[Frontiers in Bioscience 8, d143-155, January 1, 2003]

RETROVIRAL RECOMBINATION: REVIEW OF GENETIC ANALYSES

Wei-Shau Hu [1], Terence Rhodes [1,2], Que Dang [1,2], and Vinay Pathak [1]

[1] HIV Drug Resistance Program, National Cancer Institute at Frederick, Frederick, Maryland 21702, USA, [2] Department of Microbiology, Immunology, and Cell Biology, School of Medicine, West Virginia University, Morgantown, West Virginia 26506, USA

TABLE OF CONTENTS

1. ABSTRACT

Retroviruses package two copies of genomic RNA into one virion. One of the essential steps of replication is reverse transcription, in which the virally encoded enzyme reverse transcriptase (RT) uses the packaged RNA as a template to synthesize viral DNA. Because two copies of RNA are present in one virion, it is possible for RT to switch from one copy of the viral RNA to the other copy during DNA synthesis, thereby generating a recombinant containing some genetic information from each of the RNAs. Recombination occurs at high frequencies during retroviral replication. This frequent recombination has a significant impact on the current human immunodeficiency virus type 1 (HIV-1) epidemic as well as the development of retrovirus-based systems for gene therapy. In this review, the rates, mechanisms, and properties of retroviral recombination are summarized from recent genetic studies. Implications of these studies are also discussed.

2. INTRODUCTION

Frequent retroviral recombination was observed in studies performed as early as the 1960s and 1970s (1-3). In many of these studies, two mutant viruses were introduced into cultured cells and the emergence of wild-type viruses provided evidence for the occurrence of recombination events (1). The ability to recombine frequently provides an efficient mechanism for retroviruses to reassort mutations generated during reverse transcription. This ability to redistribute mutations provides an additional mechanism to increase variation in the viral population. High variation in the viral population increases the chance of survival of the population when encountering various environmental challenges.

From the viewpoint of prevention and treatment of diseases caused by retroviruses, high variation in the viral population creates multiple problems. First, the virus can evolve to escape the host immune system (4); this ability increases the pathogenicity of the virus and presents a challenge for vaccine development. Second, a more diverse viral population also increases the probability that some variants in the population can escape antiviral treatments. Both of these problems are clearly present in the current fight against the most devastating pathogen in human history, human immunodeficiency virus type 1 (HIV-1), which causes acquired immunodeficiency syndrome (AIDS) (5). Anti-HIV-1 immune responses have been detected in many infected patients; however, very few patients, if any, can ultimately control the virus replication without antiviral interventions (6). The ability of the virus to escape the immune system also impacts heavily on vaccine development (7). Although HIV-1 was identified and sequenced in the 1980s, an effective vaccine is still not yet available. Despite attempts by many talented researchers using different strategies, the variation in the

viral population continues to make it difficult to generate an effective vaccine that protects against a wide range of variants. High variation in the viral population also becomes a hindrance to antiviral treatments. For example, it has been shown that viruses that are weakly resistant to antiviral drugs can recombine and generate highly drug-resistant variants (8, 9). Similarly, viruses that are resistant to different single drugs can recombine and generate viruses that are resistant to multiple drugs (10). Even with the current combination antiviral drug therapy, there is a significant percentage of patients that fail these therapies (11, 12). Furthermore, with the emergence of the drug-resistant variants, direct transmission of these variants becomes more prevalent (13-16), which creates an even larger problem in the prevention and treatment of the infection. All of these difficulties that we are facing regarding effective antiviral treatment and vaccine development are caused by the unique replication mechanisms used by retroviruses.

Genetic recombination not only generates difficulties in the prevention and treatment of the HIV-1 epidemic, but it also generates potential pitfalls for engineering retroviruses. For example, it is possible to regenerate a replication-competent virus based on the genetic information in vector and helper constructs. Such events can be detrimental to the health of the patient receiving the treatments. However, unlike the HIV-1 epidemic, most of the factors involved in recombination can be controlled when generating and propagating engineered viruses. Understanding the mechanisms of recombination allows the design of engineered vectors to prevent the inadvertent generation of replication-competent virus or other unwanted recombinants.

This review focuses on the genetic aspect of recombination. Therefore, most of the review covers experiments using cell-based systems and studies analyzing field strains of retroviral pathogens. Many of the biochemical studies using purified RNA and proteins are not included in this review but are included in a recent review (17).

3. THE MECHANISMS OF RETROVIRAL RECOMBINATION

3.1. Frequent recombination: the genetic consequences of packaging two viral RNA copies into one viral particle

Retroviruses are unique among viruses in that they package two copies of their RNA, each containing the complete genetic information, into one virion. These two copies of RNA form a dimer in the virion. Electron microscopy (EM) studies have shown that the two RNA copies are linked near the 5' end of the genome (18, 19); biochemical analyses using nondenaturing gels also demonstrated the dimeric nature of the RNA (20). RNA dimers isolated from immature and mature virions have different properties (20). RNA dimers from immature virions have lower thermostability compared with those from mature virions, indicating that during virus maturation, not only are the polyproteins processed but the RNA dimer also undergoes maturation.

In studies reported in the 1970s, recombinants were observed not immediately after two viruses were infected into the same culture but later in the course of the experiments (21). Using modified virus vectors and a single round retroviral replication assay, the prerequisite for recombination was determined (22). Viruses were harvested either from cells containing two genetically distinct proviruses or from cells containing only one provirus. Three types of virions were produced from cells containing two proviruses: virions with two copies of RNA derived from one of the parental proviruses (homozygote), virions with two copies of RNA derived from the other provirus (homozygote), and virions with one copy of RNA derived from each parental provirus (heterozygote). In contrast, cells that only contained one provirus can only make homozygotic virions. Recombination was observed after target cells were infected with virions harvested from two-provirus-containing producer cells that contained both homozygotes and heterozygotes. However, recombination was not observed after target cells were infected with a mixture of two incoming viruses, both of which were homozygotes (22). Hence, the presence of heterozygotes determines whether a high frequency of recombination occurs. This observation demonstrated that the RNAs must be packaged in the same virus particle before recombination occurs. In addition, it also indicates that recombination occurs during reverse transcription of the copackaged genome.

3.2. Proposed mechanisms of retroviral recombination

The viral RNA packaged into the virion can also be used as mRNA; thus, it is the sense or plus-strand RNA (23). The first strand of DNA synthesized, using the RNA as a template, is minus-strand DNA; the second strand of DNA synthesized, using the newly synthesized minus-strand DNA as a template, is plus-strand DNA (figure 1A) (24). The two models that were proposed to explain the retroviral recombination mechanisms suggested that recombination occurs during the synthesis of either minus-strand DNA (figure 1B) or plus-strand DNA (figure 1C).

The forced copy-choice model (figure 1B) (2) proposed that the viral RNA contains many nicks, and that during minus-strand DNA synthesis, reverse transcriptase (RT) encounters these breaks in the RNA. At this time, RT would be forced to switch to the other RNA to continue DNA synthesis, hence the term "forced" copy-choice. This model has been generalized to include all recombination events occurring during minus-strand DNA synthesis and is referred to as minus-strand recombination or recombination during minus-strand DNA synthesis (25).

The DNA strand displacement-assimilation model suggests that recombination occurs during the synthesis of plus-strand DNA (figure 1C) (26). It was observed that plus-strand DNA synthesis in some retroviruses, such as avian sarcoma/leukosis virus (ASLV), was discontinuous and could be initiated at many locations in the viral genome. Therefore, at one stage of reverse transcription, there are multiple initiation points and short stretches of plus-strand DNA anneal to minus-strand DNA. The discontinuous plus-strand DNA could be resolved

