



Herpesvirus DNA Polymerase Mutants—How Important Is Faithful Genome Replication?

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Abstract

Purpose of Review This report broaches the topic of altered fidelity of DNA replication in herpesvirus mutants described over the past decades. Reduced genome replication fidelity of herpesvirus exonuclease mutants allows studying of virus population dynamics in the absence of exonucleolytic proofreading and can inform us on virus evolution in the face of error-prone genome replication.

Recent Findings We recently found that mutations previously described to be lethal for herpes simplex type 1 (HSV-1) caused error-prone genome replication in Marek's disease virus. This has allowed us to study the influence of augmented genetic diversity on viral population dynamics, replicative fitness, and virulence.

Summary We conclude that the use of herpesvirus fidelity mutants allows unprecedented insights into virus evolution driven by low-fidelity replication. More than that, their use allows us to observe accelerated evolution, potentially enabling time-saving screens for the rise of drug- or vaccine-resistant mutants. In addition, we can infer that lethal or suicidal phenotypes observed in low-fidelity herpesvirus mutants are likely a consequence of error-prone genome replication, ultimately leading to lethal mutagenesis of small and isolated virus populations in cell culture.

Keywords Herpesvirus · DNA replication fidelity · DNA polymerase mutant · Mutationrate · Exonuclease · Low fidelity · High fidelity

Fidelity of DNA Replication

DNA replication is a process of remarkable accuracy. Nonetheless, accuracy is not perfect and the occasional misincorporation of bases cannot be avoided. Replication errors are important, however, as they constitute the molecular basis of biodiversity and are prerequisite for evolution. Replication errors can be single-nucleotide polymorphisms (SNPs) [1], single base deletions, or single base insertions (indels) [2]. The overall fidelity of DNA replication is primarily based on (1) nucleotide selectivity and (2) exonucleolytic proofreading [2].

Nucleotide selectivity is driven by the thermodynamic barrier presented by correct versus incorrect base pairing. Forming the correct hydrogen bond is thermodynamically favored but should still allow error rates of up to 1 in 100 [3]. A much higher accuracy of RNA polymerases strongly argues for other possibilities for base discrimination. It appears that the structural properties of the holoenzyme play an important role in enhancing nucleic acid replication fidelity, for example, by excluding water from the catalytic side [4] and by controlling for steric hindrances that would result from incorrect base pairing [5].

Intrinsic exonucleolytic proofreading is a unique enzymatic function of DNA polymerases [6•]. It is executed by a 3'→5' exonuclease that is embedded in replicative DNA polymerases. The process of proofreading rests on the exonuclease removing erroneously incorporated bases, which allows the subsequent insertion of the correct nucleotide by the polymerase. The coordinated action of exonuclease and polymerase is essential for efficient and accurate DNA replication, and the proofreading process can lower the error rate of nucleic acid replication by 10–1000-fold [6•, 7, 8].

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DNA Replication Fidelity in Herpesviruses

Herpesviruses are large dsDNA viruses that encode a family B replicative viral DNA polymerase with intrinsic 3'→5' exonuclease activity, capable of exonucleolytic proofreading [9]. They are therefore believed to replicate their genomes with relatively high fidelity, comparable with other dsDNA microorganisms [10]. Most of the work on replication fidelity of herpesviruses was done for herpes simplex virus type 1 (HSV-1), and there is some ambiguous literature regarding the mutation rate of this particular alphaherpesvirus. In older reports, mutation rates of HSV-1 are estimated to be in the range of 0.003 mutations per genome and replication, equaling to a base substitution rate of around 2×10^{-8} or up to 5.9×10^{-8} based on slightly different calculations [1, 10]. These relatively low mutation rates are contrasted by a more recent study that suggests mutation rates of 3.6×10^{-4} per plaque transfer [11], the relatively rapid rise of drug-resistant mutants [12], and findings that indicate unexpected high diversity in herpesvirus infection scenarios [13–16, 17, 18, 19]. The ambiguous data on herpesvirus mutation rates seems to be partly due to the use of marker genes (often the thymidine kinase gene or foreign genetic elements) for the estimation of mutation rates, and the overall lack of well-controlled and unbiased studies that examine mutation rates based on whole virus genomes. An informative review about procedures used to address HSV-1 mutation rates in the past can be found in the literature [20].

