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Effects of Cell Cycle Status on Early Events in Retroviral Replication

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Abstract The study of retroviruses over the last century has revealed a wide variety of disease-producing mechanisms, as well as apparently harmless interactions with animal hosts. Despite their potential pathogenic properties, the intrinsic features of retroviruses have been harnessed to create gene transfer vectors that may be useful for the treatment of disease. Retroviruses, as all viruses, have evolved to infect specific cells within the host, and such specificities are relevant to both pathogenesis and retrovirus-based vector design. The majority of cells of an animal host are not progressing rapidly through the cell cycle, and such a cellular environment appears to be suboptimal for replication of all retroviruses. Retrovirus-based vectors can therefore be restricted in many important target cells, such as post-mitotic differentiated cells or stem cells that may divide only infrequently. Despite intense interest, our understanding of how cell cycle status influences retroviral infection is still quite limited. In this review, we focus on the importance of the cell cycle as it relates to the early steps in retroviral replication. Retroviruses have been categorized based on their abilities to complete these early steps in non-cycling cells. However, all retroviruses are subject to a variety of cell cycle restrictions. Here, we discuss such restrictions, and how they may block retroviral replication, be tolerated, or overcome. J. Cell. Biochem. 94: 880–889, 2005. © 2005 Wiley-Liss, Inc.

Key words: retroviruses; HIV; cell cycle; mitosis; S-phase; nuclear entry; retrovirus-based vectors

Elucidation of the relationships between retroviral replication and the cell cycle is a challenge that has long both fascinated and frustrated researchers, from two major perspectives. First, the ability to establish an infection in non-cycling cells can be a critical feature of retroviral pathogenesis; understanding such relationships can reveal novel targets for antiviral therapy. Second, although retrovirus-based vectors are in use for experimental gene transfer, as well as human gene therapy, the engineering of optimal vectors for targeting non-cycling cells has been a difficult and mostly empirical process. Further studies on the basic biology of retroviruses will undoubtedly contribute to progress in both areas. This review analyzes the impact of cell cycle status on the early steps in retroviral replication: reverse transcription of the viral RNA into DNA, DNA nuclear entry, and integration of the viral DNA into the host chromosomes. These early events can be monitored in cultured cells or experimental animals through the use of retrovirus-based vectors that encode reporter genes. Stable transduction of the reporter gene signifies that the early events have been completed successfully. By design, such vectors retain many of the biological properties that govern early steps in the replication cycle of the parental, naturally occurring virus. Accordingly, studies with retrovirus-based vectors can be used to investigate these early steps. Below, we discuss the behavior of natural retroviruses and retrovirus-based vectors interchangeably, unless otherwise noted.

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OVERVIEW OF THE CELL CYCLE

For successful propagation, viruses depend on the availability of a favorable cellular niche, and cell cycle status clearly influences this process. The cell cycle is divided into two major stages, interphase and mitosis (Fig. 1). Interphase comprises the bulk of the cell cycle, and during this period the cell grows in size and the chromosomal DNA is duplicated in preparation for cell division. During mitosis (M-phase), the nuclear membrane disassembles, and the duplicated chromosomes are separated and guided to opposite poles by the mitotic spindles. The nuclear membrane then reforms around the separated chromosomes, giving rise to two nuclei. When mitosis and nuclear duplication are completed, cell division occurs (cytokinesis).

After cell division, the new daughter cells re-enter interphase, which begins with a "gap" phase, G_1, during which the cell increases in mass and prepares for DNA replication. Cells may pause during G_1 and enter a state denoted G_0, which can last for days or even years. Cells in this state have withdrawn from the cell cycle and are referred to as quiescent, resting, or post-mitotic. The majority of vertebrate body cells are in the G_0 phase (e.g., terminally differentiated tissue cells, resting lymphoid cells, or resting stem cells). Various cues can stimulate certain cells to re-enter G_1 and continue into S-phase. S-phase is followed by a second gap phase (G_2), during which the cell prepares for mitosis.