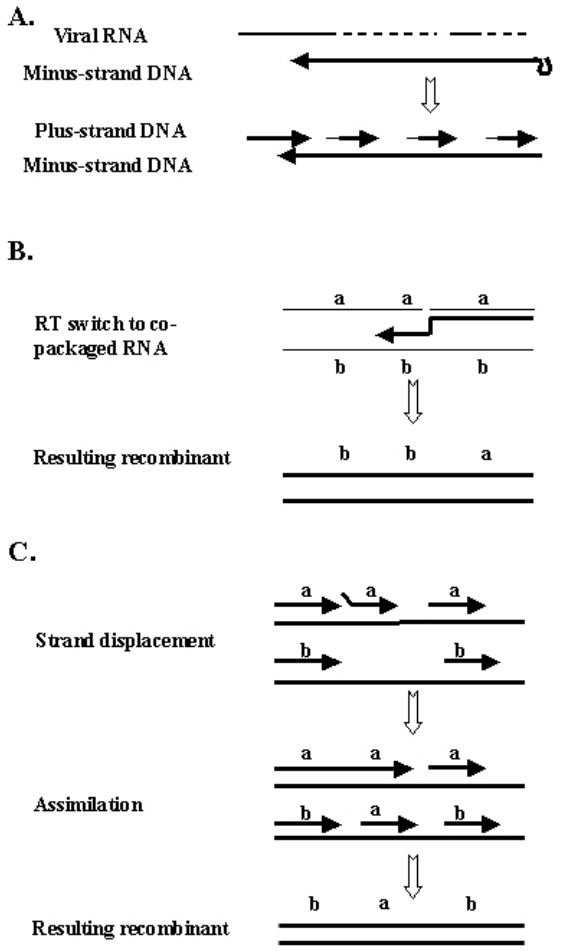

Figure 1. Minus- and plus-strand DNA synthesis and proposed models of retroviral recombination. Light lines: viral RNA, dashed lines: degraded vRNA from the RNA/DNA hybrid, heavy lines: synthesized DNA, arrows: direction of DNA synthesis. (A) Minus- and plus-strand DNA synthesis. Viral RNA is used as a template for minus-strand DNA synthesis, then minus-strand DNA is used as template for plus-strand DNA synthesis. Plus-strand DNA is synthesized in a discontinuous manner. (B) Forced copy-choice model for recombination. Minus-strand DNA synthesis uses one of the copackaged viral RNA as a template (strand "a"). When encountering a break in the strand "a" RNA, RT switches to use the copackaged strand "b" RNA as a template, and the resulting DNA is a recombinant. (C) DNA strand displacement-assimilation model for recombination. The two copackaged RNA molecules are both copied and two molecules of minus-strand DNA are synthesized (shown as "a" and "b"). Plus-strand DNA synthesis occurs in a discontinuous manner. One short stretch of plus-strand DNA from the "a" molecule is displaced by the growing point of the 5' DNA. The displaced "a" DNA fragment anneals to the minus-strand DNA "b". After repair by the host machinery a recombinant could be formed.

either by ligating the short pieces of DNA or by displacing these short pieces with continuous DNA synthesis initiated from near the 5' end of the viral genome. It was proposed that elongation of plus-strand DNA initiated near the 5' end of the viral genome could displace a portion of the short stretches of DNA, which could then anneal to (assimilate) the minus-strand DNA synthesized from the copackaged RNA. This assimilated DNA creates a heteroduplex in the viral DNA, which would be repaired by the host cell machinery after virus integration. If the repair machinery uses the assimilated DNA as a template, then a recombinant is generated.

3.3. Experimental evidence supporting the recombination models

The DNA strand displacement-assimilation model was supported by the observation of the "H" structure during EM studies (26). The H structure was composed of two double-stranded nucleic acids connected by a single-stranded nucleic acid. This provided the physical evidence for the DNA strand displacement-assimilation model.

The first conclusive evidence supporting recombination during minus-strand DNA synthesis was the observation of single cross-over recombinants (27). The recombinants generated from the two models differ in their genotypes (figure 1B, 1C). In the minus-strand recombination model, each recombination event generates a recombinant that appears to have one cross-over. However, in the DNA strand displacement-assimilation model, each recombination event involves the assimilation of one DNA fragment and generates a recombinant with two cross-overs (see resulting provirus in figure 1B and 1C). The genotypes of the recombinants were analyzed using two viral vectors with multiple restriction enzyme site markers (27). It was found that half of the recombinants had only one cross-over whereas the other half had more than one cross-over. The presence of the recombinants with one cross-over clearly indicated that they were the products of the minus-strand recombination model; this was the first supporting evidence for the model. Recombinants containing more than one cross-over could be generated either from the DNA strand displacement-assimilation model or from recombinants that underwent more than one recombination events using the minus-strand recombination model.

Whether the minus-strand recombination model is the main mechanism in retroviral recombination was addressed in a separate study, using two homologous vectors that differed in several restriction enzyme sites (25). The primer binding site (PBS) of one of the vectors was deleted; as a result, only one copy of the viral DNA could be synthesized in a heterozygotic virion. Because recombination via the DNA strand displacement-assimilation model would require the synthesis of two molecules of minus-strand DNA and plus-strand DNA, plus-strand recombination could not occur in these viruses. Therefore, this system only allowed recombination to occur during minus-strand DNA synthesis. After one round of retroviral replication, the resulting recombinants were

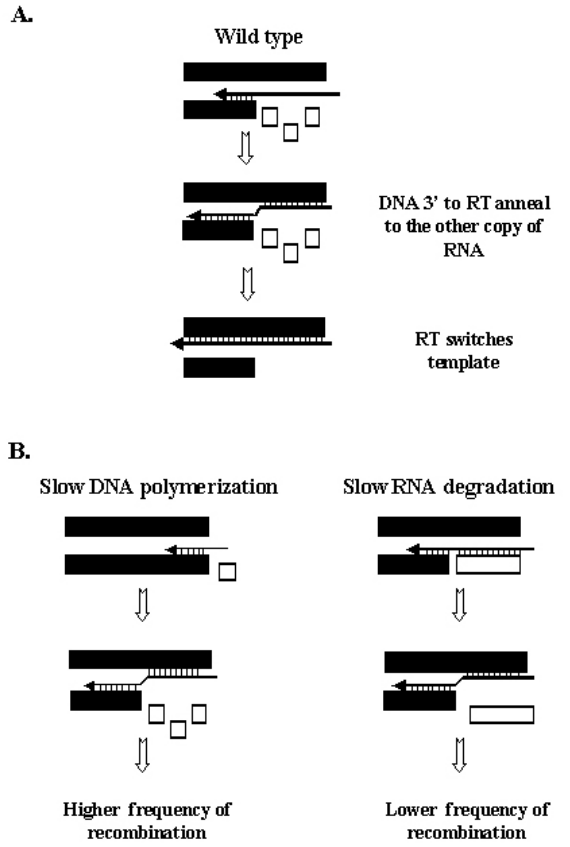

Figure 2. Dynamic copy-choice model for retroviral recombination. Bold arrows and lines: DNA synthesis, black boxes: viral RNA, white boxes: RNase H cleaved RNA, and vertical lines: hydrogen bonds between base pairs. (A) Recombination during reverse transcription using wild-type RT. Wild-type RT is efficient in both DNA polymerization and RNA degradation. RNase H removes the RNA from the DNA:RNA hybrid and exposes the newly synthesized DNA 3' to the RT. This single-stranded DNA can anneal to the copackaged RNA, thereby promoting RT to switch templates, which leads to recombination. (B) Recombination during reverse transcription with altered rate of either DNA polymerization or RNA degradation. When DNA polymerization slows down and RNA degradation remains at the normal rate, DNA 3' to the RT becomes more available to anneal to the other RNA and promotes recombination. When DNA polymerization occurs at normal rate and RNA degradation slows down, much of the DNA 3' to the RT remains in the RNA:DNA hybrid and is less available to anneal to the other RNA, which leads to a lower rate of recombination.

analyzed. It was found that again, half of the recombinants contained one cross-over whereas the other half had more than one cross-over. This study demonstrated that recombination via the minus-strand recombination model can also efficiently generate recombinants with more than one cross-over. In addition, the phenotypes of the recombinants from this study closely resembled those from the first study in which both recombination models could operate. Therefore, these studies demonstrated that the minus-strand recombination model is the major mechanism responsible for retroviral recombination. This conclusion was further supported by a later study that examined recombinants in DNA repair deficient cells (28). The DNA strand displacement-assimilation model proposed that recombinants contained a heteroduplex in their DNA, which would be repaired later by the host cell system. This heteroduplex would not be repaired in a DNA repair deficient cell; however, after cell division, a mixed colony would be generated. Because mixed colonies were not observed in DNA repair deficient cells, this observation lent further support to the conclusion that minus-strand recombination is the major mechanism of recombination.

3.4. Dynamic copy-choice model of retroviral recombination

Much of the intricate molecular mechanisms of minus-strand recombination was revealed in a series of experiments studying direct repeat deletion (29-31). Directly repeated sequences in retroviruses are unstable and are deleted at high frequencies (32-35). During DNA synthesis, RT switches its template from one copy of the repeated sequence to the other, thereby deleting one of the copies plus any intervening sequences. Both direct repeat deletion and recombination operate by the same molecular mechanism that causes template switching. During studies of the location of template switching in repeated sequences, it was found that template switching occurred more frequently toward the 5' end of the directly repeated sequences than at the 3' end of the sequences. Based on this result and other data, it was proposed that the newly synthesized DNA 3' to the RT can pair with the complementary sequences of the other RNA copy to promote RT to switch templates (29). This hypothesis also implies that the availability of the newly synthesized DNA can affect the frequency of recombination.

