Host factors in retroviral integration and the selection of integration target sites

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Abstract

In order to replicate, a retrovirus must integrate a DNA copy of the viral RNA genome into a chromosome of the host cell. The study of retroviral integration has advanced considerably in the last few years. Here we focus on host factor interactions and the linked area of integration targeting. Genome-wide screens for cellular factors affecting HIV replication have identified a series of host cell proteins that may mediate subcellular trafficking of integration complexes, nuclear import, and integration target site selection. The cell transcriptional co-activator protein LEDGF/p75 has been identified as a tethering factor important for HIV integration, and recently, BET proteins (Brd2, 4, and 4) have been identified as tethering factors for the gammaretroviruses. A new class of HIV inhibitors has been developed targeting the HIV-1 IN-LEDGF binding site, though surprisingly these inhibitors appear to block assembly late during replication and do not act at the integration step. Going forward, genome-wide studies of HIV-host interactions offer many new starting points to investigate HIV replication and identify potential new inhibitor targets.

Introduction

Retroviruses integrate a DNA copy of the viral genome into cellular DNA as an obligatory step in the viral replication cycle. Once integrated, the viral DNA is stably replicated with cellular DNA through cycles of DNA replication and cell division. The first clues regarding the mechanism of integration came from genetic experiments (1–3). Mutations at two locations within the viral genome resulted in a phenotype in which reverse transcription occured normally but the viral DNA failed to integrate. These mutations mapped to regions which we now know encode the viral integrase (IN) protein and the ends of the viral DNA sequence recognized by IN. The finding that viral DNA within extracts of infected cells efficiently integrated into exogenously added target DNA in vitro (4–6) facilitated biochemical studies of integration. This in vitro integration system enabled the DNA breaking and joining events to be unambiguously determined (6, 7). It also established that the viral DNA forms part of a large nucleoprotein complex termed the preintegration

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complex (PIC) (8). Later biochemical experiments showed that viral IN protein is necessary and sufficient to carry out the DNA cutting and joining steps of integration in the presence of a divalent metal ion (9–13). Subsequent studies established reaction conditions that facilitated efficient concerted integration of both viral DNA ends into the target DNA molecule in vitro (14–19).

This chapter focuses on mechanisms of targeting integration and the contributions of viral and cellular proteins. For structural information on nucleoprotein complexes involved in retroviral DNA integration see the chapter by Engelman and Cherepanov. For detailed discussions of the mechanisms of DNA transposition of related elements see other chapters in Mobile DNA III.

Composition of preintegration complexes

The composition and architecture of PICs remains poorly understood, largely due to their low abundance in extracts of infected cells; typically only a few PICs are present per cell. Knowledge of the protein composition is largely limited to immunoprecipitation studies which can identify protein components, but not their abundance, organization or functional roles. Even then, some identified components border on the limits of detection. Viral DNA within PICs is much easier to monitor because of highly sensitive assays such as Southern blotting and PCR. PICs have been valuable for studying the fate of the viral DNA in vitro and defining the DNA cutting and joining steps (6, 7).

A near-full complement of viral proteins have been detected within HIV-1 PICs including IN, RT, CA, NC, Vpr, and PR (20–27). Whereas IN, MA, RT, and Vpr are reported to be present in most studies, CA, NC, and PR are only detected in a few studies. These inconsistencies are likely due to differences in methods of preparation and sensitivity of the assays. Cellular proteins implied to be associated with PICs by biochemical studies include LEDGF/p75 (28), BAF (29), and HMGA1 (30). Mass spectroscopy screens have identified many additional cellular proteins potentially associated with HIV-1 PICs (31, 32). The functional role, if any, of the majority of these proteins is unknown.

The key viral protein within PICs is IN, which is responsible for the initial steps of covalent joining of viral DNA into host DNA. CA within PICs has been implicated in nuclear import (see below). Reverse transcriptase is also present in PICs but its unclear if it plays any functional role once reverse transcription is complete. Functional studies of HIV-1 and MLV PICs demonstrate a tight association of IN to the viral DNA--PICs remain active for in vitro DNA integration even after treatment with greater than 500 mM NaCl. A tetramer of HIV-1 IN forms a tight complex with viral DNA ends in vitro (33) and this likely bridges the viral DNA ends in the PIC. Footprinting of the viral DNA within MLV and HIV PICs (34–36) revealed extensive protection extending up to approximately 1 kb from the viral DNA ends. This protection is dependent on IN and is not observed in PICs derived from virus that lack IN. However, it is unclear if the protection is mediated by IN itself or recruitment of other factors by IN.

