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Viral determinants influencing intra- and intercellular communication in cytomegalovirus infection

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Cytomegaloviruses (CMVs) are typically disseminated by cell-to-cell transfer, which requires reprogramming of cellular signaling pathways and restructuring of the cell architecture. Viral particles not only transfer genetic information between cells, but also tegument proteins that enable the virus to counteract cellular defense mechanisms immediately upon entering cells. The UL25 gene family of CMVs encodes such tegument proteins and also gives rise to related nonstructural proteins expressed early in infection. Herein, we report on the functions attributed to UL25 family members of several β-herpesviruses, particularly to the M25 proteins of mouse CMV that were found to interfere with the antiviral role of the p53 tumor suppressor protein and to mediate cytoskeleton rearrangement of infected cells.

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Abbreviations: PML, promyelocytic leukemia protein; IFN, interferon; PRR, pattern recognition receptor; IRF3, interferon response factor 3; HAX1, HCLS1 (Hematopoietic Cell-Specific Lyn Substrate 1)-associated protein X-1; NCK, Non-catalytic region of tyrosine kinase adapter protein

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Introduction

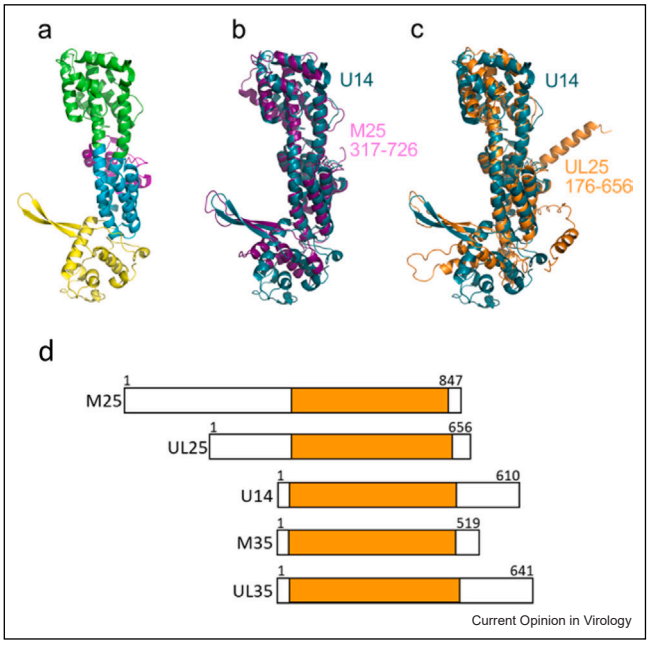
Cytomegaloviruses (CMVs) are entering an organism via mucosal surfaces and from this local infection, the virus is then disseminated throughout the body in order to colonize various tissues and organs. Although the infection is usually well-controlled by the immune system of healthy individuals, the virus is able to establish a life-long latent infection. The viral genomes persist in a state of dormancy in a fraction of the initially infected cells and can occasionally give rise to recurrent infections. CMVs are highly cell-associated and upon both acute infection and reactivation, dissemination within an infected organism occurs primarily by cell-to-cell spread. The cell association and the comparably slow infection cycle necessitate particular effort of CMVs to reprogram various cellular pathways and to keep the infected cell alive for several days. With a genome size of approximately 240 kbp and a coding potential of more than 170 proteins, CMVs are well-equipped for these tasks. Viral interference with cellular signaling pathways starts already upon attachment and binding of virus particles to the cell surface, triggering signals via cell surface molecules. This is followed by intracellular signaling mediated by tegument proteins once they are released into newly infected cells. One important function of tegument proteins is to disarm cellular defense mechanisms. Nonstructural viral proteins expressed early on are contributing to this task, paving the way to primary productive infection as well as enabling reactivation, thereby securing the persistence of the virus and chronic infection. During the infection cycle, substantial changes of cellular architecture are induced, securing the release of viral progeny and transfer to neighboring cells.

The β-herpesvirus UL25 family proteins comprise components of the tegument as well as nonstructural proteins, and exert several functions within infected cells, including effects on the cytoskeleton. We report on our findings about the mouse cytomegalovirus (MCMV) M25 proteins and summarize the facts described for UL25 orthologs of human CMV (HCMV) and related β-herpesviruses. Commonalities and differences between various UL25 family members may reflect evolutionary adaptions to the respective host species or functional needs.

