UDC 575.85:578.2'21(048.8)

MECHANISMS AND REGULATION OF RNA VIRUS RECOMBINATION

Popov N. N., Kolotova T. Yu., Davidenko M.B.

Mechnikov Institute of Microbiology and Immunology of National Academy of Medical Sciences of Ukraine

RNA recombination is a major driving force for the evolution of RNA viruses. Recombination allows for exchange of sequence blocks and thus generates mosaicism in viral RNA genomes. Rapid RNA virus evolution is a major problem due to the diseases caused by pathogenic viruses. In fact, most but not all emergences and re-emergences of human viral diseases have been associated with RNA viruses that display active recombination or reassortment.

However only in several instances it was proven that recombination led to the appearance of emergent viruses. As examples the alphavirus Western equine encephalitis virus resulted from a recombination between a Sindbis-like and a Easter-equine encephalitis-like virus [1]. New turkey coronavirus has emerged thanks to recombination of infectious bronchitis virus that has acquired a gene, coding a spike protein of another coronavirus [2]. Recombination played a key role in the generation of new geminiviruses species [3]. At the same time, in a number of cases it is left to be determined if recombination occurs in a virus after of the host change or before it.

Recombination has played the key role in the viral evolution [4]. Recombination between related viruses has been associated with the host change, increases in virulence and with the evolution of drug-resistant strains and strains escapes immune control [5-7]. But phylogenetic analysis has shown that recombination during evolution occurred not only between the representatives of the same species, genus or family, but also between very distant groups, including the viruses that had a double–stranded RNA genome and single-stranded RNA genome [8], between segmented and non–segmented viruses [9] and even between RNA and DNA viruses [10,11]. For example, there were representatives of 4 virus families taking part in the emergence of the single stranded DNA virus family Bidnaviridae. The viruses of Bidnaviridae family have developed as a result of recombination between reoviruses that contained double-stranded RNA, baculoviruses that contained double-stranded DNA, parvoviruses that contained single-stranded DNA and a DNA transposone derived from polintonviruses [11].

RNA viruses and retroviruses are the most dangerous human and animal pathogens. In the present review, the role of recombination in the generation of the virus genome changes has been summarized, as well as molecular mechanisms and regulation of the viral recombination have been discussed. The study of the recombination regulation mechanisms of RNA viruses allows discovery of the new approaches for vaccine development. The main idea of these approaches is to decrease the recombination rate of viruses being used for vaccination as much as possible.

Replicative mechanism of virus recombination

Two possible mechanisms of recombination exist for RNA viruses: a replicative or copy-choice mechanism, which depends from RNA replication, and a nonreplicative process, which involves breakage of the RNA strands and subsequent ligation reaction.

The most widespread mechanism of RNA virus recombination is replicative recombination or copy choice recombination [12]. Viral RNA-dependent RNA polymerases have low capability of copying the same template and often switches from one RNA molecule (the donor template) to another (the acceptor template) during synthesis while remaining bound to the nascent nucleic acid chain, thereby generating hybrid RNA molecule. So RNA polymerases (RdRp) are able to perform a template switch during replication.

In some viruses the frequency of template switching is very high and practically every replicated viral RNA molecule can be considered as chimera. Thus, recombinant genomes inevitably arise during replication. Considering the fact that the recombinant virus formation increases the probability of genetic changes, viral polymerases could be viewed as the tools of the natural genetic engineering at least in a number of RNA viruses.

But, how do two RNA molecules come into contact necessary for template switching? It is proposed, that the secondary structures allow the two RNA molecules to bind in trans [13]. Moreover secondary structure may promote template switching through stimulation of the RNA polymerase stalling during replication and by facilitating the transfer of the polymerase onto the acceptor RNA.

The interaction between RNA in trans could be mediated by proteins. For example, the capsid protein (CP) of brome mosaic virus (BMV) binds with the specific sequence of RNA genome and increased the recombination [14].

CP molecules likely mediate RNA interaction in trans via dimeriza- tion/oligomerization of bound CP subunits. Template switching was found to occur between related and between unrelated RNA templates, generating legitimate (homologous) and illegitimate (nonhomologous) recombinants, respectively. RNA recombination is usually homologous, as it occurs most often between regions of high sequence similarity. During homologous replicative recombination, the frequency and

DOI: 10.5281/zenodo.1000128

localization or recombination depends on the level of sequence similarity between RNA molecules.

However, exchange between different, and hence genetically dissimilar, genomic regions or between non-related RNA molecules, leading to nonhomologous recombination. The rapid decrease the incidence of template switching in case of the similarity decline between sequences limits the potential of nonhomologous replicative recombination for hybrid forms creation between phylogenetically unrelated viral species, but do not exclude it entirely. Even cellular mRNA can serve as a donor template for RNA polymerase. The result is the emergence of viruses that contain fragments of host cell genome. For example, a case of insertion of cellular mRNA into the influenza virus A was described [15].

Replicative recombination is widespread among viruses; however, rates of virus recombination vary dramatically. Among animal viruses, coronaviruses are highly recombinogenic [16]. In flaviviruses recombinant variants are much more rare [17]. It has been shown the high recombination rate in picornaviruses [18], for which recombination is a programmed process that always accompanies replication. In polioviruses that belong to the picornaviruses family, mutations usually affect the genome region that encodes structural capside proteins, whereas the recombination usually involved the region that encodes non-strictural proteins [19]. Recombination of the attenuated vaccine polioviruses used for the virus-derived poliovirus vaccine (VDPV) production with other enteroviral strains led to emergence of pathogenic chimeras [20]. Most VDPV viruses have recombined with the Coxsackie virus A (CAV).

The Astroviridae family and noroviruses that belong to the Caliciviridae, as well as picornaviruses, are not enveloped and have single-stranded RNA of positive polarity. These viruses are similar to picornaviruses also in their recombination frequency and recombination localized to the region coding non structural proteins [19].

Among plant viruses, Bromoviridae [20] and Potiviridae frequently recombine [21]. Potiviruses recombine not only with viruses of the same species or genus, but with the viruses that belong to the different genera [22]. Hybrid viruses that create by virtue of recombination between potiviruses and cellular mRNA were found [23].

Nonreplicative recombination of RNA viruses

Usually recombination between closely related strains of viruses have played a role in the emergence of new strains that are resistant to the antiviral drugs [6] or are able to escape the immune response due to new antigenic determinants [5]. But for evolutionary innovation, recombination between phylogenetically distant viral strains must happen. Accordingly, nonreplicative recombination can be considered more promising in view of the possibility of new species emergence in the course of the evolution.