The viral DNA within PICs is sensitive to endonucleases suggesting that it adopts a relatively open structure (27). In contrast, it is not sensitive to digestion with exonucleases

(27), as expected if the DNA ends are stably bridged by IN. A cellular factor termed barrier-to-autointegration factor (BAF) protects MLV PICs from autointegration in in vitro PIC integration assays (37). BAF is an essential cellular protein involved in DNA management within the nucleus (38, 39) which makes it difficult to study the effects of BAF depletion on integration in the cell. BAF is a DNA bridging protein that compacts DNA (40). Stripping BAF from MLV PICs by high-salt treatment leads to slower sedimentation in sucrose gradients (41), consistent with decompaction of the viral DNA. Addition of BAF to salt-stripped PICs restores the normal sedimentation behavior in parallel with restoring the preference for intermolecular integration and avoidance of autointegration. Consistent with these findings, treatment of MLV PICs with VRK1, the major kinase responsible for phosphorylation of BAF, promotes autointegration in a similar manner as salt- stripping (42). Since phosphorylation of BAF abrogates DNA binding (40, 43) it is expected that VRK1 would promote loss of BAF from the viral DNA within the PIC. A similar role of BAF in preventing autointegration of HIV-1 PICs has not been unambiguously demonstrated.

Nuclear import of PICs

The large size of PICs presents a formidable challenge to nuclear entry. In the case of gammaretroviruses, the chromosomes are accessed during mitosis when the nuclear enveloped is disassembled. However HIV-1 and other lentiviruses can integrate in non-dividing cells, so entry into the nucleus must be an active process involving the nuclear pores. HIV-1 PICs have comparable sedimentation properties to ribosomes (44) and an estimated Stokes radius of about 28 nM based on size exclusion chromatography (27). The mechanism has been the subject of a controversy that is still not completely resolved. Many of the protein components of the PIC possess nuclear localization signals that, when fused to normally cytoplasmic proteins result in their localization to the nucleus. MA (45);, IN (23, 46), and Vpr (24, 47, 48) have all been proposed to mediate nuclear localization of HIV-1 PICs, as has a DNA structure near the center of the viral DNA called the central flap (49). However, other studies have shown that each of these can be dispensible for nuclear entry under some circumstances. It is possible that the interpretation of the data may be complicated by redundancy of nuclear import mechanisms. More recent data strongly suggests an important role for CA in mediating nuclear entry (reviewed in (50)).

The involvement of CA in nuclear entry was suggested by the finding that infection by chimeric HIV-1 harboring an MLV CA is cell cycle dependent (51) and this conclusion has been further substantiated by subsequent studies. Certain point mutations in HIV-1 CA also confer a cell cycle dependent phenotype. Genome wide screens for cellular co-factors identified a large number proteins known to be involved in nuclear import and related functions (52–55). Of these, TNPO3, NUP358 and NUP153 are of particular interest. Knockdown of these proteins diminishes HIV-1 replication, but does not affect replication of MLV, suggesting the mechanism is specific for HIV-1 rather than an indirect effect on cellular function (53, 56–59) . Experiments with HIV-1/MLV chimeric viruses map the TNPO3 and NUP153 dependency to CA (56, 58).

Host factors affecting integration targeting

Intranuclear trafficking

Following import into the nucleus, the PIC must access the chromosomes of an infected cell. Though not fully clarified, several studies suggest that this may not be purely diffusion but rather a controlled process (60, 61). Imaging studies have suggested that integration may commonly be localized within the nucleus, particularly at the nuclear periphery (62). Consistent with this idea, data suggest that active genes may be enriched near the nuclear periphery in yeast (63); however, other data from studies in animal cells suggest that in fact active genes may be enriched more in the center of the nucleus (64–66).