Structure and Function of Herpesvirus DNA Polymerase

Like all known nucleic acid polymerases, herpesvirus DNA polymerases can be described in analogy to the structure of a cupped right hand [21, 22]. Three main regions are recognized:

1. The palm domain is home to the catalytic core harboring the highly conserved “DTDS” tetrapeptide found in all family B polymerases, where the aspartic acid residues are essential for the coordination of the divalent cations required for the “two-metal-ion-catalysis” and, thus, indispensable for the primary enzymatic function of the enzyme [23]. There is evidence for altered replication fidelity of palm domain mutants [24–26].
2. The thumb domain, which contacts the template strand and guides it into the catalytic side. It is important for the transition between the polymerase and exonuclease active site. In family A and B polymerases, the thumb domain is thought to be important for the switch between polymerization and editing (exonuclease) mode. It has been speculated that the switch function could play a role for replication fidelity [27, 28].

3. The finger domain contacts incoming nucleotides and undergoes considerable conformational changes during the catalytic process. The finger domain of herpesvirus DNA polymerases appears to be structurally different from other family B polymerases [29]. Mutations in this domain increase substrate specificity and are known to influence replication fidelity as well as conferring resistance against nucleoside analogs [30–32].

Beyond structural similarities to other polymerases, family B polymerases share seven conserved polymerase and three conserved exonuclease domains [33, 34]. These domains are named from I to VII (I to III in case of the exonuclease) according to the degree of similarity between different polymerases, I being the most conserved domain. Importantly, only the first conserved exonuclease domain, which harbors the charged amino acid residues coordinating the Mg^{2+} ion essential for catalytic activity, does not overlap with any conserved polymerase side. Exonuclease domains II and III overlap with the conserved Pol domains IV and the δ -C region, respectively (Fig. 1). Also known to be important for enzyme fidelity are the conserved polymerase domain I, located in the palm region and home to the catalytic core of the polymerase, and the conserved region III that harbors part of the finger domain of the enzyme. Another common feature of family B DNA polymerases that is shared by herpesvirus DNA polymerases is the association with an accessory subunit known as processivity factor [28]. Processivity factor is thought to influence replication fidelity by influencing DNA binding of the replication complex [20, 35–38].

DNA Replication Fidelity Variants

Mutants that affect the fidelity of DNA replication are classified based on the differences in mutation rates compared with their respective parent. Usually, low-fidelity variants are described as mutator phenotypes and high-fidelity mutants as antimutator phenotypes.

Low-Fidelity Mutants

Mutations in the polymerase gene leading to mutator phenotypes have frequently been documented (Tables 1, 2, and 3). Low-fidelity variants in herpesviruses exclusively map to one of the exonuclease domains of the protein. The lower DNA replication fidelity of some exonuclease mutants can be explained by impaired proofreading of exonuclease-deficient mutants. It is important to note that, in the case of HSV-1, the majority of exonuclease mutants have only been generated experimentally and tested *in vitro* since the introduced mutations are lethal for virus replication (Table 1). There is some

