REVIEW ARTICLE

Viral pseudoenzymes in infection and immunity

Ting-Yu Wang, Jun Zhao, Ali Can Savas, Shu Zhang and Pinghui Feng

Section of Infection and Immunity, Herman Ostrow School of Dentistry, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA

Pseudoenzymes are proteins that are evolutionarily related to active enzymes, but lack relevant catalytic activity. As obligate intracellular pathogens, viruses complete their life cycle fully dependent on the cellular supplies of macromolecule and energy. Traditionally, studies of viral proteins sharing high homology with host counterparts reveal insightful mechanisms by which host signaling pathways are delicately regulated. Recent investigations into the action of cellular pseudoenzymes elucidate diverse molecular means how enzymes are differentially controlled under various physiological conditions, hinting to the potential that pathogens may exploit these regulatory modalities. To date, there have been three types of viral pseudoenzymes reported and our understanding concerning their mechanism of regulation is rudimentary at best. However, it is clear that viral pseudoenzymes are emerging with surprising functions in infection and immunity, and we are only at the beginning to understand this new group of enzyme regulators. In this review, we will summarize current knowledge in viral pseudoenzymes and provide a perspective for future research.

Introduction

Pseudoenzymes are defined as catalytically deficient proteins that are structurally similar to active enzymes, but lack key amino acids that constitute the active site required for enzyme catalysis [1]. Although pseudoenzymes are catalytically inert, they can retain functions by (a) binding to other proteins, (b) competing for either substrate binding or assembling with active homologs of substrate, and (c) modulating the output of signaling pathways through allosteric effects [2]. Among notable pseudoenzymes, a handful of viral pseudoenzymes have been identified, but their regulatory actions remain poorly defined. Here, we summarize the three families of viral pseudoenzymes reported to date. The first family of viral pseudoenzymes from *Leporipoxvirus* consists of Cu, Zn-superoxide dismutase (SOD) homologs. These pseudodismutases were shown to regulate superoxide decomposition and affect cellular redox status during virus infection [3]. Recently, a poxvirus pseudokinase, B12, and a closely related B1 kinase were reported to regulate the phosphorylation of the cellular antiviral barrier to autointegration factor (BAF), thereby controlling DNA replication [4]. More recent studies from our group characterized several viral homologs of cellular glutamine amidotransferases (GATs), thus referred to as vGATs, from gamma herpesviruses that cause significant morbidity and mortality in immune-deficient individuals. These herpesviral pseudoenzymes hijack cellular phosphoribosylformylglycinamidine synthetase (PFAS), a cellular GAT, to deamidate the cytosolic double-stranded RNA (dsRNA) sensor RIG-I, and impede antiviral cytokine production [5]. Here, we review these three types of viral pseudoenzymes, in the context of viral infection and host responses, to gain

Abbreviations

CCS, copper chaperone for superoxide dismutase; PFAS, phosphoribosylformylglycinamidine synthetase; SOD, Cu, Zn-superoxide dismutase; vGAT, viral glutamine amidotransferase; VRK, virus-related kinase.

insight into their mechanism of regulation and biological significance.

Glutamine amidotransferases (GATs)

Cellular GATs are remarkable metabolic molecules that catalyze enzymatic incorporation of ammonia into various metabolites of biosynthetic pathways, including nucleotides, amino acids, amino sugars, and coenzymes [6,7]. These enzymes are key players in cellular metabolism and provide essential building blocks for cell proliferation and viral replication. Structurally, these enzymes contain two to three enzymatic active sites that are physically connected and functionally coordinated via a so-called molecular tunnel for ammonia transfer [8,9]. Biochemically, cellular GATs extract ammonia from glutamine to synthesize intermediates of the corresponding anabolic pathways. This extraction process is mediated by an enzymatic active site containing a catalytic triad consisting of cysteine, histine, and glutamate/asparagine. Among these three residues, the cysteine residue is essential for enzyme catalysis in a way similar to that of cysteine proteases. Alternatively, this reaction can be catalyzed by an N-terminal cysteine residue, for example, in phosphoribosyl pyrophosphate amidotransferase (PPAT) that catalyzes the rate-limiting step of the *de novo* purine synthesis pathway. Owing to the highly conserved and essential function of GATs across all kingdoms of life, the mechanism and regulation of GAT enzymes have been largely learned from prokaryotic, specifically bacterial, homologues.