A study of nuclear localization and integration targeting suggested a possible model in which retroviral integration complexes engaged a "railroad track" in the cytoplasm that transfer PICs to specific regions of the nucleus rich in favored sites of integration (57, 67). The CA mutant N74D, which alters the interaction with the cellular splicing factor CPSF6, also altered integration targeting, suggesting involvement of this system. Modulating the level or activity of the cyclophillin protein another protein that binds CA, also affected targeting. These findings point to greater involvement of CA in PIC functioning and targeting than was appreciated previously (57). Several additional nuclear-localized cell factors have been reported to influence integration (68–70) and could act at this step. More work is needed to clarify the mechanisms underlying the possible relationship between nuclear positioning of chromosomes and frequency of retroviral integration.

Favored target sites for retroviral DNA integration in cellular chromosomes

Studies mapping the distribution of integration sites in cellular chromosomes have led to a detailed picture of favored and disfavored target sites (for some previous reviews in this area see (33)(36)(71)). The first genome-wide mapping study, carried out shortly after the completion of the first draft human genome sequence, showed that HIV favored integration particularly strongly within transcription units of the SupT1 T-cell line(72). Comparison to microarray data quantifying transcriptional activity in the same cells showed that integration was particularly strongly favored in active transcription units. Subsequent studies have shown that this pattern is maintained in most cell lines studied (73, 74, 75, 76–80), with the exception of a few experimentally manipulated examples described below (Figure 1). A variety of features on the human genome correlate with active transcription units (81, 82) such as sites of histone modification including H3K4 mono and dimethylation, H3 K9 and K27 monomethylation, H3K36 trimethylation, DNAseI hypersensitive sites and sites of histone acetylation generally (67, 82, 83). In addition, gene-rich regions are associated with a syndrome of associated features, such as high G/C content, high Alu element content, low LINE element content, and Geimsa dark banding patterns, all of which are positively associated with integration (72).

The above collection of favorable features characterizes all the lentiviruses studied to date, but other retroviral genera show different patterns. The gammaretroviruses, which include the MLVs and XMRV, show favored integration close to transcription start sites, rather than throughout transcription units as is seen with lentiviruses (74, 84). The alpha-retroviruses,

which include RSV and the allied ALVs, show near random distributions (76, 85). A study of the viral determinants of integration targeting implicated IN as a major determinant.

Swapping the MLV (gammaretroviral) IN for that of HIV, in a background of an otherwise wild-type HIV provirus, resulted in chimeric viruses that showed the integration pattern expected for MLV (86). Although these viruses showed reduced ability to replicate, these studies suggested that IN was a major determinant of integration target site specificity. As is described below, targeting by the lentiviruses and gammaretroviruses can be understood as engagement of different cellular tethering factors that are distinctive for each group.

LEDGF/p75—a cellular tethering factor for HIV integration

The LEDGF/p75 protein (product of the PSIP1 gene) was first implicated in HIV replication when it was identified as a tight binder to HIV IN protein (87–89) These studies involved artificial systems, such as over-expression of IN in animal cells or yeast two-hybrid assays, leaving the significance of the interaction uncertain initially. However, subsequent experiments eventually documented that reduction of LEDGF/p75 function did have strong negative effects on viral replication (79, 90, 91), establishing the importance.

Mapping studies showed that LEDGF/p75 is comprised of several protein domains. Multiple N-terminal domains mediate chromatin binding, potentially in part to the H3 histone trimethylated at the K36 position (92, 93). The C-terminal domain binds IN ("integrase binding domain" or IBD). X-ray crystallography reveals that the LEDGF-IBD engages the catalytic and N-terminal domains of integrase. The most extensive interactions involve a pocket formed at the dimer interface of two monomers of the integrase catalytic domain and the tip of the IBD helical bundle structure (94, 95).

Given that LEDGF/p75 was a cellular protein implicated in both transcriptional regulation and HIV integration, it was of interest to test whether integration in active transcription units required LEDGF/p75. Knockdown studies showed that depleting LEDGF/p75 in fact caused a significant shift in integration targeting toward a more random pattern, consistent with the idea that the LEDGF/p75-IN interaction mediated targeting to transcription units (77, 79, 96, 97).

LEDGF/p75 is a member of a protein family, and the related protein HRP2 has also been shown to act as an integration tether. However, effects of HRP2 on HIV integration efficiency and targeting are only prominent in cells lacking LEDGF/p75, and the influence of HRP2 even in this setting is modest, supporting the idea that LEDGF/p75 is the more significant cofactor (98–101).