The ß-herpesvirus UL25 gene family gives rise to structural and nonstructural viral proteins

UL25 family proteins (also called pp85 superfamily) have first been discovered as tegument proteins present in β-herpesvirus particles [1–6]. Human herpesviruses (HHV) 6A, 6B, and 7 encode one UL25 family member (U14), while two related genes are expressed by HCMV and MCMV (UL25, UL35 and M25, M35), which probably

Figure 1



Structural comparison of HHV6B U14 to predicted models of homologs from MCMV (M25) and HCMV (UL25). **(a)** U14 domain architecture, with the four subdomains labeled in magenta, cyan, green, and yellow. Domain architecture and structure of U14 were described in Ref. [11] (pdb: 5b1q). **(b, c)** AlphaFold2-predicted structure of M25 (amino acids: 317-726) **(b)** and UL25 (amino acids: 176-656) **(c)** aligned with U14 (see **a**). **(d)** Linear representation of UL25 family proteins indicating the globular domain conserved between individual family members (orange) and flanking IDRs.

arose by gene duplication. pUL25 and pM25 represent abundant tegument components of the respective viruses [4,7], whereas the UL35 family members are present in lower amounts [7,8]. A recent spatial proteomics study of HCMV particles suggested that pUL35 is enriched within the inner and pUL25 in the outer tegument [9]. In line with the pM25 abundance, disruption of the M25 gene led to substantially reduced virion size [4]. Conversely, deletion of HCMV UL25, although encoding the third-most abundant tegument protein of this virus, did not result in a similar effect, suggesting plasticity in the incorporation of HCMV tegument proteins [10].

Comparison of the amino acid sequences of different UL25 family members revealed a conserved core (although with limited amino acid identity, 20–25%) that is either preceded or followed by less-conserved, most likely intrinsically disordered regions (IDRs). Notably, the crystal structure of the conserved part of HHV6B U14 was determined [11], disclosing an elongated helix-rich fold that can be divided into four subdomains (Figure 1a). *In silico* modeling using the AlphaFold2 program [12] indicates structural conservation of the overall architecture for other UL25 family members (Figure 1b and c). The U14 protein forms a dimer, and although there are differences in the putative interface of related UL25 family members [11], it is likely that all of them are able to homo-oligomerize. IDRs have been implicated in interaction with other proteins, suggesting that UL25 family proteins may form scaffolds for recruiting viral and cellular proteins or bind themselves to such complexes. Interactions of the HCMV UL25 and UL35 proteins with a number of other viral structural components have indeed been described, examples being the tegument proteins pUL26 and pUL82, respectively [10,13–15]. Upon virus entry into human fibroblasts, UL25-containing protein complexes can be transferred to different subcellular compartments, for instance, to the cell nucleus as reported for pUL35 and pUL82 [15], to fulfill joint functions. Besides structural virus components, the HCMV UL35 and MCMV M25 genes encode also smaller nonstructural viral proteins arising from transcripts with start sites located downstream within these open-reading frames [1,4]. Since these smaller isoforms are not incorporated into virions, it is likely that they exert regulatory functions within the infected fibroblasts, possibly sustaining the effects initiated by the related tegument proteins.

Disarming intrinsic and innate defense mechanisms of infected cells

In the early phase of infection or when overexpressed by transfection, the MCMV M25 proteins are detected in nuclear bodies of murine fibroblasts [4,16], and a similar feature was observed for the HCMV UL35 protein [15,17]. When expressed independent of viral infection, the M25 proteins as well as pUL35 colocalize with PML nuclear bodies (Ref. [17] and unpublished data). These subnuclear structures contain various cellular restriction factors and have been implicated with early defense mechanisms against DNA viruses [18]. It has been reported that nuclear body formation by pUL35 can occur independently of PML, and that restriction factors such as Daxx, Sp100, and PML are recruited to newly formed pUL35 structures [17], probably to counteract their antiviral function. In line with this hypothesis, pUL35 (in conjunction with pUL82) was able to activate the HCMV major immediate–early promoter, and an HCMV UL35 mutant displayed delayed onset of viral gene expression [19].

Once the viral DNA genome is released from incoming capsids, it becomes vulnerable for recognition by cellular pattern recognition receptors (PRR), ultimately triggering expression of type-I interferons (IFN) and other cytokines. An unbiased screen of MCMV genes revealed M35 as an antagonist of type-I IFN induction, acting downstream of several intracellular PRR as well as Toll-like receptors [8]. This function of M35 appeared particularly important for MCMV infection of macrophages and other myeloid cells, and deletion of the M35 gene

led to substantial attenuation of the respective virus mutant *in vivo*. A similar task was subsequently described for the HCMV UL35 protein, though the exact mechanisms used by the UL35 and M35 proteins may be slightly different [20]. pUL35 binds to the TANK-binding kinase 1 and prevents its phosphorylation upon activation of PRR, thereby limiting the downstream phosphorylation of IRF3 and transcription of type-I interferon genes.

