Cytomegaloviral proteins pUL50 and pUL53 are associated with the nuclear lamina and interact with cellular protein kinase C

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Human cytomegalovirus-encoded pUL50 and pUL53 belong to a group of conserved herpesviral nuclear proteins. This study describes: (i) the co-localization of pUL50 with components of the nuclear lamina such as lamin A/C and lamin B receptor by double immunofluorescent staining, (ii) a strong pUL50-mediated relocalization of pUL53 from a diffuse nuclear pattern towards a nuclear rim localization, (iii) a direct interaction between pUL50 and pUL53, as well as between pUL50 and protein kinase C (PKC), shown by yeast two-hybrid and co-immunoprecipitation analyses, (iv) in vitro phosphorylation of pUL50, which is highly suggestive of PKC activity, and finally (v) partial relocalization of PKC by pUL50/pUL53 from its main cytoplasmic localization to a marked nuclear lamina accumulation. These data suggest a role for pUL50 and pUL53 in the recruitment of PKC, an event that is considered to be important for cytomegalovirus-induced distortion of the nuclear lamina.

INTRODUCTION

Human cytomegalovirus (HCMV; family Herpesviridae, subfamily Betaherpesvirinae, genus Cytomegalovirus, species Human herpesvirus 5) is a clinically important, ubiquitous human pathogen, causing severe systemic disease in immunosuppressed patients and prenatally infected children. The most frequently applied antiviral therapies are based on treatment with nucleoside analogues such as ganciclovir (GCV), which is activated by the GCV-phosphorylating potential of the UL97-encoded viral kinase (pUL97) (Curran & Noble, 2001).

HCMV replication is restricted to specific host-cell types and is dependent on the balance of interactions between viral and cellular proteins. One of the main regulatory processes during virus infection is the intracellular trafficking of viral proteins and particles. The exchange between nucleus and cytoplasm is mediated mainly through the nuclear pore complex, and thus the integrity of the nuclear envelope, which is composed of both membrane and lamina constituents, is crucial for intracellular transport pathways. The nuclear lamina, which lies beneath the inner nuclear membrane, contains a variable number of lamin isoforms and forms a rigid, proteinaceous meshwork. During infection with herpesviruses, the nuclear lamina restricts the efficiency of nucleocytoplasmic transport of viral capsids, as the large size of herpesviral capsids (~120 nm) does not allow for transit through nuclear pores (~39 nm; Pante & Kann, 2002). Lamina destabilization requires site-specific phosphorylation of lamins and lamin-binding membrane proteins. Phosphorylation leads to lamin depolymerization and may also permit their release from lamin-binding membrane proteins, including the lamin B receptor (LBR) (Peter et al. 1990; Goldman et al., 2002). Protein kinase C (PKC) and cdc2 have been identified as kinases capable of phosphorylating lamins during mitosis (Peter et al. 1990; Collas et al., 1997). In HCMV-infected cells, in addition to cellular protein kinases, the viral kinase pUL97 also possesses lamin-phosphorylating activity (Marschall et al., 2005). pUL97 has a number of functions within the viral replication cycle and is a target for antiviral drugs (Prichard et al., 1999; Biron et al., 2002; Marschall et al., 2002; Wang et al., 2003; Herget et al., 2004; Swan et al., 2007). pUL97 has been implicated in the nuclear egress of HCMV (Wolf et al., 2001; Krosky et al., 2003; Marschall et al., 2005).