Evidence supporting the idea that LEDGF/p75 acts as a simple tether was provided by results with LEDGF/p75 chimeras (102–104). Several groups substituted new chromatin binding domains for the LEDGF/p75 N-terminal chromatin-binding domain. These chimeras, which retained the LEDGF/p75 IN binding C-terminal domain but grafted to new domains conferring new chromatin binding specificities, were introduced into cells depleted for wild-type LEDGF/p75, and analysis of HIV integration showed new favored locations. A variety of such novel tethers have now been reported, collectively supporting the idea that

LEDGF/p75 acts as a tethering protein for PICs. Studies of the yeast Ty elements Ty1-5, described elsewhere in this book, have disclosed additional examples of targeting by tethering of integrase to cellular chromatin-binding proteins.

BET proteins—cellular factors directing gammaretroviral integration

The observation that HIV targeting is directed by tethering to LEDGF/p75 raised the question of whether other retroviral genera with different targeting preferences might dock with their own group-specific tethering factors. The gammaretroviruses favor integration near transcription start sites, and within the last year cellular BET proteins (Brd2, 3 and 4) have been identified as targeting factors (BET stands for "Bromodomain and extra terminal", and Brd stands for "bromodomain containing") (105–108) Brd2-4 are known to bind to sites of histone acetylation, explaining their association with transcription start regions. Initially these proteins were identified as binders to MLV IN in yeast two-hybrid screens (109), but the importance was uncertain. Later studies found that BET proteins also bound MLV IN in affinity capture-mass spec protocols, and that the addition of BET proteins could affect the activity of MLV IN in vitro (105–108).

Definitive data on the effects of BET proteins came with experiments modulating the activity of BET proteins in cells (105–108). Reducing BET protein activity was difficult with siRNA because three proteins were involved, and full elimination of BET activity was lethal to cells. A key breakthrough came with studies based on the anticancer drug JQ1, which binds to BET proteins and competes with binding of actetylated histone tails. This allowed activity of all BET proteins to be abrogated acutely, allowing experimental analysis of cells before the onset of lethality. Using JQ1, it was possible to show that the frequency of MLV integration was reduced in the region around transcription start sites. Effects could also be detected with siRNA knockdowns of the three genes, though with lower magnitude. In follow up experiments, hybrid tethering proteins were synthesized that contained the BET IN binding region and novel chromatin binding domains. In cells depleted for normal BET proteins but containing the chimeric tethers, integration sites were notably redistributed, again strongly supporting the tethering model.

Retroviral integration and nucleosomal association

Several studies have explored the association between nucleosome packaging and integration bias. Early studies suggesting a loose association of retroviral integration sites with chromosomal sites sensitive to DNAseI digestion (reviewed in (16, 19, 20, 34)), which led to the idea that packaging DNA in nucleosomes would inhibit integration. However, once assays were available that recapitulated integration in vitro, nucleosomal wrapping was found instead to favor integration (110–114). Subsequent studies in vivo have suggested that DNA predicted to be facing outward when wrapped on nucleosomal particles is favored for integration (82, 115). However, complicating the analysis, not all methods for calling nucleosome positions in vivo agree well, and experimental mapping of nucleosome positions is imperfect and fraught with lab-specific noise. Additional studies have investigated possible roles of cellular factors in modulating interactions with nucleosomes (116, 117). Different integrating elements, described elsewhere in this book, show a wide range of responses to nucleosome binding to integration target DNA.

Role of DNA repair enzymes in completing provirus formation

The initial steps in the integration reaction are catalyzed by the viral-encoded IN enzymes, but this only takes the reaction to the point of joining one DNA strand at each end of the provirus to host DNA. The integration intermediate produced by IN has DNA gaps at each host-virus DNA junction, requiring intervention of additional enzymes to generate the fully integrated provirus. Cocktails of host-encoded DNA repair enzymes have been shown to be able to carry out the needed polymerization, branch excision, and ligation steps in vitro on substrate DNAs modeling the expected gaps (118). These enzymes are ubiquitous in cell nuclei and quite active, so their involvement seems likely. However, another possibility is that the virus-encoded RT and IN enzymes actually complete gap repair. Some in vitro evidence supports this (119), although the observed enzymatic activities may not be adequately robust. So far no studies have succeeded in creating mutant combinations that result in production of stalled gapped intermediates inside cells, leaving the activities involved incompletely clarified. In addition, several further cellular DNA repair enzymes have been proposed to act on retroviral DNA and influence integration or replication (120–127).

