

Mechanistic Features of Recombination in HIV

Román Galetto and Matteo Negroni

Unité de Régulation Enzymatique des Activités Cellulaires, CNRS-URA 2185; Institut Pasteur, Paris, France

Abstract

The importance of recombination in retroviral evolution has been acknowledged for several decades. Consequently, after the identification of HIV as the etiological agent of AIDS, it was suspected that recombination could also play a central role in the evolution of this virus. However, only recently, extensive epidemiologic studies of HIV infections worldwide have provided an estimate for the occurrence of recombination in vivo, unveiling recombination frequencies that dwarf those initially expected. Nowadays, recombination is regarded as an integral part of the infectious cycle of this retrovirus, which impacts on diagnosis and treatment of infections, especially when genetically distant viruses have been at the origin of the recombinant forms. Retroviral recombination is observed when two genetically divergent genomic RNA molecules are present in the same viral particle, and arises during the reverse transcription step. This review focuses on the mechanisms that have been proposed to account for the occurrence of recombination in retroviruses, from the strand displacement model, according to which recombination occurs during second DNA strand synthesis; to the description of the factors responsible for copy-choice recombination during first DNA strand synthesis, such as the presence of breaks, pause sites, or secondary structures in the genomic RNA. Most of these models have been supported by experimental data obtained from in vitro reconstituted systems or from cell infection studies using academic model sequences. The situation in vivo is expected to be more complex, since several factors come into play when recombination involves relatively distant isolates, as in the case of inter-subtype recombination. At present, it is clear that further studies are needed in order to evaluate whether a prevailing mechanism exists for in vivo recombination, and these studies will also be essential for understanding how the underlying mechanisms of recombination contribute to the evolution of HIV. (AIDS Reviews 2005;7:92-102)

Key words

HIV. Recombination. Molecular mechanism. Genetic variability.

Worldwide HIV genetic variability

The human immunodeficiency virus (HIV) is characterized by a high genetic variability. Two types of HIV have been identified to date, named HIV-1 and HIV-2, resulting from the introduction in humans of simian immunodeficiency viruses (SIV) from chimpanzee (SIVcpz) and from sooty mangabey (SIVsm), respectively¹. HIV-1 vi-

ruses are classified into three phylogenetic groups: M, O and N, which most likely reflect three independent events of cross-species transmission from chimpanzees². The M group (for main), representing the substantial majority of strains found worldwide, is further subdivided into nine clades or subtypes (A-D, F-H, J and K), among which subtypes A and F have been further split into two sub-subtypes^{3,4}. These subtypes present a relatively well-defined geographic distribution in different regions of the world (Fig. 1), probably reflecting founder effects rather than a higher infectivity of a given subtype with respect to others⁵. The highest degree of viral diversity is observed in the African continent, where all subtypes are represented⁵⁻⁷ (Fig. 1). The O (for outlier) and N (for non-M/non-O) groups remain essentially restricted to Central Africa, where they represent a minority of the infections^{8,9}.

Correspondence to:

Matteo Negroni
Unité de Régulation Enzymatique des Activités Cellulaires
Institut Pasteur
25, Rue du Dr Roux
75724, Paris, Cedex 15
France
E-mail: matteo@pasteur.fr

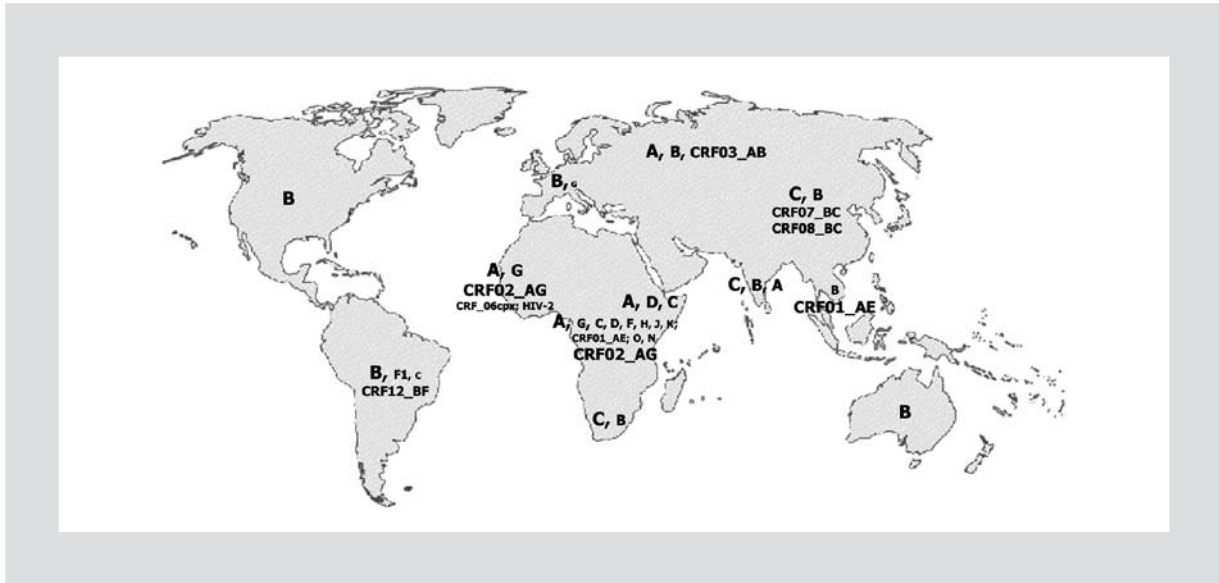


Figure 1. HIV genetic subtypes circulating in different regions of the world. The map is based on sequences of the *env* gene. Letters from A to K indicate HIV-1 subtypes of the M group. Letters O and N indicate HIV-1 groups other than M (see text). Predominant circulating recombinant forms (CRF) are also shown. The font size for each of the genetic forms indicates the approximate prevalence of various strains in each region.

HIV-2, in contrast to HIV-1, is mostly geographically restricted to West Africa, where sooty mangabeys are widely present, and has been subdivided into eight phylogenetic groups¹⁰. To date, HIV-2 appears to play a less important role in the AIDS pandemic, and it has been by far less studied than HIV-1.

Recombinant forms in the epidemics

Recent epidemiologic studies have revealed the importance of recombination as a prevalent mechanism accountable for the generation of viral diversity in the global pandemic¹¹. Indeed, apart from pure viruses belonging to groups and subtypes, other genetic variants have been detected with distinctive parts of their genome corresponding to different subtypes. These strains are assumed to have arisen by recombination between parental strains belonging to different subtypes (inter-subtype recombinants). Among these, some isolates with coincident mosaic genomes found in epidemiologically unlinked individuals are called circulating recombinant forms (CRF)⁴ and can be considered as emergent lineages. Sixteen CRF have been described to date¹², named with an identifying number and the letters corresponding to the subtypes involved in their generation. The nomenclature “cpx”, for complex, is employed when more than two subtypes had been at the origin of the CRF. In addition to these CRF, other forms of inter-subtype recombinants are found with considerable prevalence in single individuals, and are therefore called unique recombinant forms (URF).

The overall proportion of HIV-1 recombinant forms has been recently estimated to account for almost 20% of worldwide HIV-1 infections¹³.

Recombination in HIV-1 has also been shown to occur between isolates from different groups^{14,15}, or from the same subtype¹⁶. Intra-subtype recombination, even if it is now accepted as a very frequent process, has always been downplayed mostly because sequence similarity hampers its identification, while inter-subtype recombinants are easier to detect. The difficulty in identifying intra-subtype recombinants, as well as the low number of fully sequenced genomes from certain areas, suggests that the impact of recombination in the HIV epidemic is still underestimated.