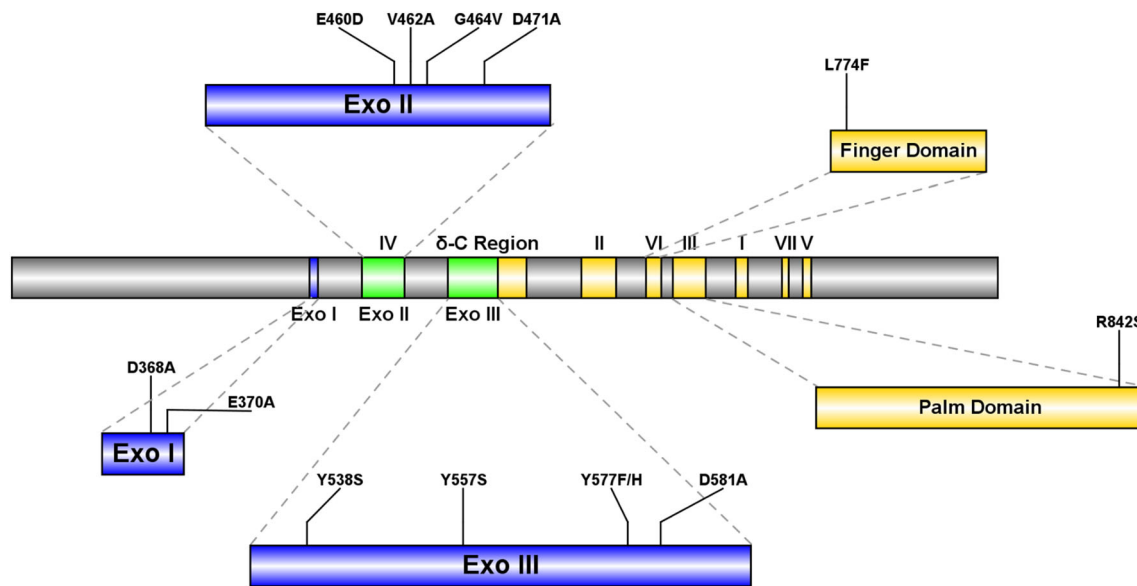


Fig. 1 Schematic representation of the HSV-1 DNA polymerase with the conserved Pol regions I–VII in yellow and the conserved Exo regions I–III in blue. Overlaps of both regions are indicated in green. Conserved

residues that were mutated and studied with respect to their influence on enzymatic activity and replication fidelity are indicated

debate on why some of the exonuclease mutations are not well tolerated by HSV-1 [40, 50, 51•].

High-Fidelity Mutants

Mutations that result in higher DNA replication fidelity relative to parental wild-type viruses have been described far less frequently (Table 1, 2, and 3). Such mutations

map to the finger and palm domains of the polymerase and cause resistance to antivirals [24, 32]. The relatively rare description of high-fidelity mutants argues for difficulties in further enhancing replication fidelity of herpesvirus DNA polymerases. For example, no exonuclease mutants with antimutator properties are described. It seems quite possible that exonucleolytic proofreading has reached an evolutionary maximum beyond which a

Table 1 Overview of HSV-1 DNA polymerase mutations known to affect exonuclease or polymerase processivity and/or DNA replication fidelity. Conserved residues that were also studied in other herpesviruses are in italics. Percent values for enzyme activity are rounded to integer values. Abbreviations: WT, wild-type; Pol, polymerase; Exo, exonuclease.

Domain	Mutation in HSV-1	Exo/Pol activity in % of WT	Mutation frequency, fold above WT	Viable virus
Exo I	<i>D368A</i>	0/103 [39]	In vitro >WT [40]	No
	<i>E370A</i>	0/111 [39]	n.d.	No
Exo II	<i>E460D</i>	n.d./0 [41]	n.d.	No
	<i>V462A</i>	n.d./53 [41]	n.d.	Yes
	<i>G464V</i>	n.d./0 [41]	n.d.	No
	<i>D471A</i>	20/32 [39]	n.d.	No
Exo III	<i>Y538S</i>	0/1,3 [39]	n.d.	No
	<i>Y557S</i>	43/73 [39]	n.d.	Yes
	<i>Y577H (Y7)</i>	n.d. ^a	5 [31]; 10–500 [42]; 800 [43]	Yes
	<i>Y577F</i>	17/71 [39]	n.d.	No
	<i>D581A</i>	6/82 [39]	n.d.	Yes
	<i>Y577H/D581A (YD12)</i>	n.d. ^a	4 [31]; 10 [44]; 300 [43]	Yes
Finger	<i>L774F</i>	n.d. ^b	15–20 [20] fold below WT (<i>high-fidelity</i>)	Yes
Palm	<i>R842S (PAAr5)</i>	62–86/	2–50 [24] fold below WT (<i>high-fidelity</i>);	Yes
		92–130 [24]	2–3 [31]	

^a Impaired in exonuclease function [43]

^b Unimpaired in exonuclease function [44]

Table 2 Overview of MDV DNA polymerase mutations known to affect exonuclease or polymerase processivity and/or DNA replication fidelity. All conserved residues mutated in this study were previously described to be important for enzymatic activity in HSV-1 (Table 1, italics). Percent values for enzyme activity are rounded to integer values. Abbreviations: WT; wild-type; Pol, polymerase; Exo, exonuclease.