The HCMV UL25 protein has a function in counteracting the innate defense response as well, though this is rather indirect via interaction with pUL26 and its stabilization [10]. pUL26 inhibits the conjugation of the interferon-stimulated gene-15 product (ISG15) to newly synthesized proteins, which would mark them for degradation [21]. A UL25 deletion mutant turned out to be much more sensitive to interferon-β, due to upregulation of ISG15 and ISGylation of viral and cellular proteins, with a negative impact on HCMV replication.

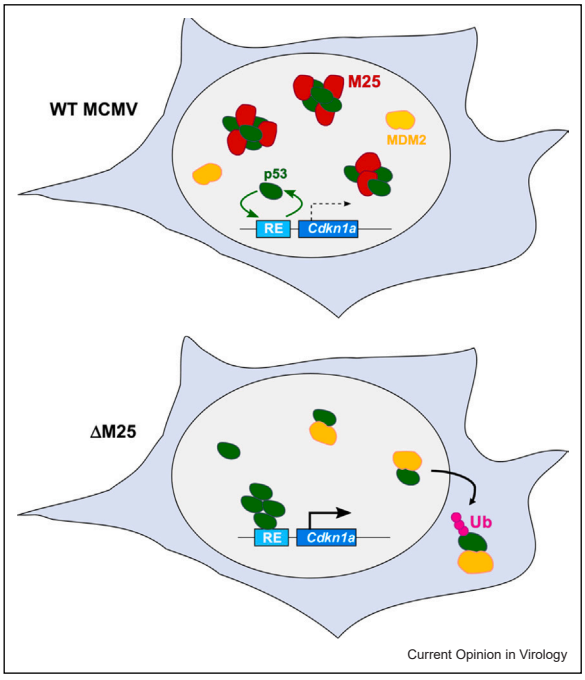
Taken together, there is a clear role of UL25 family members in blocking the activation of the type-I interferon response (pM35, pUL35) or in modulating the effects of interferon-stimulated genes (pUL25).

Interference of M25 proteins with p53

In an attempt to determine the function of the MCMV M25 protein, we expressed it in HEK293T cells and identified cellular interaction partners by immunoprecipitation and mass spectrometry [16]. The tumor suppressor protein p53 emerged as one of the most prominent M25 interaction partners. Interestingly, p53 binding was also reported for the related U14 protein of HHV6B [22]. p53 is an 'old acquaintance', well known for its protective role in oncogenesis (for review see, e.g. Ref. [23]). Upon sensing of DNA damage, p53 becomes activated and induces a plethora of effector genes. One of the immediate effects of p53 activation is cell cycle arrest and induction of DNA repair, and upon sustained p53 activation, cell death is initiated. Infection with DNA viruses leads to genotoxic stress, particularly upon replication of the viral genomes. Cell cycle arrest and cell death induced by p53 would be detrimental for virus production, and DNA viruses must therefore dampen the activity of p53. Interestingly, however, MCMV induces a DNA damage response early in infection of murine fibroblasts [24], and similar observations have been reported for HCMV and other β-herpesviruses [22,25–27]. This suggests that β-herpesviruses utilize p53 to get the productive infection cycle started, but subsequently differential regulation of p53 target genes is needed to avoid their antiviral function.

Both HCMV and MCMV sequester p53 in the nucleus of infected fibroblasts, resulting in p53 accumulation

Figure 2



Sequestration of p53 by MCMV M25 proteins and its functional consequences. Upon infection with wild-type MCMV, M25 proteins interact with p53 and sequester it to nuclear dot-like structures. This renders p53 inaccessible to its main negative regulator MDM2, which impairs its turnover and leads to gradual accumulation. As a result, the association of p53 with response elements (RE) in promoters of certain target genes is reduced, limiting their expression [16]. In the absence of the M25 proteins (i.e. after infection with the ΔM25 mutant), the natural turnover process of p53 remains unaffected. Despite lower abundance, p53 exhibits, however, stronger association with its RE and higher transcriptional activity toward specific targets in ΔM25-infected cells.

over the course of infection [16,28]. A similar effect was reported for HHV6B, although p53 accumulated in the nucleus as well as the cytoplasm of HHV6B-infected cells [22]. We could show that the M25 proteins colocalize with p53 in dot-like nuclear structures both in infected and transfected fibroblasts, leading to p53 stabilization and strongly diminished turnover (Figure 2) [16]. Deletion of the M25 gene prevented p53 accumulation during infection. Since p53 transcript levels were comparable in cells infected with wild-type MCMV or the M25 deletion mutant, the increase in p53 amounts must be primarily regulated at the protein level. Despite accumulation, binding of p53 to the promoters of its important target genes C*dkn1a* and *Mdm2* was reduced in wild-type MCMV-infected fibroblasts when compared with infection with the ΔM25 deletion mutant, going along with a lower transcription rate (Figure 2). This did not apply to other p53-regulated genes (e.g. PUMA, BAX, or p53 itself), pointing to differential regulation by the pM25-mediated sequestration. Notably, the growth deficit of an MCMV M25

