

## Modification for the Mechanism of Retroviral Recombination

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### Abstract

Three models have been proposed to explain retroviral recombination. Data support the minus strand exchange model which proposes that since the retroviral virion contains two essentially identical RNA molecules, an extended single-stranded DNA molecule is left tailing behind. This single-stranded DNA tail is free to form a hybrid duplex with another RNA molecule. This second RNA molecule will replace the original RNA as the template for continued synthesis. However, a few puzzles, such as negative interference, need to be resolved. To do so, a modified model is proposed that a recombination occurs only when the other strand RNA molecule also binds a reverse transcriptase which replaces the original one and continues the minus strand DNA synthesis using the other strand RNA as the template.

**Keywords:** Retroviral genes; Double strand DNA; Recombination; DNA synthesis

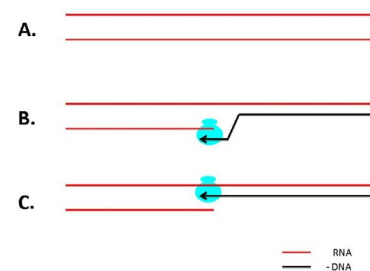
### Introduction

Mutation and recombination in retroviral genes are important components of retroviral carcinogenesis, evolution and of the AIDS epidemic. Mutation generates a pool of variants resulting in a larger pool of highly diverse variants; recombinations combine several mutations within a single viral gene, which allows for the selection of a variant with greater potential for survival. In addition, the retroviral replication cycle requires two recombinations to reverse transcribe viral single strand RNA into a double strand DNA [1].

In 1990, Hu and Temin were the first to study the mechanism of retroviral recombination during a single round viral replication cycle [2,3]. Since then, much has been learned about the mechanism of retroviral recombination, and three plausible models for retroviral recombination have been proposed. The first model proposed that recombination occurred during minus-strand synthesis using RNA as its template [4]. This model proposed that retroviral genomes contain considerable numbers of breaks within the RNA molecule and that when reverse transcriptase encounters such breaks, it switches to a homologous sequence on the other RNA molecule and continues synthesis. This is called the “forced copy choice” model. The second model proposed that recombination occurs during plus-strand DNA synthesis [5,6]. This model proposed that two minus-strand DNAs were made within one virion using both RNA templates (a retrovirus packages two RNA molecules in its virion). Since plus-strand DNA synthesis is initially discontinuous, a fragment of product DNA might be displaced by the continuous DNA synthesis. The displaced DNA fragment could then hybridize to the minus-strand DNA synthesized from the other molecule of viral RNA as a template. This is called the “plus strand displacement” model. The third model, called the “minus strand exchange” model, was first proposed by Coffin [7]. In 2000, Svarovskaia et al. proposed a similar model called the “dynamic copy choice” model [8]. The minus strand exchange model proposed that recombination occurs during minus-strand DNA synthesis; however, it does not require damage within the RNA molecule. This model proposed that since the retroviral virion contains two essentially identical RNA molecules (Figure 1a) the reverse transcriptase has a relatively low processivity, and 3) because the RNA template is degraded approximately 18 nucleotides from the point of DNA synthesis, then an extended single-stranded DNA molecule is left tailing behind. This single-stranded DNA tail is free to form a hybrid duplex with another RNA molecule (Figure 1b). If the reverse transcriptase molecule

detaches from the template, it is likely that the short (18-bp) hybrid with the original template will be displaced by branch migration of the second RNA. This second RNA molecule will replace the original RNA as the template for continued synthesis (Figure 1c). The minus strand exchange model can be further divided into several models. The initiation of the recombination event can be an interaction of hairpins within the two RNA molecules (interactive hairpin model) [9], or pause of reverse-transcription (pause dependant model). In addition, the displacement can be pause independent (active displacement model) [10].

In some recombinants, poly (A) sequences has been found [11-14]. The poly(A) tracts only exist on the RNA molecules, but not the minus-strand DNA molecules during reverse transcription, so that recombinants with poly(A) tracts must not result from the “plus strand displacement” model; therefore, some, if not all, recombination events should use an RNA molecule as a template. Furthermore, it has been determined that most, if not all, Moloney leukemia viral recombinations



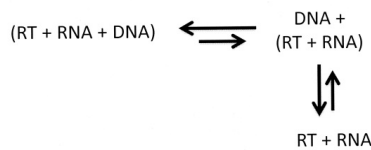
**Figure 1:** The original minus strand exchange model. (A) The retroviral virion contains two essentially identical RNA molecules. (B) An extended single-stranded DNA molecule is left tailing behind. This single-stranded DNA tail is free to form a hybrid duplex with another RNA molecule. (C) The original template will be displaced by branch migration of the second RNA. This second RNA molecule will replace the original RNA as the template for continued synthesis.