Inhibitors of HIV integration

Inhibitors acting at the active site

Antiviral agents are now available that target many steps in the HIV replication cycle, greatly improving the quality of life of HIV-positive individuals, and IN is among the targets (128). Initial work improving assays for integration allowed high-throughput screening under relatively authentic conditions, yielding starting points for drug development (129–131). All IN inhibitors targeting the active site contain chemical groups that can bind both catalytic metal atoms together with pendant groups that contact other parts of the IN-DNA complex. Several chemical families were explored before the pyrimidinone group was identified. Extensive development eventually yielded raltegravir, which received FDA approval in 2007 (132).

Mechanistic studies show that raltegravir acts by blocking binding of the integration target DNA to the PIC (133). In addition, structural studies of raltegravir bound to the PFV IN/DNA complex show that the drug displaces the LTR 3′ end from its normal position in the active site, thereby obstructing the chemical steps of DNA transesterification (134).

As with all antiviral agents, resistance mutations have arisen in viruses in subjects treated with raltegravir. The effects of these amino acid substitutions can also be understood from the PFV structure as altering the drug binding site (see section by Engelman and Cherepanov). The observation of escape mutations has motivated a search for second generation IN inhibitors, resulting in promising new compounds such as dolutegravir, which shows high potency and favorable pharmacokinetic properties (135, 136).

Inhibitors acting at the LEDGF/p75 binding site on IN

Another site on HIV IN, the LEDGF/p75 binding site, was recently shown to be a promising target. The observation that removal of LEDGF/p75 from cells or overexpression of isolated

LEDGF/p75 IBD could inhibit HIV-1 replication, motivated searches for compounds that obstructed the LEDGF/p75 interaction (79, 90, 91, 137). Further motivating this search was a report of a small molecule inhibitor of HIV integration bound to a site at the dimer interface of the HIV IN catalytic domain (138) that was later shown to also be the LEDGF/p75 IBD binding site. Several groups carried out large-scale screens and identified small molecules that bound at this site and blocked binding of the LEDGF/p75 IBD to HIV-1 IN (137, 139–143). Some of these molecules were found to have low nanomolar affinities, acceptable toxicity to cells, and inhibited virus infection quite potently. Structural studies using X-ray crystallography showed the drugs to be bound as expected in a pocket at the catalytic domain dimer interface (Figure 2). Growth of HIV in the presence of the drugs elicited escape mutations that mapped to regions encoding segments of IN near the drug binding site, genetically specifying IN as the drug target during viral replication.

Mechanistic studies of the steps affected in the viral replication cycle by these drugs yielded a surprise. Unexpectedly, the drugs were not particularly potent during the early phase of the viral replication cycle (entry through integration). In some cases the small molecules reduced targeting to transcription units, but this has been variable across the drug series. Compounds inhibited potently during the late steps (gene expression through assembly and release). It turned out that drugs of this class were promoting abnormal assembly of HIV polyprotein precursors and/or IN, so that viral particles had a disrupted structure and were fatally impaired during early steps of the next infectious cycle. LEDGF does not seem to be involved in normal assembly, because assembly proceeds normally, and the inhibitors are active in the absence of LEDGF. At the time of writing, large-scale efforts are under way in several pharmaceutical companies to complete development and bring this class of inhibitor to patients.

Controlling retroviral integration site selection with small molecules

As is described above, it is now possible to control the distributions of retroviral integration target sites by adding small molecules to cells during infections. For both lentiviruses and gammaretroviruses, addition of the drugs (some LEDGF site blockers (139–143) or JQ1 (105–108)) reduces targeting biases, so that integration site patterns more closely resemble random distributions. These molecules may have applications in human gene therapy, where targeting integration in or near transcription units is undesirable because of the risk of genotoxicity. If the efficiency of gene expression after integration is acceptable at these new locations, and if the small molecules are not too toxic to primary cells, these small molecules may be useful additives during cell transduction prior to transplantation of modified cells into patients.