Herpesviruses encode a conserved group of lamina-associated proteins, some of which recruit cellular as well as viral protein kinases to the nuclear lamina (Muranyi et al., 2002; Kato et al., 2006) and seem to be components of a functional nuclear egress complex (Sanchez & Spector, 2002). In particular, the herpes simplex virus 1 (HSV-1)-encoded proteins UL34 and UL31 have been described as essential factors for primary envelopment and thus for nuclear capsid export (Reynolds et al., 2004). A similar
functional role has been proposed for their mouse cytomegalovirus (MCMV) (pM50 and pM53) and Epstein–Barr virus (EBV) (BFRF1 and BFLF2) counterparts (Muranyi et al., 2002; Bubeck et al., 2004; Lake & Hutt-Fletcher, 2004; Farina et al., 2005; Gonnella et al., 2005; Lötzerich et al., 2006). However, relatively little information is available for the HCMV counterparts, pUL50 and pUL53. Dal Monte et al. (2002) described a lamina association of pUL53 in infected human fibroblasts. pUL53 co-localized with lamin B and was incorporated into virion tegument. These results are consistent with pUL53 having a role in nucleocapsid maturation, or egress of nucleocapsids from the nucleus to the cytoplasm (Dal Monte et al., 2002).

In this study, we analysed pUL50 and pUL53 expression in DNA transfection experiments to investigate their intracellular localization, protein interactions and whether pUL50/pUL53 complexes mediated recruitment of cellular proteins in a manner similar to that described for their homologues in other herpesviruses.

METHODS

Plasmid constructs. Expression constructs were generated by PCR amplification of the UL50 or UL53 open reading frame (ORF), respectively, using the template pCM1029 (Fleckenstein et al., 1982). In addition to full-length UL50, a truncated version, encoding aa 1–358, was generated. PCR amplification with primers carrying tag sequences resulted in fusion of the ORFs to C-terminal haemagglutinin (HA) or FLAG tags. PCR was performed using Vent DNA polymerase (New England Biolabs) for 35 cycles (denaturation for 40 s at 95 °C, annealing for 40 s at 50 °C and polymerization for 120 s at 72 °C). PCR products were inserted into the vectors pCDNA3.1 (Invitrogen), pGBT9 and pGAD424 (both Clontech) after cleavage with the restriction enzymes EcoRI/Xhol, Xhol/PstI or EcoRI/Sall, respectively. Constructs pDsRed1-N1 and pGFP-N1 expressing red (RFP) or (GFP) green fluorescent protein, respectively, were purchased from BD Clontech and used as positive controls for transfection experiments.

Oligonucleotides. Oligonucleotide primers used for PCR were purchased from Biomers; their sequences are given in Supplementary Table S1, available in JGV Online.

Cell culture and transfection. 293T and HeLa cells were cultivated in Dulbecco’s minimal essential medium containing 10 % fetal calf serum. Transient transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the protocol of the manufacturer, using 70–90 % confluent cells, with a seeding cell number of 4.2 × 10⁵ (HeLa) cells for six-well plates or 5 × 10⁶–6 × 10⁶ (293T) cells for 10 cm dishes.

Indirect immunofluorescent double staining. HeLa cells were grown on cover slips for transfection. At 2 days post-transfection, cells were fixed with 4 % paraformaldehyde (10 min, room temperature) and permeabilized using PBS/0.2 % Triton X-100 (20 min, 4 °C). Primary antibodies were incubated for 90 min at 37 °C. The secondary antibodies used for double staining were FITC-conjugated (green fluorescence; Dianova) and Cy3-conjugated (red fluorescence; Dianova), and were incubated for 45 min at 37 °C. The nucleus was counterstained with DAPI Vectashield mounting medium (Vector Laboratories). Data for immunofluorescence were collected using an Axiosvert-135 microscope (Zeiss) at magnifications of ×400 and ×630.

Co-immunoprecipitation assay (CoIP). 293T cells were transfected in six-well plates or 10 cm dishes. At 2 days post-transfection, cells were lysed in 500–1000 µl CoIP buffer [50 mM Tris/HC1 (pH 8.0), 150–300 mM NaCl, 5 mM EDTA, 0.5 % NP-40, 1 mM PMSF, 2 µg aprotinin ml⁻¹, 2 µg leupeptin ml⁻¹ and 2 µg pepstatin ml⁻¹] and used for CoIP with 1 µl (six-well plate) or 2.5 µl (10 cm dish) of anti-HA or pre-immune rabbit antiserum (anti-HA.11; HISS Diagnostics) for 2 h at 4 °C under rotation. Protein A-Sepharose beads were added to the CoIP reactions (2.5 mg, 2 h at 4 °C; Amersham Pharmacia Biotech). The precipitates were pelleted and washed before the samples were subjected to a standard Western blot analysis using mAbs specific for FLAG (M2; Sigma), PKCδ (A-3; Santa Cruz) or GFP (clones 7.1/13.1; Roche) for the detection of co-immunoprecipitates (ECL staining; New England Bio-Laboratories).