Requirements for the recombination process

The genome of retroviruses consists of an RNA molecule of positive polarity, present in two copies in each viral particle. For recombination to occur, the two copies of genomic RNA present within the capsid must be genetically different¹⁷⁻¹⁹. Cell infection results in the introduction of the viral capsid, containing the two copies of RNA and the viral enzymes, into the cytoplasm. The subsequent availability of nucleotides, to which the capsid is permeable, allows the reverse transcriptase (RT) to convert the genomic RNA into its double stranded DNA copy. Recombination results from the generation, during reverse transcription, of a chimeric DNA carrying part of

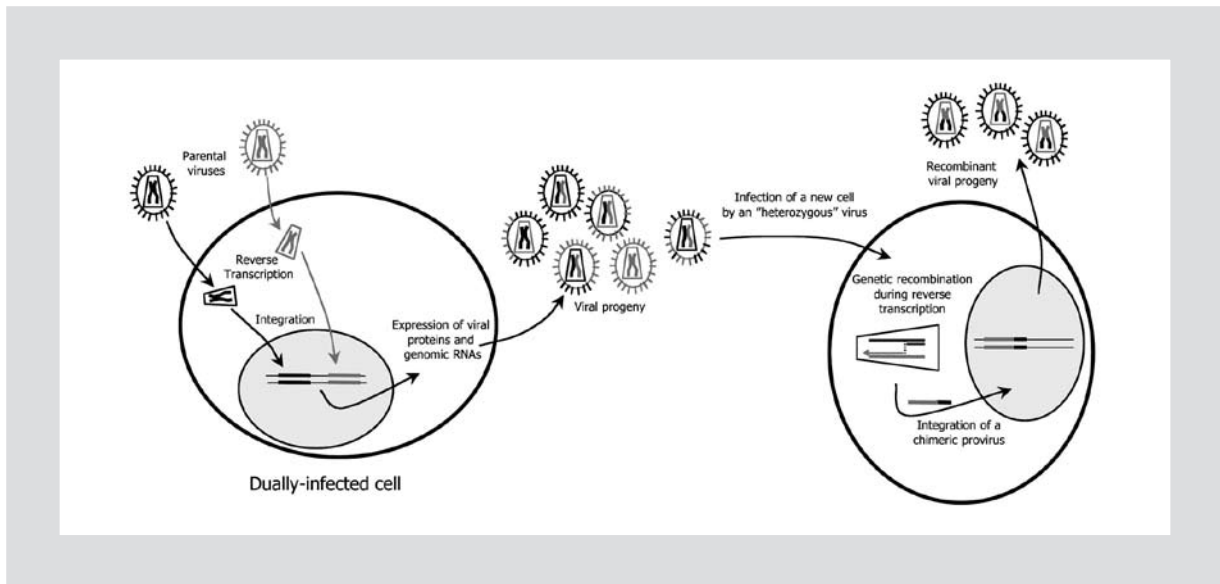


Figure 2. Outline of the recombination process. Genetically distinct viral particles are shown as virions containing black or grey genomic RNAs (the RNA dimer is shown within the viral capsid in the viral particle). Dual infection leads to integration of two proviruses in the cell's genome. The resulting viral progeny will be constituted by heterozygous and homozygous virions. Within the viral progeny, each particle is potentially assembled recruiting proteins synthesized from each of the two proviruses. This is schematically indicated by viral capsids and envelopes partially black or grey. After infection of a second target cell by a heterozygous virion, a chimeric provirus can be generated during reverse transcription (the example given is the one of recombination occurring during first-strand synthesis, by copy choice), leading to the production of recombinant viral particles.

the genetic information from each of the two genomic RNAs. As detailed below, the generation of such chimeric molecules has been proposed to occur both during synthesis of the first DNA strand (also indicated as minus DNA strand) and of the second DNA strand (plus DNA strand), through different mechanisms. In order for two genetically distinct RNAs to be present in the same viral particle, several requisites (outlined in Fig. 2) must be fulfilled. First, the two parental viruses must be able to infect the same patient and the same cellular subset, albeit not necessarily simultaneously. Second, at the level of the assembly of the viral particles, the different genomic RNAs must be able to form a functional dimer, recognized by the viral packaging machinery. A third point concerns the fact that, when two proviruses are present within the same cell, it is likely that the structural proteins of both viruses could be recruited for the assembly of the same chimeric particle. This might result in interference among these proteins during assembly.

As our knowledge of the HIV infectious cycle increases, it appears clear that HIV-1 fulfils most of these requirements. Indeed, the geographic distribution shows the presence of several areas where different M subtypes as well as isolates from O and N groups co-circulate⁷ (Fig. 1), thereby making coinfection of the same individual by genetically divergent isolates a likely event. In addition, the high replication rate of HIV-1, with the production of up to 10^{10} particles per day²⁰,

and the poor fidelity of the reverse transcription process^{21,22}, introducing on average one point mutation per infectious cycle²³, increase the probability of generating heterozygous virions even if infection occurs with a relatively homogeneous viral population.

Concerning the possibility of infection of a cell by more than one virus, a long-standing dogma was that an HIV-infected cell became resistant to superinfection, mostly because of the downregulation of the receptor for viral entry, the CD4 molecule²⁴. However, protection from superinfection does not seem to be as efficient as was originally thought. Indeed, mounting evidence for the widespread occurrence of recombination has provided overwhelming, although indirect, evidence for the frequent occurrence of coinfection. The formal demonstration for cells harboring several proviruses has come only recently from a study in splenocytes isolated from patients that showed, using fluorescence *in situ* hybridization, the presence of up to eight proviruses per cell²⁵. Coinfection can occur either simultaneously or delayed in time. Several epidemiologic studies and clinical follow up of patients have shown the occurrence of superinfection a long time after a first infection²⁶⁻²⁸ (Chohan and Overbaugh, personal communication) followed, in some cases, by the appearance of recombinant viruses between the two infecting strains²⁷. The possibility of concomitant infection of a cell with multiple viral particles also seems plausible, as deduced by a recent study of

the dynamics of recombinant HIV-1 forms generated in immunodeficient mice, where immune competence was restored by implantation of human fetal liver and thymus (humanized SCID mice)²⁹. In this work, the weak impact of viral interference on coinfection was underscored by the observation of double infection, despite a decrease in the CD4 receptor expression to approximately 10% of its normal level. This supports the idea that simultaneous coinfection can occur. HIV-1 transmission at immunologic synapses between dendritic cells and T-lymphocytes, where multiple viral particles are transmitted at the same time³⁰, is also expected to facilitate this process. Another phenomenon leading to the formation of heterozygous particles in coinfecting individuals could be the formation of syncytia between cells infected with different viruses. This process may occur in the advanced phases of disease progression, subsequent to the emergence of viruses with a syncytium-inducing phenotype³¹.

Genomic RNA dimerization is a complex process³², and the diversity of the sequences and structures found at the level of the dimerization region on the genomic RNA, as in the case of certain subtypes of the M group, is expected to hamper co-packaging of genetically divergent RNAs. Despite this assumption, studies of coinfection of cells in culture with viral isolates bearing non-homologous *versus* homologous dimer initiation sequences, indicated that the degree of sequence similarity in this region did not significantly affect the probability of co-packaging different RNAs as deduced by the detection of subsequent recombination³³. Finally, little information is available to date concerning the possible interference between structural proteins at the viral assembly step.

