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Retroviral infection of non-dividing cells: Old and new perspectives

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Abstract

The dependence of retroviral replication on cell proliferation was described as early as 1958, although different classes of retroviruses are able to infect non-dividing cells with different efficiencies. For example, the human immunodeficiency virus (HIV) and other lentiviruses infect most non-dividing cells nearly as well as dividing cells, while the gammaretroviruses such as the murine leukemia virus (MLV) cannot infect non-dividing cells, and other retroviruses have intermediate phenotypes. One exception to the ability of HIV to infect non-dividing cells involves resting CD4+ T cells in vitro where there are multiple restrictions. However, recent data show that there is massive infection of non-activated CD4+ T cell during acute infection which suggests that the situation is different in vivo. Finally, much work trying to explain the difference between HIV and MLV in non-dividing cells has focused on describing the ability of HIV to enter the nucleus during interphase. However, we suggest that events in the viral lifecycle other than nuclear import may be more important in determining the ability of a given retrovirus to infect non-dividing cells.

Contents

Early papers in Virology

Temin and Rubin published a paper in Virology in 1958 (Temin and Rubin, 1958) describing an in vitro assay to quantitatively measure titers of Rous sarcoma virus (RSV), and concluded that the physiological state of cells affects their susceptibility to infection. The following year, the same authors published another paper in Virology (Rubin and Temin, 1959) showing that productive infection of RSV requires host cell division since X-rays and UV light which inhibited cell growth also prevented RSV production if cells were treated before, but not after, infection. While the most prescient speculation in that paper centered on the parallels between retroviruses and lysogenic phage which later became the DNA provirus hypothesis and garnered Howard Temin part of the Nobel Prize (Temin, 1976), those early Virology papers also introduced the concept that some aspect of cell cycle progression is an integral part of the retroviral lifecycle. Research during the last half century has provided some answers regarding the underlying relationship between the cell cycle status of target cells and retroviral replication, although many questions remain. This relationship has important implications not only for the basic biology of retroviruses, but also for our understanding of viral pathogenesis. For example, it is the ability of the human immunodeficiency virus (HIV) to infect non-cycling cells that allows HIV replication in major viral reservoirs such as non-activated CD4+ T cells and terminally differentiated macro-

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phages in vivo. Here, we will review the involvement of cell proliferation in retrovirus replication and highlight some of the major questions in the field.

Different requirements for cell cycle progression between different retroviruses

The relationship between infectivity and cell cycle stage depends on the class of retrovirus (Katz et al., 2005). Gammaretroviruses such as the murine leukemia virus (MLV) and spleen necrosis virus (SNV) require mitosis for productive infection and are blocked from integrating into non-dividing cells (Caron and Caruso, 2005; Lewis and Emerman, 1994; Roe et al., 1993). Alpharetroviruses, such as avian sarcoma virus (ASV), differ from gammaretroviruses in that they integrate into non-dividing cells (Hatziioannou and Goff, 2001; Katz et al., 2002), but apparently require cell cycle progression for a step later in the viral lifecycle (Humphries et al., 1981; Humphries and Temin, 1974). Spumaviruses, a distinct group of retroviruses, also require cell cycle progression of their target cells for successful replication (Bieniasz et al., 1995; Patton et al., 2005; Trobridge and Russell, 2004). These cell cycle dependencies are not absolute, however, and many of these retroviruses infect non-dividing cells at reduced efficiencies. Specifically, gammaretroviruses have the poorest ability to infect non-dividing cells (less than 1% as well as dividing cells), alpharetroviruses transduce non-dividing cells 3–30% as well as dividing cells (Hatziioannou and Goff, 2001; Katz et al., 2002), while lentiviruses such as HIV show almost no difference between dividing and non-dividing cells (Lewis et al., 1992). Thus, some retroviruses are nearly totally dependent on cell cycle progression, others show an intermediate phenotype, and lentiviruses have almost no dependence on cell proliferation. Although the cell cycle dependence of many other retroviruses has not been directly tested, it is probable that other classes of retroviruses rely on cell cycle progression for productive infection since some of them induce proliferation of target cells before infection (Czarneski et al., 2003) or rely on proliferation-dependent receptors (Manel et al., 2003).