In vitro kinase assay (IVKA). The kinase activity of PKCδ–GFP was determined in vitro (2.5 µCi of [γ-32P]ATP) after immunoprecipitation of the kinase from transfected 293T cells as described previously (Marschall et al., 2001). Putative substrate proteins, such as pUL50(1–358)–HA, were co-expressed with PKCδ–GFP and co-immunoprecipitated. CoIP was performed in CoIP buffer as described above. The co-immunoprecipitates were pelleted and washed (using IVKA buffer without phosphatidylinerine and diacylglycerol) before the samples were subjected to an IVKA [IVKA buffer: 20 mM HEPES (pH 7.4), 0.03 % Triton X-100, 0.1 mg phosphatidylinerinne ml⁻¹, 10 µg diacylglycerol ml⁻¹ and 10 mM magnesium acetate]. Purified histone 2B (H2B; Roche) was added exogenously to the reaction at a concentration of 15 µM. In control settings, staurosporine (STP) was added at a concentration of 1 µM to the IVKA as an inhibitor of PKC activity.

Yeast two-hybrid screening. Protein interactions were analysed using GAL4 fusion proteins in a yeast two-hybrid system (Fields & Song, 1989). Saccharomyces cerevisiae strain Y153 expressing the proteins pUL50, pUL53 or others (in fusion with GAL4-BD as bait) was used for interaction analysis with selected expression clones (putative interactors in fusion with GAL4-AD, or vice versa) (Durfee et al., 1993). Selection for the presence of bait and interactor plasmids was achieved by cultivation on medium restricting growth to combined tryptophan/leucine prototrophy. Transformants were analysed for β-galactosidase (β-gal) activity by filter lift tests.

RESULTS

pUL50 localizes strictly at the nuclear envelope and relocates co-expressed pUL53 to this site