Based on the catalytic cysteine residue used for the amide bond cleavage, cellular GATs are classified into class I subfamily-triad GATs and class II subfamily-N-terminal nucleophile (Ntn) GATs [7,9,10]. For example, the triad GATs, including cytidine triphosphate synthetases 1 and 2 (CTPS1 and CTPS2), bacterial carbamoyl phosphate synthetases (CPSs), guanosine monophosphate synthetase (GMPS), and PFAS in nucleotide metabolism, have a highly conserved catalytic Cys-His-Glu/Asn triad that uses the thiol group of the cysteine residue for catalysis [6,11,12,13]. In contrast, the Ntn GATs, represented by PPAT in *de novo* purine biosynthesis and asparagine synthetase in amino acid synthesis, harbor the catalytic cysteine at the very N terminus. The glutaminase domains of most class I GATs share a common open α/β structure, with the catalytic triad consisting of three residues donated from distinct secondary structures. However, those of the class II GATs are composed mainly of antiparallel β sheets, presumably better exposing the N-terminal free cysteine residue for

catalysis. In general, GATs are heteromeric enzyme complexes made up of a glutaminase subunit and a synthase subunit. The glutaminase subunit hydrolyzes glutamine to glutamate and ammonia, and the latter is subsequently incorporated into the substrate of the synthase subunit [14]. In metazoans, GATs harbor both glutaminase and synthetase domains within a single polypeptide, for example, CAD and CPSs. These GATs are likely resulted from gene fusion events that may synchronize the expression of enzymes catalyzing sequential metabolic reactions of the same pathway [15], in analogy to the bacterial gene expression driven by an operon that regulates a cluster of genes of a biosynthetic pathway.

Phosphoribosylformylglycinamidine synthetase (PFAS)

PFAS, also known as FGAMS or FGARAT, belongs to the class I GAT family and is a highly conserved core enzyme across all kingdoms of life. It catalyzes the fourth step of the *de novo* purine synthesis, in which N-formylglycinamidine ribonucleotide (FGAM) is converted from the intermediate formylglycinamide ribonucleotide (FGAR) [14,16,17]. PFAS is encoded by the *purL* gene and exists in two forms known as the large PurL and small PurL. The large PurL found in most Gram-negative bacteria and eukaryotes consists of a 140 kDa polypeptide chain and contains three major domains, that is, the N-terminal domain, the FGAM synthetase domain, and the C-terminal glutaminase (GATase) domain [18]. The small PurL is found in Gram-positive bacteria and archaea, and it consists of a 66–80-kDa peptide chain that is homologous to the FGAM synthetase domain of the large PurL. For glutamine-dependent activity, the small PurL requires two additional gene products, PurQ and PurS. PurQ has a molecular weight of 25 kDa and is equivalent to the glutaminase domain responsible for the generation of ammonia, whereas the structures of the 10-kDa PurS dimer reveal homology to the N-terminal domain of the large PurL [19,20,21]. Like other cellular GATs that catalyze the synthesis of building blocks of a cell, PFAS has been solely studied for its activity in nucleotide synthesis. Remarkably, our recent work that examines herpesvirus immune evasion strategy showed that PFAS is hijacked to deamidate a cytosolic pattern recognition receptor, the dsRNA RIG-I sensor [5]. In doing so, these herpesviruses effectively derail the RIG-I-MAVS pathway to mute host innate immune activation and antiviral cytokine production. This study uncovered new activity of a metabolic enzyme in immune regulation via

deamidating a key signaling molecule, potentially coupling innate immune response to cellular metabolic status.