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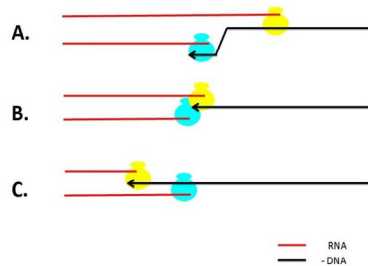
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**Figure 2:** Equilibrium of the reverse transcriptase (RT), RNAs and DNA primers. The odd of separation of the RT, the RNA template and their DNA primer is low. The odd of formation a complex of a RT and a naked RNA molecule is also low.



**Figure 3:** The modified minus strand exchange model. (A) The modified model is similar to the original model (Figure 1), except it requires another RTb (the yellow one) at its 3' end. (B) This RT then replaces the upstream RTa (the blue one) and continues to copy the DNA molecule but uses the other strand RNA as a template, and as a result, a recombination event occurs. C. Mechanism of negative interference (multiple cross overs) for retroviral recombination. After the first cross over occurs, some new upstream minus-strand DNA has been synthesized, and as a result, the RTa (the blue one) with its RNA molecule falls behind. This newly synthesized single strand DNA tail then hybridizes the RNA molecule with RTa (the blue one) on it, and the RTa (the blue one) will replace RTb (the yellow one), resulting in an additional recombination (second cross over occurs).

occur during minus strand DNA synthesis [15,16]. Therefore, either the force copy-choice or the minus strand exchange model is the mechanism of retroviral recombination. A group reported that the rate of recombination during minus and plus strand DNA synthesis were equal, suggesting that half of the recombination events result from the plus-strand displacement model [15]. However, our laboratory has conducted a similar vector that suggests most recombination occurs during minus strand DNA synthesis [16].

During retroviral reverse transcription, the minus strong stop has to jump to the 3' end of RNA, which uses the forced-copy choice mechanism [1]. The package of retroviral RNA requires that the viral RNA contain a packaging single, which locates at the upstream or the 5' end of the genome. The synthesis of the minus strand DNA uses the RNA as a template from its 3' to 5' direction, so if a break is located at the downstream or 3' end of the packaging single, the downstream part of the beaked RNA molecule would not be able to be in the virion and is not available for the recombination event. All trans-acting sequences or viral genes are located downstream from the packaging single, so we can conclude that if a recombination for viral genes occurs using the forced copy choice model, the break has to occur after the virion packaging. If that is the case, the number of breaks should be a function of time. Conversely, no report suggests the rate of retroviral recombination would increase if the virion were kept longer before the infection of its host, which suggests that the forced-copy choice model is likely not the mechanism of most retroviral recombination.

As opposed to the other two models, the minus strand exchange model is supported by data. Many reports suggest that slow polymerizations result in a high recombination rate. The slow polymerization might result from the environment [17,18], the template

[19] or a mutated polymerase [8]. According to this model, slow polymerization provides more time for the newly synthesized minus strand DNA tail to hybridize the other strand viral RNA molecule. Second, the low activity of RNaseH results in a low recombination rate [8]. Low activity of RNaseH should leave more RNA fragments on the newly synthesized minus-strand DNA molecule, which interferes with hybridization between the newly synthesized minus strand DNA and the other strand RNA.

### The Modified Minus Strand Exchange Model

However, a few puzzles remain unsolved by this model. To explain these puzzles as thoroughly as possible, we would like to modify the minus strand exchange model.

It has been well established that the reverse transcriptase, like all DNA polymerase, requires a primer to initiate its polymerization, so that after formation of a primer-template-RT complex, dNTP incorporates to the primer and synthesis continues (RT stands for reverse transcriptase). Thus, the affinity of the RT to a single strand RNA should be much lower than a primer-template (Figure 2).

Based on this fact, we propose that although there are about 50 reverse transcriptases within a single virion, few of them bind to the viral RNA unless they are at the growing point of polymerization. According to the minus strand exchange model, the naked single strand minus DNA hybridizes with the other strand RNA molecule, and it might be difficult to replace the DNA-template-RT complex from its template; since the  $K_{off}$  does not change much when the excess template strand is added to the primer/RNA template [20]. A replacement of the template for the elongation complex probably only can occur when there is another RT at its 3' end (Figure 3a). This RT would move from the 3' to 5' faster than the RT/minus DNA elongation complex since it does not need to polymerize, however, downstream RNaseH removes RNA from the DNA-RNA hybrid. This RT then replaces the upstream RT and continues to copy the DNA molecule but uses the other strand RNA as a template, and as a result, a recombination event occurs (Figure 3b). The difference in this modified model from the original minus strand exchange model is only that the modified model proposes that the replacement occurs only when a downstream RT has to replace the upstream one. In other words, one of the necessary conditions for retroviral recombination is that the two viral RNAs all have to have reverse transcriptase to bind them. There are two possible ways that an RT binds on a viral RNA molecule. The first is that this RNA molecule is reverse transcribed, which is a high frequency event (Figure 2), while the second way is that a RT binds to the viral RNA without polymerization, which is a low frequency event.