VIRUSES AND THE CELL CYCLE

The cell cycle is regulated through the actions of protein cyclins and cyclin-dependent kinases. Many animal viruses can exploit these regulatory pathways to their own advantage, by either promoting or blocking cell cycle progression via the action of virus-encoded proteins [Op De Beeck and Caillet-Fauquet, 1997; Swanton and Jones, 2001; Schang, 2003]. As cellular components fluctuate during the cell cycle, these manipulations can force the cell into a phase that is favorable for virus replication. For example, with DNA viruses, viral genome replication may require coordination with host cell DNA replication in S-phase. DNA viruses with large genomes have the luxury of encoding numerous auxiliary gene products that may (i) drive host cells into S-phase [Schang, 2003] or (ii) provide functions that are lacking in quiescent cells [Chen et al., 2002]. Such viral trickery is highly sophisticated and can also include mechanisms to activate cell cycle-specific proteins without stimulating cell cycle progression [Schang, 2003]. Viruses that lack strategies to

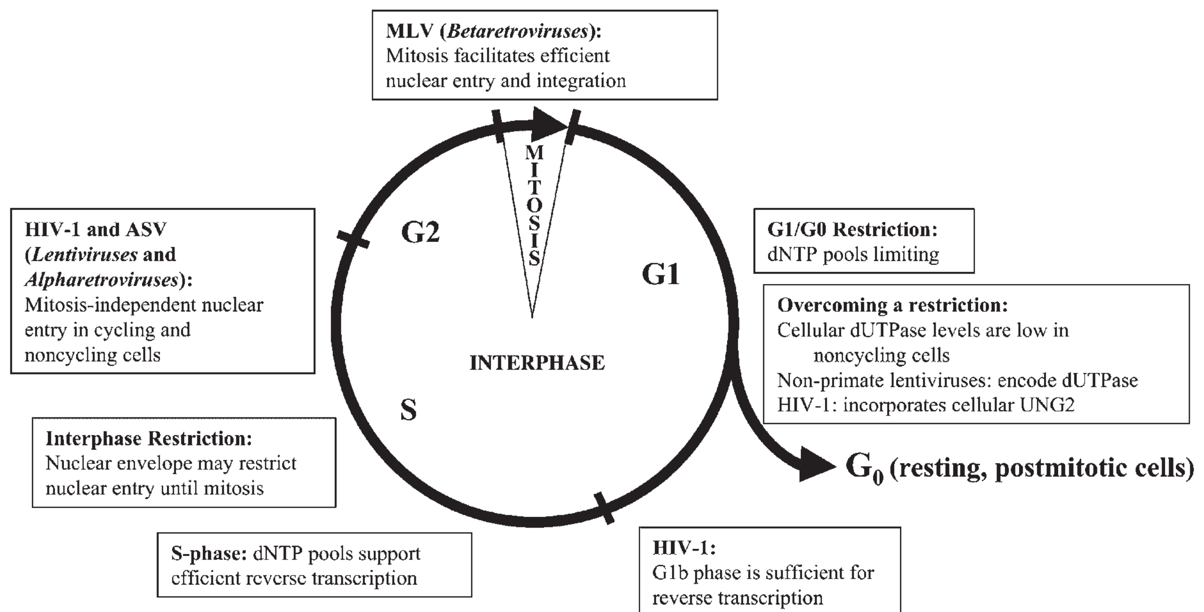

Fig. 1. Outline of the cell cycle and retrovirus-host cell cycle-specific interactions. A typical cell cycle profile is shown. Interphase and mitosis are denoted. Highlighted are restrictions, phase requirements, and viral mechanisms to overcome restrictions. The listed restrictions, requirements, and viral functions are not comprehensive, and are discussed in the text.

manipulate the cell cycle are presumably more restricted. In contrast to large DNA viruses, retroviruses (which contain relatively small RNA genomes) do not encode a large number of genes and may therefore be more limited in dealing with these cell cycle restrictions.

RETROVIRAL GENOME STRUCTURE, FUNCTION, AND REPLICATION

The prototypic retrovirus contains a "simple" genome, comprising a single-stranded RNA that encodes three genes: *gag*, *pol*, and *env* [Coffin et al., 1997; Flint, 2004]. The *gag* and *pol* gene products include the core structural proteins and the enzymes that reverse-transcribe the RNA genome into DNA (reverse transcriptase, RT) and catalyze the integration of viral DNA into the host chromosomes (integrase, IN). The *env* gene encodes the proteins that mediate binding of the virus to receptors on the host cell surface and promote entry into the cytoplasm. "Early" steps in the retroviral replication cycle include receptor binding, entry, uncoating, reverse transcription of the viral RNA into DNA, nuclear entry, and integration of the viral DNA into the host DNA (Fig. 2) [Coffin et al., 1997; Nisole and Saib, 2004]. The "late" steps are defined as transcription and