Viral glutamine amidotransferases (vGATs)

Gamma herpesviruses establish life-long latent infection in lymphoid cells, although they are capable of infecting other cell types and persisting in those cells. Epstein–Barr virus (EBV) and KSHV are associated with diverse malignancies and cancers of lymphoid, endothelial, or epithelial cell origin, particularly in individuals with immune-suppression, such as AIDS patients and organ transplantation recipients. In addition to the set of genes dedicated to support viral replication in infected cells, a large portion of these herpesviral genomes is expressed to modulate host cellular pathways, which collectively function to achieve their persistence within host [22]. Viral mimicry of key cellular signaling molecules is a common feature of these viral proteins that are evolutionarily selected and remarkably fine-tuned to achieve maximal efficiency in choreographing cellular biological processes. Pseudoenzymes, perhaps, represent one example of those proteins that are crafted by millions of years of virus–host interaction.

vGAT proteins, encoded by the open reading frame 75 (ORF75), are highly conserved within all gamma herpesviruses, including human EBV and KSHV, nonhuman primate herpesvirus saimiri (HVS) and rhesus monkey rhadinovirus (RRV), and murine herpesvirus 68 (MHV68) [23]. Interestingly, EBV and KSHV encode one homolog of vGAT, the genome of HVS and MHV68 contain two and three copies of vGAT, respectively. vGAT proteins share limited, but significant, homology with cellular PFAS. Thus, these vGAT genes are likely pirated from their natural hosts and undergo duplication during evolution. The fact that these genes are amplified in gamma herpesviruses of nonhuman primates and rodents implies their pivotal roles during viral infection. Indeed, these vGAT proteins are involved in viral capsid trafficking to the nucleus and evasion of intrinsic nuclear immunity via degrading the component of promyelocytic leukemia (PML)-associated nuclear body (ND) [24,25]. vGAT proteins are tightly associated with nucleocapsids and are resistant to detergents that normally dissociate tegument proteins. Such tight association with nucleocapsids may facilitate their delivery to the nucleus, permitting the inactivation of the intrinsic immunity of the nuclear PML-ND system. Packaged in the tegument compartment, vGAT proteins are released into the cytosol immediately after virus–host cell fusion

