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Review

HIV-1 regulation of latency in the monocyte-macrophage lineage and in CD4+ T lymphocytes

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ABSTRACT

The introduction in 1996 of the HAART raised hopes for the eradication of HIV-1. Unfortunately, the discovery of latent HIV-1 reservoirs in CD4+ T cells and in the monocyte-macrophage lineage proved the optimism to be premature. The long-lived HIV-1 reservoirs constitute a major obstacle to the eradication of HIV-1. In this review, we focus on the establishment and maintenance of HIV-1 latency in the two major targets for HIV-1: the CD4+ T cells and the monocyte-macrophage lineage. Understanding the cell-type molecular mechanisms of establishment, maintenance, and reactivation of HIV-1 latency in these reservoirs is crucial for efficient therapeutic intervention. A complete viral eradication, the holy graal for clinicians, might be achieved by strategic interventions targeting latently and productively infected cells. We suggest that new approaches, such as the combination of different kinds of proviral activators, may help to reduce dramatically the size of latent HIV-1 reservoirs in patients on HAART. *J. Leukoc. Biol.* 87: 575–588; 2010.

Introduction

The HIV-1 that causes AIDS, identified in 1983 [1], remains a global health threat with 33 million persons infected worldwide (data from United Nations, 2008). The introduction of HAART in 1996 has improved quality of life greatly and extended survival. This therapy is based on a combination of three or more drugs selected from 22 compounds that belong to three drug classes (inhibitors of RTs, proteases, and gp41) [2–4]. According to clinical trials, HAART can reduce plasma virus levels below detection limits (≤50 copies/ml plasma), raising hopes for the eradication of HIV-1. Initially, HAART induces a biphasic decline of plasma HIV-1 RNAs—a rapid decline of infected cells of the CD4+ T cells (half-life 0.5 day) is followed by a decline in plasma virus RNAs originating from infected tissue macrophages (half-life 2 weeks) [5]. Based on these studies showing biphasic decay in the level of viremia after HAART treatment, some authors predicted that the eradication might be achieved by a 3-year treatment [4]. Unfortunately, HIV-1 RNA returned to a measurable plasma level in less than 2 weeks when HAART was interrupted [6, 7]. Indeed, the long-term suppression of HIV-1 replication has unveiled the presence of latent HIV-1 reservoirs.

The first reservoir found was resting CD4+ T cells [4, 8–11]. It comprises two populations: the naïve and the memory CD4+ T cells. This latent reservoir is established very early during acute infection and therefore, limits the efficiency of HAART, even when introduced at the onset of HIV-1 infection [10, 12, 13]. Viral latency is a feature of many viruses, including other retroviruses as well. It appears that HIV-1 latency occurs at a low frequency (1 to 10^6–10^7 infected cells). As this infection is established early with X4-tropic viruses and as CD4+ T cells are CCR5-negative [14], it is difficult to understand how these cells are infected and why they are latently infected. Some investigators proposed that naive CD4+ T cells express a low level of CCR5 and therefore, are permissive for R5-tropic viruses [15]. It has been demonstrated that interaction of naïve CD4+ T cells with follicular DCs also renders them permissive to HIV-1 infection [16]. However, their relatively short lifespan is not consistent with the fact that they can function as a long-term reservoir of HIV-1. On the other hand, the presence of latent proviral HIV-1 in the memory cells has

Abbreviations: CBP=CREB-binding protein, CDK9=cyclin-dependent kinase 9, COUP-TF=chicken OVA upstream promoter transcription factor, CTIP2=COUP-TF-interacting protein 2, CycT1=cyclin T1, DC=dendritic cell, ELMO=engulfment and cell motility, GTF=general transcription factor, H3K9=histone 3 lysine 9, HAART=highly active antiretroviral therapy, HAT=histone acetylase, HDAC=histone deacetylase, HEXIM1=hexamethylenebisacetamide-inducible 1, HP1=heterochromatin protein 1, HPC=hematopoïetic cell, IKK=IκB kinase, LTR=long-terminal repeat, miRNA=micro-RNA, Nuc-1=nucleosome 1, PCAF=p300/CBP-associated factor, pTEFb=positive transcription elongation factor b, RIP=receptor-interacting protein, RNAi=RNA interference, siRNA=small interfering RNA, Sirt1=sirtuin 1, Sp1=specificity protein 1, SUV39H1=suppressor of variegation 3-9 homolog 1, TAR=transactivation response, TI=transcriptional interference, TRADD=TNFR-associated death domain, TRAF2=TNFR-associated factor 2, Ttm=transitional memory T cells

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been proven [8]. As for naïve cells, these memory cells are CCR5-negative. It is now assumed that HIV-1 latency occurs in activated CD4+ T cells, which are in the process of reverting to the resting state [17]. A recent report from the laboratory of R. P. Sékaly [18] showed the existence of two subpopulations of memory T cells serving as reservoirs for HIV-1. These cells are believed to be a major cellular reservoir for HIV-1 with different mechanisms involved to ensure viral persistence. The first reservoir—central memory T cells—can persist for decades. It is maintened through T cell survival and low-level-driven proliferation. By contrast, the second reservoir—Ttm—persists by homeostatic proliferation of infected cells and could be reduced by using drugs preventing memory T cells from dividing.

There is now evidence for the existence of other reservoirs. Some phylogenetic analyses suggest that a sustained production of the virus could indeed originate from another reservoir than the CD4+ T cell [19, 20]. It has been proposed that peripheral blood monocytes, DCs, and macrophages in the lymph nodes and hematopoitic stem cells in the bone marrow can be infected latently and therefore, contribute to the viral persistence [19, 21–25].

The monocytes are the precursors of the APCs, including macrophages and DCs. Initial studies have shown that monocytes harbor latent HIV-1 proviral DNA [26]. The detection of HIV-1 in circulating monocytes from patients under HAART [22, 27] confirmed this observation. It appears that compared with the more abundant CD14++, CD16– monocyte subsets [28], only a minor CD16+ monocyte subset is more permissive to the infection. Even if the HIV-1 proviral DNA is present only in <1% of circulating monocytes (between 0.01% and 1%), these cells constitute an important viral reservoir. Indeed, the trafficking of these bone marrow-derived monocytes is involved in the dissemination of HIV-1 into sanctuaries such as the brain [22, 27, 29, 30].

Mature myeloid DCs located in lymph nodes can sustain a very low-level virus replication and therefore, have a potential role in HIV-1 latency. The mechanisms involved in this latency are not yet known [31–33].

It is less clear whether macrophages have a potential role in HIV-1 latency [25, 34]. Patients on HAART have few lymph node macrophages infected (approximately five in 10^5). However, the finding of in vivo reactivation of these infected macrophages in response to opportunistic infections argues for macrophages as HIV-1 reservoirs [35, 36]. Although infected monocytes and macrophages contribute only a few percent to the total viral load, it is thought that these cells are important in the transmission and the pathogenesis of HIV-1 infections [35, 37–39].