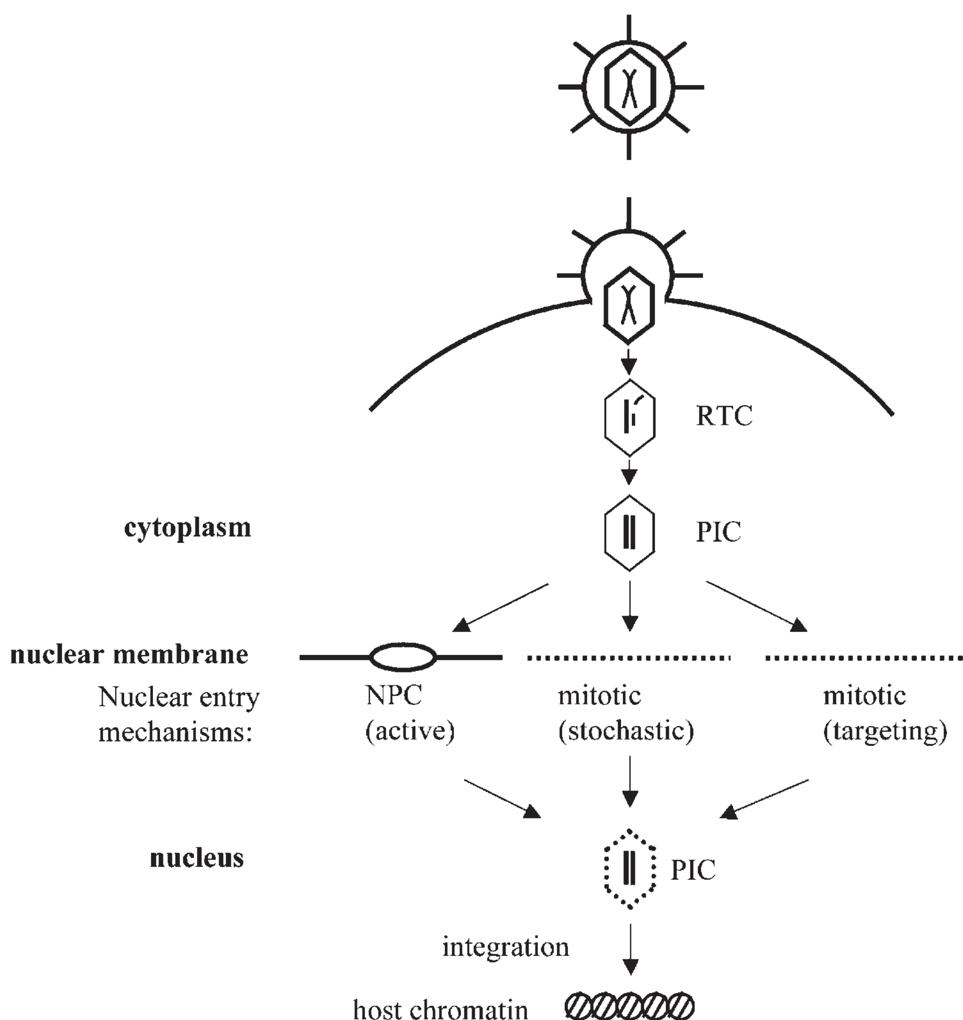

Fig. 2. Diagram of early steps in retroviral replication [Coffin et al., 1997; Flint, 2004; Nisole and Saib, 2004]. The retroviral particle is depicted at the top, showing the diploid RNA genome. Reverse transcription occurs in an ill-defined complex here referred to as the "reverse transcriptase complex" (RTC). The DNA product is depicted as thicker line. The DNA-containing complex is denoted the "pre-integration complex" (PIC). The lower portion of the diagram depicts three models for nuclear entry. Entry through the nuclear pore complex (NPC) is implicated in non-cycling cells, but may also occur in cycling cells [Katz et al., 2003]. Dashed lines indicated nuclear membrane breakdown during mitosis. Nuclear entry and retention of the PIC during mitosis may be facilitated by targeting mechanisms (e.g., binding to mitotic chromosomes) or may be stochastic.

translation of viral RNAs as well as assembly of progeny virus particles.

The RT and IN enzymes are assembled into progeny viral particles in the previously infected cell and as such are positioned to orchestrate the early steps culminating in DNA integration in the newly infected cell. These critical steps can be sensitive to the cell cycle status, but if successfully completed, the viral DNA is stably integrated and thereafter known as the "provirus." If a permissive state exists, the proviral DNA may be expressed at high levels leading to production of new virus particles. DNA integration provides a critical "sanctuary" for the viral genome. Therefore, this step is not left to chance, but is catalyzed by the viral IN protein. In the newly infected cell, the IN protein is found in protein-viral DNA assemblages referred to as the "reverse transcription complex" (RTC) and the "preintegration complex" (PIC). The latter complex is normally poised to perform integration. However, these subviral complexes face many challenges en route to integration [Nisole and Saib, 2004], including restrictions imposed by cell cycle status.