When viral RNAs are reverse-transcribed, only a small part of transcriptions result in recombinations because in most cases the other strand RNA does not bind an RT. However, when one RNA molecule is reverse-transcribed and the other strand RNA is also, as a result of the two syntheses starting off relatively at the same time or the two reverse transcriptions being synchronized, recombinations are able to occur. However, if the two syntheses are not synchronized, a recombination occurs only when the other strand RNA molecule simply binds a RT, which is an even lower frequency event than the two syntheses are synchronized.

### Multiple cross overs

It has been observed that after a recombination event occurs, the frequency of additional recombination is much higher than expected [2,21]. Hu and Temin determined that the rate of recombination of

SNV during a single replication cycle is only 4% over 1 kb distance. However, many recombinants contained several cross over events, referred to as negative interference [2]. They observed that about half the recombinations contained only one cross-over while the other half contained multiple cross overs. So after a recombination event occurred, the rate of recombination became extremely high. In addition, the rate of non-homologous recombination was only about 1/1000 the time of homologous recombination, which was about  $5 \times 10^{-5}$  per replication cycle. However, 5 out of the 27 non-homologous recombinants that were analyzed contained more than one cross over [14]. Furthermore, oncogene viruses resulted from non-homologous recombinants that occurred naturally; 13 out of the 31 analyzed contained multiple cross overs [22].

According to the modified model, a necessary condition for the recombination to occur is an RT located on the other strand of the RNA molecule. The rate is low since this condition is a low frequency event. For further examination, let's designate the originally faster RT as  $RT_a$  (Figure 3a), the blue one], and the slow RT as  $RT_b$  (the yellow one). When a recombination occurs, the  $RT_b$  replaces the  $RT_a$  and continues the minus strand DNA synthesis using the other strand of the RNA molecule as its template (first cross over occurs). After a while, some new upstream minus-strand DNA has been synthesized, and as a result, the  $RT_a$  with its RNA molecule falls behind (Figure 3c). This single strand DNA tail then hybridizes the RNA molecule with  $RT_a$  on it, and the  $RT_a$  will replace  $RT_b$ , resulting in an additional recombination (second cross over occurs). It can continue, so that  $RT_b$  then replaces the  $RT_a$  and so on, resulting in multiple cross overs. In summary, the mechanism of the negative interference is due to a recombination event, turning the low frequency event (an RT binding to the other strand RNA) into a high frequency event.

### Low rate of recombination between a normal viral RNA and RNA without the viral 3' terminal

When a virion packages a normal viral RNA and an RNA without the viral 3' terminal, the recombination rate is only 10% of that between two full-length viral RNA [23]. To explain this fact, as mentioned above, an RT located within the other strand of RNA results from two possible events. First, an RT directly binds to the viral RNA; this is a low frequency event because the affinity of RT/RNA is low (Figure 2). Second, reverse transcription occurs on both the RNA strand molecules, in other words, before reverse transcription for one strand of RNA is finished, and the other strand synthesis has begun and the first primer jump has completed or the two reverse transcriptions are synchronized. However, for MLV or SNV, this synchronized condition is a low frequency event (only less than 4% reverse transcriptions are, while the other 96% are virions in which one minus strand DNA synthesis almost finishes, but the other one might just start off). The 96% of reverse transcription would not have any cross-over unless there was also an RT binding to its opposite naked RNA molecule. However, the frequency for an RT directly binding to the viral RNA is even much less than 4%, so that most recombinations are the result of synchronization. When the viral 3' end within one of the two RNA molecules is deleted, or one viral RNA is without the 3' R region, the first primer jump becomes almost impossible for this RNA molecule and the only possibility for an RT on this RNA molecule will be the RT directly binding to the RNA molecule, which is of very low frequency. Therefore, the recombination rate between a viral RNA and a 3' deleted viral RNA is very low.

### Pause results high rate of recombination

A pause during reverse transcription results in a high rate of

recombination [8,17-19]. The original exchange model and our modified model all agree with the pause resulting in a high rate of recombination. According to the original model, the rate becomes higher, resulting from a longer time for hybridization between single strand DNA and other strand RNA. It would be difficult to explain that the hybridization should not take very long, but in fact it increased 5 times, resulting from a pause [8]. However, it is easily understood, according to the modified model, that the pause in one strand synthesis is a result of waiting for the other strand synthesis to catch up. Since most recombination results from synchronized minus DNA synthesis, and only about 4% of reverse transcriptions are synchronized, the delay of the faster synthesis will greatly increase the percentage of synchronized polymerization.

### Recombination rate increases with length of sequence identity

Although the retroviral recombination rate depends on the length of sequence identity, the increase is not a linear function [23,24]. For example, when the length of sequence identity is increased to almost 3 times, the rate only increased by 10% [23]. Since only a small portion of the other strand RNA contains the RT/RNA complex, the maximum recombination would never be more than this portion, and more length of sequence identity will result in higher rate, which has to be less than the portion with two RT/RNA complexes occurring on both strands of RNA, so that the increase would not be a linear function.

### Conclusions

We have proposed a modified minus-strand exchange model. This modified model can well explain many observations that were previously misunderstood.

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