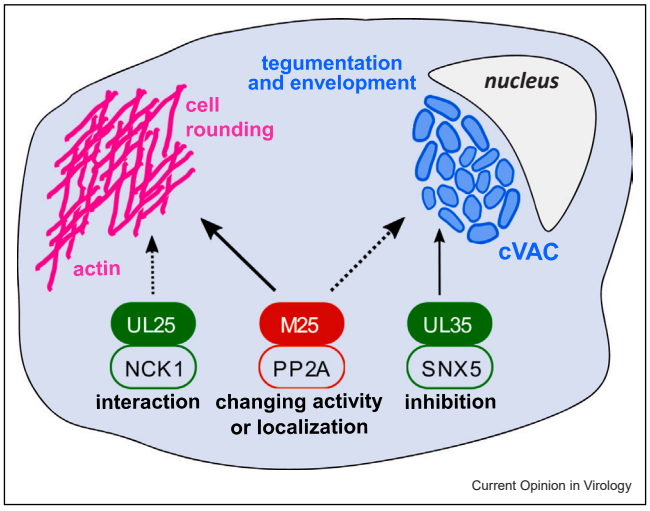
deletion mutant was substantially rescued upon propagation on p53-deficient fibroblasts [16]. Along the same line, HHV6B mutants with U14 deletion or mutation of amino acids required for p53 interaction were either not viable or severely growth-impaired [11,29]. These results indicate that p53 has an overall antiviral function in MCMV and HHV6 infection, different from HCMV infection, for which a proviral role of p53 was proposed [25,30], questioning a comparable role of HCMV UL25.

The findings for the MCMV and HHV6 UL25 family proteins underline their importance for modulating the activity of p53 in infected cells. Moreover, since p53 is incorporated into HHV6B virions (together with U14) [22], it is taken to newly infected cells and may support the 'kick-start' of the next round of infection.

Role of UL25 proteins in changing the cellular architecture

An MCMV M25 deletion mutant displayed a severe defect in inducing cell rounding [4], indicating that the M25 proteins are involved in altering the morphology of infected fibroblasts (Figure 3). Further, expression of M25 proteins alone was sufficient to induce cytoskeletal changes [4]. CMVs elicit a profound modification of the cellular architecture, leading to cell rounding, also referred to as cytopathic effect. This includes remodeling of the cytoskeleton as well as reorganization of intracellular trafficking [31–33]. Vesicles derived from the Golgi apparatus, the trans-Golgi network (TGN), and endosomes create a new compartment adjacent to the cell nucleus called the cytoplasmic virion assembly compartment (cVAC), where capsids are surrounded by the tegument protein layer and finally by a lipid membrane. Recent findings indicate that the cVAC serves as a microtubule-organizing center that not only controls the positioning of the nucleus next to it, but also facilitates release of newly generated virus particles [34–36]. We observed that an MCMV M25 deletion mutant displayed reduced cell-to-cell spread as evidenced by a small plaque phenotype [4], and similarly, a defect in virion assembly was described for a HCMV UL35 deletion mutant [19]. Moreover, the large isoform of the M25 proteins and the HCMV UL35 protein translocates to the cVAC during the late phase of infection, in line with their incorporation into new virions [4,19,37]. The described phenotypes of the M25 and UL35 virus mutants suggest that the function of these UL25 family members goes beyond being a structural component in particle formation, pointing to additional roles in reorganization of the cellular architecture or transport pathways. pUL35 was found to interact with sorting nexin 5 (SNX5) [37] (Figure 3), a component of the retromer protein complex that participates in cargo transport from endosomes to the TGN. Interference of pUL35 with this transport pathway leads, for instance, to accumulation of the viral glycoprotein B within the cVAC, thereby ensuring efficient incorporation into the viral envelope. Notably, reduced virus release observed for a UL35 mutant with defective SNX5 binding could be rescued by depletion of SNX5 [37].