Domain	Amino acid change in MDV DNA Pol	Exo/Pol activity % of WT	Mutation frequency, fold above WT	Viable virus
Exo I	<i>D358A</i>	n.d.	n.d.	Yes ^a
	<i>E360A</i>	6/99 [45]	102 [45]	Yes ^a
	<i>D358A/E360A</i>	5/100 [45]	96 [45]	Yes ^a
Exo II	<i>D461A</i>	n.d.	n.d.	Yes ^{a,b}
Exo III	<i>Y547S</i>	85/94 [45]	3 [45]	Yes
	<i>Y567F</i>	20/87 [45]	80 [45]	Yes ^a
Finger	<i>L755F</i>	105/94 [45]	Not significantly different ^b	Yes ^b

^a Suicidal phenotype

^b Unpublished data

further increase in exonuclease activity can interfere with efficient DNA replication.

Over the past decades, a number of herpesvirus fidelity mutants have been described. Most descriptions are based on HSV-1, but there is evidence in the literature for fidelity mutants in HSV-2, human cytomegalovirus (HCMV), and Marek’s disease virus (MDV). Since relatively little is known about replication fidelity in other herpesviruses, this report will focus on the viruses named above.

Herpes Simplex Viruses (HSV-1 and HSV-2)

In alphaherpesviruses, the DNA polymerase is encoded by UL30, the processivity factor by UL42 [52]. Before the structure and function of the viral polymerase were entirely understood, the discovery of fidelity mutants was based on mutations conveying resistance to antiviral drugs [24, 53]. This is still reflected in the nomenclature of some of the mutants described in the earlier literature (Table 1). With advancements in DNA sequencing and protein expression analyses, more systematic mutational analyses of the DNA polymerase became possible, which also included in vitro measurements of both enzymatic activities of the enzyme [39]. Most of the described mutants map to one of the exonuclease domains (Fig. 1, Table 1). Importantly, only relatively few exonuclease mutants can be reconstituted as viruses in cell culture, indicating a lethal effect of most of the

mutations. Different reasons for the observed lethal phenotypes are discussed [20, 50, 51]. The first and most obvious reason applies to Exo II and III mutants. Within these regions exists an interdependence of polymerase and exonuclease function, the exchange of conserved residues in these regions always affects both enzymatic activities [39, 41]. This interdependence seems to be more pronounced in Exo II mutants that always show strong reductions in polymerase processivity. Most Exo III mutants exhibit less of a reduction in polymerase activity (Table 1). The loss of polymerase function is an obvious explanation for detrimental or lethal effects observed in some of the described mutants. While large defects in polymerase processivity always have lethal outcomes, the more subtle reductions observed in most Exo III mutants do not seem to have a strong negative effect on virus growth. In fact, most published exonuclease mutants that gave rise to viable viruses in HSV-1 are Exo III mutants (Table 1). Although viruses with these mutations can replicate in cell culture, suppression of the mutator phenotype seems to be commonly observed [44]. In contrast to both Exo II and III mutations, mutations that reside within the first conserved exonuclease domain Exo I do not negatively affect polymerase processivity [39]. Instead, exonuclease function is completely abolished in Exo I mutants. These mutations affect the catalytic core of the exonuclease and replacement of the charged residues with other amino acids prevents coordination of Mg²⁺ and disables catalytic function. The reason for the lethality of these mutations is discussed extensively, but so far is

Table 3 Overview of HCMV DNA polymerase mutations known to affect exonuclease or polymerase processivity and/or DNA replication fidelity. Conserved residues also mutated in studies related to other herpesviruses are highlighted in italics

