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Disruption of Host Antiviral Resistances by Gammaherpesvirus Tegument Proteins with Homology to the FGARAT Purine Biosynthesis Enzyme

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Abstract

All known gammaherpesviruses encode at least one conserved tegument protein that contains sequence homology to the cellular purine biosynthesis enzyme: phosphoribosylformylglycineamide amidotransferase (FGARAT, or PFAS). While no enzymatic activity have been found on these viral FGARAT-homology proteins (vFGARAT), they are important for disarming host intrinsic antiviral machinery. Most vFGARAT proteins disrupt the intrinsic antiviral response -associated cellular subnuclear structure: ProMyelocytic Leukemia (PML) associated nuclear body (PML-NB). vFGARATs from different viruses target different components of PML-NB to prevent cellular repression of viral infection. In addition, vFGARATs of rhadinoviruses were recently found to oligomerize with the cellular FGARAT to deaminate RIG-I and repress inflammatory cytokine production. In this review we discuss the diverse mechanisms of antiviral response disruption by gammaherpesvirus vFGARATs and the significance of the enzyme homology domain.

Keywords

Epstein-Barr Virus; Kaposi sarcoma herpesvirus; Murine gammaherpesvirus 68 (MHV68); Herpesvirus saimiri; gammaherpesvirus; tegument; PML; ND10; Daxx; ATRX; Sp100; histone; H3.3; BNRF1; ORF75; ORF75c; vGAT; phosphoribosylformylglycineamide amidotransferase; FGARAT; PFAS

Introduction

Herpesvirus tegument proteins constitute a major component of the viral particle, and are important for multiple steps of the viral infection life cycle. Delivered pre-made into the host cell, tegument proteins are well positioned to regulate the intracellular infection environment immediately after entry. Members of the gammaherpesvirus subfamily encode tegument

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proteins that contain conserved domains of sequence homology with the cellular purine biosynthesis enzyme phosphoribosylformylglycineamide amidotransferase (FGARAT, also called PFAS). Members of this gene family include the Epstein-Barr virus (EBV) major tegument protein BNRF1, Kaposi's sarcoma associated herpesvirus (KSHV) ORF75, murine gammaherpesvirus 68 (MHV68 or MuHV4) genes ORF75a, ORF75b, and ORF75c (or viral glutamine amidotransferase, vGAT); and herpesvirus saimiri (HVS) ORF3, collectively referred to as viral FGARATs (vFGARATs). To date, efforts to elucidate the functions of vFGARATs have yet to reveal any enzymatic activity or interactions related to purine biosynthesis or metabolism. Instead, these tegument proteins disrupt various PML-NB-associated innate antiviral resistance pathways. Below, we discuss the importance of these tegument proteins in the herpesvirus life cycle, what is known about the FGARAT enzyme, the different ways that vFGARATs and other herpesviral tegument proteins disrupt innate antiviral resistance pathways, and what vFGARATs may teach us about the gammaherpesvirus infection strategy.

Tegument proteins in herpesviral biology

Herpesviruses package a collection of tegument proteins in the viral particle. Tegument proteins that are attached to the nuclear capsid are generally associated with the transport of viral capsid to the cell nucleus; while other tegument proteins can diffuse into the cytosol upon cell entry and regulate host processes during early infection, including the inhibition of several branches of intrinsic immunity pathways. Host cells possess multiple forms of intrinsic antiviral resistances that may abrogate early infection, including interferon induction, inflammasome activation, and heterochromatin assembly [1]. Herpesvirus tegument proteins have been well characterized for their ability to target and disarm these host intrinsic defenses. For example, EBV LF2 and KSHV ORF45 targets interferon regulator IRF7, to block IRF7 transactivation of type I interferon genes [2,3]. KSHV tegument protein ORF63, interacts with the host detector of viral nucleotides NLRP1, preventing its association with active components of inflammasomes, attenuating production of pyroptosis-associated cytokines IL-1 β and IL-18 [4]. HSV-1 VP22 interacts with the cellular chromatin remodeler TAF-1, preventing TAF-1-associated nucleosome assembly [5]. These examples demonstrate the numerous cellular pathways targeted by viral tegument proteins during the early stages of viral infection to regulate antiviral resistances and ensure a productive viral infection.

Purine biosynthesis homology in the gammaherpesvirus tegument

All gammaherpesviruses encode at least one essential tegument protein that contains extensive homology to the cellular enzyme formylglycineamide ribonucleotide aminotransferase (FGARAT). As this homology is not present on any alpha- or beta-herpesviruses tegument proteins, FGARAT-homology tegument proteins likely provide a function uniquely important to the gammaherpesvirus life cycle. Cellular FGARATs catalyze the fourth step in the ten-step *de novo* purine biosynthesis pathway, a pathway that synthesizes the purine precursor inosine 5'-monophosphate (IMP) from phosphoribosyl pyrophosphate (PRPP) (Figure 1). FGARAT hydrolyses ATP to power the transfer of an amino group (NH₃⁺) from glutamine to formylglycineamide ribonucleotide (FGAR),

forming the intermediate product formylglycineamidine ribonucleotide (FGAM) [6]. The product FGAM is also called phosphoribosylformylglycinamide, thus the enzyme FGARAT is alternatively named phosphoribosylformylglycinamide synthetase (PFAS). FGARAT is highly conserved across prokaryotes and eukaryotes, alternatively named PurL in bacteria. The solved crystal structure of the *Salmonella typhimurium* FGARAT (StPurL) provides the best insight into the structure of this enzyme [7]. StPurL consists of a four-domain structure: 1) an N-terminal domain of unclear function, 2) a linker region, 3) the FGAM synthetase domain, and 4) a C-terminal glutaminase (GATase1) domain (Figure 2). The FGAM synthetase domain has a pseudo-2-fold symmetry structure of two aminoimidazole ribonucleotide synthetase (AIR_S/PurM) homology domains, with the first half (PurM1) containing the FGAM synthetase active site, and a catalytically inactive second half (PurM2) of similar structure that holds an ADP for functions that are not yet understood. The C-terminal GATase1 domain removes the amine group from glutamine and channels it to the FGAR substrate in the PurM1 active site.