Figure 3



Putative roles of UL25 family members in host cell cytoskeleton reorganization and intracellular transport. CMV infection leads to extensive reshaping of the actin cytoskeleton. Moreover, intracellular vesicles are remodeled to form a new compartment called cytoplasmic virion assembly compartment (cVAC), where the final stages of particle assembly, tegumentation, and envelopment take place. Impairment of these processes leads to reduced viral cell-to-cell spread as evidenced by MCMV M25 and HCMV UL35 deletion mutants [4,19]. The MCMV M25 proteins affect cell rounding [4], probably mediated by binding to protein phosphatase 2A (PP2A) PP2A complexes, thereby interfering with their activity or localization. This could also alter the function of PP2A in Golgi morphogenesis and influence cVAC formation. The HCMV UL25 protein interacts with the adapter protein NCK1, which could contribute to cytoskeletal alteration [14]. pUL35 of HCMV binds to SNX5, inhibiting a retrograde transport pathway and ensuring proper localization of glycoprotein B within cVAC and production of HCMV virions [37].

HCMV pUL25 interacts with the adapter proteins NCK1 and NCK2 [14], which regulate actin polymerization (besides performing other tasks) [38,39], suggesting that pUL25 is involved in cytoskeletal remodeling too (Figure 3). The mechanism by which the MCMV M25 proteins influence the cytoskeleton of fibroblasts is less clear. For instance, we could not detect an interaction of the M25 proteins with NCK proteins. Another candidate protein, HAX1, known to regulate cell adhesion and motility [40] and potentially linking pM25 with cytoskeleton rearrangements, was discovered by mass spec analysis [16]. pM25 and HAX1 were observed to colocalize during infection (unpublished data), yet direct interaction could not be validated. Preliminary results suggest that the M25 proteins bind to protein phosphatase 2A (PP2A) complexes (unpublished data).

PP2A is the most abundant cellular protein phosphatase, and PP2A complexes control many different pathways, including regulation of the actin cytoskeleton and Golgi morphogenesis [41,42]. Interference of viral proteins with the activity of PP2A is not unprecedented, with the small tumor antigen of polyomaviruses being the best-known example [43–46]. The functional consequences of the pM25–PP2A interaction are subject of our current investigation. We hypothesize that the M25 proteins influence the enzymatic activity of PP2A, displace specific regulatory subunits of PP2A complexes, thereby altering substrate specificity, or change the subcellular localization of PP2A complexes (Figure 3). Similar to what has been described for the polyomavirus small tumor antigen, the M25 proteins may alter cell morphology in this way and influence release and cell-to-cell spread of MCMV.

These data suggest that several UL25 family members have a second function late in the infection cycle, changing the cellular architecture and secretory transport pathways to secure efficient production and transmission of progeny virus.

Conclusions

UL25 family proteins have been linked with a number of functions. At first glance, one might assume that these proteins have evolved in different directions and have little in common. However, the overall structural conservation of the UL25 core suggests that at least some functions are shared. For example, all UL25 genes encode tegument proteins delivering an 'additional message' to newly infected cells. As exemplified by U14, they may even recruit cellular proteins into viral particles (such as p53), giving them a piggyback ride to new cells. The incoming tegument proteins can act immediately, to overcome, for instance, early cellular defense mechanisms. An example is the interference of M35 and UL35 proteins with signaling cascades initiated by PRR, preventing or limiting the innate immune response [8,20]. The phenotype of UL25 virus mutants as well as the identified cellular interaction partners point also to functions in disarming intrinsic defense mechanisms [15–17].

Despite the abovementioned partial structural conservation, UL25 family proteins of different β-herpesvirus species have also diversified, as indicated by low overall sequence identity and acquisition of IDRs with different lengths. Consistent with this, several functions have been ascribed specifically to individual UL25 members and it would be of interest to comprehensively analyze all UL25 family proteins in this respect as well as their ability to substitute for each other. Preliminary data indicated that the HCMV UL25 protein is not able to rescue the 'cell rounding' phenotype of an MCMV M25 deletion mutant, pointing to divergent roles. Since the M25 proteins are substantially larger than other UL25 family proteins, probably resulting from fusion of another genetic unit to a primordial UL25 gene [47], they might have adopted additional functions.

Several of the UL25 family members are multifunctional and switch roles during the viral infection cycle. This goes along with alteration of their subcellular localization. In line with their incorporation into the tegument layer of virions, the UL25 proteins are present in the cVAC late in infection, contrasting with their nuclear localization at early time points. The different spatial distribution could result from interaction with other viral or cellular proteins, or post-translational modification. An important role of UL25 proteins during the late infection phase appears to be the remodeling of the cellular architecture, possibly including the shaping of the cVAC. One has to point out that most studies on UL25 proteins have been performed in fibroblast cells, yet CMVs are able to infect a wide range of different cell types, including myeloid cells, which are central for dissemination of the infection [48]. It will therefore be important to investigate the functions of UL25 family proteins in other cell types as well. In view of the connection between cytoskeletal alterations and the migration of cells, it will be of interest to learn how UL25 proteins influence the migration of myeloid cells that mediate CMV dissemination.