Domain	Mutation in HCMV	Exo/Pol activity % of WT	Mutation frequency, fold above WT	Viable virus
Exo I	<i>D301N</i>	< WT/n.d. [46]	n.d.	Yes
Exo II	F412V	< WT/n.d. [46]	n.d.	Yes
	<i>D413A</i>	n.d.	> WT [47]	Yes
Exo III	L501F	116/152 [48]	n.d.	Yes
	K513N	0–2/23–61 [49]	n.d.	Yes
	L545S	< WT/n.d. [46]	n.d.	Yes

not entirely understood [51•]. The lethal phenotype could be due to one or a combination of the following factors: (1) Important functions of the exonuclease other than proofreading are affected [41, 50]. (2) Certain mutations impact on the expression or folding of the protein [51•] (3) Mutagenesis is lethal due to a lack of proofreading in DNA replication [50]. We will discuss later why the latter explanation is favored by the authors of this report.

Fidelity measurements for the specific Exo III mutants Y577H and Y577H/D581A in HSV-1 vary dramatically and reflect the problems of mutation rate estimation in viruses. A good example is mutant Y577H, which proves to be highly mutagenic in a viral thymidine kinase (TK) assay [43], but shows only a slight increase in mutation frequencies in a *supF* mutagenesis assay [54] where mutations within a foreign genetic element inserted into the virus genome are measured. Another interesting case is the palm domain mutant R842S (PAAr5) that is described to be an antimutator based on a TK assay [55], but exhibits mutation frequencies slightly above wild-type (WT) in the *supF* assay [54]. This inconsistency in determining mutation frequencies has made the exploration of fidelity variants difficult and led to the conclusion that “the outcome of the fidelity of DNA replication is assay-dependent” [42]. With the advancements in sequencing technologies, a less-biased evaluation of mutation frequencies in HSV-1 has now become possible.

Two high-fidelity variants of the HSV-1 DNA polymerase are described to date. One, R842S that was originally described as phosphonoacetic acid-resistant clone 5 (PAAr5), maps to the palm domain of the polymerase and is thought to increase replication fidelity through enhanced nucleotide selectivity [20, 24, 56]. As discussed above, the antimutator properties of this mutant are not supported by a *supF* mutagenesis assay. The L774F high-fidelity variant has a mutation in the finger domain of the DNA polymerase, which is thought to increase substrate specificity through a slight structural change in the targeted domain [20, 32, 44]. Interestingly, this

mutant was selected from a population established by hypermutator Y577H/D581A in cell culture [32] and was shown to at least partially restore DNA replication fidelity in the absence of exonucleolytic proofreading [44, 57].

In contrast to HSV-1, relatively little is known about fidelity variants in HSV-2. There are few descriptions of high-fidelity and low-fidelity mutants in literature [12, 58–61], each without detailed examination of the enzymatic properties of the mutants. Overall, spontaneous mutation rates in HSV-2 appear to be higher than those in HSV-1 [61].

In clinical isolates, a large number of polymerase mutations is found [62, 63]; some of them map to exonuclease domains and could potentially affect replication fidelity. However, a detailed examination of the phenotype of individual mutants from clinical samples is typically not available.

Marek’s Disease Virus

Marek’s disease virus (MDV), the gallid alphaherpesvirus type 2 (GaAHV-2), is the causative agent of a lymphoproliferative disease in chickens and known for its continuous evolution towards higher virulence [64]. Recently, DNA replication fidelity of the WT virus and a number of exonuclease mutants was examined by our laboratory [45]. To study the effects of error-prone genome replication, we constructed MDV polymerase mutants based on in vitro data for HSV-1 polymerase mutants with synonymous changes [39] (Fig. 2). We found all of the tested mutants to result in viable viruses upon reconstitution in cell culture (Table 2). Among the tested mutants are the Exo I mutants that are reported to be lethal to HSV-1 virus replication. A suicidal phenotype of Exo I mutants D358A and E360A and the double mutant D358A/E360A as well as Exo III mutant Y567F was observed. The progressive growth deficit of these viruses correlated with drastically elevated mutation frequencies that were between 80 and 100 times higher when compared with WT virus. An Exo III mutant with only slightly impaired