A BLAST alignment of EBV BNRF1 against *Homo sapiens* FGARAT and *Salmonella typhimurium* StPurL showed 32% and 24% identity, respectively. All viral vFGARATs are of similar size with human FGARAT and StPurL at approximately 1300aa. Viral-host homologies are most conserved at the FGAM synthetase subdomain and the C-terminal glutaminase (GATase1) domain (702–954aa and 1040–1306aa in BNRF1 coordinates, Figures 2 and 3). In other domains, the homology can be aligned sporadically across the entire protein, suggesting that the overall protein structure is conserved. Calculating the phylogenetic tree of cellular and viral FGARAT homologues (Figure 4) from the alignment in Figure 3, shows that all viral vFGARATs can be grouped into a cluster that is further diverged from the cellular (human and *S. typhimurium*) FGARATs, suggesting that the host FGARAT gene was copied by an early common ancestor of gammaherpesviruses. Since then, vFGARATs may have divergently evolved from cellular FGARAT/PurL, while retaining the overall structural fold (Figure 5).

Despite this overall homology, key residues of the conserved ATP and glutamine - binding motifs of cellular FGARAT [8] are missing on BNRF1, as is the case in MHV68 ORF75c [9]. However, an ADP-holding pocket in the second PurM domain of StPurL is highly conserved in vFGARATs (Figure 3). Specifically, all of the residues of StPurL that form hydrogen bonds with ADP [7] in the auxiliary ADP site (K649, E718, N722, D884, D887) can be found in BNRF1 (K627, E729, N733, D883, D886), while most of the magnesium ion ligands that stabilize ADP are also conserved on BNRF1. These ADP interacting residues are conserved in human FGARAT and all vFGARATs that we examined by sequence alignment. In the glutaminase domain, only two (G1093, Q1139 of StPurL) of the five glutamine-interacting residues are conserved in BNRF1 (G1089, Q1137); the conserved glycine and glutamine residues could also be found in all vFGARATs except HVS ORF3. Thus, vFGARATs may have lost key FGARAT catalytic residues in the FGAM synthetase and GATase1 domains, yet retaining the ADP-holding site.

Diverse Functions of viral FGARAT homologues

The imperfect conservation of catalytic residues suggests that the viral proteins have lost or modified the enzymatic activities of cellular FGARAT. Indeed, to date there are no reports of intrinsic enzymatic activity associated with vFGARAT protein. Instead, a recent study found that MHV68 ORF75c functions as a pseudo-enzyme through oligomerization with cellular FGARAT to bind and deamidate the antiviral response protein RIG-I [10]. Prior to this study, almost all gammaherpesvirus vFGARATs were reported to interact with components of PML nuclear bodies and abrogate their antiviral activities. Thus, it remains possible that vFGARATs have additional targets and functions in the viral life cycle (summarized in Figure 6).

PML-NBs and antiviral resistances

The majority of vFGARAT studies reveal a common function of disrupting components of the PML nuclear bodies (PML-NBs, also named ND10). PML-NBs are dynamic nuclear structures [11], where the PML protein forms a cage-like shell surrounding associated components [12,13], including Sp100, Daxx, ATRX, and HP1. PML-NBs impact diverse cellular functions linked to gene expression, telomere regulation, chromatin remodeling, apoptosis, post-translational protein modifications, and antiviral resistance [14–16].

Numerous experiments indicate that PML-NB components confer antiviral resistance. PML-NBs increase in size and number in response to viral infection [17,18], and viral-associated interferon induction increases expression of PML [19] and Sp100 [20]. Studies from HSV-1 and HCMV indicate that viral genomes localize to PML-NBs after nuclear entry [21] [22], suggesting that PML-NBs act as a site where host antiviral resistance effectors confront incoming viral genomes. PML overexpression inhibits VZV infectivity [23], PML and Sp100 knockdown rescues HSV-1 virus lacking ICP0 [24], and both PML and Daxx knockdown results in increased HCMV replication [25]. Consistent with its antiviral function, PML has been found to form spherical cage-like structures that interact with the VZV capsid surface protein ORF23, entrapping VZV nuclear capsids in PML-oligomeric enclosures in the host cell nucleus, preventing nuclear egress and infectious virion production [23,26].