During nonreplicative recombination viruses genome RNAs are cleaved at specific points and ligated to form hybrid molecules [24, 25]. Therefore non-replicative recombination mechanism, in fact, enables non–homologous RNA to recombine easily and unconstrained. Experiments in tissue culture with synthetic RNA fragments derived from poliovirus clearly proved the existence of a nonreplicative RNA recombination. It was found that RNA poliovirus can assemble after injection of synthetic genome fragments. The assembly of a virus from fragments takes place before the viral RNA polymerase translation. These data show that recombination can occur without participation of the RNA polymerase and consequently without replication. With the help of similar method, nonreplicative recombination was discovered in a virus from the pestiviridae family – bovine diarrhea virus (BVDV) [24], and in hepatitis C virus (HCV) in the tissues culture [26].

More recently, it has been convincingly shown that nonreplicative recombination of BVDV virus does not require translation of viral proteins, including RNA polymerase [27]. For nonreplicative recombination of polioviruses, effective translation of viral proteins is not required as well. But in course of the experiments with HCV genome fragments, in which viral proteins translation before the RNA recombination was suppressed, not infectious progeny was obtained [26].

The mechanism of nonreplicative recombination is poorly understood. It is likely that recombinant may have resulted from endoribonucleolytic cleavage by cryptic ribozyme activity of RNA molecules or action of cellular endoribonucleases, followed by a ligation reaction of the RNA fragments. The breakpoints formed in the single stranded RNA regions that do not acquire secondary structure. The frequency of recombination is increased in case of modifications of 5’-triphosphate and 3’-hydroxyl groups that respectively transform into 5’-hydroxyl and 3’- monophosphoryl ends. After the insertion of the RNA fragments with modified ends into the cell, up to the 80% of them participate in recombination. But previously it has been shown that RNA fragments with such modified ends are formed by cellular endoribonucleases [28]. Hence, it is conceivable that non-replicative RNA recombination is based on cleavage of the virus RNA genomes by cellular endoribonuclease. The involvement of endonucleases in recombination supported also by the fact that enriched with endonucleases cytoplasm bodies, such as P-bodies and stress granules, participate in the lifecycle of some viruses [29].

On the second stage, RNA fragments ligate with the help of trans-etherification reaction. This process can be carried out without the enzyme participation, but it is not excluded cellular ligase involvement on this stage of the nonreplicative recombination.

DOI: 10.5281/zenodo.1000128

Sometimes in course of the ligation cellular RNA insert into the viral genome [30, 31]. For example, BVDV virus persists in the host organism for several years as consequence of the replication failure due to virus RNA synthesis dependency on limiting amounts of a cellular cofactor. Persistence often leads to the animal death, caused by new virus variants that emerge as a result of non-homologous replication, during which cellular RNA fragments insert into viral genome [31]. As the consequence of the recombination virus loose dependence on cellular factors for replication. The recombinant RNAs of BVDV can be occasionally retrotranscribed and integrated into the host genome.

Nonreplicative recombination is much less often that replicative recombination with template switch. But due to the ability to shuffle the viral RNA and assembly of the viral RNA from viral fragments of different origin, including those that come from other viruses and from cellular RNA, nonreplicative recombination may play a key role in the viral evolution [32, 33].

It would like to emphasize that the existence of nonreplicative recombination among RNA viruses indicates that viruses RNA fragments may be covalently joined with each other. These findings raise the question of the putative ability of the RNA encoded in cellular DNA to ligate with each other. The answer to this question has fundamental biological significance.

Retroviral recombination

According to the experimental data present today, the most widespread recombination mechanism of retroviruses is replicative recombination. Recombination takes place as the result of the template switch by the reverse transcriptase during the synthesis on the RNA template the DNA molecules. Usually, it is estimated that 3–12 template switches occur per genome of human immune deficiency virus (HIV-1) per replication cycle [34]. In fact, such properties of reverse transcriptase make the recombination process programmed.

But on order to recombination have biological significance, during DNA synthesis the switch to the genetically different template must occur. So, for biologically significant recombination, several processes have to take place in retroviruses: 1) the cell must be infected by two genetically different viruses; 2) both virus genomes have to be expressed in the same cell; 3) heterozygotic genomes must be copacked in one viral particle. Then the recombinant viruses emerge inevitably during the new cell infection [35].

However, hypermutagenesis of HIV-1, with the help of APOBEC family enzymes and high rate of mutagenesis during viral replication, may provide heterozygosity even if the new virions derived from two genome RNA molecules which are offspring of the single virus.

Not all viruses form biologically significant recombinants with a frequency as high as that of lentiviruses HIV-1 and HIV-2. Gamma-retroviruses recombination is far rarer. For example, murine leukemia virus MLV forms biologically significant recombinants with the rate that is 10-1000 lower than that of HIV-1 [36, 37]. This occurs despite the fact that reverse transcriptases of all viruses switch template with the same rate during replication [38]. For biologically significant recombination it is very important that one cell is infected with two or more genetically different viruses or 'doubly infected' cells. Previously it was thought that virus co-infection of the cell is a rare process. On the cellular level, viruses developed the mechanism of superinfection exclusion, which makes the cell infected by one virus refractory to the second infection [39].

But in the number of cases cells can be infected multiple times [40]. Experimentally, infection by two different HIV viruses of $CD4^+$ T cells was shown [41].

Double infection does not develop randomly [42, 43]. Most $CD4^+$ T cells of peripheral blood are resistant towards HIV infection. But small population is susceptible towards infection with two genetically different viruses. Mostly, double infection affects central $CD4^+$ memory T cells. Therefore it is suggested that long-living memory cells serve as “archives”, containing multiple viruses that ongoing to recombine in vivo [43]. Consequently, the rate of biologically significant recombination is determined to a large extent by the factors that either suppress or support superinfection.

When a host cell is coinfected with two genetically distinct viruses, progeny viral particles with heterodimeric gRNAs would be produced. Upon subsequent infection of a new cell by these heterodimeric progenies, recombination events during reverse transcription would result in a recombinant virus.