In addition to the two main targets, i.e., CD4+ T cells and cells from the monocyte-macrophage lineage, HPCs have been proposed to serve as a viral reservoir, as HIV-1 CD34+ HPCs have been detected in some patients [40, 41]. Interestingly, a HPC subset (CD34+ CD4+) has an impaired development and growth when HIV-1 is present. This HPC will then generate a subpopulation of monocytes permissive to HIV-1 infection with a low level of CD14 receptor and an increase of CD16 receptor (CD14+ CD16++). It is not yet well understood whether the abnormalities leading to the generation of this permissive cell population are a result of a direct or an indirect interaction with HIV-1. A further investigation is needed, as these HPCs generate an infected cell lineage that may spread HIV-1 to sanctuaries. The importance of HPCs as a potential reservoir for HIV-1 has been highlighted by the recent report of G. Hütter et al. [42]. They showed that it may be possible to eradicate the reservoirs for HIV-1 by myeloablation and T cell ablation. Moreover, this patient became resistant to HIV-1 infection, as this treatment was followed by a repopulation of cells of the immune system deficient in CCR5 [42].

In this review, we focus on the molecular mechanisms of the establishment and maintenance of HIV-1 latency in the two major targets of HIV-1: the CD4+ T cells and the monocyte-macrophage lineage. The two virus-infected targets are different. In CD4+ T cells, viral particles assemble at the plasma membrane and bud out of the cell, and in macrophages, a substantial amount of virus particles is budding into the intracytoplasmic compartments [43, 44]. In contrast to infection in CD4+ T cells, HIV-1 infection of macrophages is generally not lytic [45, 46]. These latter cells are also more resistant to cytopathic effects, and they are able to harbor viruses for a longer period. Sometimes, infected tissue macrophages produce viruses during the total lifespan. Deciphering the molecular mechanisms of HIV-1 latency is therefore essential for developing original strategies based on HIV-1 reactivation in association with an efficient antiretroviral therapy with, as an ultimate goal, the eradication of the virus from infected patients.

ESTABLISHMENT AND MAINTENANCE OF HIV-1 LATENCY

Models of HIV-1 latency studies

Several models have been created to study the molecular mechanisms of HIV-1 latency. These are mainly in vivo and in vitro models. Latency in primary cells is studied less because of the difficulties to grow and maintain them in ex vivo conditions in such a high density that allows HIV latency to occur even with a low frequency. Below, we discuss briefly only the most important models of latency that made it possible to better understand the molecular basis of HIV-1 latency.

In vitro models of latently infected cells. The first three model cell lines developed (Ach-2 T cell line, U1 promonocytic cell line, and JΔT T cell line) were characterized by a small constitutive expression of HIV-1, and in these, virus gene expression could be increased by treatments with cytokines and/or mitogens [47–50]. These cell lines contained virus genes that were mutated in Tat protein (U1 promonocytic cell line), TAR-RNA (Ach-2 T cell line), and the NF-κB-binding site (JΔT T cell line). Experiments suggested that these factors are important in establishing latency [51, 52]. Indeed, these elements are involved in the initiation (NF-κB) or the elongation of transcription (Tat via interaction on the TAR). Other cell lines, without mutations in the Tat gene, were also created, which better reproduce the physiology of infected cells. Marcello et al. [53] used a proviral construct containing an intact HIV-1 promoter driving GFP expression and a LTR-

driven Tat, which allowed the selection of several clonal cell lines (J-LAT) that showed no detectable GFP expression in basal conditions but a high level of GFP expression when treated with cytokines and/or mitogens [54]. In other models, cells were infected with HIV-1-based reporter viruses. In these cells, HIV-1 expression was shut down, but they were sensitive to cytokine-induced reactivation [55]. Various in vitro cell line models, i.e., myeloid cell lines at different degrees of differentiation, have also been created to reproduce HIV-1 macrophage latency [56].

Ex vivo models of latently infected cells. In vitro models do not necessarily reflect latency under in vivo conditions. Ex vivo models of primary T lymphocyte and primary-derived macrophage cell models have been developed to examine the relevant signaling pathways of HIV latency in more physiological conditions [57, 58]. However, these models are technically difficult to establish and to maintain, explaining why they are still confidential. Development of these ex vivo models is needed.

Animal models of HIV-1 latency. Animal models play an important role in understanding HIV pathogenesis. Indeed, these models provide an in vivo system to investigate the importance of the HIV-1 reservoirs. For example, Brooks et al. [58] have developed a SCID mouse model, which provided new insight into the establishment and maintenance of HIV-1 latency. Advances have also been made with reconstituted murine models harboring stem cells of human origin. For example, studies of humanized SCID models show that the function of hematopoietic stem cells is affected indirectly by HIV-1 [59]. This model is also a powerful in vivo system for preclinical studies directed toward the development of original strategies in HIV-1 reactivation. The SIV-macaque model was used to show the establishment of transcriptional HIV latency in macrophages resident in the CNS (the microglial cells) and provided the first mechanism of HIV latency in the brain [60]. This model has also been used in clinical trials to test suppressive antiretroviral therapy [61–63]. Finally, it has been confirmed in SIV macaques that the monocyte progenitor CD34+ CD4+ is affected early during the virus infection [64, 65], as it was found previously in patients infected with HIV-1 [66].

Establishment of HIV-1 latency

Two different forms of HIV-1 latency have been described in CD4+ T cells. The first one is observed in naïve cells with an unintegrated DNA and is called preintegration latency [67]. This latency is characterized by a poor RT activity, and therefore, it is unable to synthesize the provirus DNA. Several mechanisms are involved in this form of latency, such as hypermutation of the DNA induced by the restriction factor APOBEC3, a low level of the dNTP pool, and an impaired nuclear importation of the preintegration complex associated with a low level of the ATP pool [68–71]. However, this form of latency is clinically not relevant, as cells carrying a full-length integral-competent HIV-1 have short half-lives (1 day) and therefore, cannot account for the long-term latency observed during HAART. This might not be true for macrophages [72, 73].

A latent reservoir, i.e., a stable postintegration of the virus genome, is required for the formation of a long-lived population of latently infected cells. This has been observed in resting T cells, which cannot proceed to integration after a direct infection, suggesting that latency arises in infected CD4+ T cells that have reverted from an activated to a quiescent state [9, 74, 75]. These cells have a specific set of surface markers together with an integrated form of the HIV-1 genome. Moreover, in these cells, cytokine and/or mitogen activation are able to induce HIV-1 virus production [76].

