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Invited Review

UL24 herpesvirus determinants of pathogenesis: Roles in virus-host interactions

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ABSTRACT

Members of the *UL24* herpesvirus gene family are determinants of pathogenesis. The gene is widely conserved across the *Orthoherpesviridae* family, also commonly referred to as *Herpesviridae*. In this review, the impact of *UL24* homologs on pathogenesis as studied with different model systems is presented, as well as mechanistic aspects related to the different roles of UL24 proteins in virus-host cell interactions. The targeting of *UL24* for the development of therapeutic applications is also discussed.

1. Introduction

Members of the *Orthoherpesviridae* family, also known as *Herpesviridae*, infect mammals, birds and reptiles. *UL24* orthologs are not essential genes, however, experimental infections have revealed an important role for members of this gene family in the development of disease. Herpesvirus acute replication is followed by a latent infection whereby the genome persists in the host cell but there is a highly restricted pattern of gene expression, and no new virus particles are produced. Periodically, the virus can reactivate from latency, which can cause new lesions, and which promotes transmission to new hosts. Mechanistic studies have shown that *UL24* affects the host-virus relationship at various points in infection, from viral gene expression to virion morphogenesis.

1.1. *UL24* conservation among herpesviruses

UL24 orthologs have been identified in all *Orthoherpesviridae* members; however, no *UL24* orthologs are annotated in the National Center for Biotechnology Information (NCBI) database of genomes for members of the *Alloherpesviridae* family, which infect frog and fish species, or of the *Malacoherperviridae* family, which infect mollusk species (Davison, 1992, 1998; Davison et al, 2005, 2006; Divilov, 2024; van Beurden et al., 2010). Five clusters of highly conserved amino acid residues, termed homology domains (HD) were originally identified in the N-terminal portion of herpes simplex virus 1 (HSV-1) UL24 (Jacobson et al., 1989) (Fig. 1). The C-terminal portion of the protein is poorly conserved for the most part, both in amino acid composition and in length, although a limited amount of sequence homology can be detected (recent alignment in (Ruan et al., 2023)). Within the N-terminal portion of the protein is a PD-(D/E)XK endonuclease motif (Knizewski et al., 2006). This motif is found in several restriction endonucleases as well as in DNA recombination enzymes (Kosinski et al., 2005). Of note, there are certain members of the PD-(D/E)XK family that contain the motif, but for which no catalytic activity has been detected. In these cases, the conserved sequences may nevertheless have structural relevance, and thus be important for the activity of the protein (Kosinski et al., 2005). The ExK residues of the motif, which correspond to the catalytic domain in members of this family for which structural-function studies have been performed, are perfectly conserved among all UL24 proteins, strengthening the notion that these residues are important for some of the functions of the protein. To date, there has been no direct biochemical evidence of endonuclease activity for any UL24 protein. Proteomic analysis of a macrophage cell line infected with HSV-1 identified T195 as a phosphorylation site (Bell et al., 2013); however, substitution of this residue with alanine does not affect replication in epithelial cells in culture (Gonzalez et al., 2023). There have been several reports of UL24 proteins detected in herpes virions, as a component of the tegument or

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associated with capsids (Bortz et al., 2003; Hong-Yan et al., 2001; Wang et al., 2004), although UL24 was not detected in a proteomic analysis of extracellular HSV-1 virions (Loret et al., 2008). The absence of UL24 in the proteome of extracellular HSV-1 particles may represent a genuine difference in the biology of the virus compared to other herpesviruses; however, one cannot rule out that this negative result reflects technical limitations of detecting HSV-1 UL24, which has a propensity to precipitate out of solution (A. Pearson, unpublished data).

2. UL24, a determinant of pathogenesis

Studies regarding the importance of *UL24* orthologs in disease have been carried out mainly for certain members of the *Alphaherpesvirinae* subfamily of neurotropic viruses. The impact of *UL24* mutations on virus yields in epithelial cells in culture is modest. For HSV-1, reductions of approximately 1 $\log_{10}$ in viral yield have been measured (Jacobson et al., 1989). Similar decreases in replication are also seen for *UL24* mutants of pseudorabies virus (PRV), a pig pathogen (Ye et al., 2019). In contrast, for several other herpesviruses such as HSV-2 and bovine herpesvirus 1 (BHV-1), no statistically significant reduction in viral yield was seen for *UL24*-deficient strains (Blakeney et al., 2005; Whitbeck et al., 1994). Moreover, the effect of *UL24* mutations on viral replication is cell-type dependent. For example, a *UL24*-null equine herpesvirus 1 (EHV-1) strain showed no replication defect in epithelial cells but replicated poorly in neurons in culture (Kasem et al., 2010). The modest reduction in viral yield for *UL24*-mutant strains does not reflect the full importance of *UL24* in infection, which only becomes evident in animal models of infection.

2.1. UL24 orthologs in latency and reactivation

Most *UL24* pathogenesis studies have focused on HSV-1 and HSV-2 in rodent models of infection. In an ocular mouse model of HSV-1 infection, a *UL24*-null mutant exhibits an approximate 1 $\log_{10}$ reduction in viral titers in the eye but a drastic reduction in titers within trigeminal ganglia (TG) of between 2 and 4 $\log_{10}$ (Jacobson et al., 1998). In mouse and guinea pig models of vaginal HSV-2 infection, clinical signs such as the formation of lesions or inflammation are observed. These signs are greatly reduced when infection is conducted with a *UL24*-null strain (Blakeney et al., 2005). Structure-function analyses have revealed that substitution of the highly conserved E99 and K101 residues of HSV-1 UL24, which are present within homology domains 2 and 3, is sufficient to confer a *UL24*-null phenotype in a mouse model of ocular infection (Leiva-Torres et al., 2010). These residues are present in the catalytic domain of PD-D/E)XK endonuclease motif family members with enzymatic activity (Kosinski et al., 2005).