Resting T cells and HIV infection

Generally, HIV can infect cells that are arrested in the cell cycle. An exception, however, is that HIV cannot infect naive quiescent CD4+ T cells or monocytes isolated from peripheral blood that are in the G0 stage of the cell cycle. Likewise, other retroviruses are also restricted in G0 resting cells (Fritsch and Temin, 1977; Harel et al., 1981; Varmus et al., 1977). However, HIV and other lentiviruses (as well as ASV; Greger et al., 2004) are able to infect cells in G0 that are terminally differentiated and post-mitotic. These observations indicate that withdrawal from the cell cycle alone is not sufficient to make cells resistant to HIV and that other factors are important.

Importantly, subtle differences in cell "activation" play a significant role in the ability of cells to support HIV replication. For example, resting CD4+ T cells can be infected with HIV (or HIV-derived vectors) when treated with cytokines such as IL-2, IL-4, IL-7, or IL-15, even though such treatment induces neither cell proliferation nor other markers of T cell activation (Unutmaz et al., 1999). Similarly, complex interactions of resting T cells with B cells and factors secreted by macrophages can also render the T cell infectable by HIV without inducing cell activation (Swingler et al., 2003). However, while neither proliferating nor "activated", cells treated with these factors may be pushed out of G0 and into the G1b part of the cell cycle; a state defined by increased RNA synthesis in the absence of both DNA synthesis and activation markers (Dardalhon et al., 2001; Ducrey et al., 2002). This hypothesis is consistent with studies showing that resting T cells treated with mitogens could be infected when they entered G1b (Korin and Zack, 1998). In contrast, these cells remain refractory to gammaretroviral infection unless they are fully activated. Thus, there is some physiological state of cells rendering them permissive to lentiviruses as they emerge from quiescence but before they acquire markers of activation or begin proliferation.

There are likely multiple mechanisms to explain why fully quiescent cells cannot be infected by HIV, while "partially activated" ones can. Complete reverse transcription of the HIV genome is very slow in resting CD4+ T cells infected in vitro (Pierson et al., 2002; Spina et al., 1995; Swiggard et al., 2004). Quiescent cells contain low dNTP levels which might explain the slow kinetics of reverse transcription. However, addition of excess dNTPs to cells increases the amount of reverse transcriptase products, but does not overcome the block to infection (Korin and Zack, 1999). Thus, low dNTP pools in quiescent cells do not fully explain the phenotype. Interestingly, lentiviral reverse transcriptases have a higher binding affinity to dNTPs than do gammaretroviral reverse transcriptases (RT) (Operario et al., 2005; Skasko et al., 2005), and HIV infection of cells where dNTP levels are low, such as non-dividing macrophages, depends on an RT with a high Kd for dNTPs (Diamond et al., 2004). These results imply that lentiviruses evolved an enzyme to allow them to replicate in environments where dNTP levels are low since cellular DNA synthesis is absent.

Significantly, these data suggest that additional host cell proteins or processes are necessary to mediate the completion of HIV infection during the transition from quiescent cells to "partially activated" cells. Initially, it was suggested that a transcriptional factor, NFAT, whose activation was thought to enhance reverse transcription, might be a key regulator of HIV infection of quiescent cells (Kinoshita et al., 1998). Subsequent data, however, have shown that NFAT is not necessary for any of the early steps of HIV replication (Ducrey et al., 2002; Oswald-Richter et al., 2004). Instead, recent data have identified the APOBEC3G protein as a factor that may actively inhibit HIV infection in quiescent cells. APOBEC3G is a cytidine deaminase that is inactivated by the HIV Vif protein in producer cells. More recently, it has been suggested that this protein might play an additional role in inhibition of HIV replication as part of a low-molecular-mass (LMM) ribonucleoprotein complex in resting T cells that blocks HIV infections by impairing the formation of late products of reverse transcription via the RNA binding ability of APOBEC3G (Chiu et al., 2005). However, at this point, the

relationship between the LMM complex and the antiviral effects of APOBEC3G in resting cells is still correlative rather than causal.

