4B). These results indicate that the formation of the large nuclear foci-like structures by ICP27 RGG-box mutants is not due to their nucleolar localization.

We finally tested whether the hypomethylation of ICP27 RGG-box is related to the shuttling of ICP27 from the nucleus to the cytoplasm or the formation of large nuclear foci. Cytoplasmic shuttling of hypomethylated Sam68, a positive control [13,20], following treatment with 1 mM AdOx was observed, but hypomethylated ICP27 was not detected in the cytoplasm (Fig. 3D). Interestingly, the large nuclear foci-like localization of hypomethylated ICP27 was observed following inhibition with AdOx, whilst WT ICP27 remained evenly localized in the nucleus in the absence of this treatment (Fig. 3D). These findings indicate that the hypomethylation of ICP27 is closely related to the formation of large nuclear foci, but it remains to be conclusively determined whether a small portion of the ICP27 proteins can be shuttled to the cytoplasm as a result of a hypomethylated state.

HSV-1 replication is inhibited under hypomethylation conditions caused by general PRMT inhibitors or arginine substitutions

As a preliminary experiment, we established a plaque assay with WT HSV-1 (KOS1.1) or d27–1 mutant virus (ICP27-deletion mutant) and tested whether the expression of the PRMT1 gene is regulated by the increased ICP27 expression in Vero cells infected with HSV-1 or vice versa. No viral plaques formed following d27–1 infection but were efficiently formed by WT HSV-1 infection at an MOI range from 0.0002 to 0.02 (Supplementary Fig. 5A). The level