The cell cycle dependencies for retroviruses, may include: S-phase, minimally to provide sufficient concentrations of dNTP substrates for reverse transcription of viral RNA to DNA; mitosis, for nuclear entry by some retroviruses; and perhaps other phase requirements for repair of the retroviral DNA integration intermediate [Daniel et al., 1999]. Below, we consider in more detail the importance of cell cycle status to early steps in the retroviral replication cycle, which culminate in DNA integration. We focus on those cell cycle dependencies that can lead to a partial or complete restriction of early events, as well as viral mechanisms that help overcome these restrictions. Although we have highlighted early events in retroviral replication, it should be noted that a broad array of retroviral gene products, including oncoproteins [Coffin et al., 1997; Flint, 2004] and accessory proteins [Amini et al., 2004; Chen et al., 2004; Goh et al., 2004] can also influence the cell cycle in many ways.

QUESTIONS, METHODS, AND IMPLICATIONS

One active area of research has been focused on the identification of retroviral genes that play a role in overcoming cell cycle restrictions. Prototypic retroviruses with relatively simple genomes, such as the "oncoretroviruses," include the Alpharetroviruses (e.g., avian sarcoma virus, ASV) and Gammaretroviruses (e.g., murine leukemia virus, MLV). As noted above, these viral genomes include only the three common genes (*gag*, *pol*, and *env*) that encode essential enzymes and structural proteins. In contrast, the Lentiviruses (e.g., human immunodeficiency virus, HIV), contain more "complex" genomes that include additional genes. For example, the HIV-1 genome encodes six regulatory and accessory proteins. One of these, Vpr, is a multifunctional protein that may facilitate viral propagation in non-cycling cells [Cullen, 2001; Greber and Fassati, 2003], as discussed below. It has been a widely accepted dogma that "lentiviruses can infect non-cycling cells, whereas oncoretroviruses cannot." It is also commonly stated that all "oncoretroviruses" require mitosis (and thus cell cycling) for nuclear entry of their DNA, whereas lentiviruses can enter the nucleus through the nuclear pore complex (NPC) (Figs. 1 and 2). These seemingly unique properties of lentiviruses have been thought to be attributable, at least in part, to the additional or novel gene products. Although these widely held notions have recently been shown to be inappropriately broad [Hatziioannou and Goff, 2001; Katz et al., 2002], it is clear that lentivirus-based vectors show significantly higher potential for gene delivery to non-cycling cells than the traditional MLV-based (oncoretroviral) vectors [Naldini et al., 1996]. However, HIV-based vector systems that have been stripped of all auxiliary and regulatory and proteins can still complete early events in non-cycling cells [Trono, 2000]. This finding suggests that some HIV functions required for infection of non-cycling cells are encoded in *gag* and/or *pol*.

The relationship between retroviruses and the cell cycle is frequently considered in the context of this simple question: Can the retrovirus stably infect a non-cycling cell? This ability is typically measured by transduction of a viral reporter gene, and successful transduction of non-cycling cells implies that all early events can be completed independently of S-phase and mitosis. Caution must be noted in interpreting these results, as a subset of target cells may be cycling at the time of infection [Schuitemaker et al., 1994; Trobridge and

Russell, 2004]. Furthermore, because cell cycling can be blocked by a variety of treatments or natural conditions, "noncycling" is not a uniform phenotype. For example, cells can be arrested in different phases of the cell cycle (e.g., with chemical inhibitors), may be quiescent (resting), withdrawn from the cell cycle (e.g., serum-starved), or post-mitotic (differentiated). It is therefore dangerous to draw broad conclusions based on resistance of non-cycling cells in a particular situation.

A second and distinct experimental question can be asked with cycling cells: Are reverse transcription, nuclear import, and DNA integration dependent on a specific cell cycle phase? To address this question, cycling cells are synchronized and then infected at different points in the cell cycle [Roe et al., 1993; Lewis and Emerman, 1994; Katz et al., 2003]. Such studies can reveal whether execution of specific early steps in viral replication require progression through defined cell cycle phases. To interpret the results of such experiments, the target cells must be highly synchronous. This approach contrasts with infecting cells that are blocked at a chosen step in the cell cycle, as all subsequent cell cycle phases are also blocked.