occurs, enabling the evasion of host defense at the critical time when no viral polypeptides are newly synthesized during *de novo* infection. Interestingly, our work identified one of the vGAT homologs of MHV68, namely that encoded by ORF75c, as a pseudoenzyme to derail innate immune sensing by RIG-I [5] (Fig. 1). The vGAT proteins, although sharing homology with cellular PFAS, lack the residues constituting the catalytic triad that is required for the glutamine-hydrolyzing activity. Although three MHV68 ORF75 proteins share homology with cellular PFAS, these viral homologs failed to complement the enzyme function in purine synthesis in PFAS-deficient CHO cells [25]. These observations support the conclusion that viral ORF75 homologs, at least those encoded by MHV68, are pseudoenzymes in *de novo* purine synthesis, although whether these viral proteins have other intrinsic enzymatic activity remains unknown. In a functional screen to identify viral components that regulate the RIG-I innate immune pathway, our group discovered that vGAT encoded by ORF75c, but not ORF75a and ORF75b, induced RIG-I deamidation to mute cellular antiviral cytokine production [5]. In doing so, vGAT recruits cellular PFAS to deamidate RIG-I and aberrantly activate RIG-I signaling, which is hijacked by MHV68 to avoid cytokine production [5]. This study uncovers an intrinsic enzyme activity of PFAS in deamidating proteins, in addition to its previously recognized glutamine-hydrolyzing activity in purine synthesis. The ability of vGAT to deflect cellular PFAS to deamidate key signaling proteins implies a potentially ubiquitous function of protein deamidation in metazoans, as previous deamidation studies have involved largely bacterial effectors that function as *bona fide* deamidases [26,27,28]. Interestingly, PFAS appears to deamidate both glutamine and asparagine residues of RIG-I and there are three deamidation sites with flanking sequences showing no apparent similarity. It remains unknown how this cellular deamidase targets specific residues for deamidation, since there are presumably a number of asparagines and glutamines on the surface of RIG-I. Conceivably, a consensus sequence may enable protein deamidation in ways similar to kinases that target proteins for phosphorylation. In addition to RIG-I, PFAS also deamidates the viral replication and transcription activator (RTA, also known as ORF50) that is crucial for gamma herpesvirus lytic replication [29]. Remarkably, deamidation of two asparagine residues flanking the nuclear localization signal (NLS) of RTA impedes its nuclear import. The import of nuclear proteins is mediated by the importin complex that serves as a receptor to deliver proteins into the nucleus. Deamidation of RTA,
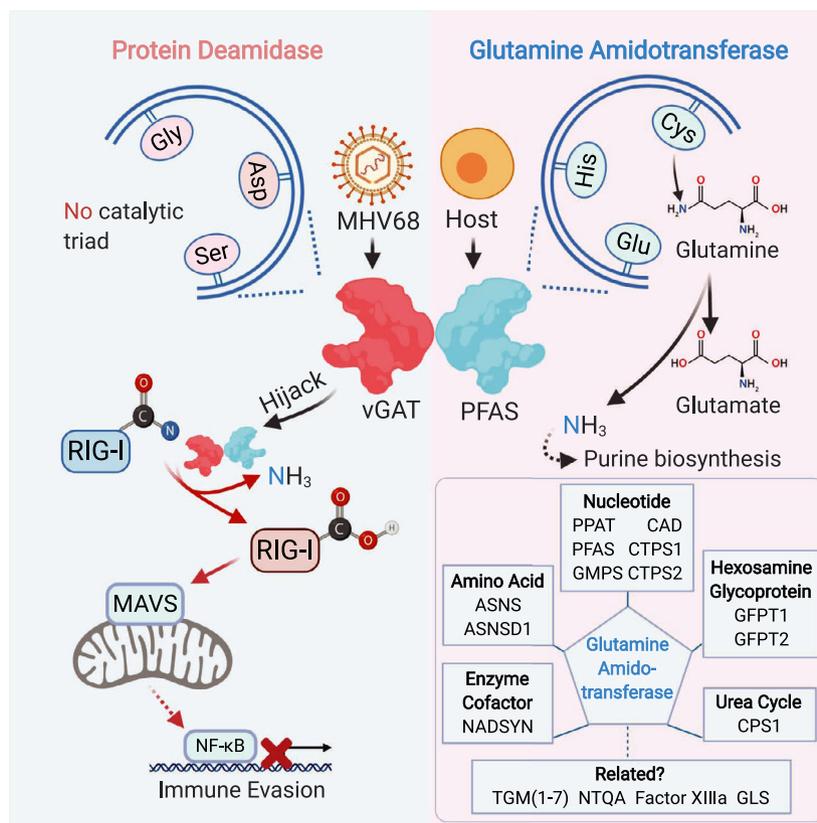
Fig. 1. Pseudoglutamine amidotransferase of herpesvirus. Glutamine amidotransferases (GATs) deamidate cellular glutamine and utilize the ammonia for diverse metabolic activities. As a GAT, phosphoribosylformylglycinamidine synthetase (PFAS) possesses a signature catalytic triad that deamidates glutamine for *de novo* purine biosynthesis. During murine gamma herpesvirus 68 (MHV68) infection, the vGAT pseudoenzyme hijacks PFAS to deamidate RIG-I, thereby avoiding antiviral cytokine production to evade host immune defense.