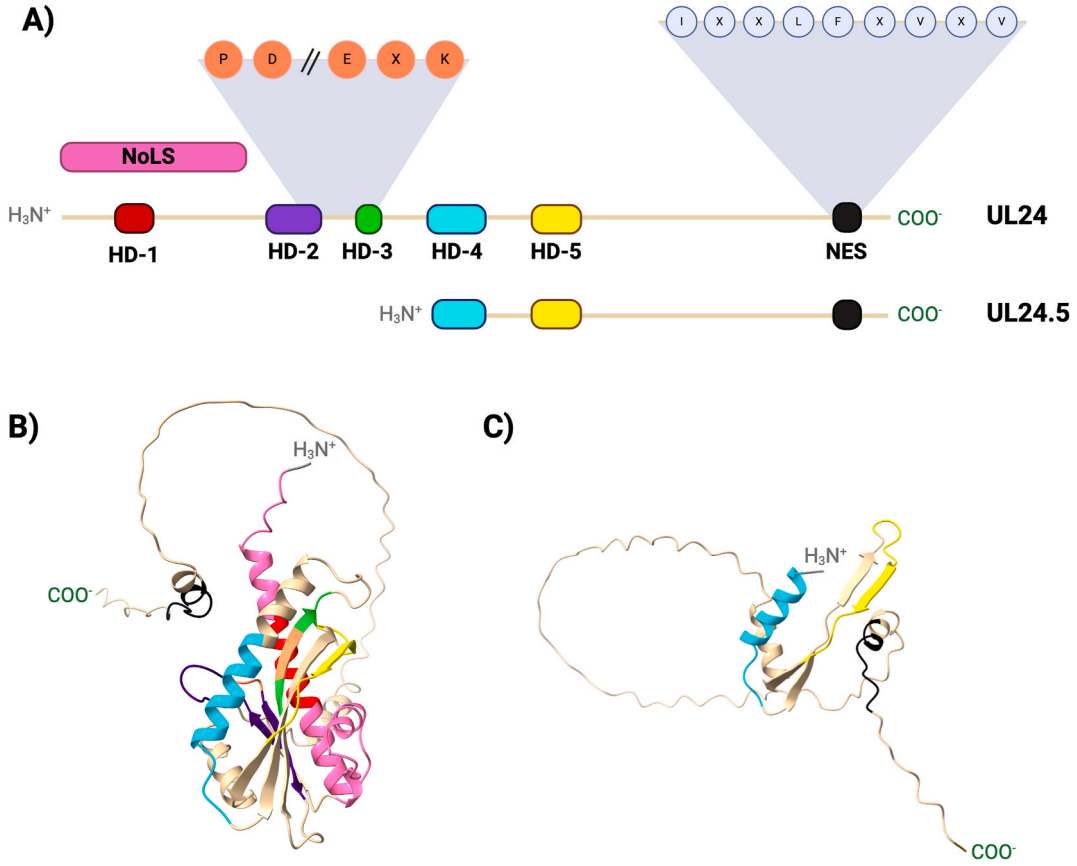

Fig. 1. Structural and functional domains of HSV-1 UL24 and UL24.5 proteins.
A) Schematic representation of UL24 and UL24.5 proteins, highlighting functional domains and the endonuclease motif. The sequence containing a nucleolar localization signal (NoLS) is shown in pink, homology domain 1 (HD-1) in red, HD-2 in purple, HD-3 in green, HD-4 in cyan, and HD-5 in yellow. The endonuclease motif is shown in coral and the nuclear export signal (NES) is in black, with corresponding amino acids indicated in the circles above. B) 3D structural model of the HSV-1 UL24 protein was generated using AlphaFold2 (Jumper et al., 2021; Mirdita et al., 2022), and visualized in ChimeraX. Domains are color-coded as in panel A. The HSV-1 UL24 sequence used in the predictions is from strain 17: P10208 · UL24_HHV11 (UniProt). C) 3D structural model of the UL24.5 protein, generated and visualized using the same methods as in panel B. Portions of the predicted structures of UL24 and UL24.5 that cover the homology domains were modeled with very high or high confidence (predicted local distance difference test (pLDDT) > 90 and 90 > pLDDT >70 respectively). Molecular graphics and analyses performed with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from National Institutes of Health R01-GM129325 and the Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases (Meng et al., 2023). Created in BioRender. Pearson, A. (2025) https://BioRender.com/r13b500.