Viral-associated disruption of PML-NBs was first described for alpha- and beta-herpesvirus proteins that are not homologues to vFGARATs (summarized in Figure 6). HSV-1 protein ICP0 is an immediate early gene that is also found in small amounts in the tegument [27,28]. ICP0 prevents PML and Sp100 from forming nuclear bodies [29,30], through inducing degradation of PML protein and certain isoforms of Sp100 [31]. ICP0-null mutant virus is deficient in viral gene expression while knockdown of PML and Sp100 resulted in a partial rescue of viral gene expression [24]. The VZV-encoded ICP0 ortholog, ORF61, disperses both PML and Sp100 away from nuclear bodies [23,32]. Interestingly, this disruption is less effective in human cell xenografts in mice, where PML entrapment of VZV nucleocapsids is dominant [23,26]. HCMV targets the PML-NB-associated transcription co-repressor Daxx through its tegument protein pp71. Upon infection of permissive cell types, pp71 localizes to PML-NBs [33], binds Daxx [34], induces Daxx degradation [35], and disperses the Daxx-

interaction partner ATRX away from PML-NBs [36]. Daxx knockdown resulted in increased immediate early (IE) gene expression along with active histone markers on the viral genome (presence of H4 acetylation, absence of H3K9 di-methylation); while Daxx over-expressing cells are refractory to HCMV IE gene expression [37], emphasizing the role of Daxx in the repression of viral gene expression.

PML disruption by viral FGARAT-homology tegument proteins

MHV68 belongs to the rhadinovirus genus of gammaherpesviruses, along with the squirrel monkey virus HVS and human virus KSHV. MHV68 encodes three FGARAT homologues, ORF75a, ORF75b, and ORF75c, with ORF75c essential for viral infection in fibroblasts [38]. ORF75c can induce PML degradation (but not Daxx) in fibroblasts [9], which was attributed to its inherent ubiquitin-E3 ligase activity [39]. ORF75c protein may be multifunctional as ORF75c knockout virus also showed reduced transport of viral capsids into the nucleus [40]. Intriguingly, a recent report found another MHV68 gene ORF61, a ribonucleotide reductase (RNR) large subunit homolog, that reorganizes PML-NBs into track-like structures [41].

The squirrel monkey rhadinovirus HVS, encodes two vFGARATs: ORF3 and ORF75 [42]. Studies of HVS infection in both human and rhesus macaque fibroblasts show that. ORF3, but not ORF75, can induce proteosomal degradation of Sp100, while sparing PML and Daxx [43]. Knockdown of Sp100 partially rescues ORF3-null virus infection, while PML knockdown resulted in a stronger rescue. This suggests that while Sp100 can repress viral infection, the structural support by PML formation of nuclear bodies may aid in localizing Sp100 onto viral DNA.

The human rhadinovirus KSHV encodes one vFGARAT homologue, ORF75. A recent study showed that ORF75 can induce the degradation of ATRX, and the dispersion of Daxx, PML, and Sp100 away from nuclear bodies [44]. Knockdown of PML and Sp100 resulted in increased KSHV infection in fibroblasts. Degradation of ATRX could be induced by tegument-delivered ORF75 as *de novo* viral gene expression was not needed. In contrast, dispersion of PML and Sp100 was only observed with transfected ORF75, but not during viral infection. These findings suggest that KSHV ORF75 may affect PML-NB components differently at distinct stages of viral infection.

EBV encodes a single vFGARAT termed BNRF1, which is essential for the early infection of B-lymphocytes by EBV. BNRF1 null virus (BNRF1) is incapable of viral gene expression and induction of B cell proliferation, although capable of lytic replication in transfected 293 cells [45]. Unlike rhadinovirus vFGARATs, BNRF1 did not induce detectable degradation of any PML-components. Rather, BNRF1 interacts with Daxx at the PML-NBs, and disperse ATRX away from Daxx and the PML nuclear bodies [46]. The Daxx-interaction domain of BNRF1 (360–600aa) [46] spatially maps to the first PurM domain of StPurL, overlapping with the putative FGAR synthetase active site. This suggests that the FGAR substrate holding domain may have divergently evolved to hold Daxx instead.

The complex of Daxx and ATRX was recently reported to form a histone variant H3.3 chaperone [47,48]. Using FRAP to measure histone mobility, BNRF1 was found to increase the free histone H3.3 pool in the nucleus, and thus have histone chaperone-like activity [49]. These data suggest a model where BNRF1 displaces ATRX to form an alternative histone chaperone complex of BNRF1/Daxx/H3.3/H4, decreasing the deposition of repressive chromatin on viral genomes [49]. This ensures the presence of permissive chromatin on the first activated viral promoter Wp, permitting viral gene expression during the earliest phase of nuclear infection. Thus, BNRF1 functions to disarm a form of antiviral repression-associated with viral chromatin assembly.

vFGARAT-regulation of the cellular RIG-I antiviral response pathway

While most gammaherpesvirus vFGARATs converge on functions involving the disruption of PML-NBs, it is not clear how this relates to FGARAT. A recent study provides the first report of a vFGARAT recruiting the enzymatic activity of cellular FGARAT to regulate the cellular antiviral gene RIG-I [10].

RIG-I belongs to the RIG-I-like receptor (RLR) family of pattern recognition receptors (PRRs), which recognizes viral RNA in the cytosolic compartment and trigger the activation of innate immune responses [50,51]. Upon binding viral RNA, RIG-I activates the downstream adaptor MAVS/Cardif/IPS-1/VISA[52,53]. Through downstream signaling cascades, RLR-MAVS signaling activates transcription factors such as interferon-regulator factor 3 and 7 (IRF3/7) and NF- κ B [54], to induce transcription of interferons and inflammatory cytokines. While RIG-I is an RNA sensor, it may also respond to DNA virus infection. The EBV encoded EBER family of non-coding RNAs was reported to interact with and activate RIG-I signaling, activating NF- κ B and inducing interferon production [55]. RIG-I is also important for the induction of interferons upon KSHV and HSV-2 infection [56,57], while the recognized viral ligand is yet unknown in both cases.