Mechanisms of recombination

Recombinant DNA molecules have been proposed to arise during synthesis of either the first³⁴⁻³⁷ or the second³⁸⁻⁴⁰ DNA strand. Recombination during second-strand synthesis was proposed on the basis of the observation that plus DNA synthesis is discontinuous in retroviruses, and that “H-like” structures were observed by electron microscopy of reverse transcription products generated in permeabilized avian sarcoma-leucosis virions⁴¹. These two observations led to the proposal of the “strand displacement assimilation model”⁴², extensively described in reference³⁸ and outlined in figure 3. According to this model, the ability of the RT to unwind double-stranded portions of templates ahead the growing DNA strand^{43,44} would give rise to a strand displacement process. This event would generate a branched DNA structure that would

then be resolved by cellular DNA ligases and nucleases, as indicated in figure 3. An implicit assumption of this model is that both genomic RNA molecules present in a virion are copied into a full-length minus DNA strand (Fig. 3). Data supporting the occurrence of recombination during second-strand synthesis have been provided^{40,45}; nevertheless, mounting evidence suggests that recombination during synthesis of the first DNA strand is largely the predominant mechanism, at least in HIV-1 and when considering isolates sharing a high level of sequence identity³⁴⁻³⁷.

Recombination occurring during first DNA strand synthesis is known as copy choice⁴⁶, and it is thought to result from template switching during reverse transcription. This process appears much simpler than recombination during second-strand synthesis, as suggested by the evidence that efficient copy choice is observed in simple reconstituted *in vitro* systems using purified RT, RNAs and nucleotides. In the case of copy choice, the RNA template that is copied before the switch is defined as the donor RNA, while the one onto which synthesis is transferred is called the acceptor. Several mechanisms (detailed below) have been proposed to explain copy choice. The common feature among these mechanisms is to be constituted basically by two steps: the annealing of the nascent DNA to the acceptor RNA, a process often referred as “docking”, and then the transfer of the growing 3' end of the nascent DNA onto the acceptor RNA. The presence of an RT-encoded RNaseH activity, responsible for the degradation of the donor RNA once it has been copied⁴⁷⁻⁵⁰, is mandatory for the generation of recombinant molecules during first-strand DNA synthesis⁵¹. In fact, the RNaseH activity makes the trailing end of the nascent DNA largely in a single-stranded form available for annealing to the other copy of genomic RNA (docking step). For the transfer to occur, the acceptor RNA must not have been reverse transcribed in the region of transfer (Fig. 3). This would ultimately lead to the transfer of DNA synthesis on the acceptor RNA, and it is essentially at this step that the various mechanistic models proposed differ among them, as detailed below.

Recombination during synthesis of the minus DNA strand

Copy choice as a consequence of genomic RNA breaks

The presence of breaks on the genomic RNA was the earliest hypothesis proposed to account for copy-choice recombination⁵². In fact, the frequent isolation of frag-

mented RNA molecules from retroviral preparations has suggested that the genomic RNA can be discontinuous within the viral particle, thereby hampering the generation of a full-length DNA by copying only one of the genomic RNAs. It has therefore been proposed that each break on the RNA template would force DNA synthesis to be transferred onto the other copy of genomic RNA (Fig. 4, top panel). Consequently, this model is known as “forced copy choice” (Fig. 4). The transfer would result from the stalling of reverse transcription imposed by the break, which would allow extensive degradation of the RNA template by the RT-encoded RNaseH activity. This is possible since, apart from degradation coupled to reverse transcription, the RNaseH can hydrolyze the RNA template independently from polymerization^{50,53,54}, leading to shortening of the heteroduplex and, eventually, to the melting of the nascent DNA from the donor RNA. The nascent DNA would then anneal onto the acceptor RNA, and DNA synthesis would be restored. An alternative possibility is that stalling of the RT favors an active displacement of the donor RNA in the heteroduplex by the acceptor RNA⁵⁵. From the standpoint of the mechanism, this process is similar to transfer on the terminal repeated sequence R during minus DNA synthesis (strong stop minus DNA strand transfer). This mechanism is certainly plausible, providing a valuable means to rescue impaired reverse transcription⁵². It is, however, difficult to predict its contribution to genetic variability in HIV as long as the extent of RNA degradation within the viral particles remains unknown. The role of RNA fragmentation on recombination has been addressed by single cycle infection studies in spleen necrosis virus (SNV) after exposure of the virions to gamma radiations. However this approach did not lead to conclusive results⁵⁶.

Pausing of reverse transcription as a trigger for recombination

An alternative to forced copy choice has been proposed, based on results obtained from reconstituted *in vitro* systems, which indicated that template switching during reverse transcription could be efficiently achieved also in the absence of manifest breaks on the RNA template⁵⁷. The presence of a strong pause site in the studied region^{57,58}, and the concomitant decrease in strand transfer observed when this pause was removed⁵⁹, led to the suggestion that pausing constitutes a trigger for template switching (Fig. 4). Conceptually, the role of pause sites would be the same as the one of RNA breaks in the forced copy choice model, discussed in the previous section. Even in this case, the two sce-

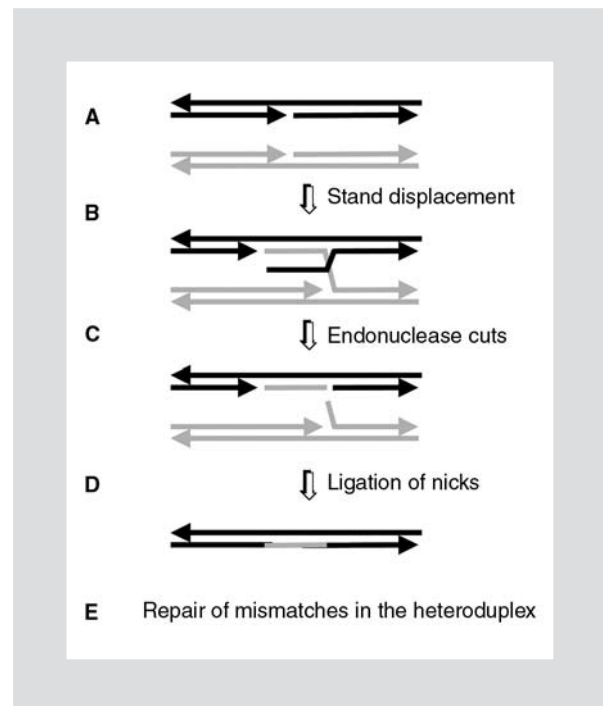


Figure 3. Strand displacement assimilation model for retroviral recombination. **A:** The arrows indicate the polarity of the DNA molecules 5' to 3'. Black and grey arrows indicate reverse transcription products from different viruses. Second-strand synthesis (the one going rightward) is shown to be discontinuous. **B:** Strand displacement by RT is shown for the “grey virus”. Its displaced strand invades the double-stranded region of the “black virus”. In consequence, one strand of the black virus is also partially displaced. For simplicity, it is assumed that this strand does not generate other displacement reactions and it is not drawn in the subsequent steps. After endonucleolytic cut (**C**) and ligation of the nicks (**D**), the resulting product will be a DNA heteroduplex that will be corrected to obtain a homoduplex by cellular repair enzymes. Stochastic repair of the mismatches present in one or the other strand will result in the fixation of mutations originally present in one or the other virus.

narios discussed for the forced copy choice process (melting of the nascent DNA followed by annealing on the acceptor RNA, or active displacement by invasion of the heteroduplex) have been proposed^{57,59}. Several studies in cell-free systems have shown that the viral nucleic acid chaperone protein nucleocapsid (NC)⁶⁰, present on the genomic RNA during reverse transcription, influences these processes, leading to an enhancement of strand transfer⁶¹⁻⁶⁴.