It has been proposed soon after the discovery of HIV-1 latency that proviral DNAs integrated into heterochromatin regions are responsible for the phenomenon of proviral silencing (**Fig. 1**) [54, 77]. Heterochromatin regions are known to be nonpermissive to proviral transcription. Different forms of proviral silencing mechanisms have been suggested. One hypothesis is that the virus takes a latent form right after DNA integration, without being transcribed. Another one suggests that some delayed forms of silencing occur only after a period of transcription of the provirus. The discovery that virus DNAs preferentially integrate into actively transcribed genes [78, 79] is in favor of this second hypothesis. This has been demonstrated in a T cell line infected in vitro and confirmed by in vivo analysis in resting CD4+ T cells [80]. Moreover, it was shown by reverse PCR that 93% of the 74 integration sites found in CD4+ memory T cell genomes were in introns of transcriptionally active genes. As many delayed forms of latency occur at transcriptionally active sites, this raises the question of why and how latency is acquired. A possible explanation is that viral DNA production is silenced by a mechanism involving different factors that interfere with viral transcription. TI is a Cis effect of one transcriptional process on a second transcriptional process [81]. It can be grouped into three different types of molecular mechanisms (Fig. 1) [82, 83]. The mechanism of promoter occlusion suggests that the polymerase complex from the upstream host promoter reads through the integrated downstream promoter of the virus. This mechanism has been confirmed recently in a CD4+ T cell line (J-LAT), which is a model for postintegration latency [84]. Another explanation for TI-promoted gene silencing is that the virus and the host genes are competing for transcription factors required for the promoter activity. TI is also possible when two transcription units—the host gene and the HIV-1 promoter—are oriented in a convergent manner. Such an orientation-dependent regulation of integrated HIV-1 transcription by a host gene transcriptional readthrough was described recently [81]. TI, especially promoter occlusion, may be an important virus-silencing mechanism, as it disables the cryptic promoter to function as a transcriptional starting-point of the virus gene [82].

A recent paper by Duverger et al. [85] suggests that below a NF-κB threshold level, the virus will integrate in a transcriptionally silent state. This means that infection leading to the establishment of latency could occur in low-level, activated CD4+ T cells or even in resting CD4+ T cells, as suggested already [86, 87]. In the view of these authors [82], once latency is established, the central mechanism maintaining latency is based on TI, as was already suggested by Han et al. [81]. This original and new view of how latency is established

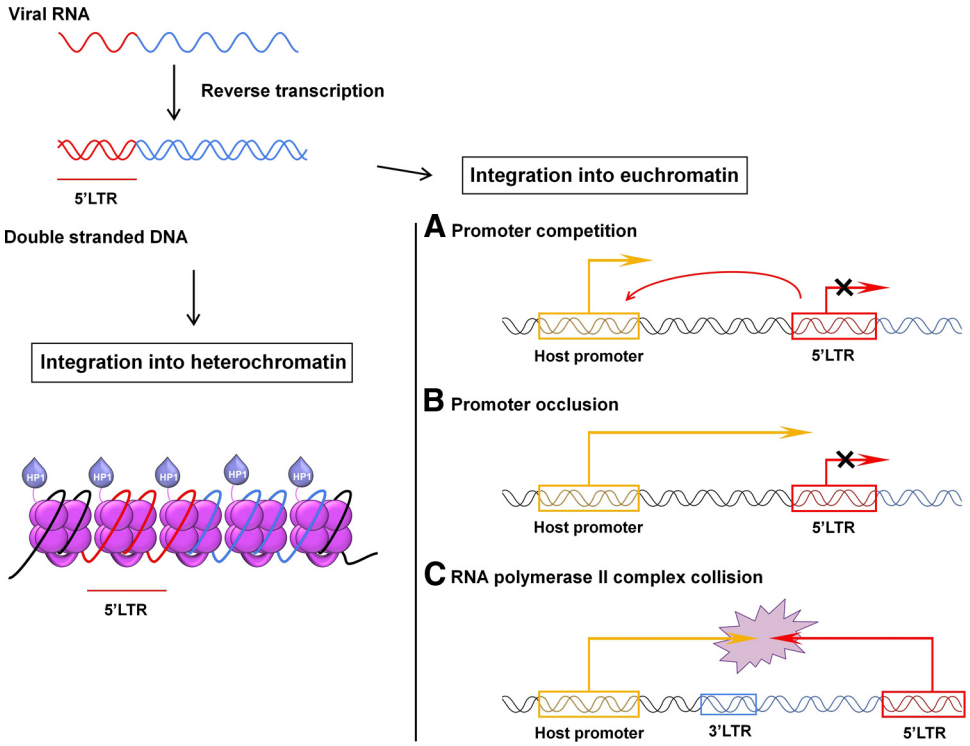

Figure 1. Establishment of postintegrational latency. After RT, viral RNA is converted into double-stranded DNA, which is then transferred into the host cell nucleus. Once the HIV-1 DNA reaches the nucleus, it is integrated into the host genome, and depending on the integration spot, postintegrational latency may occur. Different causes are distinguished to explain this phenomenon. First, the provirus ends in heterochromatin or euchromatin. In the first case (left panel), provirus lay merely in a nonreplicative part of the host genome, and so, the viral DNA is not transcribed. In the euchromatin (right panel), although the DNA is not tightly packed, the virus might enter in a latent state. Mechanisms have been described to interpret the origin of such latency set up: (A) Promoter competition: A host promoter, close to the integration site, hijacks the enhancer region of the 5′LTR, leading to transcription interferences. Enhancers interacting with the LTR preferentially induce the host gene at the expense of the provirus. (B) Promoter occlusion: Provirus is located nearby another host gene, which appears to have a stronger promoter than HIV-1. Thus, during its own transcription, the host gene prevents or displaces RNA polymerase II complexes to bind onto the proviral promoter. (C) RNA polymerase II complex collision: Promoters from the HIV-1 genome and a host gene are convergently arranged, which provokes the collision between both RNA polymerase II complexes processing the host gene and provirus.

and maintained needs further investigation, as if true, the therapeutic strategies will have to be revisited.

As it is believed that at least in CD4+ T cells, HIV-1 latency occurs in cells switching from an activated to a quiescent state, a question remains unanswered. What triggers latency in infected cells that actively produce viruses? Oscillations in NF-κB expression or stochastic fluctuations in Tat expression have been proposed to trigger the establishment of latency in CD4+ T cells [88, 89]. Weinberger et al. [89–92] have used a nice, integrated computational-experimental approach to understand the establishment of latency. Notably, they have shown that stochastic fluctuation in Tat level is a critical event, which may switch the cell from a lytic-productive to a latent-nonproductive fate. Moreover, they showed that perturbation of the Tat feedback circuit (by overexpression of the deacetylase Sirt1, which decreases Tat) changes cell fate. The probability to switch from a lytic state to a latent state increases in these cells. The probability of such events is small, but this might explain why some of the viral-producing cells become silent [89–92]. Furthermore, HIV-1 is progressively silenced in CD4+ T cells that are transfected with lentiviral vectors expressing Tat in Cis. The silencing was even greater if Tat were mutated. However, some authors suggested that the regulatory circuit involving Tat is not enough to explain cell entry into latency, as this model did not take into account the impact of other mechanisms such as epigenetic silencing [93]. Pearson et al. [93] showed that the progressive shutdown of HIV-1 expression is a result of epigenetic modifications, which affect the compaction of chromatin and thus limit HIV-1 transcription. Down-regulation of HIV expression then decreases Tat production below the level required to sustain HIV-1 transcription [93]. In the model of Weinberger et al. [89–92], overexpression of Sirt1 targets Tat acetylation, but as Sirt1 belongs to the acetylase class III family, epigenetic modifications at the HIV-1 promoter may not be excluded. We believe that any mechanism able to weaken the feedback loop of Tat can be responsible for the entry of cells into latency. In the future, mechanisms weakening the Tat circuit under physiological conditions should be explored. The epigenetic modifications observed in noninfected cells or in newly infected cells may be important in this phenomenon. Further investigations are needed in this direction.