RT is a dual-function enzyme; it has a DNA polymerase domain plus an RNase H domain (23). During minus-strand DNA synthesis, the polymerase domain of RT copies the RNA template to synthesize DNA. The RNase H domain of RT degrades the RNA in the RNA-DNA complex; RNase H has two modes of action: polymerase dependent and polymerase independent (31). In a series of experiments studying template switching, it was demonstrated that the dynamic steady state between the rates of DNA polymerization and RNase H degradation dictates the frequency of recombination (30, 31). The rate of DNA synthesis was manipulated by either disadvantageous mutations in the polymerase domain or by treatment with drugs to lower the nucleotide pool concentration in the cells to slow down DNA synthesis. The rate of RNA degradation was altered by mutations in the RNase H domain. The rate of template switching by RT that had wild-type DNA polymerase and RNase H activities was defined as the wild-type rate (figure 2A). When DNA synthesis slowed down and RNase H activity remained wild-type, the rate of template switching was higher than the wild-type rate (figure 2B). In contrast, when the DNA synthesis remained wild-type and RNase H

degradation slowed down, the rate of template switching became lower than the wild-type rate (figure 2B). This observation led to the hypothesis that the dynamic steady state between the DNA polymerization and RNA degradation rates dictates the availability of the newly synthesized DNA 3' to the RT for pairing with the other RNA template, and ultimately affects the frequency of template switching (30, 31). For example, if DNA polymerization slows down and RNase H degrades RNA at the normal rate, the newly synthesized DNA becomes more available than those from wild-type to pair with the other RNA template. This increased pairing could promote template switching. In contrast, when DNA synthesis occurs at the normal rate but RNase H activity is lower, the newly synthesized DNA is less available to pair with the other copy of the template and therefore causes a reduction in template switching.

4. THE PROPERTIES OF RETROVIRAL RECOMBINATION

4.1. Recombination rates and the effects of homology on recombination

The rate of homologous recombination during one round of retroviral replication has been measured in both spleen necrosis virus (SNV) and murine leukemia virus (MLV) (22, 36). Using vectors that are highly homologous to each other, the recombination rates between markers that were 1 kb apart were 4% and 4.7% per replication cycle for SNV and MLV, respectively (22, 36). In MLV, recombination rates with markers that were 1.3, 1.9, and 7 kb apart were also measured to be 5%, 7.4%, and 8.2%, respectively (22, 36). This result indicates that when the distance between two markers is less than 2 kb, the recombination rate increases with larger marker distances; however, the recombination rate appears to plateau after 2 kb, and does not increase proportionally with marker distances. Although recombination rates have been measured in this manner and it is important to know this information, the recombination rate is only part of the story. Retroviral recombination has high negative interference (see later) (25, 37), which should be considered when estimating the frequency and pattern of recombination.

In addition to homologous recombination, nonhomologous recombination can also occur during reverse transcription (38, 39). The rate of nonhomologous recombination has been measured in retroviruses by using vectors that do not contain significant homology. It was determined that the overall rate of nonhomologous recombination during one round of retroviral replication is approximately 5×10^{-5}; this is approximately 0.1% of the rate of homologous recombination (38, 39). The rate of recombination between two viruses becomes more frequent with the presence of short stretches of homology between the two viral genomes; in these cases, recombination occurs within the homology (40). The frequency of direct repeat deletion is also proportional to the size of the directly repeated sequences, although the frequency reaches a plateau when the direct repeat is approximately 1 kb (29).

If recombination can occur with short stretches of homology, what is the minimum length of homology needed to mediate template switching events? During analyses of deletion mutants, it was shown that the junctions of deletion often contained short stretches of homology (41). Recent studies revealed that minus-strand DNA transfer could occur with a very short stretch of homology at low efficiencies (42). A systematic study analyzing the lengths necessary for minus-strand DNA transfer indicated that 12 nt is sufficient for accurate and efficient minus-strand DNA transfer (43). Although 12 nt homology allows efficient minus-strand DNA transfer, indicating that a short stretch of homology is physically capable of mediating template switching, this length is not sufficient to allow efficient recombination to occur. The difference is the equilibrium of DNA polymerization and RNA degradation rates as described in the dynamic copy-choice model (31). In minus-strand DNA transfer, DNA polymerization is completely halted at the end of the template, thereby tipping the equilibrium to favor template switching. In contrast, in recombination, with continuous DNA polymerization, a short stretch of homology such as 12 nt does not have sufficient opportunity to allow template switching.

4.2. Retroviral recombination has high negative interference

The recombination rate of SNV was measured to be 4% when two markers were located 1 kb apart (22). With this rate, if recombination events were independent, it would be uncommon for a virus to undergo two recombination events. For example, in an engineered vector with a 3.6-kb genome, only 1 in 7 recombinants and 1 in 49 recombinants are expected to have two or three recombination events, respectively. However, upon examining these viruses, it was found that approximately half of the analyzed recombinant viruses had more than one cross-over, and some had three or four cross-overs (27). This pattern of recombination was also observed when only minus-strand recombination was allowed to generate recombinants (25). Because minus-strand recombination generates only one cross-over per recombination event, it is clear that recombinants with two or three cross-overs were generated by two or three recombination events; thus, recombinants with two or more recombination events were generated far more frequently than expected from the measured recombination rate of a single recombination event. Therefore, by definition, retroviral recombination has high negative interference, which is a term used in genetics to describe the observation of a higher-than-expected frequency of recombinants containing two or more cross-overs (44, 45). Furthermore, these studies demonstrated that high negative interference in SNV was generated by correlated recombination events during minus-strand DNA synthesis (25). In addition to SNV, high negative interference has been observed in MLV-based systems (46). Multiple cross-overs in HIV-1 recombinants were also observed with high frequencies, suggesting that recombination in HIV-1 also has high negative interference (47, 48).

High negative interference has a tremendous implication on generating diversity in a virus population. For example, if two viruses have two advantageous mutations each, the locations of the mutations were such that a minimum of three cross-overs would be needed to generate a viral genome containing all the mutations. If retroviral recombination does not have high negative interference, with a recombination rate of 4% between markers that are 1 kb apart, it would be rare to generate a recombinant with three cross-overs and all four advantageous mutations in one round of retroviral replication. However, 20 of the 47 recombinants analyzed in the aforementioned SNV-based system had 3 or more recombination events (25). Therefore, mutations can be reassorted even more frequently than previously estimated based solely on the recombination rate.

4.3. Position and accuracy of recombination events

Retroviral recombination events have been analyzed using retroviruses with authentic viral sequences as well as with engineered vectors encoding genes derived from other organisms. In both cases, recombination events have been observed throughout the viral genome and occur at various primary sequences (25, 27, 47, 48). Therefore, retroviral recombination events are not dependent on specific sequences or positions in the viral genome.

Although recombination can occur throughout the viral genome, it is unclear whether there are sequences that have enhanced recombination frequencies, i.e., recombination hot spots. It has been reported that recombination occurs preferentially within a stretch of sequences in the 5' untranslated region (5' UTR) termed the "kissing-loop", which was thought to be important in RNA dimerization (49-51). This conclusion was generated while studying recombination between MLV-based vectors with a mutated PBS and endogenous retroviruses. In this study, recombination could only occur in the 5' UTR. Upon examination of multiple recombinant viruses, it was concluded that many of the recombination events occurred at the kissing loop region.

Other studies have also examined recombination events in the 5' UTR. In several independent studies using SNV, MLV, and HIV-1 model systems, the 5' UTR did not appear to experience an increase in recombination events compared with other parts of the viral genome (25, 27, 47, 48). Because the available markers were located further apart, these studies examined the overall recombination events in 5' UTR rather than directly determining the sites of recombination at the kissing- loop region. One possible interpretation for the data from all these studies is that the kissing-loop region has enhanced recombination locally among the sequences in the 5' UTR; however, the entire 5' UTR does not experience more recombination events compared to the other part of the viral genome. Therefore, the kissing-loop region may be a local hot spot but does not increase the overall recombination rate of the 5' UTR.

The fidelity of the template switching events was once suggested to be low with the frequent nontemplate-directed nucleotide addition (52). However, multiple genetic studies later determined that recombination events are accurate. During template switching, RT copies the genetic information from the template without excess errors. Recombination studies analyzing the junctions of multiple recombinants demonstrated the accuracy of the recombination events (40, 50). Other template switching events have also been shown not to be error-prone. For example, direct repeat deletion can be used to reconstitute functional genes, which can achieve close to 100% efficiency (29). The reconstitution of a functional gene indicated that direct repeat deletion is an accurate process since nontemplate addition mutations would create a frameshift, thereby rendering the gene nonfunctional. The accuracy of minus-strand DNA transfer has also been examined. One study reported the presence of non-template addition at the minus-strand DNA transfer junctions in a significant portion of viruses (53). In contrast, several other studies have reported that minus-strand DNA transfers are accurate events, based on analyses of DNA sequences in the transfer junction in several different viruses and the ability to efficiently reconstitute functional genes during minus-strand DNA transfer (43, 42, 54).

4.4. Double infection can indirectly affect the occurrence of recombination

As described earlier, the prerequisite for recombination is the generation of heterozygotic virions that contain copackaged RNA derived from the two different viruses (22). Heterozygotic virions are generated by cells containing two genetically distinct proviruses. Therefore, the frequency with which cells are infected by two viruses has a direct impact on the generation of recombinant viruses.

The fact that frequent recombination is observed in clinical strains of HIV-1 (see later) indicates that infection by more than one virus must occur in infected individuals. Using *in situ* hybridization, one study analyzed infected cells from the germinal center of the spleen from HIV-1-infected patients (55). Sections of infected cells were isolated by microdissection, then proviral genomes were amplified by PCR and subjected to sequence analyses. These analyses indicated that some cells were infected by more than one virus. This result directly demonstrated the presence of multiply infected cells; however, it was difficult to quantify the frequency with which cells are infected by multiple viruses. Current unpublished work in our laboratory examining HIV-1 infection demonstrates that double infection (infection by two different viruses) is not random and occurs more frequently than expected.