Fig. 2 Alignment of conserved exonuclease domains of herpesviruses (Exo I–III). Highly conserved amino acids that were studied in more than one virus are displayed in color. Highlighted in red are conserved residues that were examined in all three viruses represented in this figure, the function of residues highlighted in yellow was examined in HSV-1 and MDV only. Shown in gray are conserved residues found in HCMV DNA polymerase that were not studied despite their identity to studied residues in HSV-1 and MDV

	Exo I							
HCMV	----	QQGRASTCDIEVDCDVS	DLVAVPDDSSWPRYRCLSF	DI	ECMSG---EGGFPCAES	317		
HSV-1		QPRAPMAFGTSSDVEFNCTADN	LAIIEGGMSDLPAYKLMCF	DI	ECKAGGEDELAFVAGHP	387		
MDV		QVRAPCHHCTSCDIEINCTVDN	LIGYPEDDAWPDYKLLCF	DI	ECKSGGVNECAFPCATNE	377		
		::*:*:*:*	..*:		. * * : . : * * * * * : * * . * * * :			
	Exo II							
HCMV	-----	GPDVDVVEYFPSEYELL	LGFMFLFQRYAPAFV	TGYNINSF	FLKYILTRLELYLK	426		
HSV-1		NELAARGLPTPVVLEFDSE	FEMLLAFMTLVKQYGP	EFVTGYNI	INFDWPFLLAKLTDIYK	484		
MDV		ETFQSSYNILPIVLEFDSE	FELLAFMTFIKQYAPE	FVTGYNIVNF	WAFIVTKLTTVYN	474		
		* * * * * : * : * * * * * :	..*:*:		* * * * * : * * * * * : * * : * * * * * : * * :			
	Exo III							
HCMV		YPVCMAKTNSPNYKLN	TMAELYLRQRKDDLS	YKDI	PRCFVANAEGRAQVGR	YCLQDAVLV	546	
HSV-1		YGIITDKIKLSSYKLN	VAEAVLKDKKDDLS	YRDI	PAYYAAGPAQRGVIGE	YCIQD	SLLV	585
MDV		YSIATEKLLQSYKLDV	VAEAAALGERKKELS	YKEI	PSHFAAGPEKRGII	IGEYCLQD	SLLV	575
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exonuclease activity was found to replicate stably and cause disease with WT-like kinetics while causing slightly elevated mutation frequencies of around $3\times$ above WT. Interestingly, even strong hypermutators could overcome their suicidal phenotype and adopt WT-like growth as extremely diverse populations with only partially restored DNA replication fidelity. This result indicates the possibility of survival of herpesviruses in the presence of drastically elevated mutation rates and highlights the potential for using such mutants to study the implications and dynamics of low-fidelity nucleic acid replication in viruses.

From our work with MDV Pol mutants, we also conclude that lethal phenotypes of exonuclease mutants are most likely attributable to lethal mutagenesis caused by an accumulation of detrimental mutations generated through low-fidelity replication. Small and isolated populations of hypermutators are prone to extinction in the case of MDV. Considering that this virus replicates relatively slowly in cell culture, the phenotypic difference to the fast replicator HSV-1 could be explained by the speed of genome replication and thus speed of mutation accumulation. It seems possible that some HSV-1 exonuclease mutants cause an immediate collapse of virus populations through lethal mutagenesis immediately following reconstitution. We also acknowledge that there might be a considerable difference in the ability of different viruses to tolerate a substantially increased mutational load. An interesting example is the high viability of exonuclease mutants in the case of HCMV that is described in the following section.

Human Cytomegalovirus

In HCMV, the DNA polymerase is encoded by UL54, the processivity factor by UL44 [52]. Exonuclease mutations are more common in HCMV field isolates than in any other herpesvirus. One reason for this observation could be the frequent use of nucleoside analogues, mainly ganciclovir, in the treatment of HCMV infections, and the fact that certain exonuclease mutations confer resistance against nucleoside analogs. For ganciclovir, a novel mode of action was proposed that could explain resistance conferred by exonuclease-impaired mutants [46]. Upon incorporation of ganciclovir and in the presence of a functional exonuclease, the DNA polymerase is thought to switch to an idling mode where neither excision of ganciclovir by the exonuclease nor chain elongation by the polymerase is possible. In the absence of exonuclease activity, polymerization continues, allowing incorporation of ganciclovir in the nascent DNA double strand. This relatively novel mode of action for ganciclovir would explain the increased rate at which exonuclease mutations in HCMV are selected. The inevitable consequence of this mechanism would be stable incorporation of ganciclovir into the HCMV genome, and it remains unclear if and how this can be either tolerated or circumvented by the virus.