Integration and HIV latency: implications for cure research

Recent work has focused attention on the possible influence of the chromosomal environment of the integrated proviruses on HIV latency. The formation of latent (transcriptionally silent) proviruses represents a major block to eradicating HIV from infected cells (144, 145). Studies show that HIV+ subjects who have viral replication suppressed by anti-retroviral therapy to below the limit of detection nevertheless harbor rare

cells containing integrated proviruses that are transcriptionally silent. Some of these are known to be resting memory CD4+ T-cells, which, upon exposure to antigen, can resume active transcription that allows proviral induction. As a result, renewed viral replication can restart active infection.

The observation that several individuals may have been cured of HIV has resulted in intense interest in developing generalizable means of eradicating HIV from infected people (146–148). One idea holds that cells harboring latent viruses might be selectively killed if latent proviruses could be coaxed to resume transcription, provided that the host was equiped to destroy these cells efficiently once the latent cells were disclosed to the immune system. A recent clinical trial tested this by treating well-suppressed HIV+ subjects with the histone deacetylase inhibitor SAHA (vorinostat) with the goal of altering the chromatin environment in latent cells to promote HIV transcription (149). Studies to date have reported an increase in cell-associated HIV RNA following SAHA treatment, though no increase in circulating virus, providing an early suggestion that the shock-and-kill strategy might be feasible.

The idea that SAHA would boost transcription presupposes that a lack of histone acetylation is the limiting factor for transcription of the HIV provirus. This assumption can be addressed experimentally using cell-based models of latency and proviral induction (150). In such studies, cells are infected with HIV or an HIV-based vector. Cells are then fractionated based on whether or not they express the HIV provirus. The non-expressing cells can then be induced to express HIV with an agent that activates T-cells. Those cells that respond and transcribe the proviral genome are operationally defined as the inducibly latent population.

Several studies have explored integration site distributions in these cell populations (150–152). In general, inducible proviruses are found in locations similar to from typical HIV favored integration sites. A recent meta-analysis showed that no single feature characterized latent cells studied in five different cell models (150). Significant trends were seen within each model, but the chromosomal features near favored sites were mostly dissimilar between models. The most prominent shared feature of latent integration sites was proximity to cellular alphoid repeats, which was observed to be positively associated with latency in four of five models. Alphoid repeats are enriched near centromeres, so the silencing detected may resembles position effect variagation seen in early studies in Drosophila, where apposition of an active gene to centromeric heterochromatin by inversion resulted in reduction in expression (153). However, latent sites near alphoid repeats comprised only a minority of all inducible integration sites. SAHA shock-and-kill protocols predicted a negative association between latent sites and histone acetylation, but this association was not consistently observed. Latency in these models was not associated with a single integration site pattern, indicating that transcriptional latency in HIV may have many molecular mechanisms, a conclusions also reached with other experimental approaches (154). Unfortunately these findings complicate efforts to expose and remove cells harboring latent HIV.

Other studies suggest additional possible roles for integration targeting in HIV latency. Ikeda et al. reported in 2007 that rare cells from HIV+ patients on long term successful drug therapy showed clustering of integration sites (155), and recent reports have developed this picture further (156–158). Some of these integration sites were in genes related to cell

growth or persistence, such as the transcription factor BACH2, which is a lymphoid cancer-related gene. These results raise the possibility that gene disruption by HIV integration may promote growth or persistence of latently infected cells. An example of lentiviral integration correlated with outgrowth of a cell clone has been reported in a gene therapy trial. In this case, the vector integrated in the expanded cells was located in the proto-oncogene HMGA2, providing a possible analogy for clonal outgrowth during latency (159).

Genome-wide studies to identify candidate host factors

Numerous genome-wide screens have been carried out to identify host factors important for HIV replication (collected in (160–162)). A wide variety of methods have been applied. In a few cases proteins have been identified that are candidates for affecting the integration step. Loss-of-function screens have been carried out using siRNA knockdowns, in which siRNAs targeting most human genes are assayed individually for their ability to either reduce or increase HIV infection (14)(53, 54). Gain of function studies have been carried out by overexpressing cDNAs and assessing whether they increased or decreased HIV infection (163). Focused screens have queried interferon-inducible genes, a particularly rich source of anti-viral factors (164, 165).