If double infection occurs more frequently than expected in HIV-1-infected individuals, it is likely that recombination also occurs more often than previously estimated. The frequent recombination could therefore have a significant impact on the variation of the viral population in patients.

5. RECOMBINATION IN INFECTED HOSTS

Retroviral recombination has long been observed in a wide range of hosts and different viruses. Recombination can

Table 1. Summary of inter-subtype HIV-1 recombinants

Parental subtypes	Geographical locations	References
A/B	United States, Ukraine, Russia, Cameroon	64,65, 66, 67, 68
A/C	Uganda, Belgium, Tanzania, Keyna, India, Zambia, Rwanda	69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79
A/D	Uganda, Tanzania, Kenya, Denmark, Sweden	69, 80, 71, 72, 73, 81, 75, 78, 82
A/E	China, Yugoslavia, Greece, Myanmar, Finland, Cameroon, Thailand	83, 84, 85, 86, 87, 68, 88, 62, 89, 78, 79
A/G	Cameroon, Yugoslavia, South Africa, Nigeria, Senegal, Cameroon, Gabon, Djibouti, Taiwan, Netherlands, Ghana, Kenya, Denmark	90, 84, 91, 92, 93, 94, 95, 96, 97, 98, 99, 73, 100, 81, 75, 101, 68, 102, 103, 88, 89
A/J	Botswana	104
B/C	China, Myanmar	105, 106, 107, 79, 86, 78
B/D	China, Uganda	83, 69
B/F	Argentina, Brazil	108, 109,110, 111, 112, 113, 114, 89, 78, 115
B/E	Myanmar	86
B/G	Spain	116, 117, 118
C/D	Uganda, Tanzania, Denmark	69, 71, 72, 81
C/E	Myanmar	86
F/D	Belgium, Netherlands, South Africa	119, 120
D/G	Russia	121
A/J/U	Cameroon	90
A/G/K/J	West Africa	122
A/E/G/J	Central Africa	123
A/B/E	Thailand	124
A/E/G/H/J/K/U	Kinshasa	125
G/H/F	Belgium, Netherlands	119
A/H/U	Belgium	126
A/G/J	Nigeria, Burkina Faso	93, 127
A/G/I	Greece, Cyprus	128, 129
A/G/I/J	West Africa	130
Group M/Group O	Cameroon	131, 132

impact many aspects of viral pathogenesis. Recombination can reassort mutations to generate viruses with more subtle alterations as well as change properties of the viruses in more drastic manners.

Mink cell focus-inducing (MCF) viruses are examples of recombination in animal hosts that have been reported since the 1970s (1, 56). Some strains of mice have been found to have a high incidence of spontaneous T cell lymphoma. These lymphomas were not caused by an endogenous oncogenic virus but by recombinant MCF viruses that were generated during the lifespan of the mice (1, 56). MCF viruses are recombinants between MLV and endogenous polytropic viruses, and they contain at least a portion of the *env* sequences from polytropic viruses. Because MCF viruses were generated during the lifespan of the mice, various isolates of MCF viruses have different recombination patterns, which provide evidence that they were generated from independent events.

Retroviral recombination in a host was directly demonstrated using simian immunodeficiency virus (SIV) (57). Two strains of SIV, each encoding a mutation in different accessory genes, were injected into a rhesus monkey. Wild-type viruses containing all of the functional accessory genes, most likely the recombinants of the two infecting mutant viruses, were detected from the animal weeks later. The detection of the wild type virus provided evidence that retroviral recombination occurred in the infected host.

According to sequence homology, HIV-1 can be classified into groups M, N, and O. Most of the HIV-1 strains belong to group M (major), which can be further divided into subtypes designated A to K (58). Recombination can occur within the same subtype (intra-subtype) (59, 60) or among different subtypes (inter-subtype) (61). Inter-subtype recombinants have provided some of the most dramatic examples of the impact of HIV-1 recombination in the epidemic. Inter-subtype recombinants are widespread and have been frequently isolated. Some of these recombinants have become very prevalent and have caused epidemics in certain regions. For example, A/E inter-subtype recombinant viruses are responsible for the majority of the HIV-1 epidemic in southeast Asia such as Thailand (62, 63). Table 1 summarizes many of the reported inter-subtype recombinants, including the origins of the sequences and the geographic locations where these recombinants were isolated. There are several striking features in this summary. First, inter-subtype recombination occurs in all of the group M subtypes and is not limited to any specific subtypes. Second, recombinants with sequences from multiple subtypes are also observed. These recombinants are probably the result of accumulated recombination events that occurred in multiple viral replication cycles. It is likely that recombinants from two subtypes were first generated, and then went through further inter-subtype recombination to generate isolates with sequences derived from multiple subtypes. Third, inter-subtype recombinants

are distributed around the world. For example, A/B inter-subtype recombinants were isolated from three continents. Lastly, recombination could occur between diverse viruses, such as group M and group O viruses. These inter-subtype recombinant viruses present an excellent demonstration of the flexibility of the HIV-1 genome. It is the same flexibility of the HIV-1 genome that challenges the prevention and treatment of the HIV-1 epidemic.

6. CONCLUSIONS AND IMPLICATIONS

Frequent recombination occurs during retroviral replication. Many aspects of recombination were revealed from studies performed in past decades, such as the rates, mechanisms, properties, and impact of this frequent event. Understanding the factors that affect recombination allows the design of improved retrovirus-based systems for gene delivery. Insights into the molecular mechanism of recombination can also aid in the development of antiviral interventions. The dynamic copy-choice model illustrates the intricate balance that viral proteins have to achieve in order to regulate recombination. It is possible to design antiviral treatments to interfere with virus replication and simultaneously reduce the ability of the virus to recombine. For example, one such target is the RNase H domain of the RT. RNase H activity is essential for retroviral replication; therefore, anti-RNase H drugs should reduce the virus load. In addition, inhibition of RNase H activity also should reduce recombination, thereby decreasing variation in the viral population.

In summary, frequent recombination has provided retroviruses with an advantage in developing diversity in the viral population. Understanding all aspects of retroviral recombination enables us to estimate both the potential and limitation of these viruses, as well as to design strategies to control and treat retroviral infections.

7. ACKNOWLEDGEMENT

We sincerely thank Anne Arthur for her expert editorial help on this manuscript. The work conducted in the Hu laboratory and Pathak laboratory is supported by the HIV Drug Resistance Program, National Cancer Institute.

8. REFERENCES

1. Linial, M. & D. Blair: Genetics of retroviruses. Cold Spring Harbor Laboratory, New York, NY (1985)
2. Coffin, J. M.: Structure, replication, and recombination of retrovirus genomes: some unifying hypotheses. *J Gen Virol* 42, 1-26 (1979)
3. Varmus, H. & R. Swanstrom: Replication of retroviruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1985)
4. Johnson, W. E. & R. C. Desrosiers: Viral persistance: HIV's strategies of immune system evasion. *Annu Rev Med* 53, 499-518 (2002)
5. Blattner, W., R. C. Gallo & H. M. Temin: HIV causes AIDS. *Science* 241, 515-6 (1988)
6. McMichael, A. J. & S. L. Rowland-Jones: Cellular immune responses to HIV. *Nature* 410, 980-7 (2001)
7. Barouch, D. H., J. Kunstman, M. J. Kuroda, J. E. Schmitz, S. Santra, F. W. Peyerl, G. R. Krivulka, K. Beaudry, M. A. Lifton, D. A. Gorgone, D. C. Montefiori, M. G. Lewis, S. M. Wolinsky & N. L. Letvin: Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. *Nature* 415, 335-9 (2002)
8. Kellam, P., C. A. Boucher & B. A. Larder: Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. *Proc Natl Acad Sci U S A* 89, 1934-8 (1992)
9. Kellam, P. & B. A. Larder: Retroviral recombination can lead to linkage of reverse transcriptase mutations that confer increased zidovudine resistance. *J Virol* 69, 669-74 (1995)
10. Gu, Z., Q. Gao, E. A. Faust & M. A. Wainberg: Possible involvement of cell fusion and viral recombination in generation of human immunodeficiency virus variants that display dual resistance to AZT and 3TC. *J Gen Virol* 76, 2601-5 (1995)
11. Aleman, S., K. Soderbarg, U. Visco-Comandini, G. Sitbon & A. Sonnerborg: Drug resistance at low viraemia in HIV-1-infected patients with antiretroviral combination therapy. *Aids* 16, 1039-44 (2002)
12. Loutfy, M. R. & S. L. Walmsley: Salvage antiretroviral therapy in HIV infection. *Expert Opin Pharmacother* 3, 81-90 (2002)
13. Boden, D., A. Hurley, L. Zhang, Y. Cao, Y. Guo, E. Jones, J. Tsay, J. Ip, C. Farthing, K. Limoli, N. Parkin & M. Markowitz: HIV-1 drug resistance in newly infected individuals. *Jama* 282, 1135-41 (1999)
14. Sonnerburg, A., B. Johansson, S. Ayehunie & I. Julander: Transmission of zidovudine-resistant HIV-1. *Aids* 7, 1684-5 (1993)
15. Siegrist, C. A., S. Yerly, L. Kaiser, C. A. Wyler & L. Perrin: Mother to child transmission of zidovudine-resistant HIV-1. *Lancet* 344, 1771-2 (1994)
16. Angarano, G., L. Monno, A. Appice, A. Giannelli, C. Romanelli, C. Fico & G. Pastore: Transmission of zidovudine-resistant HIV-1 through heterosexual contacts. *Aids* 8, 1013-4 (1994)
17. Negroni, M. & H. Buc: Mechanisms of retroviral recombination. *Annu Rev Genet* 35, 275-302 (2001)
18. Kung, H.-J., J. M. Bailey, N. Davidson, P. K. Vogt, M. O. Nicolson & R. M. McAllister: Electron microscope studies of tumor virus RNA. *Cold Spring Harbor Quant. Biol.* 39, 827-834 (1975)
19. Kung, H. J., S. Hu, W. Bender, J. M. Bailey, N. Davidson, M. O. Nicolson & R. M. McAllister: RD-114, baboon, and woolly monkey viral RNA's compared in size and structure. *Cell* 7, 609-20 (1976)
20. Fu, W. & A. Rein: Maturation of dimeric viral RNA of Moloney murine leukemia virus. *J Virol* 67, 5443-9 (1993)
21. Wyke, J. A., J. G. Bell & J. A. Beamand: Genetic recombination among temperature-sensitive mutnats of Rous sarcoma virus. *Cold Spring Harb Symp Quant Biol* 39 Pt 2, 897-905 (1975)
22. Hu, W. S. & H. M. Temin: Genetic consequences of packaging two RNA genomes in one retroviral particle: pseudodiploidy and high rate of genetic recombination. *Proc Natl Acad Sci U S A* 87, 1556-60 (1990)
23. Vogt, V. M.: Retroviral virions and genomes. Cold Spring Harbor Press, Cold Spring Harbor, New York (1997)