The mechanisms proposed above for the establishment of latency in CD4+ T cells might be effective in cells from the monocyte-macrophage lineage, but this requires further investigation. The mechanism of HIV-1 latency in monocytes is not fully understood. It has been proposed that once integrated into the genome, HIV-1 transcription restriction is a result of the lack of Tat transactivation by the pTEFb, which is composed of two proteins: CycT1 and CDK9 [94–96]. Indeed, CycT1 was undetectable in undifferentiated monocytes and was induced in monocyte-differentiated macrophages [97]. However, CycT1 is not the only limiting factor involved in the transcription restriction of HIV-1; the phosphorylation status of CDK9 is also important, as it increases during monocyte differentiation into macrophage [98]. Some studies suggest

that the cellular signaling pathway, which involves the receptor tyrosine kinase RON, could trigger the establishment and maintenance of HIV-1 latency in monocytic cell lines. A correlation was found between RON expression and inhibition of HIV-1 transcription, which was affected at different levels, i.e., chromatin organization, initiation, and elongation [99–101]. The retinoid signaling pathway may also be involved in the inhibition of HIV-1 reactivation. The retinoid pathway inhibits Nuc-1 remodeling and transcription [102].

Apoptotic cells are part of a suppressive microenvironment that may help to establish reservoirs of latent macrophage populations [102]. It appears that the ability to suppress transcription is independent of phosphatidylserine and that a key signaling molecule, ELMO which participates in the phagocytosis of apoptotic cells, strongly inhibits HIV-1 transcription [103]. In addition, differences in the composition of NF-κB complexes between productive and latent macrophages have been proposed to explain the difference in the HIV-1 production between these cells [104, 105]. This requires further investigation, as the difference between NF-κB complexes has a potential role in the establishment of HIV-1 latency.

The strategies developed by the virus to survive longer, once the persistent infection is established in monocyte-macrophage cells or in memory T cells, include maintenance of HIV-1 latency (see the following section) and resistance of infected cells to apoptosis. HIV-1-infected cells do not undergo apoptosis following infection [106]. The mechanism inducing apoptosis resistance of persistently infected cells is not well understood yet. It has been proposed that the modulation of TNFR signaling by viral proteins such as Tat, Nef, and Vpr could explain the formation of viral reservoirs during HIV-1 infection [107]. Recently, a microarray approach has been used to investigate the gene expression signature in the circulating monocytes from HIV-1-infected patients [105]. A stable antiapoptosis signature comprising 38 genes, including p53, MAPK, and TNF signaling networks [108], has been identified. They showed that the CCR5 coreceptor bound by HIV-1 can lead to the apoptosis resistance in monocyte cultures.

As mentioned in the introduction, a HIV-1-positive patient has received bone marrow transplantation for leukemia. There was no evidence of the virus in the bloodstream after 20 months of follow-up [42]. Myeloablation and T cell ablation were suggested to favor the elimination of the long-lived reservoirs. This work therefore raised the possibility that the HIV-1 reservoir is confined to a population of radiosensitive cells, which might include mainly HPCs and circulating monocytes and lymphocytes. This study also points out the fact that reservoirs in sanctuaries could not sustain viral replication in the absence of peripherical-circulating cells. This latest remark would have profound therpeutic implications, as the main cellular target for eliminating the virus from infected patients would be the peripherical circulating cells (including the monocytes) and/or the hematopoïetic stem cells. Moreover, this report together with the one discussed above [108] underscore the central role of CCR5 during HIV-1 infection. Indeed, transplantation was done with cells from a homozygous donor for mutation in the HIV-1 coreceptor CCR5. This mutation is well known to be associated with resistance to HIV-1 infection. The importance of the CCR5 coreceptor in HIV-1 infection, demonstrated by its role in apoptosis resistance in infected cells [108] and by the observations of Hütter et al. [42], is therefore essential, and efforts at targeting this CCR5 coreceptor will be a great challenge in the next years. It will also be interesting to investigate whether the interaction between the CXCR4 coreceptor and HIV-1 could also trigger apoptosis resistance. Another mechanism has been proposed based on HIV-1 TAR miRNA, which protects cells from apoptosis and therefore, extends the life of infected cells [109]. The survival of viral reservoirs is of great importance, as it is also an obstacle to HIV-1 eradication. Deciphering the mechanisms underlying this apoptosis resistance is essential for devising new and original therapeutic strategies to purge the reservoirs.

Maintenance of HIV-1 latency

Once viral DNA is integrated into the host genome, and latency is established, mechanisms maintaining the virus in its latent form take place. We will consider two theories: One suggests that the maintenance of latency depends on the chromatin environment, and the other one put forward a mechanism that is based on the prevention of reactivation.

The chromatin environment. It is well known that the local state of chromatin influences transcription. Indeed, a heterochromatin environment, which is more compact and structured than euchromatin, is repressive for transcription (**Fig. 2**).

On the other hand, euchromatin, a relaxed state of chromatin, is associated with active transcription. The compaction of chromatin and its permissivity for transcription depends on post-translational modifications of histones such as acetylation, methylation, sumoylation, phosphorylation, and ubiquitinylation (Fig. 2) [110]. It is also well established that viral promoter activity depend on the chromatin environment [111]. Nucleosomes are positioned precisely at the HIV-1 promoter (Fig. 2) [112, 113]. Nuc-1, a nucleosome located immediately downstream of the transcription initiation site, impedes LTR activity. Epigenetic modifications and disruption of Nuc-1 are a prerequisite of activation of LTR-driven transcription and viral expression [111]. Histones of the nucleosomes Nuc-0 and Nuc-1 are constitutively deacetylated in all model cell lines of HIV-1 latency. Enzymes with deacetylase activities are recruited by several factors, including the homodimer p50, YY1, LSF, or thyroid hormone receptors [114–116].

Furthermore, transcriptional repressors such as Myc bind the HIV-1 promoter and recruit, together with Sp1, HDAC, thereby inducing proviral latency [117]. It was found recently that recruitment of deacetylases and methylases on the LTR was associated with epigenetic modifications (deacetylation of H3K9 followed by H3K9 trimethylation and recruitment of HP1 proteins) in CD4+ T cells. In these experiments, the methylase SUV39H1 and the HP1γ proteins were knocked down by siRNA. The depletion of these factors increased the level of HIV-1 expression [118].