In the case of MHV68, He et al. reports that the vFGARAT ORF75c (referred to as viral glutamine amidotransferase, or vGAT by the authors) directly activates RIG-I by recruiting cellular FGARAT to deaminate RIG-I [10]. ORF75c acts as a “pseudo-enzyme” that does not have enzymatic activity, yet utilizes its potential structural homology to oligomerize with the host FGARAT enzyme, thus recruiting cellular amidotransferase activity to deaminate and activate the host antiviral component RIG-I. FGARAT-mediated deamidation removes amine groups from three specific amino acids on RIG-I, resulting in Q10E, N245D, and N445D editing of RIG-I. ORF75c effectively alters the substrate specificity of cellular FGARAT (PFAS) from glutamine to the ORF75c bound RIG-I. ORF75c-induced RIG-I deamidation coincides with RIG-I activation signs such as K63-linked poly-ubiquitination on RIG-I, RIG-I oligomerization, and RIG-I interaction with the downstream adaptor MAVS. This signaling cascade results in activation of NF- κ B. Knockout of RIG-I or drug-mediated inhibition of FGARAT enzymatic activity results in loss of NF- κ B activation, suggesting that RIG-I and cellular FGARAT are both important for ORF75c induction of NF- κ B activation.

Although NF- κ B activation is associated with inflammatory and anti-viral responses, the ORF75c-induced deamidation of RIG-I negates inflammatory cytokine production. Knockdown of RIG-I, or FGARAT leads to an increase of inflammatory cytokines such as CCL5 and IL-6 in MHV68-infected cells. He et al. also tested a mutant construct, RIG-I-TD, where all three deamidation targets are pre-mutated into the deamidation product amino acids. RIG-I-TD is more highly K63-linked poly-ubiquitinated and oligomerized than wild type RIG-I, supporting deamidation as an alternative route of RIG-I activation. All data considered, RIG-I deamidation, RIG-I activation, and NF- κ B activation are all required for MHV68 ORF75c to abrogate inflammatory cytokine production. While NF- κ B may also activate antiviral pathways, MHV68 lytic transactivator Rta can induce degradation of the RelA component of NF- κ B to dampen the inflammatory response [58]. RIG-I deamidation and NF- κ B activation is not unique to MHV68. He et al. reported that exogenous expression of KSHV ORF75 or HSV-1 infection can also induce deamidation of RIG-I and activation of NF- κ B, while EBV BNRF1 does not [10]. This confirms a previous screen for KSHV-encoded NF- κ B-activators, which found ORF75 as the top activator of NF- κ B [59]. These studies highlight some of the complex confrontations between virus and host innate antiviral response.

Ending Remarks

Our knowledge of vFGARAT tegument proteins has increased profoundly in the last few years, yielding many new surprises. Almost all viral FGARAT homologues can disrupt PML-NBs, while the vFGARATs of KSHV and MHV68 can induce FGARAT-dependent deamidation of RIG-I. While structural homology with FGARAT is likely needed for MHV68 ORF75c to oligomerize with and recruit FGARAT, it is less clear why vFGARATs are utilized by almost all gammaherpesviruses for PML-NB disruption. vFGARAT proteins may suggest a biochemical link between PML-NB-associated anti-viral resistance and nucleotide metabolism. In support of this speculation, the MHV68 ribonucleotide reductase ORF61 involved in nucleotide metabolism can also target PML for degradation [41]. However, as alpha- and beta-herpesviruses do not utilize FGARAT-homology proteins to disrupt PML-NBs, PML-associated antiviral resistance may be linked to purine metabolism in a gammaherpesvirus-specific host cell type, such as B-lymphocytes.

There are significant functional and structural differences between vFGARATs of rhadinoviruses (KSHV, MHV68, and HVS) and lymphocryptovirus (EBV). First, deamidation of RIG-I is likely a function unique to rhadinoviruses as this was only found with MHV68 ORF75c and KSHV ORF75; but not EBV BNRF1 [10]. However, it is yet unknown if EBV BNRF1 can interact with cellular FGARAT and potentially deamidate an alternative target. Secondly, the vFGARATs of rhadinoviruses all induced degradation of at least one PML component; however there is no evidence of lymphocryptovirus vFGARAT (EBV BNRF1) inducing any protein degradation. Thirdly, while tegument-delivered BNRF1 supports early infection [45], KSHV ORF75 is *de novo* produced through out early infection, latency, and expressed at higher levels upon lytic reactivation, suggesting that functions of ORF75 are not limited to early infection [44]. He et al. used ORF75c-deleted MHV68 where the deletion virus was produced in ORF75c-stably expressing cells [10]. The ORF75c virus has the ORF75c gene deleted from the viral genome, yet most likely

packaged complementing ORF75c protein in the tegument. These deletion-complemented virus was shown to be defective in their ability to induce deamidation of RIG-I [10], suggesting that tegument-delivered ORF75c was not sufficient for this function, *de novo* produced ORF75c is required. This is in contrast to EBV BNRF1, where complementation of BNRF1 protein in the tegument can rescue viral gene expression [46]. These differences may point to important variations between the lymphocryptovirus and rhadinovirus families in life cycle, viral gene regulation, and their repression of cellular antiviral processes.

Much remains to be discovered with vFGARATs, and why they have acquired and retained the structural homology of a complex host purine biosynthetic enzyme. The recent discovery of MHV68 ORF75c recruiting cellular FGARAT enzymatic function provides a new paradigm for viral modulation of host processes. Viral mimics of host genes are always fascinating and typically instructive [60]. Further studies of the vFGARATs have potential to reveal new mechanistic links between nucleotide metabolism and gene expression, how these are linked to viral infection, and how viruses can reprogram these cellular processes.