24. Gilboa, E., S. W. Mitra, S. Goff & D. Baltimore: A detailed model of reverse transcription and tests of crucial aspects. *Cell* 18, 93-100 (1979)
25. Anderson, J. A., R. J. Teufel, 2nd, P. D. Yin & W. S. Hu: Correlated template-switching events during minus-strand DNA synthesis: a mechanism for high negative interference during retroviral recombination. *J Virol* 72, 1186-94 (1998)
26. Junghans, R. P., L. R. Boone & A. M. Skalka: Retroviral DNA H structures: displacement-assimilation model of recombination. *Cell* 30, 53-62 (1982)
27. Hu, W. S. & H. M. Temin: Retroviral recombination and reverse transcription. *Science* 250, 1227-33 (1990)
28. Zhang, J., L. Y. Tang, T. Li, Y. Ma & C. M. Sapp: Most retroviral recombinations occur during minus-strand DNA synthesis. *J Virol* 74, 2313-22 (2000)
29. Delviks, K. A. & V. K. Pathak: Effect of distance between homologous sequences and 3' homology on the frequency of retroviral reverse transcriptase template switching. *J Virol* 73, 7923-32 (1999)
30. Svarovskaia, E. S., K. A. Delviks, C. K. Hwang & V. K. Pathak: Structural determinants of murine leukemia virus reverse transcriptase that affect the frequency of template switching. *J Virol* 74, 7171-8 (2000)
31. Hwang, C. K., E. S. Svarovskaia & V. K. Pathak: Dynamic copy choice: steady state between murine leukemia virus polymerase and polymerase-dependent RNase H activity determines frequency of in vivo template switching. *Proc Natl Acad Sci U S A* 98, 12209-14 (2001)
32. Julias, J. G., D. Hash & V. K. Pathak: E- vectors: development of novel self-inactivating and self-activating retroviral vectors for safer gene therapy. *J Virol* 69, 6839-46 (1995)
33. Delviks, K. A., W. S. Hu & V. K. Pathak: Psi- vectors: murine leukemia virus-based self-inactivating and self-activating retroviral vectors. *J Virol* 71, 6218-24 (1997)
34. Pfeiffer, J. K., R. S. Topping, N. H. Shin & A. Telesnitsky: Altering the intracellular environment increases the frequency of tandem repeat deletion during Moloney murine leukemia virus reverse transcription. *J Virol* 73, 8441-7 (1999)
35. An, W. & A. Telesnitsky: Frequency of direct repeat deletion in a human immunodeficiency virus type 1 vector during reverse transcription in human cells. *Virology* 286, 475-82 (2001)
36. Anderson, J. A., E. H. Bowman & W. S. Hu: Retroviral recombination rates do not increase linearly with marker distance and are limited by the size of the recombining subpopulation. *J Virol* 72, 1195-202 (1998)
37. Hu, W. S., E. H. Bowman, K. A. Delviks & V. K. Pathak: Homologous recombination occurs in a distinct retroviral subpopulation and exhibits high negative interference. *J Virol* 71, 6028-36 (1997)
38. Zhang, J. & H. M. Temin: Rate and mechanism of nonhomologous recombination during a single cycle of retroviral replication. *Science* 259, 234-8 (1993)
39. Hajjar, A. M. & M. L. Linial: A model system for nonhomologous recombination between retroviral and cellular RNA. *J Virol* 67, 3845-53 (1993)
40. Zhang, J. & H. M. Temin: Retrovirus recombination depends on the length of sequence identity and is not error prone. *J Virol* 68, 2409-14 (1994)
41. Pathak, V. K. & H. M. Temin: Broad spectrum of in vivo forward mutations, hypermutations, and mutational hotspots in a retroviral shuttle vector after a single replication cycle: deletions and deletions with insertions. *Proc Natl Acad Sci U S A* 87, 6024-8 (1990)
42. Yin, P. D., V. K. Pathak, A. E. Rowan, R. J. Teufel, 2nd & W. S. Hu: Utilization of nonhomologous minus-strand DNA transfer to generate recombinant retroviruses. *J Virol* 71, 2487-94 (1997)
43. Dang, Q. & W. S. Hu: Effects of homology length in the repeat region on minus-strand DNA transfer and retroviral replication. *J Virol* 75, 809-20 (2001)
44. White, R. L. & M. S. Fox: On the molecular basis of high negative interference. *Proc Natl Acad Sci U S A* 71, 1544-8 (1974)
45. Chase, M. & A. Doermann: High negative interference over short segments of the genetic structure of bacteriophage T4. *Genetics* 43, 332-353 (1958)
46. Anderson, J. A., V. K. Pathak & W. S. Hu: Effect of the murine leukemia virus extended packaging signal on the rates and locations of retroviral recombination. *J Virol* 74, 6953-63 (2000)
47. Jetzt, A. E., H. Yu, G. J. Klarmann, Y. Ron, B. D. Preston & J. P. Dougherty: High rate of recombination throughout the human immunodeficiency virus type 1 genome. *J Virol* 74, 1234-40 (2000)
48. Clavel, F., M. D. Hoggan, R. L. Willey, K. Strebel, M. A. Martin & R. Repaske: Genetic recombination of human immunodeficiency virus. *J Virol* 63, 1455-9 (1989)
49. Lund, A. H., J. G. Mikkelsen, J. Schmidt, M. Duch & F. S. Pedersen: The kissing-loop motif is a preferred site of 5' leader recombination during replication of SL3-3 murine leukemia viruses in mice. *J Virol* 73, 9614-8 (1999)
50. Mikkelsen, J. G., A. H. Lund, M. Duch & F. S. Pedersen: Recombination in the 5' leader of murine leukemia virus is accurate and influenced by sequence identity with a strong bias toward the kissing- loop dimerization region. *J Virol* 72, 6967-78 (1998)
51. Mikkelsen, J. G., A. H. Lund, K. D. Kristensen, M. Duch, M. S. Sorensen, P. Jorgensen & F. S. Pedersen: A preferred region for recombinational patch repair in the 5' untranslated region of primer binding site-impaired murine leukemia virus vectors. *J Virol* 70, 1439-47 (1996)
52. Peliska, J. A. & S. J. Benkovic: Fidelity of in vitro DNA strand transfer reactions catalyzed by HIV-1 reverse transcriptase. *Biochemistry* 33, 3890-5 (1994)
53. Kulpa, D., R. Topping & A. Telesnitsky: Determination of the site of first strand transfer during Moloney murine leukemia virus reverse transcription and identification of strand transfer-associated reverse transcriptase errors. *Embo J* 16, 856-65 (1997)
54. Cheslock, S. R., J. A. Anderson, C. K. Hwang, V. K. Pathak & W. S. Hu: Utilization of nonviral sequences for minus-strand DNA transfer and gene reconstitution during retroviral replication. *J Virol* 74, 9571-9 (2000)
55. Gratton, S., R. Cheynier, M. J. Dumaurier, E. Oksenhendler & S. Wain-Hobson: Highly restricted spread of HIV-1 and multiply infected cells within splenic germinal centers. *Proc Natl Acad Sci U S A* 97, 14566-71 (2000)
56. Fan, H.: Leukemogenesis by Moloney murine leukemia virus: a multistep process. *Trends Microbiol* 5, 74-82 (1997)