One more factor that largerly determined the recombination rate of retroviruses is the ability of RNA molecules of genetically different viruses to form heterodimeric genomes and to be packed into the same virions. Retroviral particles consist of two genomic RNA molecules. The retroviruses are able to recognize and specifically capture the genomic RNA among millions of other cellular and spliced retroviral RNAs. Consequently, in case of simultaneous infection of viruses derived from two strains, different genomic RNA may be packed into the new viral particles, forming so–called “heterozygotic” virions. After the infection of new cells, close proximity of two RNA molecules of heterozygotic virions enables the template switch during reverse transcription and, as consequence, new recombinant genomes arise.

The retroviral genome is introduced into the virions as an already formed dimer rather than two RNA genomes [44]. The sequence that enables initial dimerization and packing of retroviral genome is localized in the 5’ end of the RNA molecule and called dimerization

DOI: 10.5281/zenodo.1000128

initiation site (DIS). DIS consists of palindrome sequence that forms the stem-loop secondary structures.

Retroviral dimerization begins with complementary interactions between palindrome loops formed in the DIS sites. Therefore, for creation of a "heterozygous" virion genome the complementarity of the sequences in the dimerization initiation site is important. Viruses with mutations in the complementary sequences lose their ability to form heterodimers and consequently there are fewer cases of their participation in recombination. This explains the low frequency of recombination between HIV-1 and HIV-2 despite multiple co-infection cases.

However, the complementary interactions between loops are not sufficient to produce dimers in vivo [45]. Moreover, viral genomes with DIS mutations partially preserved the ability to dimerize. Thus other interactions along the genome seem to be desirable to keep a consistent proximity that would facilitate recombination. It was supposed that starting from complementary binding between DIS elements; the interaction spreads along the RNA molecules of the linked genomes.

RNA sequences in the middle portion of the HIV-1 genome contain sites enriched with guanine (G) residues that are near the central polypurine tract (cPPT) and that form special structures named G-quartets. It was shown experimentally that the genomes of the virus dimerize with the help of G-quartets [46]. These interactions in addition with the interactions between the DIS elements, maintain the constant connection between the two RNA molecules which facilitates the template switch for the reverse transcriptase during DNA synthesis and thus increase recombination. Really, formation of dimeric G-quartet structure correlates with hotspots of recombination, exhibiting an increased rate of template switching.

On the next stage the heterodimer packaging into the viral particle requires higher order interactions of the retroviral genome RNA with the structural Gag proteins. The interactions between the Gag protein and genome RNA are specific, but absolute specificity is not required. This lack of rigidity is the reason why heterozygous genomes can be cross-packed into the virions [47].

The cross-packaging and subsequent formation of recombinant molecules between genetically distant retroviruses was shown for the first time in the avian spleen necrosis virus SNV and murine leukemia virus MLV [48, 49]. HIV-1 genome can form heterodimers with MLV and Raus sarcoma virus RSV [48]. Cross-packaging and formation of recombinant genome were shown for HIV-1 and HIV-2 viruses [50].

With the help of replicative recombination retroviruses recombine not only between themselves, but they can also acquire elements of the host genome. The following data are in favor of this observation.

Retrotransposones that contain long end repeats (LTR) are usually derived from endogenous retroviruses that have been introduced in genomes through infection of the germ-line [51, 52]. Replicative recombination of LTR retrotransposons played a significant role in evolution, facilitating the creation of functional retrocopies of genes. In Drosophila melanogaster genomes, in genomes of mammals, chickens, fishes, mosquitos, yeast and plants [53] sequences were found that are retrocopies of eukaryotic genes flanked by LTR retrotransposon [53]. Sometimes, recombinants contained two or more genes of host genome. Whereas recombinant retrocopies of the genes are flanked by LTR sequences that contain regulatory elements they are able to be transcribed after the insertion into the genome. All recombination breakpoints between mRNA and RNA of LTR retrotransposones has short homological sequences the presence of which confirmed the origin of retrocopies with help of replicative recombination. Therefore, replicative recombination between retrotransposones and cellular mRNA can be the mechanism that is actively involved in the genomic changes during biological evolution over time.

Quite frequently there are insertions of the short fragments of cellular genome in virus genomes [54, 55].

For example, in course of the study of HIV-1 strains resistant to chemotherapeutic agents, insertions of small sequences of cellular genome into the viral genome were found [54]. Another group of researchers also has demonstrated an insertion of a sequence from human chromosome 17 into the reverse transcriptase subdomain of the HIV-1 virus [55]. This insertion allows the virus to acquire multiple drug resistance. It is not clear whether the insertion of the short cellular genome fragments takes place as a result of replicative or nonreplicative recombination. But the discovery of identical sequences of human genome in the identical position of the reverse transcriptase gene of HIV-1 virus in two separate and independent cases unconnected with each other signifies that a sequence within chromosome 17 is prone to horizontal gene transfer and mechanism exists which makes the sequence insertion a regular occurrence.

It is well known that with the help of transduction, viruses are able to acquire genes, including the protooncogenes of the host genome. In course of transduction, transcripts originating in the 5' LTR of the integrated in the host genome provirus extend beyond the 3' end of the provirus, resulting in the production of chimera RNAs are containing both viral and cellular sequences [56]. The newly synthesized chimeras RNA can be packed into virus particles that infect new cells. When such particles infect a new cell, reverse transcriptase may copy the cellular sequences and viral sequences into DNA. As a result of these events cellular sequences can insert into retroviral genomes.

Usually during transduction of the cellular gene into the viral genome some viral coding domains delete, and virus loses the ability to replicate independently.

DOI: 10.5281/zenodo.1000128

Consequently, an additional helper virus is necessary for replication. But insertion of protooncogenes into the viral genome without deletion can also occur.

In chickens, a retroviral infection is widespread. Most of the retroviruses do not contain oncogenes and induce the development of leukemia due to the insertion near the cellular protooncogene followed by protooncogen expression activation. Oncogenesis caused by retroviruses is a slow process. But in case of acquisition of cellular protooncogene by the virus, the tumor arises quickly and leading to the cancer of solid tissues not leukemia. For example, the cellular gene coding the tyrosine kinase Src inserts into the Raus sarcoma virus transforming it in oncogenic virus [57].

Regulation of the recombination rate by the cellular factors

The discussed above data show that the recombination rate is largely determined by the properties of RNA replicases and features of the viral life cycle. Besides these, recombination rate is influenced by cellular metabolism.