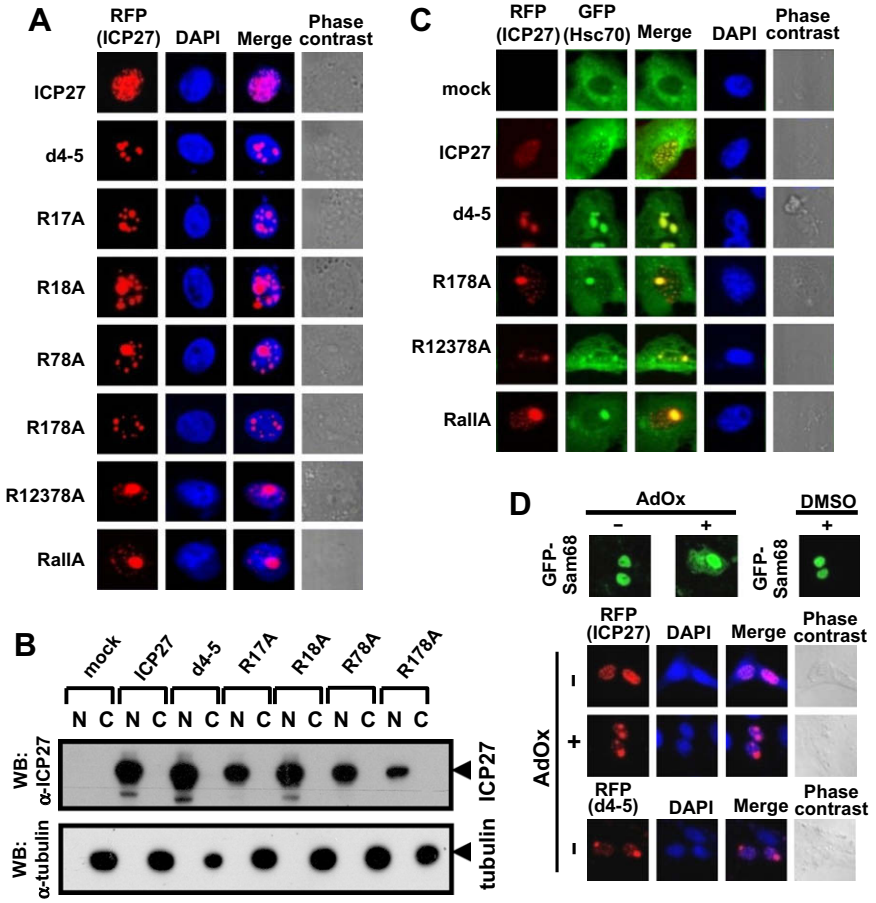

Fig. 3. The formation of nuclear foci-like structures by RGG-box mutants of ICP27. (A) The formation of nuclear foci-like structures in the double or greater point mutants of the ICP27 RGG-box. (B) Subcellular distribution of ICP27 RGG-box mutants. Tubulin was used as a marker for cytoplasmic proteins. N, nuclear fraction; C, cytoplasmic fraction. (C) Colocalization of ICP27 mutants with the nuclear foci-like structures formed by Hsc70. The RFP-tagged ICP27 mutants were cotransfected with GFP-tagged Hsc70 (Hsc70-GFP) into Vero cells. (D) Subcellular localization of ICP27 in the presence of AdOx. Cytoplasmic shuttling of Sam68 following treatment with 1 mM AdOx (top panel). DMSO (1 mM) was used as a vehicle control. The formation of nuclear foci-like structures by ICP27 following treatment with 1 mM AdOx (bottom panel).

of PRMT1 expression was not found to have changed as a result of increased ICP27 expression in Vero cells infected by HSV-1 at an MOI of 5.0 (Supplementary Fig. 5B). ICP27 expression and viral replication were also unchanged by the overexpression of PRMT1 (Supplementary Fig. 5B and C). In addition, no side-effects of the PRMT inhibitors on Vero cells were observed by cell proliferation assay or morphology testing (data not shown). To address whether arginine methylation is involved in HSV-1 replication, we carried out plaque assays in which the Vero cells infected with HSV-1 were treated with general PRMT inhibitors (AdOx or AdoHcy). The number of viral plaques had significantly decreased following AdOx or AdoHcy treatment in a dose-dependent manner (Fig. 4A and B). In addition, the viral titer was significantly reduced in infected mouse brains by treatment with 1 mM of AdOx (Fig. 4C). We further tested whether the expression of ICP27 or PRMT1 is influenced by PRMT inhibitor treatment. However, the gene expression level of PRMT1 was unchanged by exposure to AdOx and the expression of ICP27 was also unaltered by this inhibitor (Fig. 4D).

Since the activation by ICP27 of the stress kinases JNK and p38 during HSV-1 infection has been reported [14,15], we further examined the influence of the PRMT inhibitors on the cellular signaling resulting from HSV-1 infection. Signaling via JNK, p38 and ERK was not significantly altered by AdOx treatment (Fig. 4D). These results indicate that hypomethylation of ICP27 RGG-box is not directly related to the regulation of cellular signaling by HSV-1 infection. Finally, we performed an indirect plaque assay following d27–1 mutant virus infection of CRT-MG cell-lines expressing WT ICP27-GFP or its mutants (percentage of GFP^+ cells, 52–77%). Replication defect phenotypes were found for ICP27 RGG-box mutant viruses harboring three or more arginine substitutions (R178A, R12378A, or RallA; Fig. 4E). These results thus indicate that HSV-1 replication is inhibited under arginine hypomethylation conditions or by arginine mutations of critical sites in the ICP27 RGG-box.

Discussion

The arginine methylation of the RGG-box of ICP27 *in vivo* has been previously reported and suggested the possibility that protein arginine methylation participates in HSV-1 replication [11]. However, the cellular mediator(s) of this process have not yet been identified. In our present study, we show that the PRMT1 is in fact the principal cellular mediator of the arginine methylation of the ICP27 RGG-box. PRMT1 is thought to be the major form of this enzyme, accounting for ~85% of the total arginine methylation events in mammalian cells and predominantly localized in the nucleus, although some cells showed both cytoplasmic and nuclear localization of PRMT1 [21–23]. Our present data also demonstrate that ICP27 is mainly methylated in the nucleus because both the methylated and unmethylated forms of this protein are predominantly nuclear. This explains why ICP27 methylation is mainly driven by nuclear PRMT. Interestingly however, it has previously been re-