57. Wooley, D. P., R. A. Smith, S. Czajak & R. C. Desrosiers: Direct demonstration of retroviral recombination in a rhesus monkey. *J Virol* 71, 9650-3 (1997)
58. *Human Retroviruses and AIDS 1998: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences* http://hiv-web.lanl.gov
59. Saksena, N. K., B. Wang, Y. C. Ge, S. H. Xiang, D. E. Dwyer & A. L. Cunningham: Coinfection and genetic recombination between HIV-1 strains: possible biological implications in Australia and South East Asia. *Ann Acad Med Singapore* 26, 121-7 (1997)
60. Groenink, M., A. C. Andeweg, R. A. Fouchier, S. Broersen, R. C. van der Jagt, H. Schuitemaker, R. E. de Goede, M. L. Bosch, H. G. Huisman & M. Tersmette: Phenotype-associated env gene variation among eight related human immunodeficiency virus type 1 clones: evidence for in vivo recombination and determinants of cytotropism outside the V3 domain. *J Virol* 66, 6175-80 (1992)
61. http://hiv-web.lanl.gov/content/hiv-db/REVIEWS/PEETERS2000/Peeters.html
62. Gao, F., D. L. Robertson, S. G. Morrison, H. Hui, S. Craig, J. Decker, P. N. Fultz, M. Girard, G. M. Shaw, B. H. Hahn & P. M. Sharp: The heterosexual human immunodeficiency virus type 1 epidemic in Thailand is caused by an intersubtype (A/E) recombinant of African origin. *J Virol* 70, 7013-29 (1996)
63. McCutchan, F. E., P. A. Hegerich, T. P. Brennan, P. Phanuphak, P. Singharaj, A. Jugsudee, P. W. Berman, A. M. Gray, A. K. Fowler & D. S. Burke: Genetic variants of HIV-1 in Thailand. *AIDS Res Hum Retroviruses* 8, 1887-95 (1992)
64. Womack, C., W. Roth, C. Newman, J. P. Rissing, R. Lovell, D. Haburchak, M. Essex & V. C. Bond: Identification of non-B human immunodeficiency virus type 1 subtypes in rural Georgia. *J Infect Dis* 183, 138-42 (2001)
65. Liitsola, K., K. Holm, A. Bobkov, V. Pokrovsky, T. Smolskaya, P. Leinikki, S. Osmanov & M. Salminen: An AB recombinant and its parental HIV type 1 strains in the area of the former Soviet Union: low requirements for sequence identity in recombination. UNAIDS Virus Isolation Network. *AIDS Res Hum Retroviruses* 16, 1047-53 (2000)
66. Liitsola, K., I. Tashkinova, T. Laukkanen, G. Korovina, T. Smolskaja, O. Momot, N. Mashkilleyson, S. Chaplinskas, H. Brummer-Korvenkontio, J. Vanhatalo, P. Leinikki & M. O. Salminen: HIV-1 genetic subtype A/B recombinant strain causing an explosive epidemic in injecting drug users in Kaliningrad. *Aids* 12, 1907-19 (1998)
67. Bobkov, A., E. Kazennova, L. Selimova, M. Bobkova, T. Khanina, N. Ladnaya, A. Kravchenko, V. Pokrovsky, R. Cheingsong-Popov & J. Weber: A sudden epidemic of HIV type 1 among injecting drug users in the former Soviet Union: identification of subtype A, subtype B, and novel gagA/envB recombinants. *AIDS Res Hum Retroviruses* 14, 669-76 (1998)
68. Takehisa, J., L. Zekeng, E. Ido, I. Mboudjeka, H. Moriyama, T. Miura, M. Yamashita, L. G. Gurtler, M. Hayami & L. Kaptue: Various types of HIV mixed infections in Cameroon. *Virology* 245, 1-10 (1998)
69. Yirrell, D. L., P. Kaleebu, D. Morgan, C. Watera, B. Magambo, F. Lyagoba & J. Whitworth: Inter- and intragenic intersubtype HIV-1 recombination in rural and semi-urban Uganda. *Aids* 16, 279-86 (2002)
70. Snoeck, J., S. Van Dooren, K. Van Laethem, I. Derdelinckx, E. Van Wijngaerden, E. De Clercq & A. M. Vandamme: Prevalence and origin of HIV-1 group M subtypes among patients attending a Belgian hospital in 1999. *Virus Res* 85, 95-107 (2002)
71. Becker-Pergola, G., J. L. Mellquist, L. Guay, F. Mmiro, C. Ndugwa, P. Kataaha, J. B. Jackson & S. H. Eshleman: Identification of diverse HIV type 1 subtypes and dual HIV type 1 infection in pregnant Ugandan women. *AIDS Res Hum Retroviruses* 16, 1099-104 (2000)
72. Renjifo, B., P. Gilbert, B. Chaplin, F. Vannberg, D. Mwakagile, G. Msamanga, D. Hunter, W. Fawzi & M. Essex: Emerging recombinant human immunodeficiency viruses: uneven representation of the envelope V3 region. *Aids* 13, 1613-21 (1999)
73. Neilson, J. R., G. C. John, J. K. Carr, P. Lewis, J. K. Kreiss, S. Jackson, R. W. Nduati, D. Mbori-Ngacha, D. D. Panteleeff, S. Bodrug, C. Giachetti, M. A. Bott, B. A. Richardson, J. Bwayo, J. Ndinya-Achola & J. Overbaugh: Subtypes of human immunodeficiency virus type 1 and disease stage among women in Nairobi, Kenya. *J Virol* 73, 4393-403 (1999)
74. Lole, K. S., R. C. Bollinger, R. S. Paranjape, D. Gadkari, S. S. Kulkarni, N. G. Novak, R. Ingersoll, H. W. Sheppard & S. C. Ray: Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol* 73, 152-60 (1999)
75. Carr, J. K., T. Laukkanen, M. O. Salminen, J. Albert, A. Alaeus, B. Kim, E. Sanders-Buell, D. L. Birx & F. E. McCutchan: Characterization of subtype A HIV-1 from Africa by full genome sequencing. *Aids* 13, 1819-26 (1999)
76. Salminen, M. O., J. K. Carr, D. L. Robertson, P. Hegerich, D. Gotte, C. Koch, E. Sanders-Buell, F. Gao, P. M. Sharp, B. H. Hahn, D. S. Burke & F. E. McCutchan: Evolution and probable transmission of intersubtype recombinant human immunodeficiency virus type 1 in a Zambian couple. *J Virol* 71, 2647-55 (1997)
77. Kampinga, G. A., A. Simonon, P. Van de Perre, E. Karita, P. Msellati & J. Goudsmit: Primary infections with HIV-1 of women and their offspring in Rwanda: findings of heterogeneity at seroconversion, coinfection, and recombinants of HIV-1 subtypes A and C. *Virology* 227, 63-76 (1997)
78. Cornelissen, M., G. Kampinga, F. Zorgdrager & J. Goudsmit: Human immunodeficiency virus type 1 subtypes defined by env show high frequency of recombinant gag genes. The UNAIDS Network for HIV Isolation and Characterization. *J Virol* 70, 8209-12 (1996)
79. Piyasirisilp, S., F. E. McCutchan, J. K. Carr, E. Sanders-Buell, W. Liu, J. Chen, R. Wagner, H. Wolf, Y. Shao, S. Lai, C. Beyrer & X. F. Yu: A recent outbreak of human immunodeficiency virus type 1 infection in southern China was initiated by two highly homogeneous, geographically separated strains, circulating recombinant form AE and a novel BC recombinant. *J Virol* 74, 11286-95 (2000)
80. Eshleman, S. H., M. J. Gonzales, G. Becker-Pergola, S. C. Cunningham, L. A. Guay, J. B. Jackson & R. W. Shafer: Identification of Ugandan HIV type 1 variants with unique