Experimental mouse models of HSV-1 infection recapitulate all stages of a human infection except for spontaneous reactivation *in vivo* in the absence of an external stimulus. *UL24*-null mutants are able to establish latency to some degree, but exhibit a reduction in *ex vivo* reactivation. In these assays, TG are harvested post-mortem, and the capacity of the virus to reactivate and produce new infectious particles is monitored by co-culturing dissociated TG on a mono layer of susceptible cells. The reduction in reactivation appears to reflect a reduction in the amount of viral DNA present in the latently infected ganglia (Jacobson et al., 1998). This deficiency in the establishment of latency is likely linked to the reduced ability of *UL24*-null viruses to disseminate to neurons in the TG following replication in the epithelia, as demonstrated in an ocular mouse model of HSV-1 infection (Rochette et al., 2015). An HSV-2 *UL24*-null mutant can reactivate from latency *in vivo* in a guinea pig vaginal infection model; however in that report, a direct comparison of the establishment of latency and reactivation between the wildtype and mutant strains was not possible due to the lethality of the acute infection with wild type virus (Blakeney et al., 2005). The Kaposi's sarcoma associated herpesvirus (KSHV) *UL24* ortholog, known as *gene 20 or ORF20*, has also been shown to be important for reactivation. When comparing reactivation from cells with latent recombinant *ORF20*-knock out genomes or the corresponding wildtype genomes, the absence of *ORF20* was associated with a reduction in the amount of reactivated virus (Orbaum-Harel et al., 2024).

2.2. Role of UL24 in the pathogenesis of other herpesviruses

A murine model has also been used to evaluate *UL24* mutants of EHV-1 (Kasem et al., 2010). In a mouse encephalitis model where the virus is inoculated intranasally, an EHV-1 strain that does not express UL24 exhibits a drastic reduction in clinical signs. Likewise, in a mouse model for PRV, rates of mortality following intramuscular inoculation with a *UL24*-null strain are reduced significantly compared to those following infection with the corresponding wild-type strain (Ye et al., 2019). For certain viruses, severe host restriction complicates utilization of heterologous animal models of infection. For pathogenesis studies with varicella zoster virus (VZV), a humanized mouse model has been used. This model supports viral replication but does not recapitulate disease symptoms (Moffat et al., 1995). Nevertheless, the use of mice with skin and T-cell human xenographs has revealed the importance of the VZV *UL24* ortholog, *ORF35,* for efficient replication in epithelial cells *in vivo* and, to a lesser extent, in T cells (Ito et al., 2005).

Investigations testing how inactivation the *UL24* ortholog of murine gamma herpesvirus 68 (MHV-68), *gene 20*, affects pathogenesis allowed the rare use of a homologous experimental system whereby the natural host of the virus was used for the study. Following infection of mice via the intranasal route, there was no difference in the levels of virus recovered from the lungs when comparing the wild type strain to the *gene 20*-deficient strain. Likewise, no difference was noted in the establishment of latency in the spleen; however, a delay of 4 days in clearance of virus from the lung was observed for the *gene 20*-deficient strain (Nascimento et al., 2011). Thus, UL24 affects the development of clinical signs for several different herpesviruses, and often acute viral replication, the establishment of latency and viral reactivation as well. Table 1 lists results demonstrating the role of *UL24* orthologs in the pathogenesis of various herpesviruses.

Table 1
Impact on pathogenesis of mutations targeting *UL24* orthologs [a].

Virus	Mutation	Impact on pathogenesis	References
HSV-1	*UL24*-null	• Reduced viral titer in the eye and trigeminal ganglia in a mouse model of ocular infection • Decreased clinical signs • Deficiency in the establishment of latency	Jacobson et al. (1998) Rochette et al. (2015)
HSV-1	E99A and K101A substitutions in PD-(D/E)XK motif	• UL24-null phenotype in a mouse model of ocular infection	Leiva-Torres et al. (2010)
HSV-1	Substitution of the initiating methionine codon for *UL24.5*	• Increased pathogenicity in a mouse model of ocular infection	Dridi et al. (2018)
HSV-2	*UL24*-null	• Decreased clinical signs in mouse and guinea pig models of vaginal infection	Blakeney et al. (2005) Visalli et al. (2014)
VZV	*ORF35*-null	• Reduced replication in epithelial and T cells in a SCID-humanized mouse model	Ito et al. (2005)
PRV	*UL24*-null	• Reduced mortality rates following intramuscular inoculation in mice	Ye et al. (2019)
EHV-1	*UL24*-null	• Decreased clinical signs in a mouse encephalitis model	Kasem et al. (2010)
MHV-68	*gene 20*-knock out	• Delayed pulmonary clearance of virus in mice following intranasal infection	Nascimento et al. (2011)

[a] HSV- 1: Herpes simplex virus 1; HSV-2: Herpes simplex virus 2; VZV: Varicella zoster virus; PRV: Pseudorabies virus; EHV-1: Equine herpesvirus 1; MHV-68: Murine gammaherpesvirus 68.

3. Regulation of UL24 expression

UL24 of HSV-1 is associated with numerous transcripts. *UL24* transcripts utilize either the *UL24* polyadenylation (polyA) signal or the *UL26* polyA signal. The shorter transcripts that arise due to the *UL24* polyA signal are expressed with early kinetics, while those arising from use of the *UL26* polyA signal exhibit leaky-late kinetics. Furthermore, three transcription initiation sites have been detected for *UL24*, two upstream of the initiating ATG and one within the open reading frame (Cook and Coen, 1996). Despite the existence of the early *UL24* transcripts, the UL24 protein is expressed with leaky-late kinetics (Pearson and Coen, 2002). The short and long transcripts are differentially regulated at the level of nuclear export. An ICP27 mutation blocks the cytoplasmic accumulation of the long but not of the short UL24 transcripts (Hann et al., 1998; Pearson et al., 2004). A role for the transcripts originating from the start site within the HSV-1 *UL24* ORF was revealed when the UL24.5 protein was discovered. This protein corresponds to the in-frame c-terminus of UL24, and arises from use of a conserved ATG codon for translation initiation (Fig. 1). The function of UL24.5 remains to be discovered, however, blocking expression by mutating the start codon leads to an increase in clinical scores in an ocular mouse model of infection (Dridi et al., 2018).