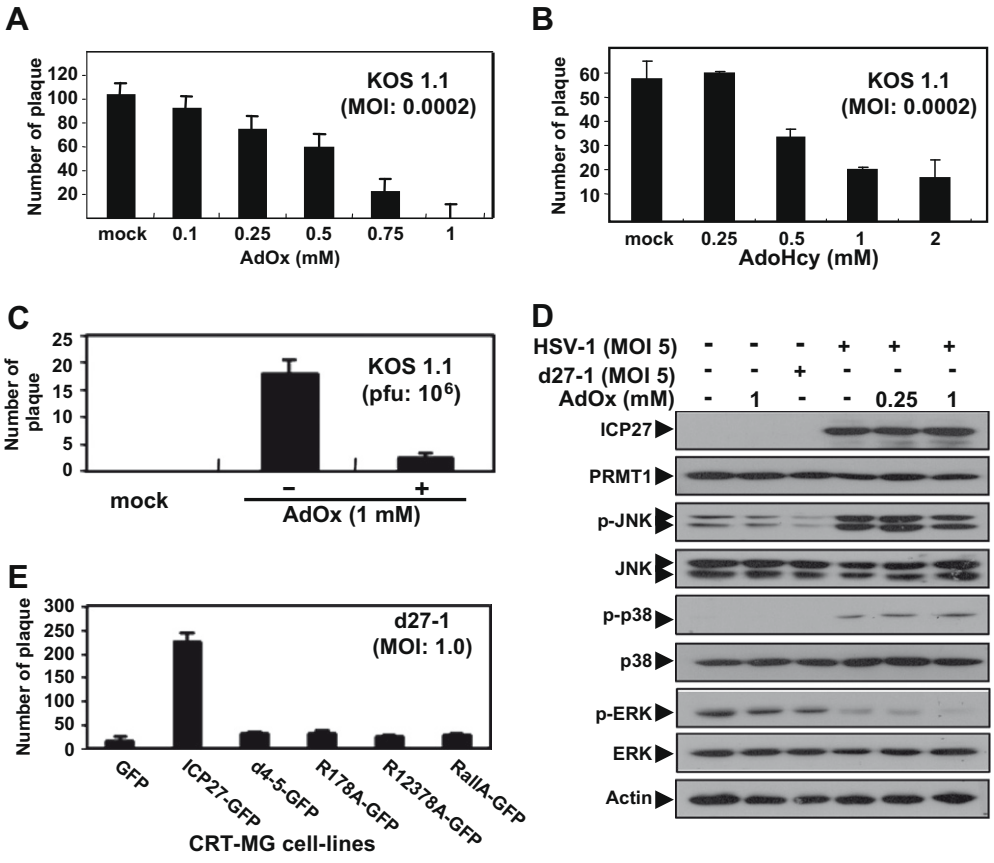

Fig. 4. Inhibition of efficient HSV-1 replication by PRMT inhibitors. (A) The effects of AdOx upon HSV-1 replication. (B) The effects of AdoHcy upon HSV-1 replication. Vero cells were infected in triplicate at an MOI of 0.0002 with WT HSV-1 in the presence of AdOx at the indicated concentrations. (C) The effects of AdOx on HSV-1 replication in brain tissue. The viral plaque number was determined using an indirect plaque assay. (D) The effects of AdOx on the signaling pathways downstream of HSV-1 infection. (E) Replication defects in the d27–1 virus in ICP27 RGG-box arginine substitution mutant cell-lines. An indirect plaque assay was carried out with d27–1 mutant virus in CRT-MG cell-lines expressing WT ICP27-GFP or its RGG-box arginine mutants.

ported that the d3–4 mutant protein, in which the NLS sequence is deleted from ICP27, is detectable only as a weakly methylated form *in vivo* [11]. Hence, we cannot exclude the possibility that cytoplasmic PRMTs play a minor roles in ICP27 methylation. During the course of our current study, a brief review and additional reports of a functional role for the arginine residues of the ICP27 RGG-box were published by Sandri-Goldin and colleagues [10,24,25]. Interestingly, the arginine residues within the ICP27 RGG-box were found to be differentially methylated in both the nucleus and cytoplasm in these reports [10,24]. However, it remains unclear how this viral protein is methylated in the nucleus and cytoplasm and it is of some interest and importance to identify the specific PRMT that is principally responsible for the methylation of ICP27. We plan to elucidate these processes further in future studies involving gene knock-down or knock-out systems for the PRMTs.