Even if reverse transcription is completed, there are additional blocks both before and after integration of the HIV genome in quiescent cells. For instance, lack of nuclear viral DNA (Bukrinsky et al., 1992) suggests that nuclear import of viral genomes is also inefficient in resting CD4+ T cells (but see Wu and Marsh, 2001). There is also one report that claims that cellular extracts from activated, but not resting CD4+ T cells, support uncoating of HIV cores in vitro (Auewarakul et al., 2005). Moreover, there are post-integration blocks to viral expression that may lead to viral latency (reviewed in more detail in Lassen et al., 2004; Williams and Greene, 2005). Thus, there appears to be multiple blocks to HIV infection of resting T cells in culture involving numerous host cell pathways.

HIV infection in vivo may be different from the in vitro models

Despite much work characterizing defects in HIV infection of resting T cells isolated from peripheral blood, the situation in whole tissues is quite different. In fact, naive CD4+ T cells in human lymphoid tissue ex vivo can be productively infected with HIV even though they have not progressed to the G1b stage of the cell cycle (Eckstein et al., 2001; Kinter et al., 2003). Moreover, in situ hybridization for viral RNA has shown that there is productive infection in non-activated cells in vivo (Zhang et al., 1999). The importance of this observations for lentiviral pathogenesis has become more clear with recent publications that show massive infection of non-proliferating memory T cells after acute infection with simian immunodeficiency viruses (SIV) (Li et al., 2005; Mattapallil et al., 2005). Many of the mucosal memory cells have gone through a recent activation stage, and hence differ from the resting T cells in periphery (Picker et al., 2004). However, even naive unactivated resting CD4+ T cells in the periphery are productively infected in animals infected with a particular highly pathogenic simian–human immunodeficiency virus (SHIV) (Nishimura et al., 2005). Thus, the microenviroments in vivo render resting cells quite permissive to infection by lentiviruses. The molecular basis for the difference between the facile infection of resting T cells in vivo (and in tissues ex vivo) and the refractory nature of resting T cells in isolation awaits further description. However, the differences between the in vitro and the in vivo results suggest that studies of blocks to HIV infection in resting CD4+ T cells in isolation may not reflect the true selective pressures that have affected lentivirus evolution.

Nuclear import or uncoating?

The complex of viral and cellular proteins that are competent for integration of the viral genome is called the pre-integration complex or PIC. Nuclear import of viral DNA as part of the PIC is an essential step for the retrovirus life cycle to carry out integration of the viral genome into the host cell chromosome. Early studies on the block to infection in non-dividing cells by MLV showed that mitosis was essential and suggested that entry into the nucleus was the rate-limiting step (Lewis and Emerman, 1994; Roe et al., 1993). It was proposed that the viral DNA of gammaretroviruses gains access to the host chromosome only when the nuclear membrane breaks down at mitosis. Since HIV can access the nucleus before mitosis, it has been assumed that the PIC of HIV contains proteins with nuclear localization signals (NLS) that are essential for the ability of HIV to infect non-dividing cells (Bukrinsky et al., 1992). Several viral elements of lentiviruses have been identified as potential components that contain an NLS and have been postulated as being important for the ability of lentiviruses to infect non-dividing cells. These include the matrix (MA), integrase (IN), and Vpr proteins in addition to a *cis*-acting element called central polypurine tract (cPPT) (Bouyac-Bertoia et al., 2001; Bukrinsky et al., 1993a; Gallay et al., 1995a, 1995b, 1997; Heinzinger et al., 1994; von Schwedler et al., 1994; Zennou et al., 2000).