Recruitment of deacetylases and methylases in the microglial cells, the CNS-resident macrophages, has also been described. These cells are major targets for HIV-1 and constitute latently infected cellular reservoirs in the brain [60]. As these infected cells are protected by the blood-brain barrier, only a few drugs

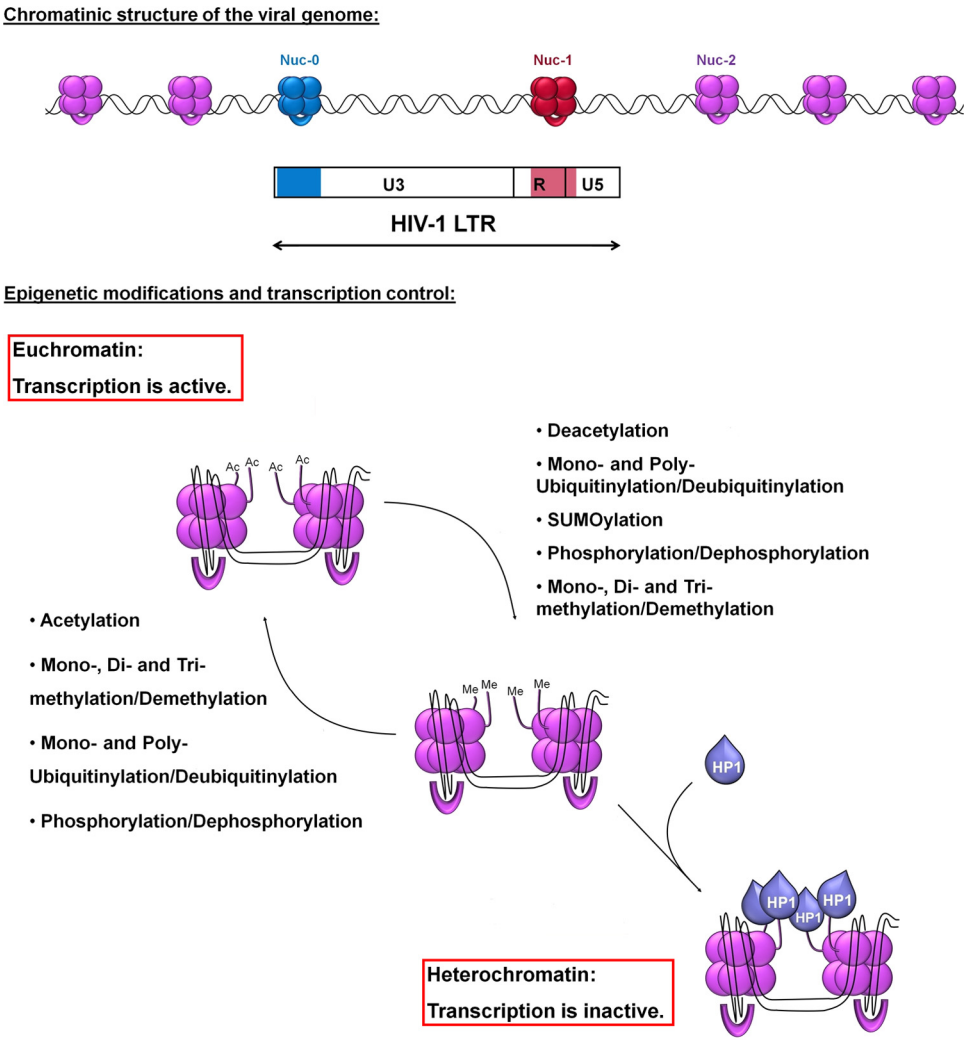

Figure 2. HIV-1 genome nucleosomal organization and epigenetic modifications of histones. 5′LTR of the HIV-1 genome presents a particular arrangement in which two nucleosomes, Nuc-0 (blue) and Nuc-1 (red), are surrounding the U3 region (upper panel). For this reason, the U3 region, which contains sites for cellular transcription factors able to modulate, enhance, and start viral replication, appears to be targetable still by chromatinic-modifying agents, even in latently infected cells. As a result of these chromatinic-modifying agents, epigenetic marks may be switched easily from repressive to activating (lower panel). Most of the epigenetic marks, carried by histone proteins, show a duality in the chromatinic state they induce. Thus, euchromatin (left) and heterochromatin (right) depend on the position rather than the presence of methylation, phosphorylation, or ubiquitinylation, and acetylation is associated with a transcriptional open state, and sumoylation is linked to a heterochromatinic closed state. Moreover, the closed state can also be stabilized through adaptor molecules such as HP1, strengthening the transcriptional repression.

are able to target them. Brain is therefore considered as a tissue sanctuary along with testes and retina. We have shown previously that CTIP2, a recently cloned transcriptional repressor that can associate with members of the COUP-TF family [119], inhibits HIV-1 replication in human microglial cells [120, 121]. CTIP2 inhibited HIV-1 gene transcription in microglial cells by recruiting a chromatin-modifying complex [122]. Indeed, our work suggests a concomitant recruitment of HDAC1, HDAC2, and methylase SUV39H1 to the viral promoter by CTIP2 (**Fig. 3**). Ordered histone modifications would allow HP1 binding, heterochromatin formation, and as a consequence, HIV silencing (Fig. 3). The heterochromatin formation at the HIV-1 promoter has been linked to postintegration latency, and this suggests that transcriptional repressors such as CTIP2 are involved in the establishment and maintenance of viral persistence and postintegration latency in the brain.

The corepressor CTIP2 has even more pleiotropic action by regulating the expression of the infected cell genes. Using a microarray analysis with a microglial cell line knocked down for CTIP2, we have seen that several genes were up- or down-regulated (unpublished results). Among these factors, the cellular CDK inhibitor CDKN1A/p21^{waf} was up-regulated. Recruited to the p21 promoter, CTIP2 silences p21 gene transcription by inducing epigenetic modifications, as described above for the HIV-1 promoter. This effect favors HIV-1 latency indirectly, as activation of the p21 gene stimulates viral expression in macrophages [123]. Moreover, CTIP2 counteracts HIV-1 Vpr, which is required for p21 expression. We suggest that all of these factors contribute together to HIV-1 transcriptional latency in microglial cells [124].

Mechanisms preventing reactivation. Mechanisms that inhibit the reactivation of HIV-1 expression also contribute to latency. Reactivation of HIV-1 from silent, infected cells requires sequential recruitment of cellular factors to the promoter region of the virus (**Fig. 4**). The initial nucleosomal organization of the HIV-1 LTR prevents the RNA polymerase II and the GTFs to access the promoter. A nucleosome remodeling by chromatin-modifying complexes is a prerequisite for transcription. These complexes disrupt the compacted chromatin and give access to the basal transcriptional machinery complex. Two major classes of chromatin remodeling complexes can be distinguished. The first class consists of ATP-dependent remodeling complexes that alter histone-DNA