KSHV *ORF20* exhibits early kinetics in the context of reactivation in cell culture from iSLK cells (Hoffman et al., 2021); however, the kinetics vary somewhat in a B-cell line cell, possibly reflecting the cell-type dependent nature of *UL24* mutant phenotypes. Like HSV-1, KSHV expresses multiple ORF20 proteins from the same gene due to the use of alternative initiation codons (Arias et al., 2014). The shortest version retains the ability to complement an *ORF20*-null strain for the production of wild type levels of virus (Orbaum-Harel et al., 2024).

In HSV-1, the *UL24* open reading frame partially overlaps that for the viral *thymidine kinase* (*tk)* gene, and the two genes are transcribed divergently. A similar organization is observed for several other herpesviruses although in some cases the region of overlap is limited to the 5' UTRs. The relevance of this organization is unclear; however, in HSV-1, genetic studies have revealed that expression of the early *tk* transcripts have a negative effect on expression of the early *UL24* transcripts (Cook and Coen, 1996). An impact on expression of neighboring genes has also been observed for the *UL24* homolog of human cytomegalovirus (HCMV), *UL76*, and the downstream *UL77* gene, which share a polycistronic mRNA. It was found that *UL76* expression hindered expression

of *UL77*, possibly through a mechanism related to translation reinitiation (Isomura et al., 2010).

4. UL24 targets the nucleus

UL24 proteins are highly basic proteins that are mainly, but not exclusively, localized to the nucleus. Among other activities, they affect the structure of the nucleolus and nuclear functions.

Upon infection of epithelial cells in culture, HSV-1 UL24 accumulates in the nucleus, but is also detected in the cytoplasmic compartment (Lymberopoulos and Pearson, 2007). By confocal microscopy, HSV-1 UL24 expressed ectopically shows nuclear staining and some cytoplasmic staining also (Bertrand and Pearson, 2008). Prominent nuclear staining has also been observed for UL24 proteins of other viruses such as HSV-2, VZV, HCMV, PRV, duck enteric virus (DEV) (Gao et al., 2017; Hong-Yan et al., 2001; Ito et al., 2005; Wang et al., 2000; Ye et al., 2019). HSV-1 UL24 plays an important role in the virus-induced remodeling of the nucleus. Both nucleolin and B23 undergo UL24-dependent dispersal from nucleoli throughout the nucleoplasm (Lymberopoulos et al., 2011; Lymberopoulos and Pearson, 2007). Transient transfection experiments demonstrated that UL24 expression is sufficient to modify the nucleolus (Bertrand and Pearson, 2008), and thus that the effect is not dependent on the infected state of the cell or on other viral proteins. In contrast, localization of the nucleolar protein fibrillarin is not affected by the ectopic expression of UL24, and the relocalization during HSV-1 infection of the RNA polymerase I transcription factor Upstream Binding Factor (UBF) is not affected by the deletion of *UL24* (Bertrand and Pearson, 2008; Lymberopoulos and Pearson, 2010). The mechanism responsible for the UL24-dependent relocalization of nucleolar proteins is unknown as is the function; however, infection with a *UL24*-null virus is associated with a defect in nuclear egress of newly formed capsids (Lymberopoulos et al., 2011).

4.1. Impact on viral gene expression

Several *UL24* family members have been shown to affect viral gene expression. A cell-culture based screen for HCMV proteins that regulate immediate early gene expression identified *UL76* (Wang et al., 2000) as a factor that, depending on experimental conditions, either increases or decreases levels of viral transcripts. Expression of HSV-1 transcripts for *ribonucleotide reductase 1* and *2* is up-regulated in cells infected with a *UL24*-null virus compared to the corresponding wildtype strain (Sanabria-Solano et al., 2016). Similarly, a KSHV strain genetically engineered to knock out expression of the *UL24* gene products exhibits an accelerated pattern of lytic gene expression (Orbaum-Harel et al., 2024). Paradoxically, the positive impact on viral gene expression is accompanied by a decrease in the production of infectious KSHV virions.

4.2. DNA damage response

Consistent with their nuclear localization, ectopic expression of UL24 proteins has been associated with stimulation of the DNA damage response. Nucleoli are the sites of ribosomal DNA transcription, and are dynamic structures that form and disassemble in response to the stage of the cell cycle (Leung et al., 2004). Pharmacological blocking of ribosomal gene transcription also alters the organization of nucleoli (reviewed in (van Sluis and McStay, 2019)). One hypothesis is that the endonuclease motif of UL24 functions in the cleavage of DNA, which blocks rDNA transcription, leading indirectly to dissociation of nucleoli. The HCMV homolog termed *UL76*, induces chromosomal breaks when expressed ectopically (Siew et al., 2009), although it has not been established if the mechanism is direct or indirect. UL76, via the unconserved C-terminal domain, induces a DNA damage response in cells, which in turn causes activation of NFκB and the production of IL-8 (Costa et al., 2013). Because DNA damage leads to cell cycle blocks, this effect may explain the G2-M block that is observed when certain *UL24* orthologs are expressed ectopically (Nascimento et al., 2009; Nascimento and Parkhouse, 2007). In a cell culture-based latency and reactivation system of KSHV, an *ORF20*-null mutant is associated with a transient increase in viral gene expression at early times post-induction of reactivation; however, one observes a defect in IL-6 and CXCL-8 expression, and an increase in cell death (Orbaum-Harel et al., 2024). The relevance of the UL24-induced cell cycle block in cell culture to pathogenesis remains unclear (Nascimento et al., 2011). Fig. 2 summarizes the major functions identified for various *UL24* orthologs in virus-host cell interactions.