indeed, diminishes its association with importin and impedes its nuclear accumulation, thereby limiting viral lytic replication. Furthermore, RTA homologs of other gamma herpesviruses appear to be deamidated and controlled for nuclear import by PFAS-mediated deamidation, suggesting a conserved mechanism in regulating gamma herpesvirus lytic gene expression and replication. Additional investigation using viruses of rodents and nonhuman primates is needed to determine the in vivo roles of PFAS-dependent deamidation. While RIG-I deamidation requires a concerted action of vGAT and PFAS, PFAS is sufficient to deamidate KSHV RTA, demonstrating intrinsic enzyme activity to deamidate proteins. These distinct activities of PFAS, in nucleotide synthesis and protein deamidation, suggest the dynamic regulation of PFAS during the infection of KSHV and likely other gamma herpesviruses, which calls for future investigation.

vGAT is shared within gamma *Herpesviridae*, whereas a functional deamidase in alpha *Herpesviridae* is the UL37 tegument protein. The vGAT and UL37 viral proteins share a similar repertoire of functions during viral infection, including being inner tegument proteins tightly associated with nucleocapsids and required for nucleocapsid trafficking, deamidating

cytosolic sensors (e.g., RIG-I and/or cGAS), and manipulating NF-κB activation [30,31,32,33,34]. However, UL37 of herpes simplex virus 1 (HSV-1), and likely its homologs of HSV-2 and Varicella-Zoster virus (VZV), is a *bona fide* deamidase that demonstrates protein-deamidating activity in cells and *in vitro* [32,33]. Comparing vGAT proteins to UL37 may reveal new insight into how vGAT proteins activate PFAS in deamidating distinct proteins to affect the cellular environment and promote viral infection. It remains unknown how vGAT alters the protein-deamidating and purine-synthesizing activities of PFAS. The latter will explore a new function of vGAT proteins in nucleotide synthesis and other metabolic pathways that are essential for viral replication. Importantly, it is clear that PFAS is sufficient to deamidate RTA *in vitro*, indicating PFAS can be a *bona fide* deamidase [29]. The deamidase activity of PFAS toward RTA suppresses KSHV lytic replication suggests an adaptation that KSHV remains latent in highly proliferative cells, which presumably have high deamidase activity of PFAS. However, PFAS deamidates RIG-I in MHV68-infected cells, namely in the presence of vGAT. Despite that these deamidation events are examined in infection of two gamma herpesviruses,

these observations imply that PFAS is regulated to deamidate distinct protein substrates during viral infection. How this is achieved is an open question. Nevertheless, these vGAT pseudoenzymes appear to influence the catalytic functions of cellular PFAS and related metabolic pathways, which collectively impinge on key biological processes underpinning herpesvirus productive infection, for example, innate immune response and nucleotide synthesis.

Viral pseudokinase

Poxviruses contain the largest genome among all viruses and encode a diverse array of factors that modulate host signaling pathways during infection [35]. A conserved family of viral Ser/Thr kinases, known as cellular vaccinia-related kinases (VRKs), is characterized by homology to the vaccinia virus B1 kinase [36]. The vaccinia B1 kinase can phosphorylate cellular barrier to autointegration factor (BAF) to suppress host immune defense, thereby promoting productive viral infection [37,38]. Cellular BAF is a highly conserved DNA-binding protein and is involved in multiple fundamental biological processes, such as mitosis, gene regulation, and genomic stability [39]. During poxvirus infection, BAF binds to viral DNA genome and inhibits DNA replication in the cytoplasm (Fig. 2, left panel). The viral B1 kinase or cellular VRK1 kinase predominantly phosphorylates Ser-4, and to much less extent Thr-2 and Thr-3, of BAF [36,40–43]. Phosphorylation of BAF greatly reduces its DNA-binding activity, thus inactivating BAF to facilitate viral DNA replication [43,44]. Herpesviruses replicate their genomes in the nucleus, and it was interesting that BAF also demonstrates antiviral activity against HSV-1. It is important to note that a phosphorylation-resistant mutant of BAF, when over-expressed, inhibits HSV-1 lytic replication, but wild-type BAF fails to do so. Upon HSV-1 infection, BAF is dephosphorylated and accumulates in the nucleus, where BAF binds to viral genome to impede DNA replication and gene expression [45]. Paradoxically, BAF was also reported to facilitate the association of SETD1A methyltransferase with promoters of viral immediately-early genes, which increases viral lytic gene expression and replication [46]. Although it is not clear what contribute to these opposite findings, this apparent discrepancy may stem from the approaches the antiviral activity of BAF was examined. It is equally possible that the role of BAF in HSV-1 infection is cell type-specific and temporally dependent. Nevertheless, BAF can bind DNA to interfere genome replication and gene expression,