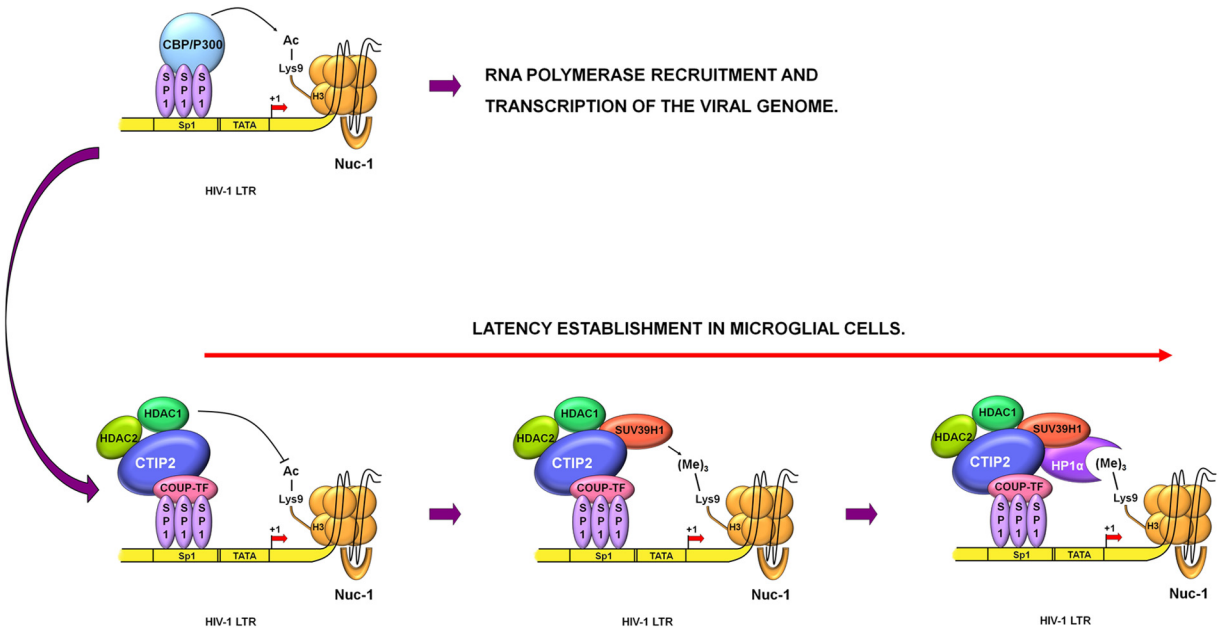

Figure 3. Postintegration latency in microglial cell. After integration in the host genome, viral DNA can be transcribed as long as the Nuc-1 is in an epigenetic open state (green). HIV-1 LTRs stay lightly packed in a euchromatinic structure, through activating epigenetic marks driven by proteins such as HAT. For instance, CBP/p300, a transcriptional coactivator, has an intrinsic HAT activity and processes Nuc-1 by interacting with Sp1, bound to HIV-1 LTR. However, it has been shown that in microglial cells, CPB/p300 can be replaced by inhibitory factors, leading to a latent HIV-1 integration. Sp1, bound to specific sites on the HIV-1 LTR, can recruit COUP-TF, which interacts with CTIP2, a known transcriptional repressor and HDAC1 and -2. Hence, histone protein 3 lysine 9 loses its acetylation in favor of a trimethylation mark induced by SUV39H1, switching Nuc-1 from a transcriptionally active to a repressive state. As a final step for latency establishment, an adaptor molecule HP1 stabilizes Nuc-1 into a heterochromatinic state (red) by targeting the newly produced trimethylation.

interactions. Enzymes of the second class regulate HAT. Cellular events, such as activation by phorbol esters (PMA, 12-*O*-tetradecanoylphorbol-13-acetate) and/or cytokines (IL-1β, IL-6, TNF-α), mitogens (PMA, prostratin), and antibodies (anti-CD3) [125], can remodel the nucleosomes (Fig. 4). HAT-regulating proteins, such as PCAF, CBP, and human GCN5, are recruited following TNF-α treatment (Fig. 4) [126]. The epigenetic modifications induced by HATs lead to better accessibility of DNA for RNA polymerase II and GTFs and allow early-phase transcription and finally, the production of Tat.

This early-phase transcription is induced by direct binding of host-cell factors to their target DNA sequences or by indirect binding via DNA-bound proteins. The ability of the viral genome to adjust its transcriptional mechanisms to specific cell types has been reviewed earlier [127]. In the early phase of transcription, multi-spliced mRNAs encoding the viral regulatory proteins (Tat, Rev, and Nef) are transcribed with low efficiency (Fig. 4). Among these viral proteins, Tat enhances gene expression by binding to TAR-RNA and associating with CycT1, which recruits Cdk9 [94–96].

The late phase of transcription is therefore under the control of Tat, which potently enhances gene expression through initiation and elongation of transcription (Fig. 4) [128]. The two steps of HIV-1 transcription occur in the two major cell types targeted by HIV-1 in a cell type-specific manner. Thus, transcription is regulated by the interplay of distinct viral and cellular transcription factors [127, 129], which bind to the LTR region of HIV-1 [130, 131]. Mechanisms preventing reactivation can act at a transcriptional and a post-transcriptional level.

Mechanisms Acting at a Transcriptional Level. One way to maintain latency of the virus is to sequestrate and/or degrade factors that would normally induce expression of the virus. MURR-1 encodes a protein involved in the cytoplasmic sequestration of NF-κB. Overexpression of this protein in memory CD4+ T cells decreases HIV-1 expression dramatically. On the other hand, knockdown of MURR-1 up-regulates HIV-1 expression. The protein Murr-1 prevents the proteasomal degradation of IκB, which interacts with NF-κB and thereby impedes reactivation of HIV expression. It is believed that this mechanism contributes to the maintenance of HIV latency [132]. Some studies point to the importance of DNA CpG methylation in the repression of transcription. These methylated sequences favor recruitment of additional factors leading to the recruitment of HDACs [133]. The use of methylase inhibitors, such as 5-aza-cytosine, induces hypomethylation and up-regulates transcription. These results strongly suggest that DNA methylation is involved in the regulation of transcription. As shown in the ACH-2 promonocytic cell lines, it may be important in the development of latency too [134, 135]. However, some reports do not support this mechanism of HIV-1 latency, as the methylase inhibitor 5-aza-cytidine induces only weak HIV-1 expression in J-LAT cells, a model cell line for latency