An important question that arises from our current findings is the precise biological function of ICP27 RGG-box methylation by PRMT1. Recent efforts by various groups have identified the functional role of ICP27 RGG-box including its regulation of RNA-binding activity, SRPK1 interaction, and viral replication [10]. Based on these findings, we speculated that the regulation of arginine methylation of ICP27 by PRMT1 might be a crucial event in the HSV-1 life cycle. We thus attempted to define the role of arginine residues in an RNA binding assay of RGG-box mutants using alanine substitutions. Interestingly, our data clearly demonstrate the crucial role of three such residues within the ICP27 RGG-box, R138, R148 and R150, in the efficient RNA binding of ICP27. We further found that these three arginine residues are the principal arginine methylation sites for PRMT1 as well as determinants of protein–protein interactions between ICP27 and the SRPK1. Furthermore, we found also that large nuclear foci-like structures are formed as a result of arginine substitutions or hypomethylation of the ICP27 RGG-box. We postulate that the formation of large nuclear foci of hypomethylated ICP27 may underlie the negative effects of this state upon HSV-1 replication. Consistent with these results, we found to our surprise that HSV-1 replication is inhibited by a hypomethylated condition of ICP27 RGG-box.

Cumulatively, our findings regarding the methylation of ICP27 RGG-box by PRMT1 indicate a new aspect of host system utilization by HSV-1 during its replication. Our current findings thus suggest that the PRMT1 plays key roles as a cellular regulator of HSV-1 replication through ICP27 RGG-box methylation.

Acknowledgments

We thank D. Knipe, M.T. Bedford, S.A. Rice, X.-D. Fu and J. Ahn for technical support. This work was supported by the Ministry of National Defense Foundation Grant and the National Research Foundation Grant from the Korean Government (ADD: 08-10-02, MEST: 2009-008146 and 2009-0072758).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.11.057.