5. Impact of UL24 on the innate immune response

UL24 orthologs have been revealed to affect expression of several genes in innate immunity pathways. When expressed ectopically in epithelial cells in culture, HSV-1 UL24 negatively regulates signaling by the DNA-sensor cGAS via the NFκB pathway. UL24 negatively affects the nuclear translocation of NFκB, which in turn inhibits expression driven by the NFκB promoter. Moreover, in human foreskin fibroblasts, the ability of exogenous DNA to stimulate expression of IFN-β and IL6 is downmodulated upon HSV-1 infection in a manner dependent on *UL24* (Xu et al., 2017). In studies using immortalized cell lines, ectopic expression of full length HSV-2 UL24 or only of the conserved N-terminal portion was shown to inhibit activation of the IFN-β promoter as well as IRF3 promoter activity induced by Sendai virus (SeV) infection (Zhang et al., 2024). In addition, siRNA-mediated knock-down of UL24 expression in the context of infection of HeLa cells was associated with a modest increase in IFN-β expression in response to SeV infection. Links between *UL24* orthologs and DNA-sensing pathways have been identified for several other herpesviruses. PRV UL24 negatively modulates expression of interferon stimulated gene (ISG)20, both upon ectopic expression, and also when comparing infection with a WT versus a *UL24*-null strain. This activity is contained within the N-terminal portion of UL24 (Chen et al., 2021). PRV UL24 also inhibits expression of ZCCHC3, a protein that drives expression of IFN-β and inhibits viral replication (Chen et al., 2022). PRV UL24 inhibition of the expression of ISGs may be related to the ability of the protein to drive the proteasomal degradation of IRF-7 (Liu et al., 2021) and of the p65 subunit of NFκB (Wang et al., 2020), which in turn reduces cGAS-STING signaling. Thus, PRV UL24 functions to down modulate the cellular antiviral response. Consistent with observations for HSV-1 and PRV UL24, in co-transfection experiments, DEV UL24 reduces expression from the IFN-β promoter in duck embryo fibroblasts (Gao et al., 2022). In addition, DEV UL24 promotes the proteasomal degradation of IRF-7 (Ruan et al., 2024).

In contrast to the negative impact of most UL24 proteins on expression of ISGs, ectopic co-expression of KSHV ORF20 with retinoic acid-inducible gene I (RIG-I) results in an increase in *interferon inducible factor 2′-5′-Oligoadenylated Synthetase Like* (*OASL*) transcript levels compared to cells overexpressing RIG-I alone (Bussey et al., 2018). In the context of infection with a DNA virus, it has been shown that OASL binds to the DNA-sensor cGAS, and inhibits production of the second messenger cyclic GMP-AMP. In this manner, OASL down regulates the innate immune response, thus favouring viral replication. However, in addition to OASL negatively affecting the expression of IFN-β, OASL downregulates expression of its own gene during infection with a DNA virus (Ghosh et al., 2019). This negative feedback function may explain how ORF20-mediated stimulation of *OASL* expression promotes replication of KSHV (Bussey et al., 2018).

Analyses of different herpesviruses point to UL24 proteins targeting components of innate immune signaling pathways—often related to DNA sensing—to promote replication of their respective virus (Fig. 2). This effect is typically manifested at the level of mRNA synthesis by blocking nuclear translocation of transcription factors or by triggering protein degradation of innate immune effectors. Alternatively, in the case of gamma herpesviruses, the effect may be indirect through the

