Roles of uracil-DNA glycosylase and dUTPase in virus replication

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Introduction

It has been known for about 15 years that herpesviruses (Caradonna et al., 1987) encode the DNA repair enzyme uracil-DNA glycosylase (UNG), an enzyme involved in the base excision repair pathway that specifically removes the RNA base uracil from DNA, while at least one retrovirus (human immunodeficiency virus type 1) packages cellular UNG into virus particles. In these instances, UNG is implicated as being important in virus replication. However, a clear understanding of the role(s) of UNG in virus replication remains elusive. Herpesviruses, poxviruses and some retroviruses encode dUTPase, an enzyme that can minimize the misincorporation of uracil into DNA. The encoding of dUTPase by these viruses also implies their importance in virus replication. An understanding at the molecular level of how these viruses replicate in non-dividing cells should provide clues to the biological relevance of UNG and dUTPase function in virus replication.

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HIV-1 Vpr and UNG

Retroviruses like HIV-1 are RNA viruses that encode reverse transcriptase (RT), an error-prone RNA-dependent DNA polymerase. Several years ago it was observed that the HIV-1 accessory protein Vpr could modulate the HIV-1 mutation rate (Mansky, 1996). Vpr is a 96 amino acid, non-structural protein, which is associated with HIV-1 particles and is localized in the nuclei of infected cells. Incorporation of Vpr into particles requires a direct interaction with the p6 region of the Gag polyprotein precursor (Bachand et al., 1999; Mansky, 1996). One interpretation of this data was that Vpr directly interacts with RT to influence enzyme fidelity. Another round of replication. Although the presence of virus-encoded UNG has been recognized, it remains unclear what the role(s) is for these viruses to encode a DNA repair enzyme that is encoded by most eukaryotic and prokaryotic cells. Herpesviruses, poxviruses and certain retroviruses also encode dUTPase, an enzyme that is involved in maintaining a low dUTP: dTTP ratio to minimize the misincorporation of uracil into DNA (Preston & Fisher, 1984; McGeoch, 1990; Elder et al., 1992). Like UNG, virus-encoded dUTPase has been recognized but is also unclear why these viruses encode an enzyme that is already encoded by most eukaryotic and prokaryotic cells. In this review, we propose that the function of UNG and dUTPase in virus replication is associated with the ability of these viruses to replicate in non-dividing cells. The study of virus replication in non-dividing cells would be the best experimental approach to determine how UNG and dUTPase function in virus replication.

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Table 1. Viruses, UNG and dUTPase

<table>
<thead>
<tr>
<th>Virus</th>
<th>Encodes UNG</th>
<th>Packages cellular UNG into virus particles</th>
<th>Encodes dUTPase</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Yes/No</td>
<td>Reference</td>
<td>Yes/No</td>
</tr>
<tr>
<td>HIV-1</td>
<td>No</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-primate lentiviruses</td>
<td>No</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>ASFV</td>
<td>No</td>
<td>Yanez et al. (1995)</td>
<td>ND</td>
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ND, Not determined.

Fig. 1. Pathways for introduction of uracil residues into DNA. (A) Function of dUTPase and misincorporation of uracil into DNA. The conversion of dUTP to dUMP and PPi occurs by dUTPase activity. This conversion helps the cells to maintain a low dUTP:dTTP ratio as well as providing a substrate (dUMP) for thymidylate synthase. If the ratio is higher, the probability of misincorporation of uracil into DNA increases. Uracil-containing DNA serves as a substrate for UNG. In non-dividing cells, dUTPase levels are lower and would result in an elevated dUTP: dTTP ratio. (B) The spontaneous deamination of cytosine residues to create uracil-containing DNA leads to base excision repair. Cytosine residues can spontaneously deaminate to create uracil-containing DNA, which can be acted upon by UNG. Higher deamination rates of cytosine residues in the DNA of non-dividing cells could contribute to higher levels of uracil-containing DNA. UNG excises the uracil residue in the DNA to create an AP site. AP endonuclease cleaves the 5’ end of the AP site. The resulting single-nucleotide gap is filled in by DNA polymerase b. The remaining nick is sealed by a DNA ligase.