Taken together, the UL25 proteins interfere with various signaling pathways within the infected cells, battling intrinsic and innate defenses. They also influence the communication between cells, as remodeling of the cytoskeleton affects cell-to-cell contacts and facilitates the spread of virions as well as of UL25 proteins themselves to neighboring cells.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

None.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Liu Y, Biegalke BJ: **The human cytomegalovirus UL35 gene encodes two proteins with different functions**. *J Virol* 2002, **76**:2460-2468.

2. Stefan A, Secchiero P, Baechi T, Kempf W, Campadelli-Fiume G: **The 85-kilodalton phosphoprotein (pp85) of human herpesvirus 7 is encoded by open reading frame U14 and localizes to a tegument substructure in virion particles**. *J Virol* 1997, **71**:5758-5763.

3. Wu CA, Carlson ME, Henry SC, Shanley JD: **The murine cytomegalovirus M25 open reading frame encodes a component of the tegument**. *Virology* 1999, **262**:265-276.

4. • Kutle I, Sengstake S, Templin C, Glaß M, Kubsch T, Keyser KA, Binz A, Bauerfeind R, Sodeik B, Čičin-Šain L, *et al*.: **The M25 gene products are critical for the cytopathic effect of mouse cytomegalovirus**. *Sci Rep* 2017, **7**:15588-x.

Description of the phenotype of the MCMV ΔM25 mutant and the role of M25 proteins in cytoskeletal changes.

5. Battista MC, Bergamini G, Boccuni MC, Campanini F, Ripalti A, Landini MP: **Expression and characterization of a novel structural protein of human cytomegalovirus, pUL25**. *J Virol* 1999, **73**:3800-3809.

6. Mahmoud NF, Kawabata A, Tang H, Wakata A, Wang B, Serada S, Naka T, Mori Y: **Human herpesvirus 6 U11 protein is critical for virus infection**. *Virology* 2016, **489**:151-157.

7. Reyda S, Tenzer S, Navarro P, Gebauer W, Saur M, Krauter S, Buscher N, Plachter B: **The tegument protein pp65 of human cytomegalovirus acts as an optional scaffold protein that optimizes protein uploading into viral particles**. *J Virol* 2014, **88**:9633-9646.

8. •• Chan B, Gonçalves Magalhães V, Lemmermann NAW, Juranić Lisnić V, Stempel M, Bussey KA, Reimer E, Podlech J, Lienenklaus S, Reddehase MJ, *et al*.: **The murine cytomegalovirus M35 protein antagonizes type I IFN induction downstream of pattern recognition receptors by targeting NF-κB mediated transcription**. *PLoS Pathog* 2017, **13**:e1006382.

This study reports on the identification of the MCMV M35 protein as inhibitor of PRR-induced signaling and type I interferon expression and its role on *in vitro* and *in vivo* MCMV infection.

9. Bogdanow B, Gruska I, Mühlberg L, Protze J, Hohensee S, Vetter B, Lehmann M, Wiebusch L, Liu F: **Spatial, quantitative and functional deconstruction of virus and host protein interactions inside intact cytomegalovirus particles**. *bioRxiv* 2022, https://doi.org/10.1101/2022.05.02.490278

10. • Zimmermann C, Büscher N, Krauter S, Krämer N, Wolfrum U, Sehn E, Tenzer S, Plachter B: **The abundant tegument protein pUL25 of human cytomegalovirus prevents proteasomal degradation of pUL26 and supports its suppression of ISGylation**. *J Virol* 2018, **92**:1180.

Mechanistic description how the UL25 protein dampens the interferon response in HCMV infection.

11. •• Wang B, Nishimura M, Tang H, Kawabata A, Mahmoud NF, Khanlari Z, Hamada D, Tsuruta H, Mori Y: **Crystal structure of human herpesvirus 6B tegument protein U14**. *PLoS Pathog* 2016, **12**:e1005594.

Crystal structure determination of the core of the HHV6 U14 protein.

12. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek A, Potapenko A, *et al*.: **Highly accurate protein structure prediction with AlphaFold**. *Nature* 2021, **596**:583-589.

13. To A, Bai Y, Shen A, Gong H, Umamoto S, Lu S, Liu F: **Yeast two hybrid analyses reveal novel binary interactions between human cytomegalovirus-encoded virion proteins**. *PLoS One* 2011, **6**:e17796.