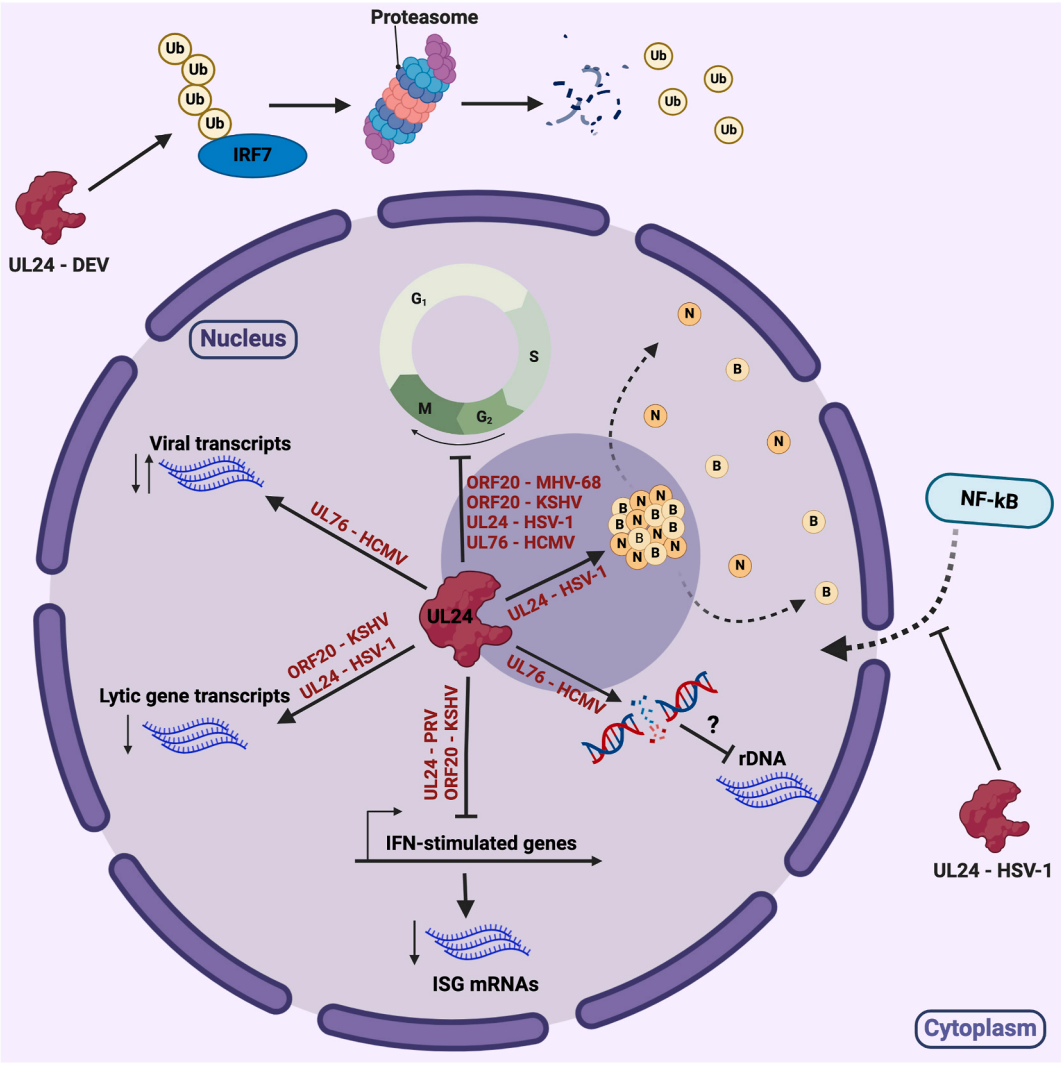

Fig. 2. Diverse roles of UL24 orthologs in disrupting nuclear processes, and in modulating innate immune responses and viral gene expression.
Diagram illustrating roles of different UL24 proteins in various cellular functions during infection. These functions include induction of the dispersal of the nucleolar proteins nucleolin (**N**) and B23 (**B**) throughout the nucleoplasm; cleavage of DNA, which may inhibit ribosomal DNA (rDNA) transcription; induction of a cell cycle block at the G2-M transition; inhibiting the nuclear translocation of NF-κB and promoting proteasomal degradation of IRF7; inhibiting interferon signaling and reducing the expression of interferon-stimulated genes (ISGs); and modulating the expression of viral genes. (DEV: duck enteritis virus; HCMV: human cytomegalovirus; HSV: herpes simplex virus; KSHV: Kaposi's sarcoma associated herpesvirus; MHV-68: murine gammaherpesvirus 68; PRV: pseudorabies herpesvirus; Ub: ubiquitin) Created in BioRender. Pearson, A. (2025) https://BioRender.com/u27s373.

stimulation of OASL, and the premature triggering of a negative feedback loop targeting innate immune pathways.

6. UL24 protein interactions

Protein-association screens and assays have been conducted for various UL24 proteins, which have identified multiple potential binding proteins. Of note, many ribosome-related proteins have been identified in the UL24-interactome, but no role for UL24 in translation has been described (Bussey et al., 2018). In addition to OASL (Bussey et al., 2018; Hoffman et al., 2021), interactions have been validated between HCMV UL76 and PARP-1 (Zhang et al., 2022), UL76 and S5a (Lin et al., 2013), KSHV ORF20 and the DNA polymerase processivity factor ORF59 (Hoffman et al., 2021), and with the DEV protein UL54, an ICP27 family member (Gao et al., 2017). The interaction with S5a, a ubiquitin receptor protein, is linked to the striking ability of UL76 to promote the formation of nuclear protein aggresomes (Lin et al., 2013; Zhang et al., 2015).

7. UL24 and the syncytial plaque phenotype

One of the first *UL24*-related phenotypes to be discovered (Jacobson et al., 1989; Sanders et al., 1982; Tognon et al., 1991) was the formation of syncytial plaques. The *UL24* syncytial plaque phenotype (*syn*) is temperature sensitive, being more prominent at 39 °C than at 37 °C, and is also cell-type dependent (Jacobson et al., 1989). *UL24* is one of only four HSV-1 genes that when mutated can lead to the formation of syncytial plaques in culture. Although the underlying mechanism remains a mystery, the *UL24 syn* phenotype differs from syncytial phenotypes related to *gB*, *UL20* and *gK* mutations in various respects. The *UL24 syn* phenotype is stimulated by the syncytia-inducing drug salubrinal (Bryant et al., 2008; Carmichael et al., 2018); however, unlike for *gB*, the effect of *UL24* mutations on syncytia formation does not appear to be synergistic with salubrinal. The viral proteins gI and gE form a heterodimer involved in cell-to-cell spread (Dingwell et al., 1994). The *UL24 syn* phenotype is dependent on the viral gE protein. Interestingly, in cells infected with *UL24 syn* mutants, gI levels are reduced. Nevertheless, the UL24 E99A/K101A mutant is unique in that it is the only *syn* phenotype