After the initial proposal, several studies have addressed the question of the importance of pausing in the promotion of template switching in cell-free systems. This was achieved by evaluating the correlation between the presence of strong pause sites detected in radio-labeled primer extension assays and the location of sites for preferential strand transfer. These analyses have shown that, although efficient strand transfer

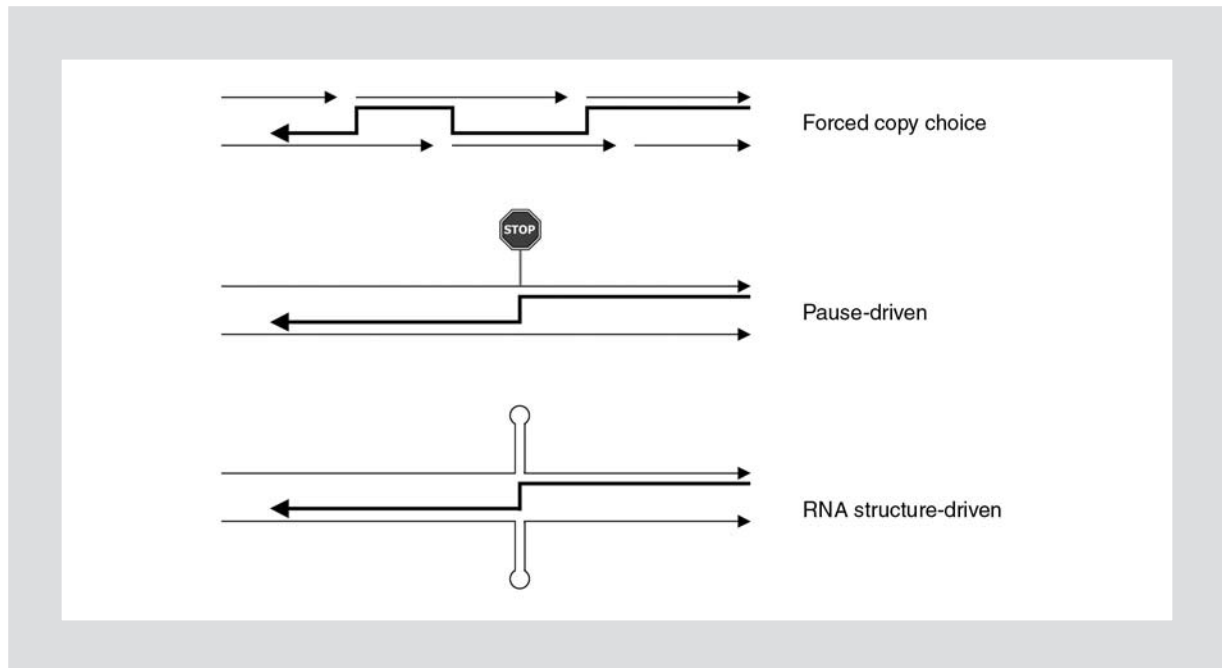


Figure 4. Models for minus DNA strand synthesis. Thin lines, genomic RNAs; thick line, minus DNA strand. The arrows indicate the 5' to 3' polarity of the nucleic acids. Forced copy choice model: the breaks on the genomic RNAs are shown as discontinuity in the lines. Pause driven model: the stop signal is drawn only on one RNA (the donor), although it might be present on both templates. RNA structure driven: a structured region of the genomic RNA is schematically indicated as a hairpin, and it is drawn on both templates, but it might be sufficient only on one of the RNAs, as detailed in the text. For the forced copy choice model a greater number of templates switches is presented, only for reasons of clarity. This does not imply a correlation between the number of template switches and the proposed molecular model.

in certain positions correlated with the nearby presence of pause sites of reverse transcription⁵⁷⁻⁵⁹, this was not the situation in the majority of the cases⁶⁵⁻⁶⁸.

The relationship between the overall rate of polymerization and the frequency of strand transfer was also investigated. *In vitro*, slowing down reverse transcription by decreasing the pool of nucleotides in the assays, led to an increase in the frequency of template switching^{57,59,69}. The same relationship was also studied in infected cells in culture using a similar approach, albeit in this case the issue was far less clear. Partial depletion of the intracellular nucleotide pool by treating the cells with hydroxyurea⁷⁰ led to an enhancement of strand transfer in Moloney murine leukemia virus (MoMLV)^{71,72}. Since hydroxyurea treatment delayed the appearance of full-length reverse transcription products⁷¹, these results were interpreted as an indication that enhancing stalling of DNA polymerization leads to an increased rate of template switching *in vivo*. Recent studies on HIV-1 have also shed light on this aspect of the question by following a different strategy²⁹. In this case, macrophages in culture, which have a low intracellular concentration of nucleotides⁷³, were supplied with exogenous nucleosides in the culture medium, a procedure known to enhance completion of reverse transcription⁷⁴. If a more efficient

process of reverse transcription correlated with a lower frequency of copy choice, a lower frequency of recombination would be expected under these conditions. However, no significant change in the frequency of recombination was observed in this study.

Overall, the emerging picture is that the presence of a pause site of reverse transcription on the donor RNA might facilitate the transfer event, independent of the underlying mechanism. However, most pause sites do not correspond to hot regions for transfer, and many hot regions do not correspond to positions of stalling of reverse transcription.

Copy choice in secondary structures of the genomic RNA

Another factor responsible for the generation of preferential sites for recombination is the presence of structured regions on the RNA template (Fig. 4). Cell free assays have indicated that the dimer initiation sequence (DIS)⁷⁵, the TAR hairpin in R^{65,76}, and a region coding for the C2 portion of the gp120⁶⁸, are regions prone to promote recombination due to the presence of secondary structures. The basic difference with respect to the mechanisms proposed for forced

copy choice and pause-driven recombination is that recombination does not occur at positions where DNA synthesis is hampered.

Experimental *in vitro* evidence suggests that, rather than docking, the limiting step for template switching in these regions is the transfer of the growing 3' end of the nascent DNA from one RNA to the other⁶⁸. In all cases studied, template switching occurred within the hairpin region and never at its base, ruling out the possibility that the role of the hairpin structures was to promote pause-driven recombination by inducing stalling of reverse transcription at the base of the stem. Stalling of reverse transcription at the base of the hairpin, however, has been suggested to increase the efficiency of transfer by favoring the docking step⁶⁷. HIV RT has been shown, indeed, to be able to polymerize through stable hairpin structures⁷⁷. Additionally, the presence of other viral proteins, such as the NC, is thought to assist in the opening of the stem portion of the hairpin *in vivo*, allowing DNA synthesis to progress within the structured region. Once opening is achieved, the whole structure is expected to be totally destabilized, and therefore the hairpin is no longer present on the donor RNA, while it should still remain on the acceptor RNA. Intriguingly, observations made in a reconstituted *in vitro* system clearly demonstrated that the presence of the hairpin on the donor RNA is dispensable, while it is required on the acceptor RNA⁶⁸. The occurrence of template switching inside a portion of acceptor template under a double-stranded form is somehow paradoxical though, since single-stranded regions would be expected to provide a more favorable condition for accepting an incoming DNA strand. In this regard, it has been proposed that the opening of the stem portion of the hairpin on the acceptor RNA would be facilitated by the formation of compensatory double-stranded regions with the donor RNA and with the nascent DNA, following a process similar to branch migration during DNA recombination⁶⁸ (Fig. 5). The stability of the hairpin also seems crucial for the efficiency of transfer, as indicated by the observation, both *in vitro* and in single-cycle infection of cells in culture, that structures with a very high stability were unable to promote template switching as efficiently as those having intermediate stability⁷⁸. This could be a reason why, even if certain regions of the genome are rich in secondary structures, such as the Rev responsive element (RRE) of HIV-1, they do not necessarily constitute hot spots for recombination²⁹. Not surprisingly, the NC protein has also been shown to play a central role in the modulation of recombination in structured regions of the genome^{65,67,68,75,78,79}. Noteworthy, the

importance of the stability of the RNA hairpins and the involvement of the NC in their destabilization has also been shown in the case of strong stop strand transfer occurring in the TAR region^{76,80-85}.