Transient expression of pUL50 and pUL53 resulted in the synthesis of abundant protein products of ~47 or 45 kDa, respectively, plus a number of additional faster-migrating species (Fig. 1a). pUL97 was included as a positive control for expression. Four specific protein modifications were reproducibly detectable for pUL53 (Fig. 1a, lanes 2 and 6, indicated by brackets), although the specific mode of modification is unknown. However, we have observed phosphorylation of both pUL50 and pUL50 by inorganic 32P in vivo-labelled of transfected cells (data not shown). Analysis of the primary sequence of the two proteins performed in silico (TMHMM server version 2.0; http://www.cbs.dtu.dk/services/TMHMM/) predicted a putative transmembrane domain for each protein.
Fig. 1. Expression of pUL50 and pUL53. (a) Western blot analysis. FLAG (F)- and HA-tagged proteins were expressed transiently in 293T cells as indicated. At 2 days post-transfection, the cells were lysed and lysates were subjected to SDS-PAGE and Western blot analysis. Protein detection was carried out using tag-specific antibodies (monoclonal anti-FLAG or polyclonal anti-HA antiserum, respectively). Protein modifications specific for pUL53 are indicated with square brackets in lanes 2 and 6. (b) Probability of membrane localization of internal regions of pUL50 and pUL53. Primary amino acid sequences were analysed by the TMHMM server version 2.0. The probability of membrane localization of each amino acid is given as a percentage; pink and blue lines indicate a theoretical ‘inside’ or ‘outside’ orientation, respectively. A marked score was found for a predicted membrane localization for aa 359–381 of pUL50. (c) HeLa cells were transfected with individual expression constructs (a–h) or co-transfected with two constructs (i–u). At 2 days post-transfection, cells were fixed and immunostained with mAbs specific for FLAG or lamin A/C or with polyclonal anti-HA. For double-staining experiments, combinations of two antibodies were applied and subsequently labelled individually with FITC or Cy3 conjugate, respectively. The merge of signals is shown on the right of each set of separate stainings. Cell nuclei were counterstained with DAPI.
transmembrane domain in pUL50 at aa 359–381 (Fig. 1b). For pUL53, no region with a probability of membrane localization was detected. To investigate intracellular localization, single transfections and co-transfections of the expression constructs were performed in HeLa cells. Both proteins were detectable exclusively in the nucleus. A putative nuclear localization signal (NLS) of ORF UL53 was predicted for aa 13–24 (PredictNLS server; http://cubic.bioc.columbia.edu/predictNLS/), but this prediction awaits experimental confirmation. No NLS was predictable for ORF UL50. Interestingly, the expressed pUL50 showed a very pronounced association with the nuclear envelope (nuclear rim staining; Fig. 1c, panels a–d), whereas pUL53 appeared to be distributed evenly throughout the nucleus (Fig. 1c, panels e–h). Importantly, upon co-expression of the two proteins, pUL53 localized almost completely towards the distinct nuclear rim staining of pUL50 (Fig. 1c, panels i–m). Thus, pUL50 was clearly dominant, altering the nuclear localization of pUL53. A deletion mutant of pUL50 lacking the predicted transmembrane domain [pUL50(1–358), Fig. 1c, panel o] exhibited a diffuse nuclear distribution and failed to influence the localization of pUL53 (Fig. 1c, panels n–q). Thus, the capacity of pUL50 to induce relocalization of pUL53 is dependent on the association of pUL50 with the nuclear envelope. Co-staining of pUL50 with endogenous lamins A/C showed a defined co-localization (Fig. 1c, panels r–u), consistent with pUL50 being associated with the inner nuclear membrane and/or the nuclear lamina.

**pUL50 interacts with pUL53 and cellular proteins**

The experiments described in Fig. 1(c) strongly implied that there is a direct interaction between pUL50 and pUL53. This potential interaction was therefore investigated in a yeast two-hybrid assay. Full-length pUL50 (including the transmembrane domain) was analysed initially, but appeared to be non-functional in the yeast two-hybrid system, probably due to its membrane-binding properties (data not shown). When a C-terminally truncated version of pUL50 was used [pUL50(1–358)], an interaction between pUL50(1–358) and pUL53 was detected (in both directions; Fig. 2a), albeit at a low level of signal intensity compared with a standard positive control (Fig. 2b, top panel). In control settings, neither protein produced any background activity (vector controls; Fig. 2b) and, moreover, did not interact with themselves (Fig. 2a, top two panels). Additional reactivity was observed between pUL50(1–358) and cellular proteins known to be transiently associated with the nuclear lamina, p32 and PKC (isoforms ε and ζ). p32 is known to interact with a range of proteins including LBR (Mylonis et al., 2004), PKC (Storz et al., 2000; Robles-Flores et al., 2002) and cytomegaloviral pUL97 (Marschall et al., 2005).

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**Fig. 2.** Yeast two-hybrid analysis of pUL50- and pUL53-specific protein interactions. (a) A GAL4-based yeast two-hybrid analysis was performed with a series of expression constructs, using co-transfections of pUL50 or pUL53 (in fusion with GAL4-BD or GAL4-AD, respectively) with putative interaction partners. A standard positive control (simian virus 40 large T antigen and tumour suppressor protein p53; Clontech) and vector specificity controls are shown in (b). Staining of selected yeast clones was performed by filter lift assay (+, β-gal positive; −, β-gal negative).
Addressing these issues of specificity, p32 did not interact with a series of other cytomegaloviral proteins including pUL53 (data not shown). pUL50, on the other hand, showed specific interactions with pUL53, p32 and PKC, but not with an N-terminal fragment of LBR (aa 1–208) or with pUL97 (Fig. 2a). Furthermore, several known interaction pairs could be reproduced in this series of experiments, i.e. the interactions of p32 with PKCα, pUL97 with p32 and LBR with p32 (Fig. 2a). No further interactions were detected for pUL53. Thus, a higher complex composed of viral and cellular lamina-associated proteins may be postulated according to the multifold interactions identified with the yeast two-hybrid system. This analysis indicates that pUL50 is a multifold interactor protein binding directly to pUL53, p32 and PKC.