that has been shown to be dependent on gI (Carmichael and Wills, 2019). The *UL24 syn* phenotype is also dependent on *UL16*, which is involved in egress of newly formed virions (Starkey et al., 2014). *UL16* encodes a tegument protein that is part of a complex involving the c-terminal domain of gE (Han et al., 2012). Double mutants for *UL16* and *UL24* can replicate, but there is a block in the formation of plaques, indicating a defect in cell-to-cell spread. *UL16* is required for *gB syn* phenotypes, but not for those related to *UL20* or *UL24* mutations, or a subset of *gK* mutations (Carmichael and Wills, 2019). Another tegument-encoding gene, *UL21*, is required for the *gB syn* phenotype, but not for syncytial plaques due to *UL24*, *UL20* or *gK* mutations (Sarfo et al., 2017).

HSV-1 *UL24* mutants often exhibit a defect in replication in the form of lower viral titers, and also produce syncytial plaques (Fig. 3). However, the formation of syncytia is not the basis for the defect in replication because two mutants have been identified, G121A and L253A/F254A, that exhibit a *syn* phenotype but do not have a replication defect (Bertrand et al., 2010; Gonzalez et al., 2023). The function of the G121 residue is unknown. The L253A/F254A double substitution impairs a nuclear export signal in the C-terminus of the protein. Although the mechanism underlying the *UL24 syn* phenotype is unknown, there are data linking UL24 to events at the plasma membrane. Nucleolin, which is relocalized from nucleoli to the nucleoplasm by UL24, is known to shuttle from nucleoli to the plasma membrane, and can serve as a receptor for certain pathogens and nucleic acids (Kitagawa et al., 2022). In addition, in human foreskin fibroblasts infected with a *UL24*-null HSV-1 strain, co-localization of viral glycoproteins with the actin cytoskeleton is reduced. Moreover, Golgi morphology is greatly altered from what is seen in cells infected with a wildtype virus (Ben Abdeljelil et al., 2013). Because herpes virions acquire their final