For virus replicase complexes assembly specific cellular proteins and factors are being used [58]. It is well documented that replicating complex assembly of three viruses TBSV, BMV and dianthovirus depend on protein-protein, protein-RNA interactions and interactions between the proteins and lipids [59]. Furthermore, replication of each of these three viruses specifically depends on pattern of these cellular factors. This leads to a very important conclusion, according to which every virus has its own unique mechanism for adaptation to the cellular environment in order to assemble the replicative complex and to be able to proliferate. But as replication of most viruses is inevitably accompanies by recombination, the dependence of replication on the specific cellular factors suggest the influence of the latter on the viral recombination rate. Therefore, the recombination rate of the virus has been determined by the cellular metabolic reactions. Consequently, the history of a cell and the environmental stress factors can indirectly influence on the viral recombination rate through cellular metabolism.
For example, in course of the study of tomato bushy stunt virus (TBSV) in the yeast cells 40 cellular factors that suppress or, conversely, enhance the recombination rate were revealed [60]. Recombination influenced by cellular endo- and exonucleases, helicases, actin network proteins, Ca^{++}/Mn^{++} pumps, Rpn11 proteosome proteinase and other factors. Due to these connections the virus recombination rate becomes affected by cellular metabolic pathways.

Endo- and exonucleases cleaves the viral RNA with the formation of so-called degradation RNA (degRNA). DegRNA induces the RNA replicase to switch the template, thus facilitating the emergence of recombinant viruses [61]. Usually, degRNA exists transiently and undergoes further degradation by 5'-3'-exoribonucleases. Respectively, endo- and exonucleases can enhance and suppress recombination. Indeed, expression of AtXrn4p 5'-3'-exoribonucleases in Nicotiana benthamiana infected with Cucumber necrosis tombusvirus (CNV) stimulated degradation of the CNV RNA but promotes rapid emergence of new recombinant variants of CNV that commonly have deletion of 5' terminal genome sequences [62].

The second metabolic pathway that regulates the TBSV recombination rate is Ca^{++}/Mn^{++} pumps that control the level of Mn^{++} in the cytosol. The rise of the Mn^{++} ions level enhances the viral replicase template switching during RNA replication [63]. Respectively, the recombination rate of the virus increases.

The replicase complex of the TBSV virus co-opts cellular helicases required for replication. DEAD box containing helicase AtRH20 suppresses the formation of the recombinant viral genomes. But the second type of the co-opting cellular helicases – eIF4AIII-similar plant helicase AtRH2 stimulates viral recombination [64]. When AtRH20 helicase is absent or AtRH2 helicase is overexpressed in the plant cells, the frequency of recombination increases.

As was noted earlier, recombination is a programmed process for retroviruses. But the recombination rate of HIV-1 is regulated by the cellular factors and metabolism [65]. The decrease in the deoxynucleotidphosphates (dNTP) in the cell increases the frequency of the reverse transcriptase stalling and template switching during replication [66]. A number of retroviruses have the ability to regulate the dNTP concentration with the help of retroviral protein Vpx. Vpx induces the degradation of cellular protein SAMHDI and thus increases concentration of dNTPs and decreases the recombination rate. But HIV-1 does not contain a gene encoding Vpx, consequently the viral recombination rate is significantly dependent on dNTP concentration in the cell. So, the recombination rate of the virus in the macrophages is 4-5 higher than in the T lymphocytes [67]. Terminally differentiated macrophages have a very low concentration of dNTP, accordingly the recombination rate in these cells is high. In the T lymphocytes, the reverse situation develops.

In the same time, HIV-2 virus contains the Vpx protein. On this basis, it can be hypothesized that the high rate of HIV-1 recombination and low recombination rate in other retroviruses can be explained by the presence or absence of Vpx protein.

Thus, the recombination rate is dependent on the developing metabolic situation and cellular factors balance. Therefore, there could be such metabolic situations in the cell that would promote and accelerate evolution of the virus, which is advantageous in the case when virus is badly adapted to the cellular conditions. Consequently, the assumption that emergent recombinant viruses can appear as a result of interaction between the

DOI: 10.5281/zenodo.1000128

virus and the host and that in this process the host, in fact, plays an active role can be quite possible. In other words, the host organism can actively participate in the new virus emergence through regulation of the recombination.

Factors that determined the origin of the recombination hotspots

A comparative analysis of complete picornaviruses and retroviruses genomes suggested that recombination sites were not randomly distributed, instead being located in hotspots flanking genomic regions with very low rates of recombination [68]. What is the nature of the recombination hotspots and how are they formed?

Usually it is thought that the distribution of recombination hotspots in vivo is the result of the selection process and not the primary recombination breakpoints localization. According to this scenario, recombination could be distributed along the whole genome with equal probability. But only those recombinants that emerged as a result of the breakpoints generations at the specific sequences and are a combination of defined genome fragments retain the ability to give viable progeny. There have been many studies showing that even in the absence of selection, recombination does not occur randomly on the virus genome, highlighting the presence of additional factors governing the recombination process. A number of experimental data confirmed the assumption, according to which genomes of at least some viruses have acquired specific structure during evolution, and this structure determines the localization of the recombination hotspots before selection of the recombinants.

Polioviruses of the enteroviridae group are highly recombinogenic. It has been revealed that localization of the recombination sites of polioviruses in the cell culture and in the cell-free system depends on the temperature [69]. Specifically, recombination in vivo under 37 and 40 degrees Celsius is localized in the region that coding non-structural proteins. In contrast both in vitro and in vivo recombination sites at 34 degrees were distributed more evenly along the genome. Formation of the recombination pattern by selection forces was ruled out by analyses of the growth kinetics of the recombinants.

Subsequently, it was mapped over 50 thousand breakpoints throughout the genome of poliovirus [70]. This map shows that the majority of recombination spots are localized in a small number of hotspots that have specific primary and secondary RNA structure.

The association between the primary and secondary RNA structure and RNA breakpoints supports the hypothesis according to which localization of recombination hotspots is determined not only by the selective forces but by the genome structure as well. This suggestion confirmed by the mapping of the RNA breakpoints which formed before recombination in the conditions that minimize the influence of the selective forces on the recombination pattern. In order to do that, first, all produced virions were studied, not only viable isolates. Second, the descendants of the viruses after one infectious cycle were investigated. Therefore the mapped hotspots of recombination reflect the viral genome architecture and not only selection processes.

5’ untranslated region (UTR) of poliovirus required for viral replication and translation. Polioviruses with a defect in 5’ UTR of the VDPV vaccines recombine with enteroviral genomes and hundreds of recombinants were isolated. Homologous and nonhomologous recombinants were revealed. The majority of the homologous and nonhomologous recombination sites clustered in three positions in 5’ UTR, forming recombination hotspots [71]. The location of three recombination hotspots is likely to be determined by secondary RNA structure. Moreover, three hotspots of recombination flank three functional domains that may be considered to constitute recombination modules.