Cell cycle restrictions to infection may be traced to the inhibition of a specific step in retroviral replication, such as reverse transcription or nuclear entry, which can be distinguished by analysis of viral DNA. Cytoplasmic viral DNA is linear, whereas the nuclear forms of viral DNA are either integrated or can accumulate as dead-end, circular DNA species. Completion of integration can be measured by covalent attachment of viral DNA to host DNA via PCR-based assays, or by the transduction assays that measure viral reporter gene expression. The fact that a substantial percentage of viral particles may not be competent for completion of early steps of infection can be a complicating factor in all such assays. Indeed, a large proportion of the unintegrated viral DNA in infected cells may not be in productive complexes [Butler et al., 2001] or may be destined for degradation by innate cellular immunity [Nisole and Saib, 2004]. It also seems possible that only a subset of target cells may be in the appropriate cell cycle phase that is optimal for completion of early steps in replication.

The interplay between HIV replication and the cell cycle has major implications with respect to AIDS pathogenesis. The primary target cells of HIV-1 infection in a human host are CD4+ T-cells and macrophages. The majority of CD4+ T-cells are non-cycling and are resting in the G_0 phase. This phase can restrict early steps in HIV-1 replication including reverse transcription, nuclear import, and integration [Stevenson et al., 1990; Zack et al., 1990; Bukrinsky et al., 1991; Pierson et al., 2002; Swiggard et al., 2004]. However, intact unintegrated DNA may be a significant "latent" form of HIV in the resting T-cell [Bukrinsky et al., 1991]. In contrast to the restrictions observed in G_0 T-cells, nuclear import [Bukrinsky et al., 1992] and integration [Weinberg et al., 1991] were demonstrated in G_1/S-phase arrested T-cell lines and non-cycling monocyte/macrophages, respectively. These seminal studies and others [Lewis et al., 1992; Lewis and Emerman, 1994] established that HIV-1 can complete the early steps, including integration, in these non-cycling cells. The ability of HIV-1 to productively infect non-cycling macrophages (see below) is thought to be an important step in establishing human infection, as well perhaps providing a long term virus reservoir.

HISTORICAL PERSPECTIVES AND DOGMA

In light of the more recent focus on the relationship between the cell cycle, HIV biology, and retroviral vector design, it is worthwhile to briefly consider some historical studies that relate to the current dogma. Many of the earliest, pioneering studies of retroviral replication by Temin and co-workers investigated the influence of cell cycle status on productive infection by Rous Sarcoma Virus, which is the type species for the ASV group. In the 1960s, Temin found that passage through mitosis was required for productive infection by ASV, but these experiments did not identify, which step in viral replication was limiting in arrested cells [Temin, 1967]. It was later shown that cells arrested by serum starvation (G_0) could not support completion of the reverse transcription step [Fritsch and Temin, 1977]. Further studies by Humphries [Humphries et al., 1981] demonstrated that if cells were infected with ASV at the time of release from serum starvation (G_0), reverse transcription could be completed and integration could occur prior to mitosis. Results from these studies suggested that passage through mitosis was only required to "activate" the newly integrated DNA for virus production

[Humphries et al., 1981]. These findings were consistent with earlier studies by Varmus and co-workers, who also concluded that ASV DNA could be integrated prior to mitosis [Varmus et al., 1977].

One implication of this "mitosis-independent" DNA integration, which was not noted at the time, is that viral DNA must enter the nucleus through the NPC via an active process during interphase. However, more than a decade later, studies by Brown and co-workers [Roe et al., 1993], and others [Lewis and Emerman, 1994], provided evidence that the DNA nuclear entry and integration steps of another "oncoretrovirus," MLV, were highly dependent on mitosis [Roe et al., 1993]. This requirement for mitosis was then extrapolated to all "oncoretroviruses," [Lewis and Emerman, 1994] essentially discounting the earlier findings with ASV that demonstrated mitosis-independent DNA integration. This extrapolation seems to have been driven by a comparison with HIV-1. The discovery that HIV-1 could complete early events in some non-cycling cells and that HIV-1 DNA could enter the nucleus through the NPC [Weinberg et al., 1991; Bukrinsky et al., 1992], appeared to distinguish HIV-1 from the prototype "oncoretrovirus," MLV, which required mitosis. However, recent studies with ASV have demonstrated its capacity for mitosis-independent integration [Hatziioannou and Goff, 2001; Katz et al., 2002, 2003; Greger et al., 2004], consistent with the earlier findings [Humphries et al., 1981]. Thus, mitosis-independent integration of viral DNA in non-cycling cells is not limited to HIV and lentiviruses. Furthermore, as noted in detail below, even with HIV, the early steps in infection can be restricted in certain non-cycling cells. A less dogmatic view of the role of cell cycle in retroviral replication seems warranted and fresh experimental approaches should be helpful to more accurately describe the retroviral replication cycle and for the design of retrovirus-based vectors [Pages and Bru, 2004].