Overall, it seems that copy choice can occur following several mechanisms. Fragile sites in the genomic RNA, pause sites of reverse transcription, and structured regions of the RNA could all constitute hot spots for recombination. This list is certainly not exhaustive though, and understanding the role of recombination in the shaping of the viral population now requires an evaluation in infected cells of the mechanisms proposed mainly from observations in reconstituted *in vitro* systems.

From academic studies to HIV infection

Most studies aimed at the dissection of the mechanism of recombination in reconstituted systems have been performed using sequences chosen for their ability to efficiently carry out the transfer process. A crucial question, though, is whether recombination actually occurs preferentially at certain positions *in vivo*, or if it is rather homogeneously widespread along the genome. Recombinant isolates found in patients suggest that recombination is widespread along the genome, although preferential sites have been recently identified⁴⁰. The fact that all the regions of the genome are susceptible to undergo recombination is supported by mapping recombination breakpoints after a single infection cycle of cells in culture^{29,37,86}. However, since in these cases recombination was studied throughout the whole genome, addressing the question of the existence of hot regions for recombination would have required the analysis of an extremely large number of recombinant isolates. A study of recombination on a restricted region of the HIV-1 *env* gene after a single cycle of infection of cells in culture⁷⁸, instead, supported the idea of the existence of circumscribed preferential sites for recombination.

In the vast majority of the cases, the mechanisms described in the previous sections have been proposed on the basis of experiments where sequences sharing a high degree of identity were employed. It is known that the degree of sequence divergence plays a crucial role in the efficiency of the recombination process⁸⁷⁻⁹⁰. Therefore, the local degree of sequence identity must also be taken into account when considering recombination involving divergent strains.

It has also been suggested that recombination might be influenced by cellular factors. This idea is supported by the observation that the frequency of recombination in infected cells in culture is ten times higher in macro-

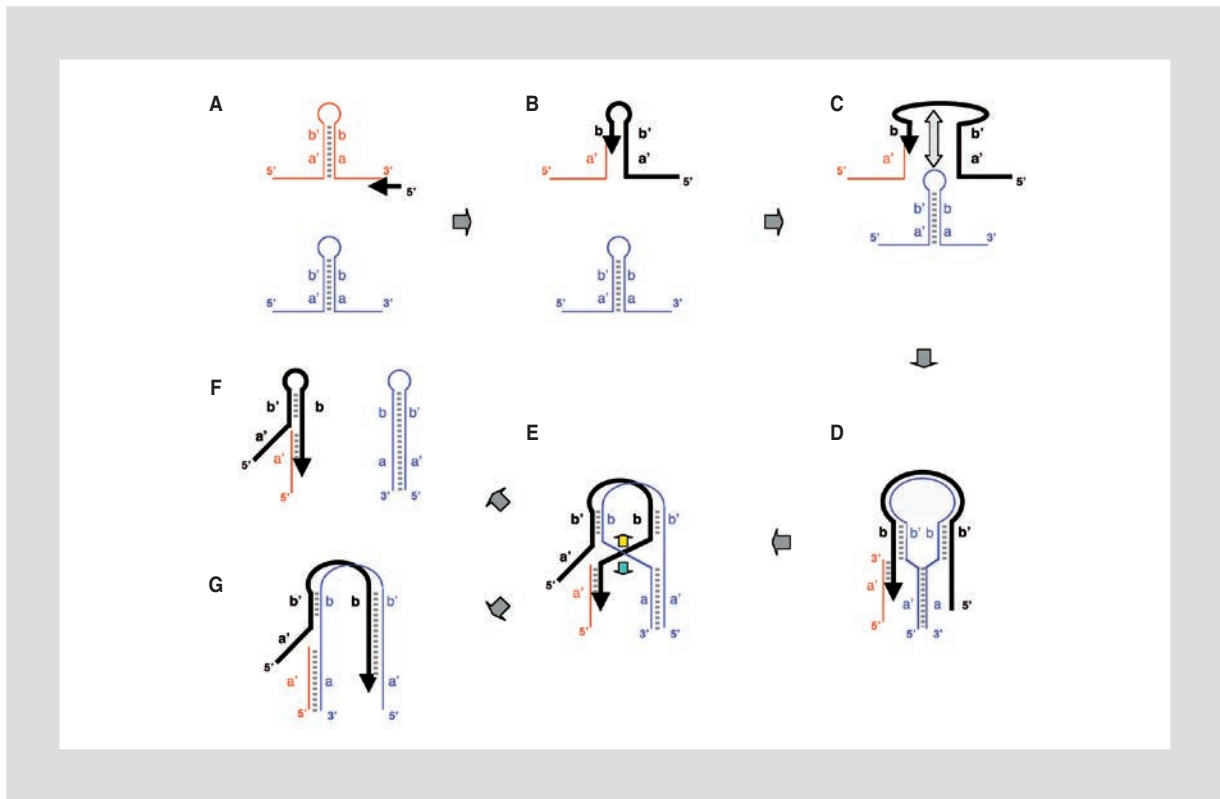


Figure 5. Model proposed⁶⁸ for template switching in internal regions of the RNA template through branch migration. Donor and acceptor RNAs are drawn as red and blue thin lines, respectively; the nascent DNA is drawn as a thick black line. **A:** Regions a and b are complementary to regions a' and b'. **B:** Reverse transcription progresses within the donor RNA, copying regions a, b, and b' into their complementary sequences. **C:** The nascent DNA docks on the acceptor RNA (double-headed light grey arrow) in a region arbitrarily indicated as the loop portion of the hairpin, since this region is under a single-stranded form on the acceptor RNA. **D:** Opening of the stem on the acceptor RNA as a zipper can occur, generating two double-stranded regions, through annealing to the nascent DNA. An alternative representation of the drawing is shown in **E**. The cross-over point can then move through branch migration following the direction indicated either by the yellow or by the blue arrow (**E**), and the resulting structures are shown in panels **F** and **G**, respectively. Branch migration following the direction indicated by the yellow arrow does not lead to strand transfer (**F**), since DNA synthesis continues on the donor RNA (red). Migration following the blue arrow (**G**), in contrast, leads to the transfer of the growing DNA strand on the acceptor RNA (blue).

phages than in peripheral blood monocytes, possibly due to myeloid differentiation²⁹. In infected individuals it is therefore likely that the extent of recombination could also vary according to the cell type that is infected.

Concluding remarks on recombination in AIDS

In HIV-1, genetic variability and, as a consequence, recombination have been correlated with the progression of the disease within patients^{91,92}, and to the spreading of the AIDS epidemic both in defined geographic areas as well as worldwide^{11,93}. Concerning the correlation between disease progression and increased genetic variability, the question of which is the cause and which is the consequence still remains unclear⁹⁴. An exception to this case is certainly constituted by the influence of recombination in the generation of resistant viruses in re-

sponse to highly active antiretroviral therapy (HAART). Despite the efficacy of these treatments in decreasing viral load, residual ongoing replication in treated patients allows the introduction of sequential mutations, leading to the generation of resistant viruses able to efficiently replicate even under treatment⁹⁵⁻⁹⁷. In this case, a precise pattern of mutations is required to generate the resistant phenotype, and recombination probably just shortens the time required to go from viruses carrying a set of mutations that already confer a partial resistance, to fully resistant viruses⁹⁸. However, this is an extreme case where the equilibrium between the contribution of the mechanism of recombination and the one of selective pressure acting on the recombinant forms is particularly unbalanced toward the latter. The situation is probably different when considering the issue of the influence of genetic variability in the emergence of viruses able to elude the immune system. In this case, the molecular mechanism

of recombination might contribute to the generation of some specific recombinant forms among the many expected to be possible, and could potentially influence the composition of the viral population that is submitted to natural selection. These considerations remain speculative, however, since almost all epidemiologic studies on HIV-1 rely, to date, on the analysis of recombinant variants once they have become dominant in the population. This does not allow teasing apart the relative roles of immune selection and of the mechanisms of recombination in the process of selection of the successful recombinant forms. This remains an important issue for future research aimed at understanding the dynamic interplay between virus evolution and the host's immune system.