References

[1] M.T. Bedford, S. Richard, Arginine methylation an emerging regulator of protein function, Mol. Cell 18 (2005) 263–272.
[2] F.O. Fackelmayer, Protein arginine methyltransferases: guardians of the Arg?, Trends Biochem Sci. 30 (2005) 666–671.
[3] M.E. Torres-Padilla, D.E. Parfitt, T. Kouzarides, M. Zernicka-Goetz, Histone arginine methylation regulates pluripotency in the early mouse embryo, Nature 445 (2007) 214–218.
[4] V. Christen, F. Duong, C. Bernsmeier, D. Sun, M. Nassal, M.H. Heim, Inhibition of alpha interferon signaling by hepatitis B virus, J. Virol. 81 (2007) 159–165.
[5] D.C. Iacovides, C.C. O'Shea, J. Oses-Prieto, A. Burlingame, F. McCormick, Critical role for arginine methylation in adenovirus-infected cells, J. Virol. 81 (2007) 13209–13217.
[6] Y.J. Li, M.R. Stallcup, M.M. Lai, Hepatitis delta virus antigen is methylated at arginine residues, and methylation regulates subcellular localization and RNA replication, J. Virol. 78 (2004) 13325–13334.
[7] B. Xie, C.F. Invernizzi, S. Richard, M.A. Wainberg, Arginine methylation of the human immunodeficiency virus type 1 Tat protein by PRMT6 negatively affects Tat interactions with both cyclin T1 and the Tat transactivation region, J. Virol. 81 (2007) 4226–4234.
[8] J. Rho, S. Choi, Y.R. Seong, W.K. Cho, S.H. Kim, D.S. Im, Prmt5, which forms distinct homo-oligomers, is a member of the protein-arginine methyltransferase family, J. Biol. Chem. 276 (2001) 11393–11401.
[9] J. Rho, S. Choi, Y.R. Seong, J. Choi, D.S. Im, The arginine-1493 residue in QRRGRTGR1493G motif IV of the hepatitis C virus NS3 helicase domain is essential for NS3 protein methylation by the protein arginine methyltransferase 1, J. Virol. 75 (2001) 8031–8044.
[10] R.M. Sandri-Goldin, The many roles of the regulatory protein ICP27 during herpes simplex virus infection, Front. Biosci. 13 (2008) 5241–5256.
[11] W.E. Mears, S.A. Rice, The RGG box motif of the herpes simplex virus ICP27 protein mediates an RNA-binding activity and determines in vivo methylation, J. Virol. 70 (1996) 7445–7453.
[12] J. Lengyel, C. Guy, V. Leong, S. Borge, S.A. Rice, Mapping of functional regions in the amino-terminal portion of the herpes simplex virus ICP27 regulatory protein: importance of the leucine-rich nuclear export signal and RGG Box RNA-binding domain, J. Virol. 76 (2002) 11866–11879.
[13] J. Rho, S. Choi, C.R. Jung, D.S. Im, Arginine methylation of Sam68 and SLM proteins negatively regulates their poly(U) RNA binding activity, Arch. Biochem. Biophys. 466 (2007) 49–57.
[14] P.A. Gillis, L.H. Okagaki, S.A. Rice, Herpes simplex virus type 1 ICP27 induces p38 mitogen-activated protein kinase signaling and apoptosis in HeLa cells, J. Virol. 83 (2009) 1767–1777.
[15] D. Hargett, T. McLean, S.L. Bachenheimer, Herpes simplex virus ICP27 activation of stress kinases JNK and p38, J. Virol. 79 (2005) 8348–8360.
[16] S.H. Chen, H.W. Yao, I.T. Chen, B. Shieh, C. Li, Suppression of transcription factor early growth response 1 reduces herpes simplex virus lethality in mice, J. Clin. Invest. 118 (2008) 3470–3477.
[17] K.S. Sciabica, Q.J. Dai, R.M. Sandri-Goldin, ICP27 interacts with SRPK1 to mediate HSV splicing inhibition by altering SR protein phosphorylation, EMBO J. 22 (2003) 1608–1619.
[18] L. Li, L.A. Johnson, J.Q. Dai-Ju, R.M. Sandri-Goldin, Hsc70 focus formation at the periphery of HSV-1 transcription sites requires ICP27, PLoS ONE 3 (2008) e1491.
[19] K. Murano, M. Okuwaki, M. Hisaoka, K. Nagata, Transcription regulation of the rRNA gene by a multifunctional nucleolar protein, B23/nucleophosmin, through its histone chaperone activity, Mol. Cell. Biol. 28 (2008) 3114–3126.
[20] J. Cote, F.M. Boisvert, M.C. Boulanger, M.T. Bedford, S. Richard, Sam68 RNA binding protein is an in vivo substrate for protein arginine *N*-methyltransferase 1, Mol. Biol. Cell 14 (2003) 274–287.
[21] M.T. Bedford, S.G. Clarke, Protein arginine methylation in mammals: who, What, and why, Mol. Cell 33 (2009) 1–13.
[22] F. Herrmann, J. Lee, M.T. Bedford, F.O. Fackelmayer, Dynamics of human protein arginine methyltransferase 1(PRMT1) in vivo, J. Biol. Chem. 280 (2005) 38005–38010.
[23] J. Tang, A. Frankel, R.J. Cook, S. Kim, W.K. Paik, K.R. Williams, S. Clarke, H.R. Herschman, PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells, J. Biol. Chem. 275 (2000) 7723–7730.
[24] S.K. Souki, P.D. Gershon, R.M. Sandri-Goldin, Arginine methylation of the ICP27 RGG box regulates ICP27 export and is required for efficient herpes simplex virus 1 replication, J. Virol. 83 (2009) 5309–5320.
[25] S.K. Souki, R.M. Sandri-Goldin, Arginine methylation of the ICP27 RGG box regulates the functional interactions of ICP27 with SRPK1 and Aly/REF during herpes simplex virus 1 infection, J. Virol. 83 (2009) 8970–8975.