14. Nobre LV, Nightingale K, Ravenhill BJ, Antrobus R, Soday L, Nichols J, Davies JA, Seirafian S, Wang EC, Davison AJ, *et al*.: **Human cytomegalovirus interactome analysis identifies degradation hubs, domain associations and viral protein functions**. *Elife* 2019, **8**:e49894, https://doi.org/10.7554/eLife.49894

15. Schierling K, Stamminger T, Mertens T, Winkler M: **Human cytomegalovirus tegument proteins ppUL82 (pp71) and ppUL35 interact and cooperatively activate the major immediate-early enhancer**. *J Virol* 2004, **78**:9512-9523.

16. •• Kutle I, Szymańska-de Wijs KM, Bogdanow B, Cuvalo B, Steinbrück L, Jonjić S, Wagner K, Niedenthal R, Selbach M, Wiebusch L, *et al*.: **Murine cytomegalovirus M25 proteins sequester the tumor suppressor protein p53 in nuclear accumulations**. *J Virol* 2020, **94**:e00574-20.

Description of the interaction between MCMV M25 and p53 and of interference with the antiviral function of p53.

17. Salsman J, Wang X, Frappier L: **Nuclear body formation and PML body remodeling by the human cytomegalovirus protein UL35**. *Virology* 2011, **414**:119-129.

18. Tavalai N, Stamminger T: **Intrinsic cellular defense mechanisms targeting human cytomegalovirus**. *Virus Res* 2011, **157**:128-133.

19. Schierling K, Buser C, Mertens T, Winkler M: **Human cytomegalovirus tegument protein ppUL35 is important for viral replication and particle formation**. *J Virol* 2005, **79**:3084-3096.

20. Fabits M, Gonçalves Magalhães V, Chan B, Girault V, Elbasani E, Rossetti E, Saeland E, Messerle M, Pichlmair A, Lisnić VJ, Brinkmann MM: **The cytomegalovirus tegument protein UL35 antagonizes pattern recognition receptor-mediated type I IFN transcription**. *Microorganisms* 2020, **8**:790.

21. Kim YJ, Kim ET, Kim Y, Lee MK, Kwon KM, Kim KI, Stamminger T, Ahn J: **Consecutive inhibition of ISG15 expression and ISGylation by cytomegalovirus regulators**. *PLoS Pathog* 2016, **12**:e1005850.

22. Takemoto M, Koike M, Mori Y, Yonemoto S, Sasamoto Y, Kondo K, Uchiyama Y, Yamanishi K: **Human herpesvirus 6 open reading frame U14 protein and cellular p53 interact with each other and are contained in the virion**. *J Virol* 2005, **79**:13037-13046.

23. Hafner A, Bulyk ML, Jambhekar A, Lahav G: **The multiple mechanisms that regulate p53 activity and cell fate**. *Nat Rev Mol Cell Biol* 2019, **20**:199-210.

24. Marcinowski L, Lidschreiber M, Windhager L, Rieder M, Bosse JB, Radle B, Bonfert T, Gyory I, de Graaf M, Prazeres da Costa O, *et al*.: **Real-time transcriptional profiling of cellular and viral gene expression during lytic cytomegalovirus infection**. *PLoS Pathog* 2012, **8**:e1002908.

25. Hannemann H, Rosenke K, O'Dowd JM, Fortunato EA: **The presence of p53 influences the expression of multiple human cytomegalovirus genes at early times postinfection**. *J Virol* 2009, **83**:4316-4325.

26. Salsman J, Jagannathan M, Paladino P, Chan PK, Dellaire G, Raught B, Frappier L: **Proteomic profiling of the human cytomegalovirus UL35 gene products reveals a role for UL35 in the DNA repair response**. *J Virol* 2012, **86**:806-820.

27. Scherer M, Read C, Neusser G, Kranz C, Kuderna AK, Muller R, Full F, Worz S, Reichel A, Schilling EM, *et al*.: **Dual signaling via interferon and DNA damage response elicits entrapment by giant PML nuclear bodies**. *Elife* 2022, **11**:e73006, https://doi.org/10.7554/eLife.73006

28. Fortunato EA, Spector DH: **p53 and RPA are sequestered in viral replication centers in the nuclei of cells infected with human cytomegalovirus**. *J Virol* 1998, **72**:2033-2039.

29. Mori J, Tang H, Kawabata A, Koike M, Mori Y: **Human Herpesvirus 6A U14 is important for virus maturation**. *J Virol* 2016, **90**:1677-1681.

30. Casavant NC, Luo MH, Rosenke K, Winegardner T, Zurawska A, Fortunato EA: **Potential role for p53 in the permissive life cycle of human cytomegalovirus**. *J Virol* 2006, **80**:8390-8401.

31. Sanchez V, Greis KD, Sztul E, Britt WJ: **Accumulation of virion tegument and envelope proteins in a stable cytoplasmic compartment during human cytomegalovirus replication: characterization of a potential site of virus assembly**. *J Virol* 2000, **74**:975-986.