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References

* of special interest

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1. Bieniasz PD. An intrinsic host defense against HIV-1 integration? *J. Clin. Invest.* 2007; 117:302–304. [PubMed: 17273551]
2. Wu L, Fossum E, Joo CH, Inn K-S, Shin YC, Johannsen E, Hutt-Fletcher LM, Hass J, Jung JU. Epstein-Barr virus LF2: an antagonist to type I interferon. *Journal of virology.* 2009; 83:1140–1146. [PubMed: 18987133]
3. Zhu FX, King SM, Smith EJ, Levy DE, Yuan Y. A Kaposi's sarcoma-associated herpesviral protein inhibits virus-mediated induction of type I interferon by blocking IRF-7 phosphorylation and nuclear accumulation. *Proceedings of the National Academy of Sciences of the United States of America.* 2002; 99:5573–5578. [PubMed: 11943871]
4. Gregory SM, Davis BK, West JA, Taxman DJ, Matsuzawa S-I, Reed JC, Ting JPY, Damania B. Discovery of a viral NLR homolog that inhibits the inflammasome. *Science.* 2011; 331:330–334. [PubMed: 21252346]
5. van Leeuwen H, Okuwaki M, Hong R, Chakravarti D, Nagata K, O'Hare P. Herpes simplex virus type 1 tegument protein VP22 interacts with TAF-I proteins and inhibits nucleosome assembly but not regulation of histone acetylation by INHAT. *The Journal of general virology.* 2003; 84:2501–2510. [PubMed: 12917472]
6. Zhang Y, Morar M, Ealick SE. Structural biology of the purine biosynthetic pathway. *Cell. Mol. Life Sci.* 2008; 65:3699–3724. [PubMed: 18712276]
7. Anand R, Hoskins AA, Stubbe J, Ealick SE. Domain organization of *Salmonella typhimurium* formylglycinamide ribonucleotide amidotransferase revealed by X-ray crystallography. *Biochemistry.* 2004; 43:10328–10342. [PubMed: 15301531] ** This crystal structure of *Salmonella typhimurium* FGARAT (StPurL) provides the most detailed protein domain information of FGARATs, including key residues in the ADP-binding domain and GATase1 domain.
8. Patterson D, Bleskan J, Gardiner K, Bowersox J. Human phosphoribosylformylglycineamide amidotransferase (FGARAT): regional mapping, complete coding sequence, isolation of a

- functional genomic clone, and DNA sequence analysis. *Gene*. 1999; 239:381–391. [PubMed: 10548741]
9. Ling PD, Tan J, Sewatanon J, Peng R. Murine gammaherpesvirus 68 open reading frame 75c tegument protein induces the degradation of PML and is essential for production of infectious virus. *Journal of virology*. 2008; 82:8000–8012. [PubMed: 18508901] * First report of gammaherpesvirus vFGARAT disrupting PML-NBs. The discussion of this paper includes a good analysis of the sequence homology with cellular FGARAT.
 10. He S, Zhao J, Song S, He X, Minassian A, Zhou Y, Zhang J, Brulois K, Wang Y, Cabo J, et al. Viral Pseudo-Enzymes Activate RIG-I via Deamidation to Evade Cytokine Production. *Mol. Cell*. 2015 ** This study of MHV68 ORF75c reports the interaction of gammaherpesvirus vFGARAT interacting with cellular FGARATs to deaminate RIG-I. The first report of vFGARAT function related to cellular FGARAT instead of PML-NBs.
 11. Ascoli CA, Maul GG. Identification of a novel nuclear domain. *The Journal of cell biology*. 1991; 112:785–795. [PubMed: 1999457]
 12. Dellaire G, Kepkay R, Bazett-Jones DP. High resolution imaging of changes in the structure and spatial organization of chromatin, gamma-H2A.X and the MRN complex within etoposide-induced DNA repair foci. *Cell Cycle*. 2009; 8:3750–3769. [PubMed: 19855159]
 13. Luciani JJ, Depetris D, Usson Y, Metzler-Guillemain C, Mignon-Ravix C, Mitchell MJ, Megarbane A, Sarda P, Sirma H, Moncla A, et al. PML nuclear bodies are highly organised DNA-protein structures with a function in heterochromatin remodelling at the G2 phase. *Journal of cell science*. 2006; 119:2518–2531. [PubMed: 16735446]
 14. Lallemand-Breitenbach V, de Thé H. PML nuclear bodies. *Cold Spring Harb Perspect Biol*. 2010; 2:a000661–a000661. [PubMed: 20452955]
 15. Negorev D, Maul GG. Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot. *Oncogene*. 2001; 20:7234–7242. [PubMed: 11704851]
 16. Eskiw CH, Bazett-Jones DP. The promyelocytic leukemia nuclear body: sites of activity? *Biochem. Cell Biol*. 2002; 80:301–310. [PubMed: 12123283]
 17. Chelbi-Alix MK, Pelicano L, Quignon F, Koken MH, Venturini L, Stadler M, Pavlovic J, Degos L, de Thé H. Induction of the PML protein by interferons in normal and APL cells. *Leukemia*. 1995; 9:2027–2033. [PubMed: 8609713]
 18. Lavau C, Marchio A, Fagioli M, Jansen J, Falini B, Lebon P, Grosveld F, Pandolfi PP, Pelicci PG, Dejean A. The acute promyelocytic leukaemia-associated PML gene is induced by interferon. *Oncogene*. 1995; 11:871–876. [PubMed: 7545807]
 19. Stadler M, Chelbi-Alix MK, Koken MH, Venturini L, Lee C, Saïb A, Quignon F, Pelicano L, Guillemain MC, Schindler C. Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. *Oncogene*. 1995; 11:2565–2573. [PubMed: 8545113]
 20. Guldner HH, Szosteki C, Grotzinger T, Will H. IFN enhance expression of Sp100, an autoantigen in primary biliary cirrhosis. *J. Immunol*. 1992; 149:4067–4073. [PubMed: 1281200]
 21. Ishov AM, Maul GG. The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition. *The Journal of cell biology*. 1996; 134:815–826. [PubMed: 8769408] * Establishes PML-NBs as an intra-nuclear localization site of DNA viral genomes, characterized SV-40, Ad5, and HSV-1 disruption of PML-NBs.
 22. Koriath F, Maul GG, Plachter B, Stamminger T, Frey J. The nuclear domain 10 (ND10) is disrupted by the human cytomegalovirus gene product IE1. *Experimental cell research*. 1996; 229:155–158. [PubMed: 8940259]
 23. Reichelt M, Wang L, Sommer M, Perrino J, Nour AM, Sen N, Baiker A, Zerboni L, Arvin AM. Entrapment of Viral Capsids in Nuclear PML Cages Is an Intrinsic Antiviral Host Defense against Varicella-Zoster Virus. *PLoS Pathog*. 2011; 7:e1001266. [PubMed: 21304940] * High resolution microscopy suggesting that PML-NBs may form a cage-like structure to trap viruses.
 24. Everett RD, Parada C, Gripon P, Sirma H, Orr A. Replication of ICP0-null mutant herpes simplex virus type 1 is restricted by both PML and Sp100. *Journal of virology*. 2008; 82:2661–2672. [PubMed: 18160441]