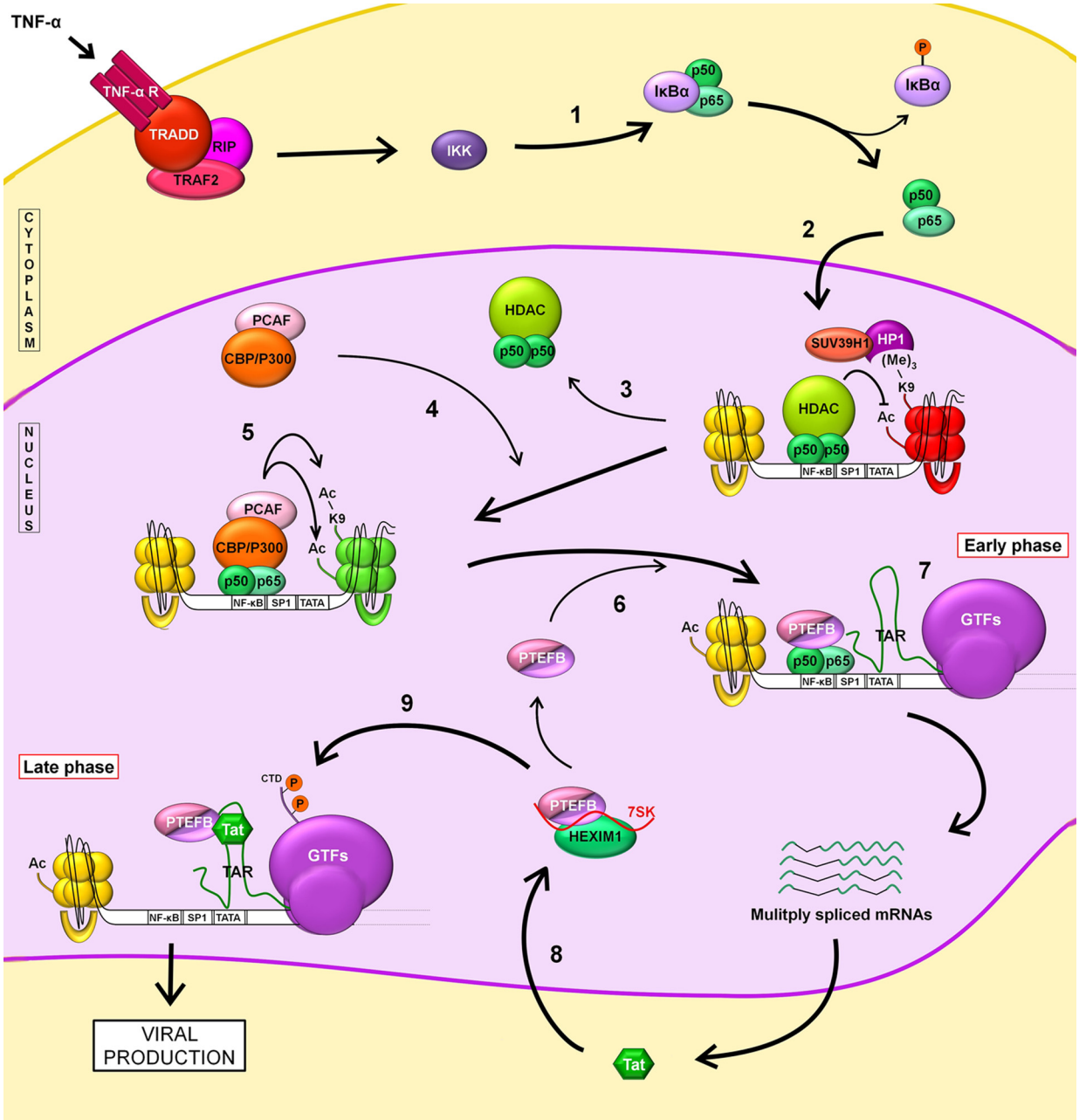

Figure 4. Mechanisms of latent HIV-1 reactivation in T lymphocyte. Reactivation of HIV-1 provirus in latently infected cells may be mediated through extracellular signals, such as the TNF-α molecule. Extracellular TNF-α triggers activation of IKK by direct interaction with TNF-αR, located at the T lymphocyte surface. The TRADD/RIP/TRAF2 complex, associated with the TNF-αR, induces a reaction cascade leading to IKK activation. Following its activation, IKK phosphorylates IκBα, protein sequestering the heterodimer p50/p65 in the cytoplasm (1). p50/p65, a transcription factor, is then released and so, migrates into the nucleus (2). Latent HIV-1 integration is a result of a tight packing of the nucleosomes on the 5′LTR, responsible for a heterochromatin structure formation (red nucleosome). The transcriptional-inactive environment is maintained through repressive epigenetic marks, for instance, histone 3 lysine 9 trimethylation or general deacetylations of the nucleosome. As described, these marks are processed, respectively, by SUV39H1 and HDACs. Moreover, trimethylation is also recruiting packing proteins, such as HP1, which enhance the chromatin-closed state. HDACs are bound onto the HIV-1 LTR by interacting wih the p50/p50 homodimer, also targeting the NF-κB sites but unlike p50/p65, acts as a repressor. Thus, free p50/p65 removes p50/p50 and HDACs simultaneously (3) and recruits PCAF/CBP, a complex of coactivator and HAT, onto the HIV-1 LTR (4). This complex switches the epigenetic marks from a repressive to active form by acetylating the nucleosome, currently blocking the TATA box (5). As a result, the HIV-1 genome adopts a relaxed euchromatin form (green nucleosome). For this reason, viral DNA is accessible to other factors, such as pTEFB (6) as well as the GTFs (7), the starting point of the HIV-1 replication early-phase. Production of multiply spliced viral mRNAs following the beginning of the replication produces first, viral proteins, such as the transactivator Tat. Once synthesized, Tat recruits more pTEFB from the inactive pool of pTEFB/Hexim1/7SK RNA complexes available in the nucleus (8). The Tat/pTEFB complex, on one hand, binds to the TAR element on native viral mRNA and on the other hand, phosphorylates the RNA polymerase II, hence speeding up the process (9). The viral replication enters into the late phase, during which viruses are produced at a high rate. Viral DNA has gotten out its latent state completely.

[136]. On the other hand, some investigators conducted a cDNA screen in J-LAT cells for genes that reactivate HIV-1 and have identified a transcriptional repressor methyl-CpG-binding domain protein 2, which binds methylated DNA. Furthermore, they have shown that the association of the DNA methylation inhibitor with prostratin, a NF-κB inducer, or TNF-α has a synergistic effect on reactivation of HIV-1 expression [137]. In vivo studies are required to assess the importance of DNA CpG methylation in more physiological conditions.

Mechanisms Acting at a Post-Transcriptional Level. Several post-transcriptional mechanisms are important in maintaining HIV-1 latency. The discovery of RNAi has initiated many studies of transcriptional mechanisms. In the pioneering work of Craig Mello (University of Massachusetts Medical School) and Andrew Fire (Stanford University School of Medicine), RNAi was identified as a post-transcriptional response to exogenous double-stranded RNA in *Caenorhabditis elegans*. They also showed that RNAi is involved in the regulation of gene expression. There are two classes of RNAi. One group of RNAi has an exogenous origin and is called siRNA. These are cleavage products from enzymatic complexes such as Dicer and Drosha. RNAi of the second class is encoded by the host genome and is thought to be an endogenous regulator of gene expression. These miRNAs are short, single-stranded RNAs of 19–25 nucleotides that are involved in various biological processes in eukaryotic cells [138, 139]. The miRNAs interact with a complementary sequence in the 3′-untranslated region of target mRNAs, which lead to mRNA degradation or more often, to translational inhibition [139]. miRNAs are involved in the regulation of virus expression as well [140]. Recently, it was shown that miRNAs regulate the expression of the HAT Tat cofactor PCAF and HIV replication [141]. Moreover, in an elegant experiment using microarray technology, an enrichment of miRNAs in clusters has been observed only in resting CD4+ T cells and not in active CD4+ T cells [142]. Huang et al. [142] found that several of the miRNA clusters inhibited HIV replication. They suggested that miRNAs contribute to HIV latency in resting primary CD4+ T cells. As multispliced RNAs for Tat and Rev are present in resting T cells, even if in small amounts, it may be that degradation of these RNAs by miRNAs contributes to the maintenance of HIV latency [143]. Such a mechanism based on cellular miRNA has also been described in circulating monocytes [144]. Another post-transcriptional mechanism involves the regulation of the exportation of viral RNAs. Nuclear retention of multi-spliced RNAs has been found in memory CD4+ T cells. The mechanism underlying this nuclear retention is unclear but may involve the down-regulation of the protein poly track-binding, which interacts with RNAs. This factor is present in active CD4+ T cells, and overexpression of this protein in resting cells allowed reactivation of HIV-1 expression [145].