interpretation was that Vpr interacts with other proteins that influence the accuracy of the reverse transcription process. To investigate this mutation phenotype, HIV-1 replication with Vpr mutants containing single amino acid substitutions was analysed in order to map the determinants responsible for the ability of Vpr to influence replication fidelity (Mansky et al., CDEA...
Fig. 2. Role of the Vpr–UNG complex in the HIV-1 life cycle. Virus-infected cells that express the viral proteins will accumulate HIV-1 Vpr protein complexed with the nuclear form of cellular UNG at the nucleus. Vpr–UNG complexes can then associate with the HIV-1 Gag polyprotein precursor by association of Vpr with the p6 region of Gag. The Vpr–UNG complex is incorporated into HIV-1 particles by the Vpr–Gag interaction. Virus is subsequently released from infected cells. Virus infection of a permissive cell will lead to particle disassembly and release of the virion core. The core contains many molecules, including the viral RNA, RT, Vpr, UNG and the nucleocapsid and capsid proteins. Many of these molecules in the core become the reverse transcription complex. Reverse transcription of the viral RNA occurs, after which, viral DNA and associated proteins (now called the preintegration complex) are transported to the nucleus where the viral DNA is integrated into the host cell DNA. After integration, DNA repair can occur in the provirus DNA sequence. For simplicity, incorporation of RT and other viral proteins into HIV-1 particles is not shown. Also for simplicity, only one Vpr–UNG complex is shown in particles associated with viral core.

2000, 2001). Vpr mutants were selected based on their ability to interact with two different proteins in the yeast two-hybrid assay, namely UNG and HHR23A, a human homologue of RAD23 derived from yeast (Bouhamdan et al., 1996, 1998; Selig et al., 1997). HHR23A is presumed to function with HHR23B in a nucleotide excision DNA repair pathway as part of a multiprotein complex associated with the xeroderma pigmentosum complementation group C protein (van der Spek et al., 1996). One Vpr mutant, in which tryptophan at position 54 was changed to arginine (Vpr*W54R), led to the same mutation phenotype observed during HIV-1 replication in the absence of Vpr (Mansky et al., 2000).

Vpr*W54R had a phenotype that is comparable to wild-type Vpr in its ability to arrest cells in the G_s-M phase of the cell cycle, to localize to the nucleus, to be efficiently incorporated into HIV-1 particles and to interact with HHR23A (Selig et al., 1997; Mansky et al., 2001). In contrast, the W^454 → R substitution prevented Vpr from interacting with UNG and HIV-1 expressing Vpr*W54R led to a 4-fold increase in the rate of G → A mutations. In addition, Vpr*W54R did not allow efficient packaging of UNG into HIV-1 particles. The nuclear form of UNG was preferentially packaged into HIV-1 particles, presumably because both Vpr and UNG are targeted to the nucleus where they could subsequently associate with viral RNA that is destined for packaging into virus particles. This indicates that the interaction and virion-incorporation of Vpr and UNG into HIV-1 particles correlates with the influence of Vpr on the HIV-1 mutation rate. Other studies have also implicated the interaction of UNG with the HIV-1 integrase as an interaction required for UNG incorporation into virus particles (Willetts et al., 1999). It has not been determined yet if the enzymatic activity of UNG is associated with the mutation rate phenotype. Work with UNG-deficient mice has indicated that the nuclear form of UNG has a specialized role in preventing uracil misincorporation and that another cellular UNG is involved in the removal of uracil in DNA created by cytosine deamination (Nilsen et al., 2000). Several other Vpr variants, which do not associate with HHR23A showed that the interaction of Vpr with HHR23A is not associated with the ability of Vpr to influence the HIV-1 mutation rate (Mansky et al., 2001).

A current working hypothesis regarding a possible role for UNG enzymatic activity in HIV-1 replication is that to efficiently remove uracil bases in HIV-1 DNA during synthesis in non-dividing cells, UNG is incorporated into particles via interaction with Vpr (Fig. 2). An intriguing question regarding
the incorporation of UNG into HIV-1 particles is whether other repair enzymes are incorporated into virions. The excision of uracil from DNA by UNG will cause abasic (apurinic or apyrimidinic) sites (AP sites). The base excision repair pathway is considered to be the major mechanism to repair AP sites; this pathway is initiated by AP endonuclease (known as HAP, APEX or Ref-1), which catalyses the incision of DNA at AP sites (Friedberg et al., 1995). Attempts to identify AP endonuclease in HIV-1 particles have failed to date (Mansky et al., 2000). A DNA ligase activity in HIV-1 particles has yet to be analysed. A lack of enzymes involved in base excision repair (Fig. 1B) in virus particles may suggest that these enzymes are recruited after virus entry, either in the cytoplasm or, more likely, in the nucleus following integration of viral DNA.

dUTPases and non-primate lentiviruses

dUTPase is a cellular enzyme and, similar to UNG, is well conserved in prokaryotic and eukaryotic cells. By hydrolysing dUTP to dUMP and PPi, dUTPase plays the dual role of both providing a substrate for thymidylate synthase (an enzyme that converts dUMP to TMP) in a major biosynthesis pathway to TTP and maintaining a low dUTP:dTTP ratio to minimize the misincorporation of uracil into DNA (Fig. 1A). Uracil can also occur in DNA by the spontaneous deamination of cytosine residues (Fig. 1B). The expression of cellular dUTPase is regulated by the cell cycle, being at high levels in dividing, undifferentiated cells and at low levels in terminally differentiated, non-dividing cells (Miller et al., 2000).