whereas phosphorylation prevents its association with DNA to release BAF-mediated inhibition.

The vaccinia B12 gene encodes a paralog of the B1 kinase and share 36% amino acid identity with the B1 kinase. Unlike B1, the B12 kinase lacks catalytic activity due to amino acid variations of key catalytic residues [47]. Specifically, the D167G substitution of the active site in the subdomain VII of B1 disrupts a salt bridge forming with Mg^{2+} ions that orient the γ phosphate of the bound ATP molecule for phosphor transfer. Furthermore, the vaccinia B12 protein demonstrates a potent inhibitory activity to dampen BAF phosphorylation, which can be antagonized by B1 kinase. Although B1 kinase can phosphorylate BAF, B12 reduces BAF phosphorylation in cells infected with B1-deficient virus, suggesting that B12 acts in a B1-independent pathway [4]. Intriguingly, B12 primarily localizes to the nucleus where dephosphorylated BAF and subsequent antiviral activity are increased by B12, thereby extending the period of viral production in susceptible cells. These results show that B1 and B12 constitute a paired kinase–pseudokinase system to regulate BAF phosphorylation and inhibition of viral DNA replication. However, the molecular mechanism how B12 pseudokinase functions, either in pair with B1 and VRKs or independent of any kinases, to modulate the DNA-binding activity of BAF remains unclear.

It is generally postulated that the vaccinia B12 gene may have arisen via a gene duplication event of a B1-like ancestor [47,48]. There are a few lines of evidence supporting this postulate. First, B12 is restricted to members of the *Orthopoxvirus* genus and only present in viruses with the B1 kinase, suggesting potentially paired function and inter-regulation [38]. Second, both vaccinia B1 and B12 genes are expressed during early viral infection, coinciding with BAF-mediated regulation of DNA replication. Whether the BAF-mediated binding to viral genome also impacts the expression of viral genes remains a legitimate question. Finally, B1 and cellular VRK2 kinase inhibit viral pseudokinase B12 in infected cells to restrict BAF's antiviral function in a phosphorylation-dependent manner [49], providing direct biochemical evidence for the kinase–pseudokinase pair in DNA replication. These results offer compelling evidence that BAF-mediated restriction of DNA replication is delicately coordinated by the B1 kinase–B12 pseudokinase pair. Additionally, B12 may function through a distinct and BAF-independent pathway [4], although the nature of such a pathway remains unknown. Thus, B1 kinase and B12 pseudokinase present an unprecedented example of a pair of epistatic paralogues serving to enhance gene