The molecular mechanisms of the establishment and the maintenance of latency in the two main targets for HIV-1 are multi-factorial and are involved at various stages of the viral lifecycle, as discussed above. Possibly, some forms of latency are the result of the counter-reaction of the infected cell to silence HIV-1. It is tempting to speculate that defensive mechanisms arise in cells after a long history of coevolution of mammalian cells and retroviruses. Ironically, these mechanisms contribute to the persistence of HIV-1 in infected patients.

NEW PERSPECTIVES OF THERAPEUTIC INTERVENTION IN HIV-1 INFECTION

Since the discovery of HIV-1, vast research has been done to prevent and to cure AIDS. Much research concentrated on the development of vaccines against HIV. Unfortunately, all HIV-1 vaccines have failed in the past 25 years, showing that in practice, this approach is not working. Many actors of the scientific world, including the new Nobel Prize winner in physiology and medicine, Francoise Barre-Sinoussi, voiced the need for a new direction in HIV immunology research [146–149]. The failure of the current HAART to eradicate the virus originates partly in the truly latent reservoirs [19, 150, 151]. However, phylogenetic approaches also point to an ongoing replication at a low level in certain infected cells. Moreover, these cells are often found in tissue sanctuary sites, where penetration of drugs is restricted, as in the brain [152–154]. Furthermore, several RT inhibitors are ineffective in chronically infected macrophages [29], and protease inhibitors have significantly lower activities in these cells compared with lymphocytes [155]. Finally, emergence of multidrug-resistant viruses has been reported increasingly in patients receiving HAART [102, 156, 157]. All of these considerations (existence of true latent reservoirs, tissue-sanctuary sites, and multidrug resistance) encourage the development of new and original anti-HIV-1 treatment strategies.

Today, the therapy for HIV-1-infected patients is based on a combination of HIV gp41, RT, and protease inhibitors. We believe that new drugs should target other steps of the HIV-1 cycle. For example, they could be directed against proteins involved in the transcription of the inserted virus genome. Tat has a critical role in transcription and constitutes a major target in therapeutic intervention in the HIV replicative cycle [158–160]. Moreover, drugs could be designed to target cellular cofactors involved in the activation of transcription. This strategy should be able to bypass drug resistance, which arises with viral proteins. An increasing number of studies suggest that inhibitors of cellular LTR-binding factors, such as NF-κB and Sp1, repress LTR-driven transcription [158, 160–163]. Proteins such as p27, purified from St. John's Wort, which belongs to the DING family [164], are good candidates. p27 suppresses transcription and replication of HIV-1 by interfering with the function of cellular factors (i.e., NF-IL-6) and the viral protein Tat [165, 166]. However, these drugs do not cross the blood-brain barrier easily [152], and their structures must be changed to render them accessible to the brain.

We also believe that understanding the molecular mechanisms involved in the establishment, maintenance, and reactivation is critical for designing new treatment strategies. HIV-1 transcription inhibitors could be used to prevent reactivation and keep the virus in its dormant state. However, it will not allow the complete eradication of HIV-1 and a lifelong multitherapy associated with drug side-effects and development of

drug resistance, and high treatment cost would still be required.

Recently, a new and original strategy has been proposed to eradicate the virus from infected patients. The main idea is to facilitate the reactivation of viruses from latent reservoirs, which are then destroyed by HAART. Many factors have been involved in reactivation, including physiological stimuli such as TNF-α, IL-1β, IL-2, IL-6, IFN-γ, or CD154 [49, 167–171], chemical compounds such as phorbol esters (PMA and prostratin), HDAC inhibitors (trichostatin, valproic acid, sodium butyrate, and suberoylanilide hydroxyamic acid) [112, 172–180], caspase inhibitors [181], p-TEFb activators [182], hydroxyurea [183], and some activating antibodies (anti-CD3) [11, 184]. A recent antifungal agent, amphotericine B, reactivates viruses from the THP89GFP monocytic cell line, a model macrophage cell line of HIV-1 latency [185]. This compound is specific for macrophages, but in T cells, it may induce reactivation in trans when cocultured with the amphotericine B-mediated macrophages [186]. Many eradication protocols passed preclinical studies [5], but to date, all failed in clinical trials. Valproic acid, which is a HDAC inhibitor, was promising, as it was able to reactivate virus expression in the two major reservoirs (CD4+ T cells and macrophages). Indeed, valproic acid was described as effective in the first clinical trial [179, 187], but recent clinical trials did not confirm these results [188, 189]. The p-TEFb activator hexamethylenebisacetamide is also a promising molecule currently under study. In pilot studies, it was able to reactivate latently infected cells and prevent reinfection by down-regulating CD4 receptor expression [182]. Finally, some protocols failed as a result of the potential toxicity of treatments based on nonspecific CD4+ T cell activation such as IL-2. The recent discovery that an alternatively spliced form of the cellular transcription factor Ets-1 can activate latent HIV-1 in a NF-κB-independent manner has highlighted the therapeutic potential of cellular factors for the reactivation of latent HIV-1 [190]. Future eradication protocols should combine several drugs able to reactivate HIV-1 from latently infected cells. Such an approach has been found to be promising, as the association of a HDAC inhibitor or a DNA methylation inhibitor with prostratin has a synergistic effect on the activation of HIV-1 expression [137, 191]. Viral eradication might also be achieved by strategic interventions targeting the resistance of infected cells to apoptosis and molecules involved in latency reactivation. Finally, according to recent data highlighting the existence of two subsets of memory T cells serving as a reservoir [18], a combined use of strategic intervention targeting viral replication (through reinforced HAART) and antiproliferative drugs (such as anti-cancer drugs) has been proposed. Moreover, this treatment has to be introduced very early in the course of infection (by the way, confirming the importance of early therapeutic interventions), as it reduced the constitution of the proliferative reservoir drastically (Ttm memory cells).

CONCLUSION

The latent HIV-1 reservoirs established early during infection present a major obstacle for virus eradication. Understanding the molecular mechanisms of HIV-1 latency is a prerequisite for designing new treatments that aim to eliminate the reservoirs. The mechanisms involved in the establishment and maintenance of HIV-1 latency are diverse and not understood fully. Eradication of the virus from infected patients will require a drastic reduction of the reservoirs associated to HAART, reinforced with new, potently antiretroviral drugs targeting chronically infected cells. These new drugs need to access tissue-sanctuary sites, such as the brain. As the development of a HIV-1 vaccine remains a major challenge for immunologists, we believe that future directions for a HIV-1-curative therapy will rely more on the development of original therapeutic strategies, which take into account latency, the nature of the latent reservoir, chronic replication, and accessibility to tissue sanctuary.

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