Another issue for which recombination must be considered concerns the development of a vaccine strategy against HIV-1 infection⁹⁹. Even if the breadth of cross-subtype immune protection provided by the actual vaccine strategies is still unclear¹⁰⁰⁻¹⁰³, the continuous appearance of new recombinant strains potentially threatens strategies aimed at providing protection against "non-recombinant" viral subtypes. As an example, since discontinuous epitopes overlapping the CD4 binding site of the gp120 protein seem to constitute a frequent target for neutralizing antibodies¹⁰⁴⁻¹⁰⁸, it is conceivable that recombination could efficiently reshuffle these fragments of epitopes in new combinations able to evade immune responses¹⁰⁹. Since humoral immunity seems crucial for preventing the establishment of a chronic infection¹¹⁰, it must be considered that recombination could thwart the development of a successful vaccine.

In conclusion, many crucial features of the interplay between the immune system and HIV can be affected by the frequent occurrence of recombination. Ever since the first identification of recombinant forms in 1995¹¹¹, it has become clear that recombination must be regarded as an integral part of the replication process of HIV, rather than as a sporadic event increasing its genetic variability. Determining its contribution to reshuffling different regions of the genome, particularly when dealing with genetically distant strains, will be essential to understand the rules that govern the generation of the recombinant forms that could potentially frustrate future efforts in the development of a medical strategy for the control of AIDS.

Acknowledgments

We thank Judith Levin and Henri Buc for critical reading of the manuscript; Bhavna Chohan and Julie Overbaugh for communicating results prior to publica-

tion; and Michel Véron for constant support. Work in our laboratory is supported by funding from the French Agency for AIDS Research (ANRS; grant 02172 to M.N.) and from Sidaction (fellowship to R.G.).

References

- Hahn B, Shaw G, De Cock K, Sharp P. AIDS as a zoonosis: scientific and public health implications. *Science* 2000;287:607-14.
- Gao F, Bailes E, Robertson D, et al. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 1999;397:436-41.
- Robertson D, Anderson J, Bradac J, et al. HIV-1 Nomenclature Proposal. In *Human Retroviruses and AIDS 1999*. Kuiken C, Foley B, Hahn B, et al. (eds.). Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, 1999; 492-505.
- Robertson D, Anderson J, Bradac J, et al. HIV-1 nomenclature proposal. *Science* 2000;288:55-6.
- Thomson M, Perez-Alvarez L, Najera R. Molecular epidemiology of HIV-1 genetic forms and its significance for vaccine development and therapy. *Lancet Infect Dis* 2002;2:461-71.
- Vidal N, Peeters M, Mulanga-Kabeya C, et al. Unprecedented degree of HIV type 1 (HIV-1) group M genetic diversity in the Democratic Republic of Congo suggests that the HIV-1 pandemic originated in Central Africa. *J Virol* 2000;74:10498-507.
- Peeters M, Toure-Kane C, Nkengasong J. Genetic diversity of HIV in Africa: impact on diagnosis, treatment, vaccine development and trials. *Aids* 2003;17:2547-60.
- Peeters M, Gueye A, Mboup S, et al. Geographical distribution of HIV-1 group O viruses in Africa. *Aids* 1997;11:493-8.
- Ayoub A, Souquieres S, Njinku B, et al. HIV-1 group N among HIV-1-seropositive individuals in Cameroon. *Aids* 2000;14:2623-5.
- Damond F, Worobey M, Campa P, et al. Identification of a highly divergent HIV type 2 and proposal for a change in HIV type 2 classification. *AIDS Res Hum Retroviruses* 2004;20:666-72.
- Rambaut A, Posada D, Crandall K, Holmes E. The causes and consequences of HIV evolution. *Nat Rev Genet* 2004;5:52-61.
- The Circulating Recombinant Forms (CRFs). Los Alamos National Laboratory. HIV Sequence Database. (last accessed, March 2005). <http://hiv-web.lanl.gov/content/hiv-db/CRFs/CRFs.html>
- Osmanov S, Pattou C, Walker N, Schwardlander B, Esparza J. Estimated global distribution and regional spread of HIV-1 genetic subtypes in the year 2000. *J Acquir Immune Defic Syndr* 2002;29:184-90.
- Takehisa J, Zekeng L, Ido E, et al. HIV type 1 intergroup (M/O) recombination in Cameroon. *J Virol* 1999;73:6810-20.
- Peeters M, Liegeois F, Torimiro N, et al. Characterization of a highly replicative intergroup M/O HIV type 1 recombinant isolated from a Cameroonian patient. *J Virol* 1999;73:7368-75.
- McVean G, Awadalla P, Fearnhead P. A coalescent-based method for detecting and estimating recombination from gene sequences. *Genetics* 2002;160:1231-41.
- Vogt P. Genetically stable reassortment of markers during mixed infection with avian tumor viruses. *Virology* 1971;46:947-52.
- Hu W, Temin H. Genetic consequences of packaging two RNA genomes in one retroviral particle: pseudodiploidy and high rate of genetic recombination. *Proc Natl Acad Sci USA* 1990; 87:1556-60.
- Clavel F, Hoggan M, Willey R, Strebel K, Martin M, Repaske R. Genetic recombination of HIV. *J Virol* 1989;63:1455-9.
- Ho D, Neumann A, Perelson A, Chen W, Leonard J, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123-6.
- Preston B, Poesz B, Loeb L. Fidelity of HIV-1 reverse transcriptase. *Science* 1988;242:1168-71.
- Hu W, Temin H. Retroviral recombination and reverse transcription. *Science* 1990;250:1227-33.