HIV-1 and related retroviruses (called lentiviruses) can be subdivided into viruses that infect primates (e.g. HIV) and viruses that infect non-primates (e.g. equine infectious anaemia virus, EIAV). Previous work has indicated that EIAV and most non-primate lentiviruses encode and package a dUTPase into virus particles (Table 1) (Elder et al., 1992). Studies on caprine arthritis–encephalitis virus (CAEV) with in-frame nucleotide insertions or deletions in the dUTPase gene have indicated that the replication of dUTPase-minus mutants are severely affected in non-dividing host cells (e.g. primary macrophages) and the virus loads can be decreased 10- to 100-fold in comparison with wild-type virus (Turelli et al., 1997). The frequency of G → A transition mutations in viral DNA increases during replication of dUTPase-minus CAEV and feline immunodeficiency virus and eventually leads to replication-defective proviruses (Lerner et al., 1995; Turelli et al., 1997). Uracil misincorporation into DNA could influence DNA conformation and sequence-specific protein binding and may explain the decrease in virus production and replication. However, replication of dUTPase-minus CAEV mutants in dividing cells (e.g. mitogen-stimulated T-cells and continuous T-cell lines) is only minimally decreased, suggesting that actively dividing cells could have sufficiently high endogenous dUTPase activity, which compensates for the lack of virion-associated dUTPase activity (Turelli et al., 1997).

Herpesviruses, poxviruses and UNG

UNG is encoded and expressed by DNA viruses of two main families, the Herpesviridae and the Poxviridae (Table 1). Herpesviruses replicate their viral DNA and assemble virus capsids in the nucleus of infected cells. The ability of UNG to influence virus replication of different herpesviruses has implied a role for viral UNG in the replication of virus in the host, particularly in non-dividing cells (e.g. terminally differentiated cells), where levels of cellular UNG are believed to be low. Viral UNG has been shown to be dispensable for replication in cell culture (Mullaney et al., 1989) but herpes simplex virus type 1 (HSV-1) UNG-minus mutants replicated and spread poorly in mice (Pyles & Thompson, 1994b).

A more recent study suggests that the elimination of both viral and cellular UNG activity does not affect the efficiency of replication for another herpesvirus, varicella-zoster virus (VZV) (Reddy et al., 1998). A VZV mutant with a deletion of the gene encoding UNG was shown to replicate as efficiently as the parental virus in cell culture. A natural inhibitor of UNG from the Bacillus subtilis bacteriophages PBS1 and PBS2 (uracil-DNA glycosylase inhibitor, UGI) inactivates UNG activity from a variety of organisms, including herpesviruses. The replication of UGI-expressing VZV, in either the presence or the absence of viral UNG, was as efficient as that in the parental virus. This implies that cellular UNG cannot functionally replace viral UNG when it is not expressed. This provides an indication that UNG may be dispensable for replication in actively dividing cells in culture.

Human cytomegalovirus (CMV) UNG was first reported to delay viral DNA synthesis and replication (Prichard et al., 1996). More recent studies have suggested that UNG excises uracil residues from replicating CMV DNA to create sites that can serve as substrates for initiation of recombination-dependent replication late in infection (Courcelle et al., 2001). CMV DNA replication is thought to switch from a bidirectional (theta structure) mode early in infection to a rolling-circle mode of replication late in the infection process. The nicks in the DNA generated by the removal of uracil residues by UNG and cleavage of AP sites by AP endonuclease could serve a functional role in the switch from bidirectional to rolling-circle replication (Fig. 3). In quiescent, non-dividing cells, the lack of CMV UNG expression leads to a delay in replication for 48 h (Courcelle et al., 2001). In actively dividing cells, virus replication of the UNG mutant occurs without delay (Courcelle et al., 2001). This indicates that the role of virus-encoded UNG is particularly important in non-dividing cells. In summary, studies with herpesvirus UNG indicate that there is some debate as to whether viral UNG is important for virus replication in dividing cells; viral UNG appears to play an important role for replication in non-dividing cells.