Another approach involves genome-wide studies to identify host proteins that bind to viral proteins (32). In these protocols, HIV proteins are expressed in cells, then captured using appropriate tagging systems. Bound proteins are then eluted and analyzed by mass spectrometry approaches. Recent comprehensive studies have identified many more cellular proteins binding to the HIV proteins, in some cases with strong statistical support for specificity. Another approach involves yeast two-hybrid assays, in which candidate interactors are expressed together as fusions in yeast, and interactions scored by effects on yeast transcription. For both LEDGF/p75 and BET proteins, early studies used yeast two-hybrid assays to document binding.

Given the gigantic size of the genome-wide data sets, the development of useful summaries and convenient web browers becomes as important as generating the data to begin with. Table 1 summarizes some of web-enabled sites for investingating these data. The GuavaH web site allows one to query HIV and human genotypes and their relationship to phenotypes (166). The GeneOverlapper site can be used to query lists of genes in groups to find genes in common (162). Over 40 lists are included, which can be queried in over 200 trillion combinations, so a blog site is included for readers to post comments on their findings, allowing "crowd sourcing" of discovery. The NCBI keeps a list of HIV proteins and their interactions. So far, with respect to integration biology, these large data sets have helped identify tethering factors and a variety of cellular factors implicated in PIC trafficking and nuclear import. A rich array of additional cellular proteins have been implicated, and some bind IN, offering many new starting points for investigating the roles of cellular proteins in integration.

Other fates of the viral DNA

The normal fate of retroviral DNA is integration into the host genome. However, forms of viral DNA are also found that are thought to be abortive products not on the integration

pathway (167, 168). Some viral DNA is found in the form of 2-LTR circles that likely arise by non-homologous end joining of the linear viral DNA after preintegration complexes have fallen apart (125). 1-LTR circles appear to be the product of homologous recombination between the two LTRs in the linear viral DNA. Other types of aberrant product, including full length circular viral DNA with a rearrangement and smaller circles with deletion, are the result of autointegration.

Several models have suggested that the unrepaired integration intermediate and/or unintegrated forms of viral DNA may trigger pathways leading to cell death. Retroviral infection was reported by some to induce cell death in NHEJ-deficient cell lines (120, 121, 169). Cell death in these cell lines was reported to be dependent on the presence of functional IN, suggesting that the integration intermediate is recognized as DNA damage. Consistent with this idea, inhibition of DNA-PK by wortmannin makes normal cells sensitive to killing by retroviral vectors with an active IN, but not by vectors with an inactive IN. Results with these models have varied (125, 170). In another study, infection of activated primary CD4$^+$ lymphocytes by HIV-1 also induced cell killing (171). Killing was not observed in the absence of active IN or the presence of IN inhibitors, suggesting a requirement for integration. However, in a recent study from another group, unintegrated viral DNA was implicated as the signal in killing of CD4$^+$ lymphocytes (172). Knockdown of the interferon–γ-inducible protein 16 (IFI16), a DNA sensor, abrogated cell killing in this model, suggesting that the integrated viral DNA triggers an innate immune response that leads to caspase 1 activation and pyroptosis. Killing was still observed with virus with an active site mutation in IN, but not in the presence of a nonnucleoside reverse transcriptase inhibitor, demonstrating a requirement for DNA synthesis but not integration. Further work is required to reconcile and advance work in this area.

Conclusions

Retroviral host factors and their roles in integration targeting has been a very active area of study. Genome-wide screens have yielded many new starting points for investigating HIV biology, including HIV integration. The discoveries of tethering factors for HIV and MLV provide a simple mechanism. In contrast, the finding that drugs targeting the LEDGF/p75 binding site in fact disrupt assembly in a fashion that is mostly independent of LEDGF/p75 was not at all expected, and focuses attention on the apparent role of IN in late replication steps. The role of integration in latency, and the role of replication intermediates in cell toxicity and immunodepletion are further areas where additional investigation would be valuable.