23. Mansky L, Temin H. Lower in vivo mutation rate of HIV type 1 than that predicted from the fidelity of purified reverse transcriptase. *J Virol* 1995;69:5087-94.
24. Piguet V, Schwartz O, Le Gall S, Trono D. The downregulation of CD4 and MHC-I by primate lentiviruses: a paradigm for the modulation of cell surface receptors. *Immunol Rev* 1999;168:51.
25. Jung A, Maier R, Vartanian J, et al. Multiply infected spleen cells in HIV patients. *Nature* 2002;418:144.
26. Jost S, Bernard M, Kaiser L, et al. A patient with HIV-1 superinfection. *N Engl J Med* 2002;347:731-6.
27. Fang G, Weiser B, Kuiken C, et al. Recombination following superinfection by HIV-1. *Aids* 2004;18:153-9.
28. Ramos A, Hu D, Nguyen L, et al. Intersubtype HIV type 1 superinfection following seroconversion to primary infection in two injection drug users. *J Virol* 2002;76:7444-52.
29. Levy D, Aldrovandi G, Kutsch O, Shaw G. Dynamics of HIV-1 recombination in its natural target cells. *Proc Natl Acad Sci USA* 2004;101:4204-9.
30. McDonald D, Wu L, Bohks S, KewalRamani V, Unutmaz D, Hope T. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* 2003;300:1295-7.
31. Berger E, Doms R, Fenyo E, et al. A new classification for HIV-1. *Nature* 1998;39:240.
32. Paillart J, Shehu-Xhilaga M, Marquet R, Mak J. Dimerization of retroviral RNA genomes: an inseparable pair. *Nat Rev Microbiol* 2004;2:461-72.
33. St Louis D, Gotte D, Sanders-Buell E, et al. Infectious molecular clones with the nonhomologous dimer initiation sequences found in different subtypes of HIV type 1 can recombine and initiate a spreading infection in vitro. *J Virol* 1998;72:3991-8.
34. Anderson J, Bowman E, Hu W. Retroviral recombination rates do not increase linearly with marker distance and are limited by the size of the recombining subpopulation. *J Virol* 1998;72:1195-1202.
35. Yu H, Jetzt A, Ron Y, Preston B, Dougherty J. The nature of HIV type 1 strand transfers. *J Biol Chem* 1998;273:28384-91.
36. Zhang J, Tang L, Li T, Ma Y, Sapp C. Most retroviral recombinations occur during minus-strand DNA synthesis. *J Virol* 2000;74:2313-22.
37. Jetzt A, Yu H, Klarmann G, Ron Y, Preston B, Dougherty J. High rate of recombination throughout the HIV type 1 genome. *J Virol* 2000;74:1234-40.
38. Boone L, Skalka A. Strand Displacement Synthesis by Reverse Transcriptase. In *Reverse Transcriptase*. Skalka A and Goff S. (eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993;119-33.
39. Hu W, Pathak V, Temin H. Role of Reverse Transcriptase in Retroviral Recombination. In *Reverse Transcriptase*. Skalka A and Goff S. (eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993;251-74.
40. Magiorkinis G, Paraskevis D, Vandamme A, Magiorkinis E, Sypsa V, Hatzakis A. In vivo characteristics of HIV type 1 intersubtype recombination: determination of hot spots and correlation with sequence similarity. *J Gen Virol* 2003;84:2715-22.
41. Junghans R, Boone L, Skalka A. Products of reverse transcription in avian retrovirus analyzed by electron microscopy. *J Virol* 1982;43:544-54.
42. Junghans R, Boone L, Skalka A. Retroviral DNA H structures: displacement assimilation model of recombination. *Cell* 1982;30:53-62.
43. Huber H, McCoy J, Seehra J, Richardson C. HIV 1 reverse transcriptase. *J Biol Chem* 1989;264:4669-78.
44. Hottiger M, Podust V, Thimmig R, McHenry C, Hubscher U. Strand displacement activity of the HIV type 1 reverse transcriptase heterodimer and its individual subunits. *J Biol Chem* 1994;269:986-91.
45. Bowman R, Hu W, Pathak V. Relative rates of retroviral reverse transcriptase template switching during RNA- and DNA-dependent DNA synthesis. *J Virol* 1998;72:5198-206.
46. Vogt P. The genome of avian RNA tumor viruses: a discussion of four models. In *Proceedings of the Fourth Lepetit Colloquium: «Possible episomes in eukaryotes»*. Silvestri L. (ed.). North Holland Publishing Co., Amsterdam 1973;35-41.
47. Oyama F, Kikuchi R, Crouch R, Uchida T. Intrinsic properties of reverse transcriptase in reverse transcription. Associated RNaseH is essentially regarded as an endonuclease. *J Biol Chem* 1989;264:18808-17.
48. Krug M, Berger S. Ribonuclease H activities associated with viral reverse transcriptases are endonucleases. *Proc Natl Acad Sci USA* 1989;86:3539-43.
49. Wisniewski M, Balakrishnan M, Palaniappan C, Fay P, Bambara R. The sequential mechanism of HIV reverse transcriptase RNaseH. *J Biol Chem* 2000;275:37664-71.
50. Schultz S, Zhang M, Champoux J. Recognition of internal cleavage sites by retroviral RNaseH. *J Mol Biol* 2004;344:635-52.
51. Telesnitsky A, Goff S. Two defective forms of reverse transcriptase can complement to restore retroviral infectivity. *EMBO J* 1993;12:4433-8.
52. Coffin J. Structure, replication, and recombination of retrovirus genomes: some unifying hypotheses. *Journal of General Virology* 1979;42:1-26.
53. Wisniewski M, Balakrishnan M, Palaniappan C, Fay P, Bambara R. Unique progressive cleavage mechanism of HIV reverse transcriptase RNaseH. *Proc Natl Acad Sci USA* 2000;97:11978-83.
54. Ben-Artzi H, Zeelon E, Amit B, Wortzel A, Gorecki M, Panet A. RNaseH activity of reverse transcriptases on substrates derived from the 5' end of retroviral genome. *J Biol Chem* 1993;268:16465-71.
55. Peliska J, Benkovic S. Mechanism of DNA strand transfer reactions catalyzed by HIV-1 reverse transcriptase. *Science* 1992;258:1112-8.
56. Hu W, Temin H. Effect of gamma radiation on retroviral recombination. *J Virol* 1992;66:4457-63.
57. DeStefano J, Mallaber L, Rodriguez-Rodriguez L, Fay P, Bambara R. Requirements for strand transfer between internal regions of heteropolymer templates by HIV reverse transcriptase. *J Virol* 1992;66:6370-8.
58. DeStefano J, Bambara R, Fay P. The mechanism of HIV reverse transcriptase-catalyzed strand transfer from internal regions of heteropolymeric RNA templates. *J Biol Chem* 1994;269:161-8.
59. Wu W, Blumberg B, Fay P, Bambara R. Strand transfer mediated by HIV reverse transcriptase *in vitro* is promoted by pausing and results in misincorporation. *J Biol Chem* 1995;270:325-32.
60. Levin J, Guo J, Rouzina I, Musier-Forsyth K. Nucleic acid chaperone activity of HIV-1 nucleocapsid protein: critical role in reverse transcription and molecular mechanism. *Prog Nucleic Acids Res Mol Biol* 2005;80:217-86-.
61. Rodriguez-Rodriguez L, Tsuchihashi Z, Fuentes G, Bambara R, Fay P. Influence of HIV nucleocapsid protein on synthesis and strand transfer by the reverse transcriptase *in vitro*. *J Biol Chem* 1995;270:15005-11.
62. Raja A, DeStefano J. Kinetic analysis of the effect of HIV nucleocapsid protein (NcP) on internal strand transfer reactions. *Biochemistry* 1999;38:5178-84.
63. DeStefano J. HIV nucleocapsid protein stimulates strand transfer from internal regions of heteropolymeric RNA templates. *Arch Virol* 1995;140:1775-89.
64. Derebail S, Heath M, DeStefano J. Evidence for the differential effects of nucleocapsid protein on strand transfer in various regions of the HIV genome. *J Biol Chem* 2003;278:15702-12.
65. Kim J, Palaniappan C, Wu W, Fay P, Bambara R. Evidence for a unique mechanism of strand transfer from the transactivation response region of HIV-1. *J Biol Chem* 1997;272:16769-77.
66. Negroni M, Buc H. Copy-choice recombination by reverse transcriptases: reshuffling of genetic markers mediated by RNA chaperones. *Proc Natl Acad Sci USA* 2000;97:6385-90.
67. Roda R, Balakrishnan M, Kim J, Roques B, Fay P, Bambara R. Strand transfer occurs in retroviruses by a pause-initiated two-step mechanism. *J Biol Chem* 2002;277:46900-11.
68. Moumen A, Polomack L, Unge T, Veron M, Buc H, Negroni M. Evidence for a mechanism of recombination during reverse transcription dependent on the structure of the acceptor RNA. *J Biol Chem* 2003;278:15973-82.
69. Negroni M, Buc H. Recombination during reverse transcription: an evaluation of the role of the nucleocapsid protein. *J Mol Biol* 1999;286:15-31.