Poxviruses are large, complex DNA viruses that replicate in the cytoplasm of vertebrate and invertebrate cells. Poxvirus-encoded UNG has been found to be more closely associated
with maintaining virus replication in cell culture. A vaccinia virus UNG mutant was able to replicate viral DNA at approximately 2% of the level of the parental virus (Millns et al., 1994). Attempts to inactivate the UNG gene of Shope fibroma virus were not successful and resulted in a loss of virus viability (Stuart et al., 1993). A temperature-sensitive (ts) mutant of the D4R gene (which encodes UNG) of vaccinia virus has been isolated, indicating that D4R is essential for replication (Ellison et al., 1996). Interestingly, bacteriophage UGI does not inhibit vaccinia virus UNG (Ellison et al., 1996). A conserved leucine residue in UNG which is part of the DNA-binding groove that interacts with UGI within a hydrophobic pocket based on the crystal structure is replaced by an arginine residue in the vaccinia virus UNG protein sequence (Mol et al., 1995; Ellison et al., 1996). The presence of this amino acid substitution has been used to explain why UGI does not inhibit vaccinia virus UNG (Ellison et al., 1996). Mutations in the active site of vaccinia virus UNG that eliminated the ability of the enzyme to excise uracil residues from DNA but still allowed the enzyme to bind DNA were introduced into the UNG gene and then transfected into cells infected with the ts mutant virus. Genetic and DNA sequence analyses indicated that the only viruses that survived were recombinants that had eliminated the active site mutations (Ellison et al., 1996). Since poxviruses replicate their viral DNA in the cytoplasm, their dependence on viral UNG during replication.

Herpesviruses and poxviruses encode dUTPase (Table 1), as do some other DNA viruses (Baldo & McClure, 1999) such as African swine fever virus (ASFV) (Dixon et al., 1994; Yanez et al., 1995). ASFV is a member of the Asfarviridae family and has some similarities to poxviruses. ASFV mutants that do not express dUTPase have been associated with inefficient replication in non-dividing cells (Oliveros et al., 1999). Herpesvirus dUTPase-minus mutants have been reported to be attenuated for neurovirulence, lack the ability to reactivate virus replication from latency (Pyles et al., 1992) and possess an increased frequency of mutant formation (Pyles & Thompson, 1994a).

Orf virus is a member of the Parapoxvirus genus of the family Poxviridae. The orf virus NZ2 strain deletion variant, isolated after serial passage in primary bovine testis cells, had a deletion of the E3L gene, which encodes a protein related to the HSV dUTPase, indicating that this gene is dispensable for replication in dividing cells (Fleming et al., 1995). However, an attenuated variant of the orf virus D1701 strain, which is used as a live vaccine against contagious ecthyma in sheep, contains an intact E3L gene (Cottone et al., 1998). This gene has been shown recently to encode a functional dUTPase (Cottone et al., 2002).

Concluding remarks

Why UNG and dUTPase are needed to maintain virus replication in non-dividing and non-cycling cells requires more intensive investigation. These cells are thought to express low levels of cellular UNG and dUTPase and therefore having a virus encode these enzymes or package the cellular enzymes into virus particles provides a means to supply the virus with UNG and/or dUTPase. The study of virus replication in non-dividing cells with low or no UNG and/or dUTPase expression will be the best experimental approach for determining the

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**Fig. 3.** A model for UNG function in the late phase of CMV DNA replication. The creation of viral DNA with uracil residues occurs by either misincorporation or cytosine deamination. Uracil residues are excised by UNG and AP sites are cleaved by AP endonuclease. The resulting nicks in the viral DNA can serve as initiation sites for rolling-circle replication and recombination-dependent replication. The double-stranded viral DNA is represented by two circular or linear black lines; single-stranded viral DNA is represented by single black lines. Newly synthesized DNA is indicated by a dashed line. Uracil residues in DNA are indicated by the letter ‘U’ alongside the black circular line. AP sites are indicated by gaps in the outer circle. The virus replication complex is indicated by the shaded, overlapping ovals. This figure was adapted from Courcelle et al. (2001).
underlying properties and mechanisms of how these enzymes aid in virus replication. An advantage for viruses to be able to replicate efficiently in non-dividing cells may allow these viruses to persist more effectively in the host. However, given the high mutation rate of HIV, it seems unlikely that a virus that has evolved a high rate of mutation and evolution would utilize a cellular repair enzyme to lower this rate. One possible role for UNG function may be in the repair of viral DNA during periods of latency in non-dividing cells when there is no active virus replication. In summary, future studies of UNG and dUTPase function in virus replication should focus on studies using non-dividing cells.

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References


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