The central protein interactions identified with the yeast two-hybrid system were confirmed by CoIP analysis using transfected 293T cells. The expression of suitable amounts of proteins was monitored by Western blot analysis (Fig. 3b, d). In CoIP experiments, a strong positive signal was obtained for the interaction between pUL50(1–358) and pUL53 (Fig. 3a), whilst interaction of pUL50(1–358) with another cytomegaloviral protein, pUL97, was negative. Using the full-length version of pUL50 in a parallel CoIP setting, an interaction with co-expressed pUL53 was also detectable, but the signal intensity was lower (possibly due to the loss of an insoluble membrane-bound fraction of full-length pUL50; data not shown). In addition, the interaction between pUL50 and cellular PKC was confirmed (Fig. 3c). Using a GFP-fused construct of PKCα, CoIP was positive for pUL50, but negative for pUL53 and pUL97 tested in parallel (Fig. 3c, lanes 3–5). When Western blot detection of the CoIP samples was performed with a mAb recognizing both recombinant and endogenous PKCα, two PKC-specific bands were obtained, with the recombinant form being dominant over a faint band for endogenous PKC (Fig. 3c, lane 8).

**PKC is probably the kinase phosphorylating pUL50 in vitro and is recruited by pUL50/pUL53 to the nuclear lamina in co-transfected cells**

The finding of a protein–protein interaction between pUL50 and PKC raised the question of whether pUL50 and its viral interactor, pUL53, were substrates of PKC-mediated phosphorylation. Therefore, we performed an IVKA using the protein kinase and putative substrate proteins immunoprecipitated from transfected cells. First, pUL50–HA, pUL53–HA and PKCα–GFP were expressed

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**Fig. 3.** Interactions of pUL50 with pUL53 and PKC. (a) HA-tagged pUL50(1–358) was transiently co-expressed in 293T cells with FLAG (F)-tagged pUL53 or pUL97, respectively. pUL97 was used as a CoIP negative control (lane 3). At 2 days post-transfection, cells were lysed and pUL50(1–358)–HA was precipitated using anti-HA antibody. Pre-immune serum was used as a negative control to monitor specificity. Co-immunoprecipitates were subjected to Western blot analysis using a mAb specific for FLAG. (b) Interaction of pUL50–HA or pUL53–HA with recombinant and endogenous PKCα. CoIP analysis was performed as in (a). Detection of co-precipitates was performed on Western blots using the antibodies indicated below the blots. Note that the mAb for PKCα detects both the recombinant and endogenous forms of PKCα. (c, d) Expression controls. Lysates of transfected cells shown in (a) and (b), respectively, were subjected to Western blot analysis for demonstration of protein expression using the indicated antibodies. Specific bands are indicated by arrows [note the cross-reactive band for the immunoglobulin heavy chain (lg-HC)].
separately, immunoprecipitated and added to IVKA reactions (twofold excess of substrate precipitates compared with kinase precipitates). Numerous weakly phosphorylated protein species were detected that potentially co-migrated with pUL50–HA and pUL53–HA, but these did not allow a conclusive interpretation (data not shown). Therefore, an improved setting was performed using a combined CoIP/IVKA strategy. PKC and putative substrates, such as pUL50(1–358)–HA, were co-expressed with PKCz–GFP and co-immunoprecipitated using a tag-specific antibody (anti-HA; Fig. 4a). In this case, clear phosphorylation of pUL50(1–358)–HA by PKCz–GFP was detectable (Fig. 4a, lanes 5 and 7). pUL53–HA did not co-immunoprecipitate PKCz–GFP (Fig. 4b, upper panel, lanes 6 and 9); thus, no signals for phosphorylation of pUL53–HA by PKCz–GFP were obtained (Fig. 4a, lane 6).