These data suggest that in enteroviruses the recombination could be localized by the primary and secondary structure of the genome and recombination modules that can partially overlap with functional modules may be the structural unit of the recombination.

These data have enormous practical significance. VDPV polioviruses have caused epidemics of paralytic poliomyelitis in 15 regions of the world. Therefore, for live attenuated vaccines development, it is necessary to take into account their ability to recombine. The modification of the recombination hotspot sequences could be used for the development of recombination-deficient, genetically stable live vaccine strains.

Successive recombination events involving modules would lead to the construction of mosaic intertypic genomes. This new concept of modular evolution for virus requires further evaluation.

The process of recombination in the HIV-1 is organized in similar way. It has been shown that the recombination is not constant along the genome. 7 hot spots and 3 cold spots in gag genes (code for the core structural proteins) and 5 hot spots and 7 cold spots in pol genes (code for the RNA-dependent DNA polymerase) have been identified [73].

The RNA secondary structures can induce stalling of reverse transcriptase at sites which form the RNA secondary structures and thereby enhance the probability of template switching regardless of selection forces [72, 73]. For example, G-quartets, that, as was mentioned above, facilitated the RNA genome dimerization, keep the RNA genomes in proximity and promoting template switching by reverse transcriptase from the donor template to the acceptor during DNA synthesis, thus increasing the recombination rate in numerous hot spots [46].

The significant influence of genome organization on the recombination hotspots localization is also

DOI: 10.5281/zenodo.1000128

confirmed by the following data. First, analysis the breakpoint distribution shows that breakpoints are preferentially located within RNA hairpins. These results show direct involvement of secondary RNA structures in promoting breakpoints and recombination hotspots distribution.

Second, genome sites within 30 nucleotides of gene borders have significantly higher breakpoint densities than internal gene sites. The highly stable secondary structures determine the recombination hotspots localization near the linker segments that separate individual proteins and that define domains within the HIV-1 Gag, Gag-Pol, and Env proteins. In other words, RNA secondary structures that serve as signals for recombination are mapped in the regions that divide the genome into the functional modules [73]. Consequently, RNA recombination hotspots localization in the areas between the genes is determined by the secondary RNA structure, i.e. genome architecture, not only by the selection.

Therefore, the determination of recombination localization by the viral genome architecture allows the appearance of new combinations of genes or gene domains which code for autonomous protein domains that acquire the 3D structure independently. The modular assembly of genomes through recombination reduces the risks of fitness losses associated with genetic exchanges between divergent genomes and the emergence of nonviable variants. Consequently, RNA structures within the genome directly enhance the adaptive value of recombination without selection influence.

In noroviruses recombination hotspots are also predominantly clustered in the intergenic regions [74]. Noroviruses are the major cause of acute gastroenteritis. Norovirus genome consists of single–stranded RNA that contains three open reading frames (ORF), ORF1 encodes non–structural proteins, including the RNA polymerase, ORF2 encodes the main capsid protein and ORF3 – minor capsid protein.

The analysis of recombination in 555 genomes of norovirus has revealed a recombination hotspots in the intergenic region between ORF1 and ORF2 open reading frames [75]. Recombination hotspot localization is seemingly determined by the secondary structure. But it is not proven experimentally and conclusively.

The propensity of recombination hotspots to localize in the intergenic regions in noroviruses facilitates the creation of new gene combinations with adaptive advantages. Biological significance of recombination in noroviruses is confirmed by the fact that every three years new recombinant strains emerge that able to cause a pandemic [74].

Another factor that can to localize the recombination hotspots is transcription that promotes the template switch. For example, a recombination hotspot was found in the transcription promoter of the brome mosaic virus (BMV) [76]. The removal or extension of the poly(U) tract of the promoter reduced or enhanced recombination, respectively. Deletion of the hairpin forming site or its replacement by a different stem-loop structure inhibited recombination. But how the transcription promoter enables the template switch and thus stimulates recombination is not clear. Therefore, RNA virus recombination can be controlled by the cis-elements of the genome, as it occurs in the eukaryotic and prokaryotic cells.

Not only genome architecture can influence the localization of recombination hotspots, but the environment as well. Bromoviruses also have mosaic genomes, in which recombination domains partially coincide with functional domains. Recombination hotspots are found in all RNA segments of BMV virus. Some recombination hotspots of BMV are mapped in similar location in both the whole plants and protoplasts. But a different type of recombination hotspots exist –the hotspots that are specific for the infected organism species [77]. Consequently, localization of recombination hotspots can be influenced by the host organism as well, not only by the genome architecture.

However now, it is unclear what determines the dependence of the recombination hotspots on the infected plant species. It may be the consequence of selective pressure of host organism, but alternately it may be the result of the ability of the organism specific factors to induce new recombination hotspots.

Conclusions

Drawing conclusions, it is necessary to note that for a number of RNA viruses recombination is an inevitable process that accompanies the viral replication, i.e. it is programmed by the virus itself. But not every recombination is biologically significant, because in case of biological significance genetically different variants should emerge.

The recombination rate is determined, first of all, by the virus itself, the polymerase it encodes, the genome structure and the life cycle features. Second, the rate of biologically significant recombination depends on the factors that control superinfection of the cell by two genetically different viruses and also from viral mutation rate. Third, the recombination rate is significantly determined by the cellular factors. Cellular factors can actively participate in natural engineering of the new viruses depending on the metabolic situation developing in the cell.

Recombination hotspots at least in the number of viruses divide the genome into recombination modules. Moreover recombination modules can overlap with the functional modules that compose the genome. Therefore, the organization of recombination by the viral genome architecture increases the probability of appearance of new combinations of functional modules, which in turn

DOI: 10.5281/zenodo.1000128

increases the emergence of viable recombinant variants. It should be emphasized that the localization of recombination hotspots is determined not only by the selective forces, but also directed by the viral genome sequences and, probably, cellular factors.

This conclusion has a big practical significance. If the viral genome consists of modules that participate in recombination, and contains recombination hotspots, this allows construction of the strains for vaccination that are devoid of recombination hotspots. In such strains the recombination would be hampered, and probability of appearance of new viable strains as a result of recombination would be significantly decreased.