Acknowledgments

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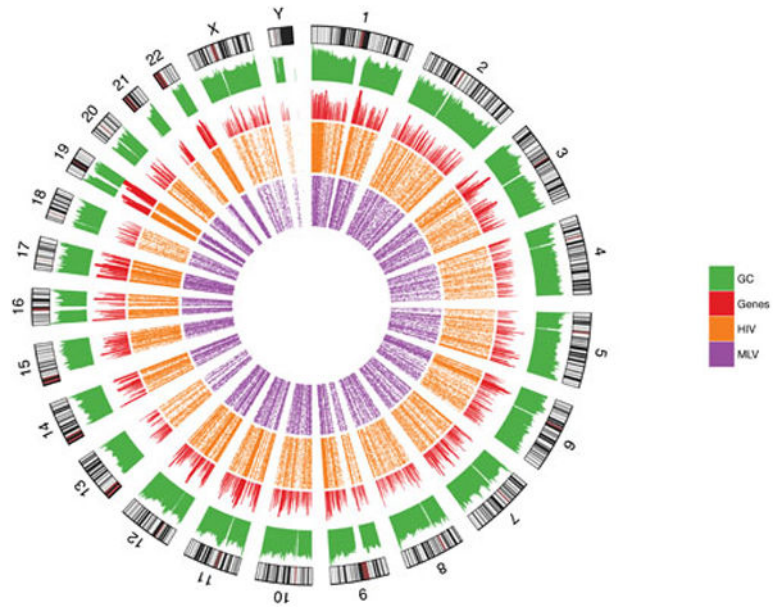
Figure 1.
Sites of HIV DNA integration in chromosomes. The human chromosomes are shown in the outermost ring. The green histograms indicated relative G/C content; red indicates gene density, orange indicates density of HIV integration sites in T cells (78), and purple indicates MLV integration site density in T cells(115).
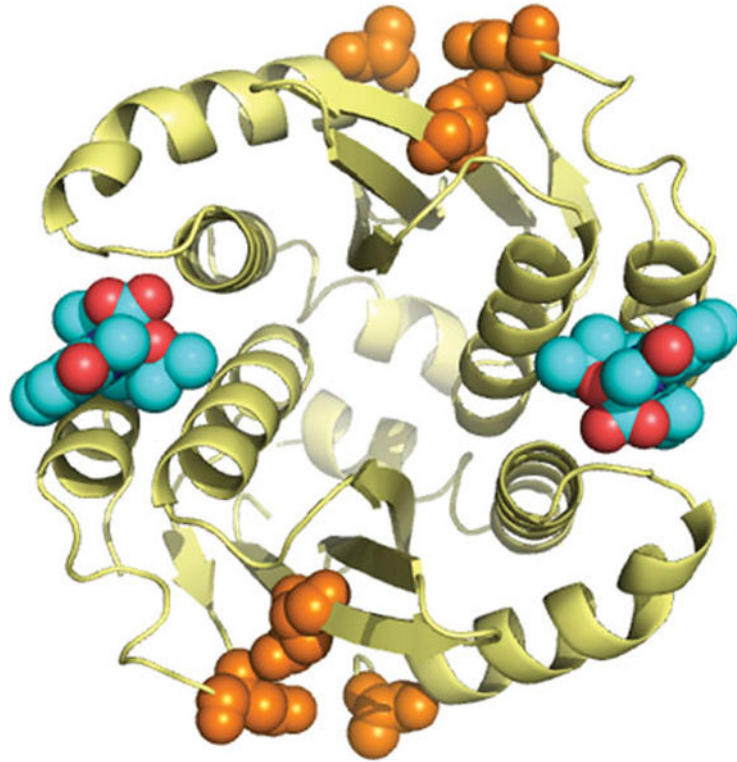
Figure 2.
The inhibitor GSK1264 bound to the LEDGF-binding site on the HIV IN catalytic domain dimer. The alpha carbon backbone of the IN catalytic domain dimer is shown in gold. The GSK1264 compound is show in cyan (carbons) and red (oxygens). Active site residues are shown in orange. Details on the structure and function of GSK1264 are reported in (137).

Table 1

Resources for working with genome-wide data on HIV.

Table 1. Some resources for working with genome-wide data from HIV research

Name of resource	Link
GuavaH	http://www.GuavaH.org
NCBI	http://www.ncbi.nlm.nih.gov/
Los Alamos HIV Databases	www.hiv.lanl.gov/
UNAIDS	http://www.unaids.org/en/
Stanford University HIV Drug Resistance Database	http://hivdb.stanford.edu/index.html
GPS-prot	http://www.gpsprot.org
HIV Replication Cycle	http://www.hivsystemsbiology.org/wiki/index.php/Introduction
Gene Overlapper	http://www.hivsystemsbiology.org/GeneListOverlapper/