The co-immunoprecipitation of PKCz–GFP by pUL50(1–358)–HA was clearly detectable (Fig. 4b, lower panel, lanes 5, 7 and 8). Autophosphorylation of PKCz–GFP (which was not chemically stimulated in transfected cells) was below the threshold of detection (Fig. 4a, lanes 5 and 7). The strongest signals for in vitro phosphorylation of the standard substrate H2B were demonstrated for those samples containing PKCz–GFP (Fig. 4a, lanes 5 and 7). Other detectable signals for H2B phosphorylation most probably resulted from the co-immunoprecipitation of endogenous PKC or an unknown associated protein kinase. As a specificity control, a known inhibitor of PKC activity, STP (1 μM), was applied to the reactions, resulting in complete suppression of phosphorylation signals for pUL50(1–358)–HA and H2B (Fig. 4a, lanes 8 and 9). Thus, phosphorylation of pUL50 was detectable in vitro and PKCz is the most probable candidate for this activity.

Finally, we analysed the effects of expressing recombinant pUL50 and/or pUL53 on the intracellular localization of PKC in co-transfection experiments performed with HeLa cells. The cytoplasmic distribution of PKCz–GFP (Fig. 5, panels a–d) changed to favour a nuclear or perinuclear rim distribution when pUL50 and pUL53 were co-expressed (Fig. 5, panels e–h). Thus, PKC was partially recruited to the nuclear envelope as a consequence of pUL50/pUL53 expression. pUL50 alone, in the absence of co-expressed pUL53, was sufficient to relocalize PKCz–GFP (data not shown). Following relocalization, PKCz–GFP was observed not only to co-localize with endogenous LBR, but also to be associated with a reduced LBR immunofluorescent signal in individual cells (Fig. 5, panels i–m). Comparable immunofluorescence experiments performed in parallel confirmed the reproducibility of the finding. Thus, the reduction of detectable levels of LBR associated with the co-localization and accumulation of PKC might be the result of a reduction in the integrity of the nuclear lamina. From these results, we concluded that the viral protein complex pUL50–pUL53 mediates relocalization of PKC and speculate that this is associated with a disintegrative process in the nuclear lamina. Similar effects have been described for HSV-1 UL34-mediated recruitment of PKC (Park & Baines, 2006).

**Fig. 4.** IVKA demonstrating phosphorylation activity of PKCz. (a) pUL50(1–358)–HA, pUL53–HA and PKCz–GFP were co-expressed in transfected 293T cells (6×10^6–12×10^6) as indicated, co-immunoprecipitated from cell lysates using an anti-HA antiserum and subjected to an IVKA. As a standard kinase substrate, H2B (15 μM) was added directly to all reactions. PKC activity could be inhibited by the addition of 1 μM STP. (b) After phospoinmagner exposure, the IVKA blot was used for Western blot immunostaining with anti-HA (upper panel) and anti-GFP (lower panel) antibodies, respectively, to demonstrate recombinant expression of the proteins. Specific bands for pUL50(1–358), pUL53 and PKCz–GFP are indicated by arrows or brackets, respectively [note the cross-reactive band for the immunoglobulin heavy chain (lg-HC)].