References

1. Weaver SC., Kang W., Shirako Y., Rumenapf T., Strauss EG., Strauss JH. Recombinational history and molecular evolution of western equine encephalomyelitis complex alphaviruses // J. Virol. 1997. Vol.71. P.613-636.
2. Jackwood MW., Hall D., Handel A. Molecular evolution and emergence of avian gammacoronaviruses // Infect. Genet. Evol. 2012. Vol.12(6). P.1305-1316.
3. Lefeuvre P., Moriones E. Recombination as a motor of host switches and virus emergence: geminiviruses as case studies // Curr. Opin. Virol. 2015. Vol.10. P.14–19.
4. Koonin EV., Dolja VV., Krupovic M. Origins and evolution of viruses of eukaryotes // The ultimate modularity Virology. 2015. Vol.479–480. P.2-25.
5.Khatchikian D., Orlich M., Rott R. Increased viral pathogenicity after insertion of a 28S ribosomal RNA sequence into the haemagglutinin gene of an influenza virus // Nature. 1989. Vol.340. P.156–157.
6.Malim MH., Emerman M. HIV-1 sequence variation: drift, shift, and attenuation // Cell. 2001. Vol.104. P.469–472.
7. Nora T., Charpentier C., Tenaillon O., Hoede C., Clavel F., Hance A.J. Contribution of recombination to the evolution of human immunodeficiency viruses expressing resistance to antiretroviral treatment // J. Virol. 2007. Vol.81. P.7620-7628.
8. Krupovic M., Dolja VV., Koonin EV. Plant viruses of the Amalgaviridae family evolved via recombination between viruses with double-stranded and negative-strand RNA genomes // Biol Direct. 2015. Vol.10. P.12.
9. Qin XC., Shi M., Tian JH., Lin XD., Gao DY., He JR., Wang JB., Li CX., Kang YJ., Yu B., Zhou DJ., Xu J., Plyusnin A., Holmes EC., Zhang YZ. A tick-borne segmented RNA virus contains genome segments derived from unsegmented viral ancestors // Proc. Natl. Acad. Sci. U S A. 2014. Vol.111. P.6744-6753.
10 Diemer GS. Stedman KM. A novel virus genome discovered in an extreme environment suggests recombination between unrelated groups of RNA and DNA viruses // Biol. Direct. 2012. Vol.7. P.13.
11. Krupovic M., Koonin EV. Evolution of eukaryotic single-stranded DNA viruses of the Bidnaviridae family from genes of four other groups of widely different viruses // Sci. Rep. 2014. Vol.4. P. 5347.
12. Simon-Loriere E., Holmes EC. Why do RNA viruses recombine? // Nat. Rev. Microbiol. 2011. Vol.9. P.617-643.
13. Nagy PD., Bujarski JJ. Targeting the site of RNA-RNA recombination in brome mosaic virus with antisense sequences // Proc. Natl. Acad. Sci. U.S.A. 1993. Vol.90. P.6390–6394.
14. Sztuba-Solinska J., Fanning SW., Horn JR., Bujarski JJ. Mutations in the coat protein-binding cis-acting RNA motifs debilitate RNA recombination of Brome mosaic virus // Virus Res. 2012. Vol.170. P.138–149.
15. Khatchikian D., Orlich M., Rott R. Increased viral pathogenicity after insertion of a 28S ribosomal RNA sequence into the haemagglutinin gene of an influenza virus //Nature. 1989. Vol.340. P.156–157.
16. Woo PC., Lau SK., Huang Y., Yuen KY. Coronavirus diversity, phylogeny and interspecies jumping // Exp. Biol. Med. (Maywood). 2009. Vol. 234. P.1117-1144.
17. Taucher C., Berger A., Mandl CW. A *trans*-complementing recombination trap demonstrates a lowpropensity of flaviviruses for intermolecular recombination // J. Virol. 2010. Vol.84. P.599–611.
18. Lukashev AN. Recombination among picornaviruses // Rev. Med. Virol. 2010. Vol.20. P.327–337.
19. Simmonds P., Welch J. Frequency and dynamics of recombination within different species of human enteroviruses // J. Virol. 2006. Vol.80(1). P.483-576.
20. Urbanowicz A. [et al.] Homologous crossovers among molecules of brome mosaic bromovirus RNA1 or RNA2 segments in vivo // J. Virol. 2005. Vol.79. P.5732–5742.
21. Gibbs A., Ohshima K. Potyviruses and the digital revolution // Annu Rev. Phytopathol. 2010. Vol.48. P.205–223.
22. Desbiez C., Lecoq H. Evidence for multiple intraspecific recombinants in natural populations of Watermelon mosaic virus (WMV, Potyvirus) // Arch Virol. 2008. Vol.153(9). P.1749-1803.
23. Tanne E. Occurrence of a DNA sequence of a non-retro RNA virus in a host plant genome and its expression: evidence for recombination between viral and host RNAs // Virology. 2005. Vol.332. P. 614-636.
24. Gallei A., Pankraz A., Thiel H-J., Becher P. RNA recombination in vivo in the absence of viral replication // J. Virol. 2004. Vol.78. P.6271–6281.
25. Gmyl AP. [et al.] Nonreplicative RNA recombination in poliovirus. J. Virol. 73:8958–8965. Gmyl AP, Korshenko SA, Belousov EV, Khitrina EV, Agol VI. 2003. Nonreplicative homologous RNA recombination: promiscuous joining of RNA pieces? // RNA. 1999. Vol. 9. P.1221–1231.