However, these results are controversial since HIV with mutations in each of the identified NLSs still remains infectious in non-dividing cells (Dvorin et al., 2002; Fouchier et al., 1997; Freed et al., 1997; Limon et al., 2002a, 2002b; Petit et al., 2000; Reil et al., 1998). Moreover, the presence of an NLS on the PIC is not sufficient for infection of non-dividing cells since addition of nuclear localization signals to gammaretroviruses does not increase their ability to infect non-dividing cells (Caron and Caruso, 2005; Deminie and Emerman, 1994; Seamon et al., 2002), and foamy virus, a retrovirus that naturally encodes at least three proteins in its core with NLSs (Imrich et al., 2000), is still not able to infect non-dividing cells (Trobridge and Russell, 2004). Other studies have focused on cellular proteins that may modulate the nuclear localization of the HIV PIC such as the host protein called LEDGF which directly binds to IN of HIV and feline immunodeficiency virus (FIV), but not MLV, and can modulate the nuclear versus cytoplasmic localization of IN (Llano et al., 2004; Maertens et al., 2003). However, the significance of LEDGF as a mediator of nuclear localization of the PIC is questionable since knockdown of LEDGF by siRNA could prevent nuclear localization of IN, but did not reduce the ability of HIV or FIV to infect non-dividing cells (Llano et al., 2004).

There are two general explanations to account for the fact that none of the described NLSs in the HIV PIC seems to be essential for infection of non-dividing cells. First, it is possible that lentiviruses have evolved to gain access to the nucleus quickly such that each of the NLSs is redundant and they can substitute for one another. However, we have recently deleted all four of the described NLSs in the HIV PIC and still find that the virus is able to infect non-dividing cells (Yamashita and Emerman, 2005). It is possible, however, that there is still another NLS that has yet to be described.

The second explanation is that nuclear import is not the rate-limiting step for infection of non-dividing cells and the focus on nuclear import has been misplaced. In support of this second idea, we recently reported that the capsid protein (CA) is a dominant determinant of retroviral infectivity in non-dividing cells (Yamashita and Emerman, 2004) although CA is not nucleophilic. The association of CA with incoming virions

is different for MLV than it is for HIV since CA is tightly associated with intracellular complexes of MLV (Bowerman et al., 1989; Fassati and Goff, 1999), but not HIV (Bukrinsky et al., 1993b; Karageorgos et al., 1993; Khiytani and Dimmock, 2002; Miller et al., 1997). Thus, one hypothesis for the dominant effect of CA on the ability of retroviruses to infect non-dividing cells (Yamashita and Emerman, 2004) is that CA affects the uncoating process due to its differential association with intracellular viral complexes of MLV versus HIV. If incoming virions can enter the nucleus only after uncoating has proceeded, then uncoating, rather than nuclear import might be the rate-limiting step in the ability to infect non-dividing cells.

While CA is not associated with isolated HIV pre-integration complexes, we know that it must be associated with the entering virion at least initially because a large fraction of intracellular incoming virions can be stained with a capsid antibody (McDonald et al., 2002). In addition, HIV CA is the target of a cytoplasmic antiviral protein called Trim5α (Lee and KewalRamani, 2004) highlighting the fact that incoming HIV virions maintain an association with CA for at least a short time and must undergo a separate uncoating process. Thus, we speculate that the interaction of CA with cytoplasmic factors may ultimately govern the ability of different retroviruses to infect non-dividing cells via regulation of the uncoating step. Nuclear import may then be mediated by the association of cellular factors with the PIC, but uncoating and cell cycle stage may determine when or how these factors are able to mediate that import. It is also possible that a downstream event such as integration can be limiting for gammaretroviruses in non-dividing cells.

Some of the confusion about nuclear import and retroviral infection of non-dividing cells is caused by the experimental systems used to monitor nuclear entry. For instance, the reduction of LTR-circles, dead-end products of viral DNA, has been widely used as evidence for a reduction in nuclear entry of viral DNA. However, there are retroviral mutants that enter the nucleus, but fail to produce LTR circles (Mannioui et al., 2005; Yuan et al., 2002). Moreover, a recent report challenged the assumption that 2-LTR circles are only found in the nucleus (Serhan et al., 2004). It may be that 2-LTR circles are better markers for the completion of reverse transcription than for nuclear import, but in any case, it is not technically correct to state that the absence of LTR circles always indicates a failure of nuclear migration of viral DNA. Thus, future studies to address the processes governing the access of retroviruses to the nucleus in dividing and non-dividing cells will likely require quantitative biochemical fractionations or real-time imaging techniques. Additionally, novel uncoating assays (Forshey et al., 2002; Narayan and Young, 2004) must be considered to address the events that may precede nuclear import.

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