Most of the described HCMV mutants are mainly characterized with respect to their drug resistance [65–69]. Some of them are, however, known to increase mutation rates compared with WT [47], for others in vitro measurements showing an exonuclease defect are available [49] (Table 3). It is remarkable that HCMV seems to tolerate mutations in the exonuclease domain of the DNA polymerase much better than HSV-1. Notably, an Exo I mutation (D301N), which affects the aspartic acid within the highly conserved “FDIE” Exo I motif present in the catalytic core of the enzyme (Fig. 2), does not seem to negatively affect virus growth [68]. In contrast, the loss of the homologous D residue at position 368 is reported to be lethal to HSV-1 [51] and the removal of the same amino acid in the MDV DNA polymerase causes a highly suicidal phenotype [45]. Mutation frequencies for Exo I mutants have not been systematically recorded and certainly require future analysis. Another interesting example that indicates that changes of conserved amino acids within the exonuclease are better tolerated by HCMV than by other herpesviruses, is Exo II D413A, originally identified in a clinical HCMV isolate [70], which results only in a mild growth deficit upon introduction into a HCMV laboratory strain [47]. The counterpart of this mutant in HSV-1 (D471A) cannot be reconstituted [50] and that of MDV (D461A) showed a severe and progressive growth deficit consistent with the expected consequences of hypermutation and loss of polymerase processivity (unpublished data). This polymerase mutant is so far the only mutant HCMV formally described to exhibit mutator properties [47], although exact mutation frequencies in this mutant were not recorded. Also, in the background of ganciclovir resistance, the only known herpesvirus mutant with enhanced exonuclease activity was described. The L501F mutation maps close to the proximal end of the conserved Exo III site and was described to show significantly enhanced exonuclease activity [48].

The viability of HCMV Pol mutants presents a very interesting difference to other herpesviruses. This difference could be a consequence of a much higher tolerance of HCMV to elevated mutation rates or could be explained by a less striking impact of these mutations on the replication fidelity accounted for by the HCMV DNA polymerase. A third possibility is that, in the case of HCMV, loss of replication fidelity can be readily compensated for by other domains of the polymerase, other viral proteins involved in DNA replication and repair, or even by other cellular gene products co-opted by the virus. The lack of whole genome sequencing studies involving virus populations established by exonuclease mutants so far prevents us from examining these possibilities.

The relative abundance of mutations in this region of the polymerase gene in clinical isolates highlights their clinical importance. It is also tempting to speculate that some of the high genetic variation in clinical HCMV isolates might be due to increased mutation rates acquired by exonuclease-deficient viruses selected under ganciclovir treatment. The formal analysis of

mutation frequencies in exonuclease mutants seems to be an important area of study in the future.

Conclusions

Mutants that affect the fidelity of viral DNA replication were initially regarded as an important tool to understand the mechanism of DNA replication and its fidelity. We believe, however, that herpesvirus fidelity mutants are important in many more aspects. Especially, low-fidelity variants allow us to interrogate the consequences of more error-prone genome replication and can be used as tools to better understand virus evolution. Stably replicating hypermutators like Y547S in MDV or D413A in HCMV can serve as models for a fast-forward evolution and could allow faster observation of adaptive changes, for example, in drug and vaccine resistance research. Finally, fidelity mutants in herpesviruses are frequently associated with altered susceptibilities to antiviral drugs. Especially in the case of HCMV, the understanding of the principles that lead to emergence of drug-resistant polymerase mutants seems of great clinical and therapeutic importance. More than that, higher mutation frequencies in these mutants may lead to accelerated development of multi-drug resistance and should be subject to close observation and systematic studies in the future.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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