RETROVIRUSES MAY STIMULATE CELLS PRIOR TO ENTRY TO PRODUCE A FAVORABLE ENVIRONMENT FOR EARLY EVENTS

At least two mechanisms can be envisioned for how retroviruses might deal with the potentially restrictive environment of non-cycling cells: (i) viral proteins may directly or indirectly supply functions to help mitigate the limiting environment of a non-cycling cell (discussed below), (ii) the retrovirus may induce cell cycle progression to create a more favorable environment. Recent studies have indicated that binding of some retroviruses to Toll-like receptors (TLRs) on the cell surface can stimulate the cell to enter into the cell cycle [Rassa and Ross, 2003]. Similarly, it has been hypothesized that engagement of the Jaagsiekte Sheep retrovirus with surface proteins of non-cycling target lung cells might cause these cells to exit the quiescent state [Rosenberg, 2001]. However, such stimulatory activities are unlikely to be universal and retroviruses clearly must deal with potential cell cycle restrictions in additional ways.

ROLE OF S-PHASE AND dNTP PRECURSOR POOLS

Obvious features of S-phase that may be important for early events in the retrovirus replication cycle are the high dNTP precursors and/or other phase-specific host co-factors that facilitate viral DNA synthesis or DNA integration. After entry into the cytoplasm of the newly infected cell, the retroviral RNA is reverse transcribed within the RTC (Fig. 2). Although the virus brings the RT enzyme with it, cellular dNTP precursors are required for DNA synthesis. The concentration of such precursors varies during the cell cycle, being highest in S-phase [Leeds et al., 1985]. As noted above, very early studies showed that in serum-starved (G_0) cells, ASV could not complete the reverse transcription step [Fritsch and Temin, 1977]. It was later demonstrated that low nucleotide pools are also limiting for reverse transcription of HIV-1 [Gao et al., 1993]. However S-phase itself is not absolutely required for completion of early steps by a variety of retroviruses, indicating that all of the early steps in the retroviral life cycles can be completed successfully in non-cycling cells that are not expected to contain peak concentrations of dNTPs [Weinberg et al., 1991; Hatziioannou and Goff, 2001; Katz et al., 2002; Greger et al., 2004]. Furthermore, dNTP levels are relatively low in post-mitotic cells that have withdrawn from the cell cycle (G_0), such as macrophages [Terai and Carson, 1991], yet HIV can propagate in these cells. It is possible that the ability to infect a cell outside of S-phase depends on the availability of threshold levels of dNTP

precursors or other factors in such cells, and different retroviruses may display different sensitivities to such conditions.

In a cellular environment where dNTPs are limiting, reverse transcription may be initiated, but not completed [Pierson et al., 2002; Swiggard et al., 2004]. The half-life of the incomplete viral DNA may be a critical determinant for successful infection, as stable replication intermediates may be completed at a later time, when cell cycle conditions are more favorable. The stability of the RTC or PIC complexes may also contribute to overcoming cell cycle restrictions. Stability differences among viral nucleoprotein complexes may account, therefore, for some of the distinctive properties of retroviruses with respect to cell cycle restrictions.

The ability of HIV-1 to propagate in post-mitotic macrophages plays an important role in establishing infection in humans. However, only a subset of cultured primary macrophages appear to be permissive for infection. This subset of terminal differentiated cells may in fact, have some proliferative potential [Schuitemaker et al., 1994]. Furthermore, late G_1 was required to support efficient HIV-1 reverse transcription in primary macrophages [Kootstra et al., 2000]. Similarly, progression of T-cells from G_0 to the late G_1 phase (G_1b) is required to complete HIV-1 reverse transcripts [Korin and Zack, 1998]. These findings may be explained by the fact that dNTP pools increase as the cell approaches S-phase in late G_1. In both "G_1-arrested" macrophages and unstimulated T-cells, the defect in HIV-1 reverse transcription can be ameliorated by increasing the cellular dNTP precursor pools [Korin and Zack, 1999; Kootstra et al., 2000]. These results support the idea that low cellular dNTP pools can limit reverse transcription. Although elevation of dNTP pools could restore reverse transcription in macrophages and unstimulated T-cells, this was not sufficient to complete early events [Korin and Zack, 1999; Kootstra et al., 2000], indicating that other blocks to virus replication can exist in particular post-mitotic or quiescent cells. As with resting T-cells, resting monocytes (macrophage precursors) also show defects in HIV-1 reverse transcription [Triques and Stevenson, 2004]. But in this case, increasing the cellular dNTP pools was not sufficient to restore efficient reverse transcription. Taken together, these studies help to distinguish requirements for sufficient dNTP pools, from other more complex cell cycle restrictions.