DOI: 10.5281/zenodo.1000128

26. Scheel TKH. [et al.] Productive Homologous and Non-homologous Recombination of Hepatitis C Virus in Cell Culture // PLoS Pathog. 2013. Vol.9. P.e1003228.
27. Kleine Büning M., Meyer D., Austermann-Busch S., Roman-Sosa G., Rümenapf T., Becher P. Nonreplicative RNA Recombination of an Animal Plus-Strand RNA Virus in the Absence of Efficient Translation of Viral Proteins // Genome Biol Evol. 2017. Vol.9(4). P.817-829.
28. Li WM., Barnes T., Lee CH. Endoribonucleases--enzymes gaining spotlight in mRNA metabolism // FEBS J. 2010. Vol.277. P.627–641.
29. Beckham CJ., Parker R. P bodies, stress granules, and viral life cycles // Cell. Host. Microbe. 2008. Vol.3. P.206–212.
30. Monroe SS., Schlesinger S. RNAs from two independently isolated defective interfering particles of Sindbis virus contain a cellular tRNA sequence at their 5' ends // Proc. Natl. Acad. Sci. US A. 1983. Vol.80. P.3279-3362.
31. Becher P., Tautz N. RNA recombination in pestiviruses: cellular RNA sequences in viral genomes highlight the role of host factors for viral persistence and lethal disease // RNA Biol. 2011. Vol.8. P.216–224.
32. Koonin EV., Dolja VV., Krupovic M. Origins and Evolution of Viruses of Eukaryotes: The Ultimate Modularity // Virology. 2015. Vol. 479-480. P. 2–25.
33. Krupovic M., Dolja VV., Koonin EV. Plant Viruses of the Amalgaviridae Family Evolved via Recombination Between Viruses with Double-Stranded and Negative-Strand RNA Genomes // Biology Direct. 2015. Vol.10. P. 12.
34. Zhuang, J., Jetzt AE., Sun G., Yu H., Klarmann G., Ron Y., Preston BD., Dougherty JP. Human immunodeficiency virus type 1 recombination: Rate, fidelity, and putative hot spots // J. Virol. 2002. Vol.76. P.11273–11282.
35. Delviks-Frankenberry K., Galli A., Nikolaitchik O., Mens H., Pathak VK., Hu WS. Mechanisms and factors that influence high frequency retroviral recombination // Viruses. 2011. Vol.3. P.1650–1680.
36. Rhodes T., Wargo H., Hu W-S. High rates of human immunodeficiency virus type 1 recombination: near-random segregation of markers one kilobase apart in one round of viral replication // J. Virol. 2003. Vol.77. P.11193–11200.
37. Flynn JA., An W., King SR., Telesnitsky A. Nonrandom dimerization of murine leukemia virus genomic RNAs // J. Virol. 2004. Vol.78. P. 12129–12139.
38. Anderson JA., Pathak VK., Hu WS. Effect of the murine leukemia virus extended packaging signal on the rates and locations of retroviral recombination // J. Virol. 2000. Vol.74. P. 6953–6963.
39. Webster B., Ott M., Greene WC. Evasion of superinfection exclusion and elimination of primary viral RNA by an adapted strain of hepatitis C virus // J. Virol. 2013. Vol.87. P.13354–13369.
40. Jung A., Maier R., Vartanian JP., Bocharov G., Jung V., Fischer U., Meese E., Wain-Hobson S., Meyerhans, A. Recombination: Multiply infected spleen cells in HIV patients // Nature. 2002. Vol.418. P. 144.
41. Hu WS., Temin HM. Genetic consequences of packaging two RNA genomes in one retroviral particle: Pseudodiploidy and high rate of genetic recombination // Proc. Natl. Acad. Sci. USA. 1990. Vol.87. P.1556–1616.
42. Dang Q., Chen J., Unutmaz D., Coffin JM., Pathak VK., Powell D. [et al.] Nonrandom HIV-1 infection and double infection via direct and cell-mediated pathways // Proc. Natl. Acad. Sci. USA. 2004. Vol.101. P.632–639.
43. Haqqani AA., Marek SL., Kumar J., Davenport M., Wang H., Tilton JC. Central memory CD4+ T cells are preferential targets of double infection by HIV-1 // Virol J. 2015. Vol.12. P.184.
44. Moore MD., Nikolaitchik OA., Chen J., Hammarskjöld ML., Rekosh D., Hu WS. Probing the HIV-1 genomic RNA trafficking pathway and dimerization by genetic recombination and single virion analyses // PLoS Pathog. 2009. Vol.5. P.e1000627.
45. Song R., Kafaie J., Yang L., Laughrea M. HIV-1 viral RNA is selected in the form of monomers that dimerize in a three-step proteasedependent process. The DIS of stem-loop 1 initiates viral RNA dimerization // J. Mol. Biol. 2007. Vol.371. P.1084–1098.
46. Piekna-Przybylska D., Sharma G., Bambara RA. Mechanism of HIV-1 RNA dimerization in the central region of the genome and significance for viral evolution // J. Biol. Chem. 2013. Vol.288(33). P.24140-24190.
47. Lizna M., Tahir A., Farah Mustafa. Cross- and Co-Packaging of Retroviral RNAs and Their Consequences // Viruses. 2016. Vol.8(10). P.E276.
48. Yin PD., Hu WS. RNAs from genetically distinct retroviruses can copackage and exchange genetic information in vivo // J. Virol. 1997. Vol.71. P.6237–6242.
49. Yang S., Temin HM. A double hairpin structure is necessary for the efficient encapsidation of spleen necrosis virus retroviral RNA //EMBO J. 1994. Vol.13. P.713–726.
50. Motomura K., Chen J., Hu W.-S. Genetic Recombination between Human Immunodeficiency Virus Type 1 (HIV-1) and HIV-2, Two Distinct Human Lentiviruses // J. Virol. 2008. Vol.82. P.1923–1933.
51. Malik HS., Henikoff S., Eickbush TH. Poised for contagion: evolutionary origins of the infectious abilities of invertebrate retroviruses // Genome Res. 2000. Vol.10. P.1307–1325.
52. Ribet D., Harper F., Dupressoir A., Dewannieux M., Pierron G., Heidmann T. An infectious progenitor for the murine IAP retrotransposon: emergence of an intracellular genetic parasite from an ancient retrovirus // Genome Res. 2008. Vol.18. P.597–609.
53. Tan S., Cardoso-Moreira M., Shi W., Zhang D., Huang J., Mao Y., Jia H., Zhang Y., Chen C., Shao Y., Leng L., Liu Z., Huang X., Long M., Zhang YE. LTR-