32. Das S, Vasanji A, Pellett PE: **Three-dimensional structure of the human cytomegalovirus cytoplasmic virion assembly complex includes a reoriented secretory apparatus**. *J Virol* 2007, **81**:11861-11869.

33. Das S, Pellett PE: **Spatial relationships between markers for secretory and endosomal machinery in human cytomegalovirus-infected cells versus those in uninfected cells**. *J Virol* 2011, **85**:5864-5879.

34. Procter DJ, Furey C, Garza-Gongora AG, Kosak ST, Walsh D: **Cytoplasmic control of intranuclear polarity by human cytomegalovirus**. *Nature* 2020, **587**:109-114.

35. Procter DJ, Banerjee A, Nukui M, Kruse K, Gaponenko V, Murphy EA, Komarova Y, Walsh D: **The HCMV assembly compartment is a dynamic Golgi-derived MTOC that controls nuclear rotation and virus spread**. *Dev Cell* 2018, **45**:83-100.e7.

36. Lyon SM, Kalejta RF: **HCMV assembly is totally tubular**. *Dev Cell* 2018, **45**:1-2.

37. •• Maschkowitz G, Gärtner S, Hofmann-Winkler H, Fickenscher H, Winkler M: **Interaction of human cytomegalovirus tegument proteins ppUL35 and ppUL35A with sorting nexin 5 regulates glycoprotein B (gpUL55) localization**. *J Virol* 2018, **92**:e00013-18.
Mechanistic description of the UL35-SNX5 interaction and the functional consequences on the cytoplasmic virion assembly compartment and HCMV particle release.

38. Bywaters BC, Rivera GM: **Nck adaptors at a glance**. *J Cell Sci* 2021, **134**:jcs258965., https://doi.org/10.1242/jcs.258965

39. Buvall L, Rashmi P, Lopez-Rivera E, Andreeva S, Weins A, Wallentin H, Greka A, Mundel P: **Proteasomal degradation of Nck1 but not Nck2 regulates RhoA activation and actin dynamics**. *Nat Commun* 2013, **4**:2863.

40. Balcerak A, Trebinska-Stryjewska A, Wakula M, Chmielarczyk M, Smietanka U, Rubel T, Konopinski R, Macech-Klicka E, Zub R, Grzybowska EA: **HAX1 impact on collective cell migration, cell adhesion, and cell shape is linked to the regulation of actomyosin contractility**. *Mol Biol Cell* 2019, **30**:3024-3036.

41. Madsen CD, Hooper S, Tozluoglu M, Bruckbauer A, Fletcher G, Erler JT, Bates PA, Thompson B, Sahai E: **STRIPAK components determine mode of cancer cell migration and metastasis**. *Nat Cell Biol* 2015, **17**:68-80.

42. Kean MJ, Ceccarelli DF, Goudreault M, Sanches M, Tate S, Larsen B, Gibson LC, Derry WB, Scott IC, Pelletier L, *et al*.: **Structure-function analysis of core STRIPAK proteins: a signaling complex implicated in Golgi polarization**. *J Biol Chem* 2011, **286**:25065-25075.

43. Nunbhakdi-Craig V, Craig L, Machleidt T, Sontag E: **Simian virus 40 small tumor antigen induces deregulation of the actin cytoskeleton and tight junctions in kidney epithelial cells**. *J Virol* 2003, **77**:2807-2818.

44. Sontag JM, Sontag E: **Regulation of cell adhesion by PP2A and SV40 small tumor antigen: an important link to cell transformation**. *Cell Mol Life Sci* 2006, **63**:2979-2991.

45. Schuhmacher D, Sontag JM, Sontag E: **Protein phosphatase 2A: more than a passenger in the regulation of epithelial cell-cell junctions**. *Front Cell Dev Biol* 2019, **7**:30.

46. Kwun HJ, Shuda M, Camacho CJ, Gamper AM, Thant M, Chang Y, Moore PS: **Restricted protein phosphatase 2A targeting by Merkel cell polyomavirus small T antigen**. *J Virol* 2015, **89**:4191-4200.

47. Brocchieri L, Kledal TN, Karlin S, Mocarski ES: **Predicting coding potential from genome sequence: application to betaherpesviruses infecting rats and mice**. *J Virol* 2005, **79**:7570-7596.

48. Farrell HE, Bruce K, Lawler C, Oliveira M, Cardin R, Davis-Poynter N, Stevenson PG: **Murine cytomegalovirus spreads by dendritic cell recirculation**. *MBio* 2017, **8**:e01264-17.