The Spumaviruses (foamy viruses, FV) are distinct from all other retroviruses in that reverse transcription is completed prior to budding from the host cell. Accordingly, FV propagation should not be affected by limiting dNTP pools in the next host cell. FV is restricted in non-cycling cells, but the block appears to be at the level of nuclear import of viral DNA, as passage through mitosis, but not S-phase, is required for efficient FV-based vector transduction of human cells. However, aphidicolin-arrested cells (blocked at S-phase) cannot support transduction by FV [Trobridge and Russell, 2004]. Thus, some feature of S-phase, other than abundant dNTP precursors, seems to be required for FV replication. These findings support the idea that there may exist a wide range of post-reverse transcription, cell cycle-specific requirements for completion of early steps for all retroviruses.

HOST AND VIRAL AUXILIARY ENZYMES CAN SUPPORT EARLY STEPS IN NON-CYCLING CELLS

A major role of cellular dUTPases is to reduce dUTP concentrations, as incorporation of dUTP into DNA can be mutagenic. The amounts of cellular dUTPases are low in non-cycling cells, where DNA synthesis is limited and this activity is apparently not required. Certain non-primate lentiviruses encode dUTPases [Payne and Elder, 2001; Chen et al., 2002], a feature shared with the herpes viruses and poxviruses. If the viral dUTPase activity is eliminated, the retroviral mutation rate increases and this correlates with reduced viral propagation in non-cycling cells [Lerner et al., 1995; Payne and Elder, 2001; Chen et al., 2002]. Retroviral dUTPase activity is not required in cycling cells, probably because cellular dUTPase levels are high enough to prevent dUTP incorporation. The cellular dUTPases can also stimulate TTP production by providing the dUMP substrate for thymidylate synthase, and thereby increase the dNTP pools. However, the small amount of virus-associated dUTPase that is brought into the infected cell would probably not be sufficient to increase cellular dNTP pools. To exploit dUTPase function in this way, the dUTPase activity, and nucleotide pools, would have to be compartmentalized with

the viral RTC [Reddy and Fager, 1993]. In any case, as the coding capacity of retroviruses is limited by virion size, the presence of the dUTPase gene in non-primate lentiviruses is highly significant. How might other retroviruses deal with this problem? The HIV-1 accessory protein, Vpr interacts with a cellular uracil-DNA glycosylase 2 (UNG2) and mediates its assembly into viral particles [Chen et al., 2002]. The assembled cellular UNG2 may facilitate repair of dUMP residues that have been incorporated into viral DNA, thereby acting as a functional equivalent of the retrovirus-encoded dUTPases [Chen et al., 2004]. These findings highlight the importance of accessory functions that affect viral DNA synthesis to facilitate viral propagation in non-cycling cells.

NUCLEAR ENTRY

In cycling cells, the nucleus disassembles transiently during mitosis, whereas in non-cycling cells, it remains intact. The general view is that the intact nuclear membrane represents a barrier for nuclear entry of the retroviral PIC in non-cycling cells and, as such, specific viral functions are required to overcome this barrier. Disassembly of the nuclear membrane during mitosis also may promote nuclear entry of the PIC. At first glance, mitotic entry would seem to be a passive process, as breakdown of the nuclear membrane would allow the PIC to enter compartments that will be included in the nucleus when it reassembles. However, distribution of cellular components between the cytoplasm and the nucleus is tightly controlled both during interphase and mitosis, and it is likely that mitotic entry would also require specific virus-host cell interactions. Both the viral DNA and IN must be delivered inside the nucleus for successful infection and it is logical that they should enter together as components of the PIC. Although viral and cellular components of the PIC likely collaborate to guide nuclear entry, it is not entirely clear, which components of the PIC engage the cellular pathways and, which are passive "cargo." Nevertheless, such trafficking toward, and into, the nucleus can involve engagement of the RTCs and PICs with both the cellular cytoskeleton [Bukrinskaya et al., 1998; McDonald et al., 2002] and nuclear import pathways [Cullen, 2001; Greber and Fassati, 2003].