DOI: 10.5281/zenodo.1000128

mediated retroposition as a mechanism of RNA-based duplication in metazoans // Genome Res. 2016. Vol. 26. P.1663-1675.
54. Takebe Y., Telesnitsky A. Evidence for the acquisition of multi-drug resistance in an HIV-1 clinical isolate via human sequence transduction // Virology. 2006. Vol.351. P.1-6.
55. Megens S., Vaira D., De Baets G., Dekeersmaeker N., Schrooten Y., Li G., Schymkowitz J., Rousseau F., Vandamme AM., Moutschen M. [et al.] Horizontal gene transfer from human host to HIV-1 reverse transcriptase confers drug resistance and partly compensates for replication deficits // Virology. 2014. Vol.456. P. 310–318.
56. Muriaux D., Rein A. Encapsidation and transduction of cellular genes by retroviruses // Front Biosci. 2003. Vol.8. P. d135-177.
57. Collett MS., Erikson RL. Protein kinase activity associated with the avian sarcoma virus src gene product // Proc. Natl. Acad. Sci. USA. 1978. Vol.75. P.2021–2024.
58. Bujarski JJ. Genetic recombination in plant-infecting messenger-sense RNA viruses: overview and research perspectives // Front Plant. Sci. 2013. Vol.26(4). P.68.
59. Mine A., Okuno T. Composition of plant virus RNA replicase complexes. Curr. Opin. Virol. 2012. Vol.2. P. 663–669.
60. Prasanth KR., Kovalev N., de Castro Martín IF., Baker J., Nagy PD. Screening a yeast library of temperature-sensitive mutants reveals a role for actin in tombusvirus RNA recombination // Virology. 2016. Vol.489. P.233-275.
61. Cheng CP., Serviene E., Nagy PD. Suppression of viral RNA recombination by a host exoribonuclease // J. Virol. 2006. Vol.80. P.2631–2640.
62. Cheng CP., Jaag HM., Jonczyk M., Serviene E., Nagy PD. Expression of the Arabidopsis Xrn4p 5'-3' exoribonuclease facilitates degradation of tombusvirus RNA and promotes rapid emergence of viral variants in plants // Virology. 2007. Nov 25. Vol.368(2). P.238-286.
63. Jaag HM., Pogany J., Nagy PD. A host Ca2+/Mn2+ ion pump is a factor in the emergence of viralRNA recombinants // Cell Host Microbe. 2010. Vol. 7. P.74–81.
64. Chuang C., Barajas D., Qin J., Nagy PD. Inactivation of the host lipin gene accelerates RNA virus replication through viral exploitation of the expanded endoplasmic reticulum membrane // PLoS Pathog. 2014. Vol.10 (2). P. e1003944.
65. Smyth RP., Davenport MP., Mak J. The origin of genetic diversity in hiv-1 // Virus Res. 2012. Vol.169. P. 415–429.
66. Nguyen LA., Kim DH., Daly MB., Allan KC., Kim B. Host SAMHD1 protein promotes HIV-1 recombination in macrophages // J. Biol. Chem. 2014. Vol.289(5). P.2489-2575.
67. Cromer D., Schlub TE., Smyth RP., Grimm AJ., Chopra A., Mallal S., Davenport MP., Mak J. HIV-1 Mutation and Recombination Rates Are Different in Macrophages and T-cells // Viruses. 2016. Vol.8(4). P.118.
68. Simmonds P. Recombination and selection in the evolution of picornaviruses and other mammalian positive-stranded RNA viruses // J. Virol. 2006. Vol.80. P.11124 –11140.
69. Duggal R., Wimmer E. Genetic Recombination of Poliovirus in Vitro and in Vivo: Temperature-Dependent Alteration of Crossover Sites // Virology. 1999. Vol.258. P.30–41.
70. Runckel C., Westesson O., Andino R., DeRisi JL. Identification and manipulation of the molecular determinants influencing poliovirus recombination // PLoS Pathog. 2013. Vol.9(2). P.e1003164.
71. Muslin C., Joffret M-L., Pelletier I., Blondel B., Delpeyroux F. Evolution and Emergence of Enteroviruses through Intra- and Inter-species Recombination: Plasticity and Phenotypic Impact of Modular Genetic Exchanges in the 5' Untranslated Region // PLoS Pathog. 2015. Vol. 11(11). P. e1005266.
72. Simon-Loriere E., Martin DP., Weeks KM., Negroni M. RNA structures facilitate recombination-mediated gene swapping in HIV-1 // J. Virol. 2010. Vol.84. P. 12675–12682.
73. Smyth RP., Schlub TE., Grimm AJ., Waugh C., Ellenberg P., Chopra A., Mallal S., Cromer D., Mak J., Davenport MP. Identifying recombination hot spots in the HIV-1 genome // J. Virol. 2014. Vol.88. P.2891-2902.
74. Jackie E. Mahar, Karin Bok, Kim Y. Green, Carl D. Kirkwooda. The Importance of Intergenic Recombination in Norovirus GII.3 Evolution // J. Virol. 2013. Vol.87(7). P.3687-3698.
75. Tsimpidis M., Bachoumis G., Mimouli K., Kyriakopoulou Z., Robertson DL., Markoulatos P., Amoutzias GD. T-RECs: rapid and large-scale detection of recombination events among different evolutionary lineages of viral genomes // BMC Bioinformatics. 2017. Vol.18(1). P.13.
76. Wierzchoslawski R., Dzianott A., Bujarski J. Dissecting the requirement for subgenomic promoter sequences by RNA recombination of brome mosaic virus in vivo: evidence for functional separation of transcription and recombination // J. Virol. 2004. Vol.78. P. 8552–8564.
77. Kolondam B., Rao P., Sztuba-Solinska J., Weber PH., Dzianott A., Johns MA., Bujarski JJ. Co-infection with two strains of Brome mosaic bromovirus reveals common RNA recombination sites in different hosts // Virus. Evol. 2015. Vol. 1(1). P.vev021.

MECHANISMS AND REGULATION OF RNA VIRUS RECOMBINATION

Popov N. N., Kolotova T. Yu., Davidenko M.B.

This review attempts to summarize the most important data concerning of molecular mechanisms and regulation of the viral recombination. Two mechanisms are

DOI: 10.5281/zenodo.1000128

responsible for RNA virus recombination, specifically replicative recombination based on replicase template switching and nonreplicative joining among fragments of viral origin. Retroviruses recombination occurs during DNA synthesis, whereby reverse transcriptase undergoes template switching between the two copackaged RNAs, However numerous questions about molecular mechanisms of RNA recombination remain unanswered. RNA recombination is one of the driving forces of genetic variability and virus evolution. But significant differences in recombination frequency were observed among various RNA viruses and retroviruses. We do not understand conclusively why the frequency of RNA recombination varies so much among RNA viruses but the data summarized in this review support the hypothesis according to which not only selection forces plays a role in the determination of the recombination rate. Numerous virus and host factors were found to affect the rate of viral RNA recombinants and the distribution of recombination breakpoints.

Key words: RNA viruses, retroviruses, replicative recombination, nonreplicative recombination, template switching, recombination hotspots

DOI: 10.5281/zenodo.1000128