25. Tavalai N, Papior P, Rechter S, Stamminger T. Nuclear domain 10 components promyelocytic leukemia protein and hDaxx independently contribute to an intrinsic antiviral defense against human cytomegalovirus infection. *Journal of virology*. 2008; 82:126–137. [PubMed: 17942542]
26. Reichelt M, Joubert L, Perrino J, Koh AL, Phanwar I, Arvin AM. 3D Reconstruction of VZV Infected Cell Nuclei and PML Nuclear Cages by Serial Section Array Scanning Electron Microscopy and Electron Tomography. *PLoS Pathog*. 2012; 8:e1002740. [PubMed: 22685402]
27. Loret S, Lippé R. Biochemical analysis of infected cell polypeptide (ICP)0, ICP4, UL7 and UL23 incorporated into extracellular herpes simplex virus type 1 virions. *The Journal of general virology*. 2012; 93:624–634. [PubMed: 22158881]
28. Loret S, Guay G, Lippé R. Comprehensive characterization of extracellular herpes simplex virus type 1 virions. *Journal of virology*. 2008; 82:8605–8618. [PubMed: 18596102]
29. Maul GG, Guldner HH, Spivack JG. Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). *The Journal of general virology*. 1993; 74(Pt 12):2679–2690. [PubMed: 8277273]
30. Maul GG, Everett RD. The nuclear location of PML, a cellular member of the C3HC4 zinc-binding domain protein family, is rearranged during herpes simplex virus infection by the C3HC4 viral protein ICP0. *The Journal of general virology*. 1994; 75(Pt 6):1223–1233. [PubMed: 8207389]
31. Chelbi-Alix MK, de The H. Herpes virus induced proteasome-dependent degradation of the nuclear bodies-associated PML and Sp100 proteins. *Oncogene*. 1999; 18:935–941. [PubMed: 10023669]
32. Walters MS, Kyratsous CA, Silverstein SJ. The RING finger domain of Varicella-Zoster virus ORF61p has E3 ubiquitin ligase activity that is essential for efficient autoubiquitination and dispersion of Sp100-containing nuclear bodies. 2010; 84:6861–6865.
33. Ishov AM, Vladimirova OV, Maul GG. Daxx-mediated accumulation of human cytomegalovirus tegument protein pp71 at ND10 facilitates initiation of viral infection at these nuclear domains. *Journal of virology*. 2002; 76:7705–7712. [PubMed: 12097584]
34. Hofmann H, Sindre H, Stamminger T. Functional interaction between the pp71 protein of human cytomegalovirus and the PML-interacting protein human Daxx. *Journal of virology*. 2002; 76:5769–5783. [PubMed: 11992005]
35. Hwang J, Kalejta RF. Proteasome-dependent, ubiquitin-independent degradation of Daxx by the viral pp71 protein in human cytomegalovirus-infected cells. *Virology*. 2007; 367:334–338. [PubMed: 17590404]
36. Lukashchuk V, McFarlane S, Everett RD, Preston CM. Human cytomegalovirus protein pp71 displaces the chromatin-associated factor ATRX from nuclear domain 10 at early stages of infection. *Journal of virology*. 2008; 82:12543–12554. [PubMed: 18922870]
37. Woodhall DL, Groves IJ, Reeves MB, Wilkinson G, Sinclair JH. Human Daxx-mediated repression of human cytomegalovirus gene expression correlates with a repressive chromatin structure around the major immediate early promoter. *The Journal of biological chemistry*. 2006; 281:37652–37660. [PubMed: 17035242]
38. Song MJ, Hwang S, Wong WH, Wu T-T, Lee S, Liao H-I, Sun R. Identification of viral genes essential for replication of murine gamma-herpesvirus 68 using signature-424 tagged mutagenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:3805–3810. [PubMed: 15738413]
39. Sewatanon J, Ling PD. Murine gammaherpesvirus 68 ORF75c contains ubiquitin E3 ligase activity and requires PML SUMOylation but not other known cellular PML regulators, CK2 and E6AP, to mediate PML degradation. *Virology*. 2013; 440:140–149. [PubMed: 23541081]
40. Gaspar M, Gill MB, Losing JB, May JS, Stevenson PG. Multiple functions for ORF75c in murine herpesvirus-4 infection. *PloS one*. 2008; 3:e2781. [PubMed: 18648660]
41. Sewatanon J, Ling PD. Murine gammaherpesvirus 68 encodes a second PML-433 modifying protein. *Journal of virology*. 2014; 88:3591–3597. [PubMed: 24371073]
42. Albrecht JC, Nicholas J, Biller D, Cameron KR, Biesinger B, Newman C, Wittmann S, Craxton MA, Coleman H, Fleckenstein B. Primary structure of the herpesvirus saimiri genome. *Journal of virology*. 1992; 66:5047–5058. [PubMed: 1321287]