Fig. 2. Pseudokinase and pseudodismutase of poxvirus. Vaccinia virus encodes an active kinase B1 and a pseudokinase B12, a paired kinase–pseudokinase system that coordinates the phosphorylation of cellular BAF to regulate viral replication. Phosphorylation status of barrier to autointegration factor (BAF) dictates its DNA-binding activity that blocks viral DNA replication (left). *Leporipoxvirus* encodes a pseudo-Cu, Zn-superoxide dismutase (SOD), which competes for intracellular copper with its host counterparts, resulting in decreased activity of cellular SODs that degrade superoxide. The increased intracellular superoxide promotes the survival and proliferation of virus-infected cells (right).
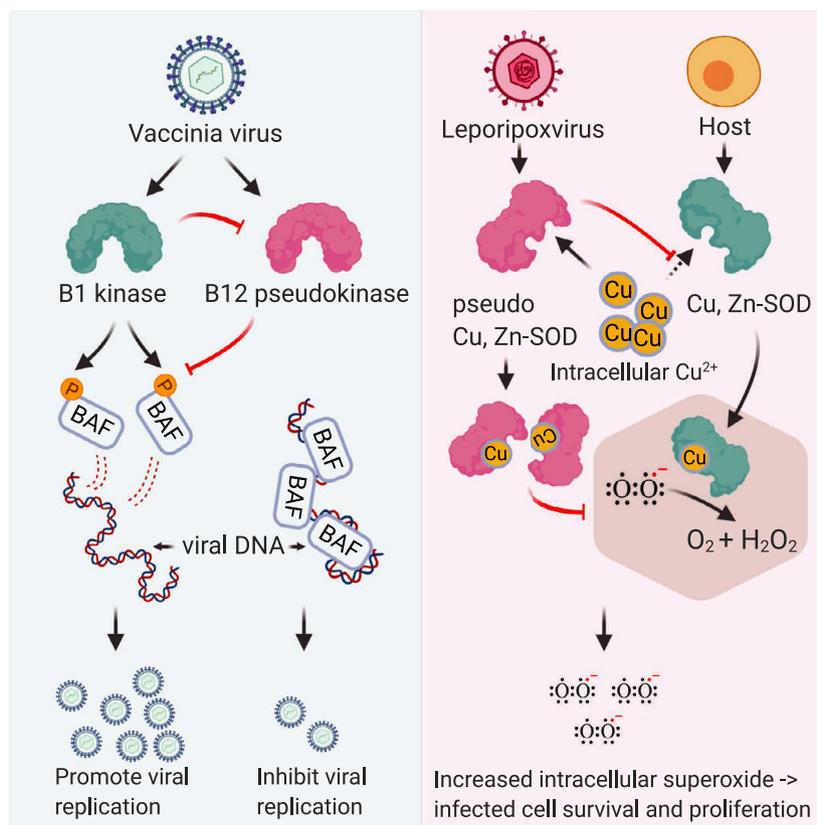
(B1) conservation during poxvirus evolution. Such paired regulatory gene system may confer growth fitness in analogy to the toxin–antitoxin (known as TA) system in bacteria to cope with conditions of stress [50].

Viral pseudodismutase

Cellular superoxide dismutases (SOD) are metalloenzymes that regulate cellular redox homeostasis by catalyzing the dismutation of two molecules of superoxide radicals into hydrogen peroxide and dioxygen [51–53]. The Cu, Zn-SOD depends upon a zinc atom to maintain the structural integrity and utilizes a copper atom as a catalytic cofactor [52]. The copper chaperone for SOD (CCS) delivers a copper atom specifically to SOD through the formation of a SOD-CCS heterodimer [54,55]. The poxviral Cu, Zn-SOD homologs are catalytically inactive and cannot decompose superoxide. On the contrary, the Cu, Zn-SOD homologs of myxoma virus (MYX) and shope fibroma virus (SFV) gradually reduce the activity of cellular Cu, Zn-SOD during infection [3,56] (Fig. 2, right panel). Mechanistically, these viral pseudodismutases demonstrate a capacity to selectively sequester cellular