There are several possible pathways by which the retroviral PIC might enter the nucleus in cycling and non-cycling cells (Fig. 2). (1) There is strong evidence that in non-cycling cells (assuming no cell-specific restrictions in earlier steps), the HIV PIC can enter the nucleus through the NPC via nuclear localization signals (NLSs) present on viral proteins (MA, IN, Vpr) and a DNA "flap" structure [Cullen, 2001; Greber and Fassati, 2003]. Similarly, an NLS in ASV IN might mediate import through the NPC during ASV infection [Kukolj et al., 1998]. (2) A second entry mechanism in non-cycling cells may involve NPC-independent penetration of the nuclear membrane, mediated by the membrane disruptive activity of the HIV-1 Vpr protein [de Noronha et al., 2001]. (3) In cycling cells the PIC could be captured passively during mitotic re-assembly of the nucleus. However, this mitotic entry model seems unlikely as large macromolecules appear to be excluded during nuclear re-assembly [Swanson and McNeil, 1987]. (4) Lastly, in cycling cells, nuclear entry and retention of the PIC could be mediated by targeting to nuclear components (i.e., chromatin) during mitotic nuclear re-assembly. This is an attractive model, as MLV DNA has been shown to accumulate in the nucleus after mitosis [Roe et al., 1993]. Such accumulation would not be expected for stochastic capture, during which only a subpopulation of viral DNA molecules should be included in the nucleus.

As described above, HIV-1 (and other lentiviruses) can transduce or propagate in certain non-cycling cells and therefore the PIC must be able to enter the nucleus independently of the nuclear membrane disassembly that occurs during mitosis. Of the NLSs and other determinants or activities that have been identified in the HIV-1 PIC, none are absolutely required for HIV propagation in non-cycling cells [Dvorin et al., 2002]. The presence of these multiple, apparently dispensable import signals may signify collaboration or redundancies of signals, or the ability of PICs to interact with various cell-type-specific nuclear entry pathways. There has been considerable focus on HIV NPC-mediated import. However, as noted above, ASV can also infect some non-cycling cells [Hatziioannou and Goff, 2001; Katz et al., 2002; Greger et al., 2004] and may therefore also encode multiple signals that promote nuclear entry through the NPC.

Although much effort has been focused on elucidating mechanisms of retroviral DNA nuclear import in non-cycling cells, few investigations have attempted to define the primary or alternate pathways in cycling cells where both the NPC and mitotic entry pathways theoretically are available. Recent studies in cycling cells have demonstrated that HIV-1 DNA can enter the nucleus and become integrated prior to mitosis [Katz et al., 2003] suggesting that the NPC pathway is used in cycling cells. Similar findings with ASV [Katz et al., 2003] are consistent with earlier studies [Humphries et al., 1981]. Although these investigations did not rule out the use of a mitotic pathway, they showed quite clearly that mitosis-independent nuclear import is a prominent pathway for both viruses in cycling cells.

SUMMARY

Once retroviral DNA is integrated, it is expressed and replicated as part of the host chromosome. Completion of early steps in the virus replication cycle is critical therefore for persistence of the infection. Here, we have discussed the ways in which S-phase and mitosis might influence these early steps, and have considered why different retroviruses may display varying degrees of dependence on these phases. Overwhelming evidence indicates that HIV-1 can complete early events in some non-cycling cells, and lentiviral vectors are less dependent on cell cycle status than are Alpha- and Gamma-retroviruses (i.e., ASV and MLV, respectively). However, several recent studies have shown clearly that ASV DNA integration can occur in a variety of non-cycling cells. Other studies have indicated that retroviruses may be subject to phase-specific restrictions at early steps that follow reverse transcription, and these findings warrant further study. The encoding of retroviral proteins, such as dUTPase, or the assembly of host proteins such as UNG2 into virus particles, appear to represent viral strategies that have evolved to overcome cell-cycle restrictions that impinge on the fidelity of viral DNA biosynthesis. Lastly, we note that potential target cells exist as populations, and the availability of a subset of cell cycle-competent cells within the population might be sufficient for retroviruses to thrive. Such considerations should not be neglected in future investigation of the effects of cell cycle status on retroviral replication.

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