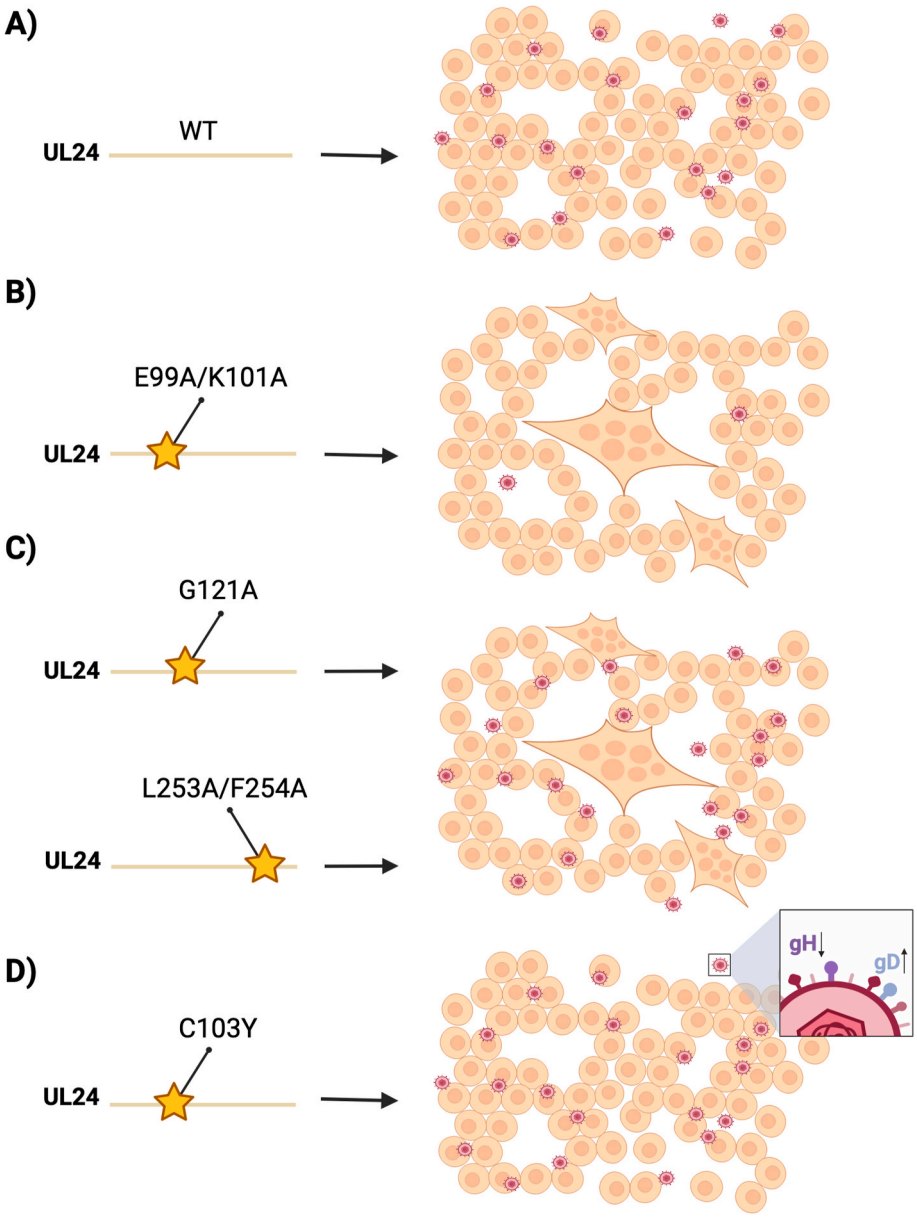

Fig. 3. UL24 HSV-1 mutants and the syncytial plaque phenotype.
A) Wild-type (WT) UL24 of HSV-1 produces non-syncytial plaques. B) The E99A/K101A mutant and *UL24*-null mutants result in a syncytial plaque phenotype characterized by large multinucleated cells, and an approximate 10-fold replication defect. C) The G121A and L253A/F254A mutants exhibit a syncytial plaque phenotype, but replicate like the corresponding wild-type strain. D) The C103Y mutant leads to an alteration in levels of gD and gH in HSV-1 virions. Cells are shown in beige and virions in red. Created in BioRender. Pearson, A. (2025) https://BioRender.com/n75y757.

envelope from Golgi-derived vesicles (Turcotte et al., 2005), this difference could affect fusion events related to egress of newly formed virus particles. Interestingly, studies investigating mutations that affect the efficiency with which different HSV-1 strains infect cells, identified a substitution mutation in UL24, C103Y, that led to a 2-fold increase of glycoprotein D (gD) protein in the viral envelope, and a 25% decrease in gH (Marzulli et al., 2023), although this strain did not have a syncytial plaque phenotype (Fig. 3). Mutation of the VZV *UL24* ortholog, *ORF35*, also leads to an altered plaque phenotype. Although plaques formed in cell culture by wildtype VZV are syncytial, mutations in *ORF35* result in modified plaques whereby the distribution of nuclei within the syncytia are disorganized (Ito et al., 2005).

8. Targeting of UL24 for therapeutics

UL24-mutant herpesviruses have been exploited in the development of potential therapeutic agents for both humans and animals. Because of the partial overlap between *UL24* and *tk*, these strains are often deleted for both genes. Many earlier vaccine attempts and gene therapy vectors focused on viruses deleted for *tk*, a viral gene necessary for sensitivity to the drug acyclovir (Elion et al., 1977). However, such *tk* mutations often resulted in a loss of *UL24* expression, for example (Krisky et al., 1998), and reviewed in (Peters and Rabkin, 2015). In most cases the phenotypic impact of the *UL24*-mutation was not established. Nevertheless, with increased understanding of how *UL24* affects pathogenesis, deliberate targeting of this gene for inactivation to engineer forms of the virus for use as vaccines or expression vectors has been investigated. Moreover, the *UL24 syn* phenotype may be beneficial for certain therapeutic purposes, as it could promote spread of the virus between cells within a tissue.

Because of the importance of *UL24* for pathogenesis, *UL24*-deleted viruses have been investigated as useful attenuated strains. Several *UL24*-null vaccine candidates have been studied. *UL24* is one of the genes that was inactivated in a quadruple-gene-deleted PRV strain (Li et al., 2024) designed as an improved vaccine for this important animal pathogen. A *UL24* deletion was also the strategy used to develop an attenuated vaccine against EHV-1. In a Syrian hamster model of intranasal infection, immunization with a EHV-1 strain deficient for *UL24*, *tk* and a viral glycoprotein, protected against the development of clinical signs better than a corresponding strain without the *UL24* mutation (Hu et al., 2024). HSV-2 *UL24* mutants have also been tested as vaccines in preclinical mouse and rat models. Immunization, either intramuscular or intravaginal, with a *UL24* HSV-2 mutant produces cell-mediated and humoral immunity, and confers protection against clinical signs (Visalli et al., 2014). Moreover, tests using a guinea pig model of vaginal infection showed a reduction in recurrent disease in challenge experiments in animals that had been vaccinated subcutaneously. This reduction was likely due to the reduction in the establishment of latency in vaccinated animals. Each of these attempts described varying degrees of success although the products are not commercially available. In addition to targeting *UL24* for the construction of attenuated strains, the UL24 protein has been developed as a vaccine against DEV. In this vaccine candidate, DEV UL24 was expressed using an attenuated *Salmonella* typhimurium strain to deliver the vaccine orally to ducklings (Liu et al., 2016; Yu et al., 2012). The vaccine produced a humoral and cellular immune response in ducks, and was partially protective in a DEV challenge experiment. This protective effect was enhanced by the use of an enterotoxin B subunit adjuvant.

9. Conclusions and future perspectives

Research on various herpesviruses has demonstrated the importance of *UL24* in pathogenesis. Functional analyses have shown that UL24 is a multifunctional protein impacting the host cell at the level of the nucleus and the plasma membrane. Expression of UL24 is associated with a downregulation of innate immune signaling. Future research questions should be directed at investigating if there is a functional link between nuclear and plasma membrane-related UL24 events, and in understanding how the UL24-related activities observed in cell culture affect pathogenesis in a host organism. Such information will be critical to exploit *UL24* as a therapeutic target for herpesviruses.

CRediT authorship contribution statement

Angela Pearson: Writing – review & editing, Writing – original draft, Conceptualization. **Amel Bouhamar:** Conceptualization, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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