43. Full F, Reuter N, Zielke K, Stamminger T, Ensser A. Herpesvirus saimiri antagonizes nuclear domain 10-instituted intrinsic immunity via an ORF3-mediated selective degradation of cellular protein Sp100. *Journal of virology*. 2012; 86:3541–3553. [PubMed: 22278248] * HVS vFGARAT (ORF3) induced degradation of PML-NB component Sp100.
44. Full F, Jungnickl D, Reuter N, Bogner E, Brulois K, Scholz B, Stürzl M, Myoung J, Jung JU, Stamminger T, et al. Kaposi's sarcoma associated herpesvirus tegument protein ORF75 is essential for viral lytic replication and plays a critical role in the antagonization of ND10-instituted intrinsic immunity. *PLoS Pathog*. 2014; 10:e1003863. [PubMed: 24453968] * KSHV vFGARAT (ORF75) disruption of PML-NB components PML, Sp100, Daxx and ATRX.
45. Feederle R, Neuhierl B, Baldwin G, Bannert H, Hub B, Mautner J, Behrends U, Delecluse HJ. Epstein-Barr virus BNRF1 protein allows efficient transfer from the endosomal compartment to the nucleus of primary B lymphocytes. *Journal of virology*. 2006; 80:9435–9443. [PubMed: 16973549]
46. Tsai K, Thikmyanova N, Wojcechowskyj JA, Delecluse H-J, Lieberman PM. EBV tegument protein BNRF1 disrupts DAXX-ATRAX to activate viral early gene transcription. *PLoS Pathog*. 2011; 7:e1002376. [PubMed: 22102817] * EBV vFGARAT (BNRF1) disruption of PML-NB components Daxx and ATRX.
47. Lewis PW, Elsaesser SJ, Noh KM, Stadler SC, Allis CD. Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:14075–14080. [PubMed: 20651253] * These two reports demonstrate the PML-NB components Daxx and ATRX as a histone chaperone complex.
48. Drane P, Ouararhni K, Depaux A, Shuaib M, Hamiche A. The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. *Genes & development*. 2010; 24:1253–1265. [PubMed: 20504901] * These two reports demonstrate the PML-NB components Daxx and ATRX as a histone chaperone complex.
49. Tsai K, Chan L, Gibeault R, Conn K, Dheekollu J, Domsic J, Marmorstein R, Schang LM, Lieberman PM. Viral Reprogramming of the Daxx Histone H3.3 Chaperone during Early Epstein-Barr Virus Infection. *Journal of virology*. 2014; 88:14350–14363. [PubMed: 25275136]
50. Chan YK, Gack MU. RIG-I-like receptor regulation in virus infection and immunity. *Curr Opin Virol*. 2015; 12:7–14. [PubMed: 25644461]
51. Schlee M. Master sensors of pathogenic RNA – RIG-I like receptors. *Immunobiology*. 2013; 218:1322–1335. [PubMed: 23896194]
52. Kowalinski E, Lunardi T, McCarthy AA, Louber J, Brunel J, Grigorov B, Gerlier D, Cusack S. Structural Basis for the Activation of Innate Immune Pattern-Recognition Receptor RIG-I by Viral RNA. *Cell*. 2011; 147:423–435. [PubMed: 22000019]
53. Seth RB, Sun L, Ea C-K, Chen ZJ. Identification and Characterization of MAVS, a Mitochondrial Antiviral Signaling Protein that Activates NF- κ B and IRF3. *Cell*. 2005; 122:669–682. [PubMed: 16125763]
54. Silverman N, Maniatis T. NF-kappaB signaling pathways in mammalian and insect innate immunity. *Genes & development*. 2001; 15:2321–2342. [PubMed: 11562344]
55. Samanta M, Iwakiri D, Kanda T, Imaizumi T, Takada K. EB virus-encoded RNAs are recognized by RIG-I and activate signaling to induce type I IFN. *The EMBO journal*. 2006; 25:4207–4214. [PubMed: 16946700]
56. Rasmussen SB, Jensen SB, Nielsen C, Quartin E, Kato H, Chen ZJ, Silverman RH, Akira S, Paludan SR. Herpes simplex virus infection is sensed by both Toll-like receptors and retinoic acid-inducible gene-like receptors, which synergize to induce type I interferon production. *The Journal of general virology*. 2009; 90:74–78. [PubMed: 19088275]
57. West JA, Wicks M, Gregory SM, Chugh P, Jacobs SR, Zhang Z, Host KM, Dittmer DP, Damania B. An important role for mitochondrial antiviral signaling protein in the Kaposi's sarcoma-associated herpesvirus life cycle. *Journal of virology*. 2014; 88:5778–5787. [PubMed: 24623417]
58. Dong X, He Z, Durakoglugil D, Arneson L, Shen Y, Feng P. Murine gammaherpesvirus-68 evades host cytokine production via replication transactivator-induced RelA degradation. *Journal of virology*. 2012; 86:1930–1941. [PubMed: 22130545]