70. Gao W, Cara A, Gallo R, Lori F. Low levels of deoxynucleotides in peripheral blood lymphocytes: a strategy to inhibit HIV type 1 replication. *Proc Natl Acad Sci USA* 1993;90:8925-8.
71. Pfeiffer J, Topping R, Shin N, Telesnitsky A. Altering the intracellular environment increases the frequency of tandem repeat deletion during Moloney murine leukemia virus reverse transcription. *J Virol* 1999;73:8441-7.
72. Svarovskaia E, Delviks K, Hwang C, Pathak V. Structural determinants of murine leukemia virus reverse transcriptase that affect the frequency of template switching. *J Virol* 2000;74:7171-8.
73. Perno C, Yarchoan R, Cooney D, et al. Inhibition of HIV (HIV-1/HTLV-III_{Ba-L}) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2',3'-dideoxynucleosides. *J Exp Med* 1988;168:1111-25.
74. O'Brien W, Namazi A, Kalhor H, Mao S, Zack J, Chen I. Kinetics of HIV type 1 reverse transcription in blood mononuclear phagocytes are slowed by limitations of nucleotide precursors. *J Virol* 1994;68:1258-63.
75. Balakrishnan M, Fay P, Bambara R. The kissing hairpin sequence promotes recombination within the HIV-1 5' leader region. *J Biol Chem* 2001;276:36482-92.
76. Moumen A, Polomack L, Roques B, Buc H, Negroni M. The HIV-1 repeated sequence R as a robust hot-spot for copy-choice recombination. *Nucleic Acids Res* 2001;29:3814-21.
77. Suo Z, Johnson K. Effect of RNA secondary structure on the kinetics of DNA synthesis catalyzed by HIV-1 reverse transcriptase. *Biochemistry* 1997;36:12459-67.
78. Galetto R, Moumen A, Giacomoni V, Veron M, Charneau P, Negroni M. The structure of HIV-1 genomic RNA in the gp120 gene determines a recombination hot spot *in vivo*. *J Biol Chem* 2004;279:36625-32.
79. Roda R, Balakrishnan M, Hanson M, et al. Role of the reverse transcriptase, nucleocapsid protein, and template structure in the two-step transfer mechanism in retroviral recombination. *J Biol Chem* 2003;278:31536-46.
80. Klasens B, Huthoff H, Das A, Jeeninga R, Berkhout B. The effect of template RNA structure on elongation by HIV-1 reverse transcriptase. *Biochim Biophys Acta* 1999;1444:355-70.
81. Hong M, Harbron E, O'Connor D, et al. Nucleic acid conformational changes essential for HIV-1 nucleocapsid protein-mediated inhibition of self-priming in minus-strand transfer. *J Mol Biol* 2003;325:1-10.
82. Beltz H, Azoulay J, Bernacchi S, et al. Impact of the terminal bulges of HIV-1 cTAR DNA on its stability and the destabilizing activity of the nucleocapsid protein NCp7. *J Mol Biol* 2003;328:95-108.
83. Beltz H, Piemont E, Schaub E, et al. Role of the structure of the top half of HIV-1 cTAR DNA on the nucleic acid destabilizing activity of the nucleocapsid protein NCp7. *J Mol Biol* 2004;338:711-23.
84. Heilman-Miller S, Wu T, Levin J. Alteration of nucleic acid structure and stability modulates the efficiency of minus-strand transfer mediated by the HIV-1 nucleocapsid protein. *J Biol Chem* 2004;279:44154-65.
85. Kanevsky I, Chaminade F, Ficheux D, et al. Specific interactions between HIV-1 nucleocapsid protein and the TAR element. *J Mol Biol* 2005;348:1059-77.
86. Zhuang J, Jetzt A, Sun G, et al. HIV type 1 recombination: rate, fidelity, and putative hot spots. *J Virol* 2002;76:11273-82.
87. An W, Telesnitsky A. Effects of varying sequence similarity on the frequency of repeat deletion during reverse transcription of a HIV type 1 vector. *J Virol* 2002;76:7897-902.
88. Pfeiffer J, Telesnitsky A. Effects of limiting homology at the site of intermolecular recombinogenic template switching during Moloney murine leukemia virus replication. *J Virol* 2001;75:11263-74.
89. Zhang J, Temin H. Rate and mechanism of nonhomologous recombination during a single cycle of retroviral replication. *Science* 1993;259:234-8.
90. Zhang J, Temin H. Retrovirus recombination depends on the length of sequence identity and is not error prone. *J Virol* 1994;68:2409-14.
91. Liu S, Mittler J, Nickle D, et al. Selection for HIV type 1 recombinants in a patient with rapid progression to AIDS. *J Virol* 2002;76:10674-84.
92. Shriner D, Rodrigo A, Nickle D, Mullins J. Pervasive genomic recombination of HIV-1 *in vivo*. *Genetics* 2004;167:1573-83.
93. Najera R, Delgado E, Perez-Alvarez L, Thomson M. Genetic recombination and its role in the development of the HIV-1 pandemic. *Aids* 2002;16(Suppl 4):S3-16.
94. Shankarappa R, Margolick J, Gange S, et al. Consistent viral evolutionary changes associated with the progression of HIV type 1 infection. *J Virol* 1999;73:10489-502.
95. Molla A, Korneyeva M, Gao Q, et al. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. *Nat Med* 1996;2:760-6.
96. Finzi D, Blankson J, Siliciano J, et al. Latent infection of CD4+ T-cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* 1999;5:512-7.
97. Martinez-Picado J, DePasquale M, Kartsonis N, et al. Antiretroviral resistance during successful therapy of HIV type 1 infection. *Proc Natl Acad Sci USA* 2000;97:10948-53.
98. Larder B, Kellam P, Kemp S. Convergent combination therapy can select viable multidrug-resistant HIV-1 *in vitro*. *Nature* 1993;365:451-3.
99. Gaschen B, Taylor J, Yusim K, et al. Diversity considerations in HIV-1 vaccine selection. *Science* 2002;296:2354-60.
100. Moore J, Parren P, Burton D. Genetic subtypes, humoral immunity, and HIV type 1 vaccine development. *J Virol* 2001;75:5721-9.
101. Seaman M, Xu L, Beaudry K, et al. Multiclade HIV type 1 envelope immunogens elicit broad cellular and humoral immunity in rhesus monkeys. *J Virol* 2005;79:2956-63.
102. Van der Groen G, Nyambi P, Beirnaert E, et al. Genetic variation of HIV type 1: relevance of interclade variation to vaccine development. *AIDS Res Hum Retroviruses* 1998;14(Suppl 3):S211-221.
103. Letvin N, Huang Y, Chakrabarti B, et al. Heterologous envelope immunogens contribute to AIDS vaccine protection in rhesus monkeys. *J Virol* 2004;78:7490-7.
104. Chen B, Vogan E, Gong H, Skehel J, Wiley D, Harrison S. Structure of an unliganded simian immunodeficiency virus gp120 core. *Nature* 2005;433:834-41.
105. Burton D, Barbas C 3rd, Persson M, Koenig S, Chanock R, Lerner R. A large array of human monoclonal antibodies to type 1 HIV from combinatorial libraries of asymptomatic seropositive individuals. *Proc Natl Acad Sci USA* 1991;88:10134-7.
106. Barbas C 3rd, Bjorling E, Chiodi F, et al. Recombinant human Fab fragments neutralize human type 1 immunodeficiency virus *in vitro*. *Proc Natl Acad Sci USA* 1992;89:9339-43.
107. Burton D, Pyati J, Koduri R, et al. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science* 1994;266:1024-7.
108. Roben P, Moore J, Thali M, Sodroski J, Barbas C 3rd, Burton D. Recognition properties of a panel of human recombinant Fab fragments to the CD4 binding site of gp120 that show differing abilities to neutralize HIV type 1. *J Virol* 1994;68:4821-8.
109. Cho M, Lee M, Chen C, Matthews T, Martin M. Identification of gp120 regions targeted by a highly potent neutralizing antiserum elicited in a chimpanzee inoculated with a primary HIV type 1 isolate. *J Virol* 2000;74:9749-54.
110. Pantaleo G, Koup R. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat Med* 2004;10:806-10.
111. Robertson D, Hahn B, Sharp P. Recombination in AIDS viruses. *J Mol Evol* 1995;40:249-59.