patterns of recombination in pol involving subtypes A and D. *AIDS Res Hum Retroviruses* 18, 507-11 (2002)
81. Iversen, A. K., G. H. Learn, L. Fugger, J. Gerstoft, J. I. Mullins & P. Skinhoj: Presence of multiple HIV subtypes and a high frequency of subtype chimeric viruses in heterosexually infected women. *J Acquir Immune Defic Syndr* 22, 325-32 (1999)
82. Leitner, T., D. Escanilla, S. Marquina, J. Wahlberg, C. Brostrom, H. B. Hansson, M. Uhlen & J. Albert: Biological and molecular characterization of subtype D, G, and A/D recombinant HIV-1 transmissions in Sweden. *Virology* 209, 136-46 (1995)
83. Yu, X. F., W. Liu, J. Chen, W. Kong, B. Liu, Q. Zhu, F. Liang, F. McCutchan, S. Piyasirisilp & S. Lai: Maintaining low HIV type 1 env genetic diversity among injection drug users infected with a B/C recombinant and CRF01_AE HIV type 1 in southern China. *AIDS Res Hum Retroviruses* 18, 167-70 (2002)
84. Stanojevic, M., A. Papa, E. Papadimitriou, S. Zerjav, D. Jevtovic, D. Salemovic, T. Jovanovic & A. Antoniadis: HIV-1 subtypes in Yugoslavia. *AIDS Res Hum Retroviruses* 18, 519-22 (2002)
85. Papa, A., E. Papadimitriou, A. Papoutsi, V. Kiosses & A. Antoniadis: HIV-1 subtypes and circulating recombinant forms (CRFs) in Northern Greece. *Virus Res* 85, 85-93 (2002)
86. Motomura, K., S. Kusagawa, K. Kato, K. Nohtomi, H. H. Lwin, K. M. Tun, M. Thwe, K. Y. Oo, S. Lwin, O. Kyaw, M. Zaw, Y. Nagai & Y. Takebe: Emergence of new forms of human immunodeficiency virus type 1 intersubtype recombinants in central Myanmar. *AIDS Res Hum Retroviruses* 16, 1831-43 (2000)
87. Liitsola, K., M. Ristola, P. Holmstrom, M. Salminen, H. Brummer-Korvenkontio, S. Simola, J. Suni & P. Leinikki: An outbreak of the circulating recombinant form AECM240 HIV-1 in the Finnish injection drug user population. *Aids* 14, 2613-5 (2000)
88. McCutchan, F. E., M. O. Salminen, J. K. Carr & D. S. Burke: HIV-1 genetic diversity. *Aids* 10 Suppl 3, S13-20 (1996)
89. Gao, F., S. G. Morrison, D. L. Robertson, C. L. Thornton, S. Craig, G. Karlsson, J. Sodroski, M. Morgado, B. Galvao-Castro, H. von Briesen & et al.: Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type 1 sequence subtypes A through G. The WHO and NIAID Networks for HIV Isolation and Characterization. *J Virol* 70, 1651-67 (1996)
90. Tebit, D. M., L. Zekeng, L. Kaptue, M. Salminen, H. G. Krausslich & O. Herchenroder: Genotypic and phenotypic analysis of HIV type 1 primary isolates from western Cameroon. *AIDS Res Hum Retroviruses* 18, 39-48 (2002)
91. Nyambi, P., L. Heyndrickx, K. Vereecken, S. Burda, K. De Houwer, S. Coppens, M. Urbanski, C. Williams, P. Ndumbe & W. Janssens: Predominance of infection with HIV-1 circulating recombinant form CRF02_AG in major Cameroonian cities and towns. *Aids* 16, 295-6 (2002)
92. Bredell, H., G. Hunt, A. Casteling, T. Cilliers, C. Rademeyer, M. Coetzer, S. Miller, D. Johnson, C. T. Tiemessen, D. J. Martin, C. Williamson & L. Morris: HIV-1 Subtype A, D, G, AG and Unclassified Sequences Identified in South Africa. *AIDS Res Hum Retroviruses* 18, 681-3 (2002)
93. Agwale, S. M., C. Zeh, K. E. Robbins, L. Odama, A. Saekhou, A. Edubio, M. Njoku, N. Sani-Gwarzo, M. S. Gboun, F. Gao, M. Reitz, D. Hone, D. Pieniazek, C. Wambebe & M. L. Kalish: Molecular surveillance of HIV-1 field strains in Nigeria in preparation for vaccine trials(1). *Vaccine* 20, 2131-9 (2002)
94. Tscherning-Casper, C., G. Dolcini, P. Mauclere, E. M. Fenyo, F. Barre-Sinoussi, J. Albert & E. Menu: Evidence of the existence of a new circulating recombinant form of HIV type 1 subtype A/J in Cameroon. The European Network on the Study of In Utero Transmission of HIV-1. *AIDS Res Hum Retroviruses* 16, 1313-8 (2000)
95. Montavon, C., C. Toure-Kane, F. Liegeois, E. Mpoudi, A. Bourgeois, L. Vergne, J. L. Perret, A. Boumah, E. Saman, S. Mboup, E. Delaporte & M. Peeters: Most env and gag subtype A HIV-1 viruses circulating in West and West Central Africa are similar to the prototype AG recombinant virus IBNG. *J Acquir Immune Defic Syndr* 23, 363-74 (2000)
96. Lee, C. N., W. K. Wang, W. S. Fan, S. J. Twu, S. C. Chen, M. C. Sheng & M. Y. Chen: Determination of human immunodeficiency virus type 1 subtypes in Taiwan by vpu gene analysis. *J Clin Microbiol* 38, 2468-74 (2000)
97. Janssens, W., M. O. Salminen, T. Laukkanen, L. Heyndrickx, A. van der, R. Colebunders, F. E. McCutchan & G. van der Groen: Near full-length genome analysis of HIV type 1 CRF02.AG subtype C and CRF02.AG subtype G recombinants. *AIDS Res Hum Retroviruses* 16, 1183-9 (2000)
98. Cornelissen, M., R. van Den Burg, F. Zorgdrager & J. Goudsmit: Spread of distinct human immunodeficiency virus type 1 AG recombinant lineages in Africa. *J Gen Virol* 81, 515-23 (2000)
99. Candotti, D., Y. Adu-Sarkodie, F. Davies, E. Baldrich-Rubio, K. Stirrups, H. Lee & J. P. Allain: AIDS in an HIV-seronegative Ghanaian woman with intersubtype A/G recombinant HIV-1 infection. *J Med Virol* 62, 1-8 (2000)
100. McCutchan, F. E., J. K. Carr, M. Bajani, E. Sanders-Buell, T. O. Harry, T. C. Stoeckli, K. E. Robbins, W. Gashau, A. Nasidi, W. Janssens & M. L. Kalish: Subtype G and multiple forms of A/G intersubtype recombinant human immunodeficiency virus type 1 in Nigeria. *Virology* 254, 226-34 (1999)
101. Andersson, S., H. Norrgren, F. Dias, G. Biberfeld & J. Albert: Molecular characterization of human immunodeficiency virus (HIV)-1 and -2 in individuals from guinea-bissau with single or dual infections: predominance of a distinct HIV-1 subtype A/G recombinant in West Africa. *Virology* 262, 312-20 (1999)
102. Carr, J. K., M. O. Salminen, J. Albert, E. Sanders-Buell, D. Gotte, D. L. Birx & F. E. McCutchan: Full genome sequences of human immunodeficiency virus type 1 subtypes G and A/G intersubtype recombinants. *Virology* 247, 22-31 (1998)
103. Takehisa, J., M. Osei-Kwasi, N. K. Ayisi, O. Hishida, T. Miura, T. Igarashi, J. Brandful, W. Ampofo, V. B. Netty, M. Mensah, M. Yamashita, E. Ido & M. Hayami: Phylogenetic analysis of human immunodeficiency virus 1 in Ghana. *Acta Virol* 41, 51-4 (1997)