**DISCUSSION**

We demonstrated that pUL50 relocalized pUL53 to a distinct rim pattern associated with the nuclear envelope. Upon co-expression, both proteins co-localized with components of the nuclear lamina such as lamin A/C and LBR. Direct interactions between pUL50 and pUL53 and between pUL50 and PKC were shown in yeast two-hybrid and CoIP analyses. In addition, pUL50 also interacted with cellular p32 in a yeast two-hybrid analysis. Furthermore, partial relocalization of PKC, in the presence of pUL50 and pUL53, from its main cytoplasmic localization to a marked nuclear lamina accumulation was demonstrated.
In prior studies on the HSV-1 homologues of UL50 and UL53, an interaction between UL34 and UL31 has been described (Liang & Baines, 2005). In addition, an interaction between positional homologues of UL34 and UL31 in EBV and MCMV has been demonstrated (Gonnella et al., 2005; Lötzerich et al., 2006). Thus, the interaction between members of the herpesviral UL34/UL31 protein homology group seem to have been conserved during herpesvirus evolution and may be crucial for their function. As it is known that UL34 is a type II transmembrane protein (Purves et al., 1992; Roller et al., 2000; Shiba et al., 2000), we performed a transmembrane prediction for its HCMV homologue, pUL50. A C-terminal region of 23 aa was identified as a potential transmembrane domain. Consistent with this finding, pUL50 localized in a rim pattern around the nuclear envelope in transfected cells. More specifically, an association with the nuclear lamina was illustrated by perfect co-localization of pUL50 with endogenous lamin A/C as well as LBR. In contrast, pUL53 showed diffuse nuclear localization when expressed alone, but was strictly relocalized by pUL50 to a lamina-associated localization. A similar situation has been shown for HSV-1 UL31, which co-localized with UL34 at the nuclear envelope upon co-expression but showed a non-defined nuclear distribution in the absence of other viral proteins (Reynolds et al., 2001).

An important common feature of UL34/UL31 and their homologues is their interaction with viral and cellular protein kinases (Muranyi et al., 2002; Reynolds et al., 2004; Ryckman & Roller, 2004; Bjerke & Roller, 2006; Kato et al., 2006; Park & Baines, 2006). In particular, cellular PKC is strongly recruited by viral lamina-associated proteins. In the present study, a direct interaction between pUL50 and PKC was demonstrated by yeast two-hybrid and CoIP analyses. In agreement with this concept was the finding that co-expressed pUL50 and pUL53 were able to relocalize PKC towards the nuclear lamina. In addition, an in vitro phosphorylation study provided strong evidence that pUL50 is a substrate of PKC.

Taken together, our studies suggest an involvement of pUL50 and pUL53 in cytomegalovirus-induced alterations of the nuclear envelope in the context of nuclear capsid export. We demonstrated properties of pUL50 and pUL53 similar to those of homologues of other herpesviruses, which seem to suggest that pUL50 and pUL53 likewise play

**Fig. 5.** Partial relocalization of PKC by pUL50/pUL53 and effects of relocalized PKC on endogenous LBR. HeLa cells were transfected with the expression construct for PKCα-GFP (a–d) or co-transfected with PKCα-GFP, pUL50-HA and pUL53-HA as indicated (e–m). PKCα-GFP fluorescence was analysed in comparison with indirect immunofluorescent staining of the two HA-tagged proteins (anti-HA, Cy3-conjugated anti-rabbit; g) or endogenous LBR (anti-LBR, Cy3-conjugated anti-rat; l), respectively.

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**Recombinant expression:**

**Immunofluorescent staining:**

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a role in the nuclear egress of viral capsids. Importantly, both proteins have been categorized as essential for virus replication in vitro (Dunn et al., 2003; Yu et al., 2003). For MCMV replication, a scenario was based on the formation of a nuclear egress complex composed of cellular and viral proteins including pM50 and pM53 (Muranyi et al., 2002; Bubeck et al., 2004; Rupp et al., 2007), which may be essential for capsid egress. It was postulated that recruitment of specific protein kinases may lead to increased phosphorylation of lamins, resulting in the depolymerization of the nuclear lamina (Muranyi et al., 2002; Sanchez & Spector, 2002; Lötzerich et al., 2006). In the case of HCMV, pUL50 also seems to possess an important recruitment function. In particular, the interaction of pUL50 with PKC and its ability to relocalize PKC to nuclear lamina sites seem to be connected with a PKC-induced reduction in detectable levels of LBR. Moreover, it is known that the cytomegaloviral kinase pUL97 is recruited to the nuclear lamina mainly through its interaction with p32 (Marschall et al., 2002; Spector, 2002; Lotzerich et al., 2006). In the case of HCMV, pUL50 and pUL53 possess highly defined properties of nuclear protein interactions and suggest that the two proteins may have particular importance for the formation of a multi-component egress complex.

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