CCS, thus reducing the copper supply to cellular SODs [57]. Such competition between viral and cellular SODs for CCS results in the decreased level of intracellular pool of metal-chelated Cu, Zn-SODs and a decline in dismutase activity of cellular Cu, Zn-SODs. Consequently, the intracellular concentrations of superoxide increase, which suppresses Fas-mediated apoptosis and fuels the proliferation of virus-infected cells [56,58,59]. Evidently, these viral pseudodismutases provide significant benefit to support *Leporipoxvirus* replication, because highly proliferative cells offer more metabolic intermediates for productive viral infection. Considering MYX and SFV can cause fibroxanthosarcoma-like tumors when infecting their natural hosts, one might speculate that these pseudodismutases may contribute to the tumorigenicity of these two *Leporipoxivureses* [60]. Surprisingly, recombinant viruses lacking these SOD homologs demonstrate no significant difference in virus replication or virulence compared to wild-type virus, raising a question concerning the physiological roles of these viral pseudodismutases in *Leporipoxvirus* infection [3]. In addition to poxvirus infection, tumor cells with differential levels of cellular SOD activity exhibit altered rate of metastasis and growth, which support a

positive role of superoxide in promoting the metastasis and growth of tumor cells [61,62]. The intracellular superoxide, regulated by cellular SODs and viral pseudodismutases, may serve as an important metabolic molecule to influence cell proliferation, transformation, and metastasis by activating the mitogen-activated protein kinases [63]. How intracellular superoxide altered by these viral pseudodismutases is coupled to other cellular processes remains to be further investigated. However, these observations suggest that virus-induced tumorigenesis may be due to the imbalanced intracellular superoxide concentration/flux mediated by viral pseudoenzymes. It also provides an example of how viral pseudoenzymes can target cellular metabolic pathways to enhance the survival of virus-infected cells en route to tumor formation, if these viral pseudodismutases, when ectopically expressed, are shown to induce tumor formation. Nevertheless, these studies collectively demonstrate that viral pseudodismutases can manipulate intracellular levels of superoxide to inhibit apoptosis and promote cell proliferation, implying their potential roles in tumorigenesis and tumor metastasis.

Perspectives and concluding remarks

To date, there have been only a few viral pseudoenzymes reported and their functions are not well understood. Viruses, even the largest DNA viruses such as herpesviruses and poxviruses, have relatively small genomes compared with those of their host cells. The fact that both poxviruses and herpesviruses encode pseudoenzymes within their genomes implies the important regulatory role of pseudoenzymes in fundamental biological processes, as viruses do not spare their genetic coding capacity. Thus, these viruses offer a useful tool and system to investigate the function and mechanism of pseudoenzymes in fundamental biology. Studies involving viral pseudoenzymes of cellular GATs, SODs, and VRKs reveal common themes and distinct actions of pseudoenzymes in regulating immune response, redox homeostasis, and DNA replication, respectively. However, the physiological roles of these pseudoenzymes are incompletely understood, particularly during in vivo infection. Although *bona fide* cellular and viral enzymes targeted by these viral pseudoenzymes are identified, mechanism of action of these pseudoenzymes (e.g., vGATs and B12) is poorly understood and additional cellular targets may exist. Applying cutting-edge multi-omics analysis to model animals infected with recombinant viruses may identify new in vivo roles of and specific pathways regulated by these viral pseudoenzymes, thus imparting life to

these seemingly dead enzymes and expanding their functional repertoire. On the other hand, it is very plausible that there are viral pseudoenzymes yet to be identified. Sequenced viral genomes will enable their identification, although requiring extensive downstream analysis and structural modeling. Needless to say, that our understanding of viral pseudoenzymes is rudimentary at best. With the importance of viral infections testified by the ongoing COVID-19 pandemic, future effort investigating viral pseudoenzymes in biology and medicine will be an even more fruitful investment. Traditionally, therapeutic agents are sought to target *bona fide* viral enzymes. The pivotal regulatory roles of pseudoenzymes attest a new class of molecules that can serve as targets of intervention for antiviral therapy.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

TW and PF conceived the paper; TW, JZ, AS, SZ, and PF wrote the paper.

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