104. Novitsky, V. A., S. Gaolekwe, M. F. McLane, T. P. Ndung'u, B. T. Foley, F. Vannberg, R. Marlink & M. Essex: HIV type 1 A/J recombinant with a pronounced pol gene mosaicism. *AIDS Res Hum Retroviruses* 16, 1015-20 (2000)
105. Yang, R., X. Xia, S. Kusagawa, C. Zhang, K. Ben & Y. Takebe: On-going generation of multiple forms of HIV-1 intersubtype recombinants in the Yunnan Province of China. *Aids* 16, 1401-7 (2002)
106. Yu, X. F., W. Liu, J. Chen, W. Kong, B. Liu, J. Yang, F. Liang, F. McCutchan, S. Piyasirisilp & S. Lai: Rapid dissemination of a novel B/C recombinant HIV-1 among injection drug users in southern China. *Aids* 15, 523-5 (2001)
107. Su, L., M. Graf, Y. Zhang, H. von Briesen, H. Xing, J. Kostler, H. Melzl, H. Wolf, Y. Shao & R. Wagner: Characterization of a virtually full-length human immunodeficiency virus type 1 genome of a prevalent intersubtype (C/B') recombinant strain in China. *J Virol* 74, 11367-76 (2000)
108. Thomson, M. M., E. Delgado, I. Herrero, M. L. Villahermosa, E. Vazquez-de Parga, M. T. Cuevas, R. Carmona, L. Medrano, L. Perez-Alvarez, L. Cuevas & R. Najera: Diversity of mosaic structures and common ancestry of human immunodeficiency virus type 1 BF intersubtype recombinant viruses from Argentina revealed by analysis of near full-length genome sequences. *J Gen Virol* 83, 107-19 (2002)
109. Vicente, A. C., K. Otsuki, N. B. Silva, M. C. Castilho, F. S. Barros, D. Pieniazek, D. Hu, M. A. Rayfield, G. Bretas & A. Tanuri: The HIV epidemic in the Amazon Basin is driven by prototypic and recombinant HIV-1 subtypes B and F. *J Acquir Immune Defic Syndr* 23, 327-31 (2000)
110. Gomez Carrillo, M., M. Avila, J. Hierholzer, M. Pando, P. L. Martinez, F. E. McCutchan & J. K. Carr: Mother-to-child HIV type 1 transmission in Argentina: BF recombinants have predominated in infected children since the mid-1980s. *AIDS Res Hum Retroviruses* 18, 477-83 (2002)
111. Thomson, M. M., M. L. Villahermosa, E. Vazquez-de-Parga, M. T. Cuevas, E. Delgado, N. Manjon, L. Medrano, L. Perez-Alvarez, G. Contreras, M. G. Carrillo, H. Salomon & R. Najera: Widespread circulation of a B/F intersubtype recombinant form among HIV-1-infected individuals in Buenos Aires, Argentina. *Aids* 14, 897-9 (2000)
112. Ramos, A., A. Tanuri, M. Schechter, M. A. Rayfield, D. J. Hu, M. C. Cabral, C. I. Bandea, J. Baggs & D. Pieniazek: Dual and recombinant infections: an integral part of the HIV-1 epidemic in Brazil. *Emerg Infect Dis* 5, 65-74 (1999)
113. Fernandez-Medina, D., M. Jansson, R. D. Rabinovich, O. Libonatti & H. Wigzell: Identification of human immunodeficiency virus type 1 subtypes B and F B/F recombinant and dual infection with these subtypes in Argentina. *Scand J Infect Dis* 31, 235-42 (1999)
114. Marquina, S., T. Leitner, R. D. Rabinovich, J. Benetucci, O. Libonatti & J. Albert: Coexistence of subtypes B, F, and as B/F env recombinant of HIV type 1 in Buenos Aires Argentina. *AIDS Res Hum Retroviruses* 12, 1651-4 (1996)
115. Sabino, E. C., E. G. Shpaer, M. G. Morgado, B. T. Korber, R. S. Diaz, V. Bongertz, S. Cavalcante, B. Galvao-Castro, J. I. Mullins & A. Mayer: Identification of human immunodeficiency virus type 1 envelope genes recombinant between subtypes B and F in two epidemiologically linked individuals from Brazil. *J Virol* 68, 6340-6 (1994)
116. Perez-Alvarez, L., E. Delgado, M. L. Villahermosa, M. T. Cuevas, V. Garcia, E. Vazquez de Parga, M. M. Thomson, A. Prieto, L. Cuevas, L. Medrano, J. A. Taboada & R. Najera: Biological characteristics of newly described HIV-1 BG recombinants in Spanish individuals. *Aids* 16, 669-72 (2002)
117. Delgado, E., M. M. Thomson, M. L. Villahermosa, M. Sierra, A. Ocampo, C. Miralles, R. Rodriguez-Perez, J. Diz-Aren, R. Ojea-de Castro, E. Losada, M. T. Cuevas, E. Vazquez-de Parga, R. Carmona, L. Perez-Alvarez, L. Medrano, L. Cuevas, J. A. Taboada & R. Najera: Identification of a newly characterized HIV-1 BG intersubtype circulating recombinant form in Galicia, Spain, which exhibits a pseudotype-like virion structure. *J Acquir Immune Defic Syndr* 29, 536-43 (2002)
118. Thomson, M. M., E. Delgado, N. Manjon, A. Ocampo, M. L. Villahermosa, A. Marino, I. Herrero, M. T. Cuevas, E. Vazquez-de Parga, L. Perez-Alvarez, L. Medrano, J. A. Taboada & R. Najera: HIV-1 genetic diversity in Galicia Spain: BG intersubtype recombinant viruses circulating among injecting drug users. *Aids* 15, 509-16 (2001)
119. Op de Coul, E., A. van der Schoot, J. Goudsmit, R. van den Burg, W. Janssens, L. Heyndrickx, G. van der Groen & M. Cornelissen: Independent introduction of transmissible F/D recombinant HIV-1 from Africa into Belgium and The Netherlands. *Virology* 270, 267-77 (2000)
120. Laukkanen, T., J. K. Carr, W. Janssens, K. Liitsola, D. Gotte, F. E. McCutchan, E. Op de Coul, M. Cornelissen, L. Heyndrickx, G. van der Groen & M. O. Salminen: Virtually full-length subtype F and F/D recombinant HIV-1 from Africa and South America. *Virology* 269, 95-104 (2000)
121. Bobkov, A., E. Kazennova, L. Selimova, N. Ladnaya, A. Kravchenko, B. Foley, I. Morrison, V. Pokrovsky, J. Weber & R. Cheingsong-Popov: HIV type 1 gag D/env G recombinants in Russia. *AIDS Res Hum Retroviruses* 14, 1597-9 (1998)
122. Montavon, C., C. Toure-Kane, J. N. Nkengasong, L. Vergne, K. Hertogs, S. Mboup, E. Delaporte & M. Peeters: CRF06-cpx: a new circulating recombinant form of HIV-1 in West Africa involving subtypes A, G, K, and J. *J Acquir Immune Defic Syndr* 29, 522-30 (2002)
123. Montavon, C., L. Vergne, A. Bourgeois, E. Mpoudi-Ngole, G. Malonga-Mouellet, C. Butel, C. Toure-Kane, E. Delaporte & M. Peeters: Identification of a new circulating recombinant form of HIV type 1, CRF11-cpx, involving subtypes A, G, J, and CRF01-AE, in Central Africa. *AIDS Res Hum Retroviruses* 18, 231-6 (2002)
124. Tovanabutra, S., V. Polonis, M. De Souza, R. Trichavaroj, P. Chanbancherd, B. Kim, E. Sanders-Buell, S. Nitayaphan, A. Brown, M. R. Robb, D. L. Birx, F. E. McCutchan & J. K. Carr: First CRF01_AE/B recombinant of HIV-1 is found in Thailand. *Aids* 15, 1063-5 (2001)

125. Vidal, N., C. Mulanga-Kabeya, N. Nzilambi, E. Delaporte & M. Peeters: Identification of a complex env subtype E HIV type 1 virus from the democratic republic of congo, recombinant with A, G, H, J, K, and unknown subtypes. *AIDS Res Hum Retroviruses* 16, 2059-64 (2000)
126. Janssens, W., T. Laukkanen, M. O. Salminen, J. K. Carr, G. Van der Auwera, L. Heyndrickx, G. van der Groen & F. E. McCutchan: HIV-1 subtype H near-full length genome reference strains and analysis of subtype-H-containing inter-subtype recombinants. *Aids* 14, 1533-43 (2000)
127. Oelrichs, R. B., C. Workman, T. Laukkanen, F. E. McCutchan & N. J. Deacon: A novel subtype A/G/J recombinant full-length HIV type 1 genome from Burkina Faso. *AIDS Res Hum Retroviruses* 14, 1495-500 (1998)
128. Nasioulas, G., D. Paraskevis, E. Magiorkinis, M. Theodoridou & A. Hatzakis: Molecular analysis of the full-length genome of HIV type 1 subtype I: evidence of A/G/I recombination. *AIDS Res Hum Retroviruses* 15, 745-58 (1999)
129. Gao, F., D. L. Robertson, C. D. Carruthers, Y. Li, E. Bailes, L. G. Kostrikis, M. O. Salminen, F. Bibollet-Ruche, M. Peeters, D. D. Ho, G. M. Shaw, P. M. Sharp & B. H. Hahn: An isolate of human immunodeficiency virus type 1 originally classified as subtype I represents a complex mosaic comprising three different group M subtypes (A, G, and I). *J Virol* 72, 10234-41 (1998)
130. Montavon, C., F. Bibollet-Ruche, D. Robertson, B. Koumare, C. Mulanga, E. Esu-Williams, C. Toure, S. Mboup, E. Saman, E. Delaporte & M. Peeters: The identification of a complex A/G/I/J recombinant HIV type 1 virus in various West African countries. *AIDS Res Hum Retroviruses* 15, 1707-12 (1999)
131. Takehisa, J., L. Zekeng, E. Ido, Y. Yamaguchi-Kabata, I. Mboudjeka, Y. Harada, T. Miura, L. Kaptu & M. Hayami: Human immunodeficiency virus type 1 intergroup (M/O) recombination in cameroon. *J Virol* 73, 6810-20 (1999)
132. Peeters, M., F. Liegeois, N. Torimiro, A. Bourgeois, E. Mpoudi, L. Vergne, E. Saman, E. Delaporte & S. Saragosti: Characterization of a highly replicative intergroup M/O human immunodeficiency virus type 1 recombinant isolated from a Cameroonian patient. *J Virol* 73, 7368-75 (1999)

Key words: Retroviral recombination, Dynamic copy-choice, High negative interference, Double infection, Heteryozygotic virion, Minus-strand DNA Synthesis, DNA Strand Displacement-Assimilation, Recombination Rate, Template Switching, Review

Send correspondence to: Wei-Shau Hu, HIV Drug Resistance Program, National Cancer Institute, FCRDC, Room 336, Building 535, Frederick, MD 21702. Tel: 301-846-1250, Fax: 301-846-6013, E-mail: whu@ncifcrf.gov