59. Konrad A, Wies E, Thureau M, Marquardt G, Naschberger E, Hentschel S, Jochmann R, Schulz TF, Erfle H, Brors B, et al. A systems biology approach to identify the combination effects of human herpesvirus 8 genes on NF-kappaB activation. *Journal of virology*. 2009; 83:2563–2574. [PubMed: 19129458]
60. Elde NC, Malik HS. The evolutionary conundrum of pathogen mimicry. *Nat. Rev. Microbiol.* 2009; 7:787–797. [PubMed: 19806153] * A thought provoking review on mimicry based host-pathogen interactions.
61. Nelson, DL.; Cox, MM. *Lehninger Principles of Biochemistry*. Macmillan; 2008. Biosynthesis of Amino Acids, Nucleotides, and Related Molecules; p. 833-880.
62. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 2011; 7:539–539. [PubMed: 21988835]
63. Kim Y-E, Lee J-H, Kim ET, Shin HJ, Gu SY, Seol HS, Ling PD, Lee CH, Ahn J-H. Human cytomegalovirus infection causes degradation of Sp100 proteins that suppress viral gene expression. *Journal of virology*. 2011; 85:11928–11937. [PubMed: 21880768]

Highlights

1. Viral encoded enzyme-like proteins function to disarm host antiviral resistance
2. Viral FGARATs interact with and alter host PML-NB components
3. Viral FGARATs link purine biosynthesis with anti-viral resistance

Figure 1. The *de novo* purine biosynthesis pathway

The synthesis of purines involves a multi-step pathway that builds the purine ring on top of the pentose ring of phosphoribosyl pyrophosphate (PRPP). In the fourth step, FGAR aminotransferase (FGARAT, or PurL in prokaryotes) catalyzes the transfer of an amino group from glutamine (Gln) on to the pre-formed ring on formylglycineamide ribonucleotide (FGAR), resulting in the formation of formylglycineamidine ribonucleotide (FGAM). The aminotransferase domain of FGARAT shares structural homology with that of the fifth step enzyme AIR Synthetase (PurM in prokaryotes), which closes the ring of FGAM to form 5-

Aminoimidazole ribonucleotide (AIR). This multi-step pathway (10 steps in eukaryotes, 11 steps in other organisms) ultimately results in the complete purine ring on the purine precursor: Inosinate (IMP). In the middle steps, R denotes the 5-phospho-D-ribosyl group. Figure adapted from [61].

Figure 2. Domain alignment diagram of viral and cellular FGARATs

Alignment of a vFGARAT (EBV BNRF1) with the known domain structure of cellular FGARAT (*Salmonella typhimurium* PurL, StPurL). All vFGARATs maintain the second PurM and GATase1 domains conserved. In the case of EBV BNRF1, the domain that aligns to the first PurM (FGAM synthetase active site) of FGARAT is responsible for Daxx interaction [46]. StPurL domain information adapted from [7].

ADP at the auxiliary ADP site as listed on StPurL [7] are marked in green; while the Glutamine interacting residues of StPurL GATase1 domain are marked in red.

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Figure 4. Phylogenetic tree of FGARAT homology proteins

The protein sequence alignment in Fig 3 was used to calculate the divergent distances between viral and cellular FGARAT homologues using the Phylip v3.696 software package, using the Probability Matrix from Blocks (PMB) model to calculate distances and the neighbor-joining method to calculate the distance matrix.

Figure 5. Computational Model of BNRF1 3D Structure

BNRF1 predicted structure was modeled on the existing FGARAT structure from *S. typhimurium* (PDB 1T3T) using Raptor X webserver (<http://raptorx.uchicago.edu>). *S. typhimurium* FGARAT (left), EBV BNRF1 (right). The amino acids 300–600 containing the BNRF1 Daxx-Interaction Domain (DID) is highlighted in red.

Gammaherpesvirus vFGARATs

MHV68

KSHV

HVS

EBV

Alpha & Betaherpesvirus PML-NB disruptors

HSV-1

HCMV

VZV

Figure 6. Functions of vFGARATs and herpesviral PML-NB disruptors

Gammaherpesvirus vFGARAT functions include RIG-I deamidation and the disruption of PML-NB components. Examples of alpha and beta-herpesviruses disruption of PML-NBs are shown below for comparison. Black circles denote viral proteins while red blocks denote cellular proteins. Proteins induced for degradation are drawn as slashed and grayed out.

* PML and Sp100 dispersion is only observed upon KSHV ORF75 overexpression, but not upon viral infection [44].

** HCMV IE1 specifically induces degradation of SUMO-modified isoforms of Sp100; while dispersing other isoforms of Sp100 [22,63].