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Recombination

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Introduction

Genetic recombination of viruses could be defined as the exchange of fragments of genetic material (DNA or RNA) among parental viral genomes. The result of recombination is a novel genetic entity that carries genetic information in nonparental combinations. Biochemically, recombination is a process of combining or substituting portions of nucleic acid molecules. Recombination has been recognized as an important process leading to genetic diversity of viral genomes upon which natural selection can function. Depending on the category of viruses, recombination can occur at the RNA or DNA levels. Since these processes are different for DNA and RNA viruses, they are described separately.

Recombination in DNA Viruses

In many DNA viruses, genetic recombination is believed to occur by means of cellular DNA recombination machinery. Cellular DNA recombination events are of either homologous (general recombination) or nonhomologous types. The nonhomologous recombination events occur relatively rarely and are promoted by special proteins that interact with special DNA signal sequences. In general, homologous recombination events occur much more often and they are most commonly known as genetic crossing-over that happens in every DNA-based organism during meiosis.

The biochemical pathways responsible for DNA crossing-over are well established. General elements involved in general recombination include DNA sequence identity, complementary base-pairing between double-stranded DNA molecules, heteroduplex formation between the two recombining DNA strands, and specialized recombination enzymes. The best-studied recombination system of *Escherichia coli* involves proteins such as recA, and RecBCD, and it has led to a large amount of literature. Interestingly, related DNA recombination proteins have been characterized in eukaryotes, including yeast, insects, mammals, and plants.

Yet certain DNA virus species encode their own recombination proteins, and some of these viruses serve as model system with those to study the recombination processes. One of the best-known systems is of certain bacteriophages that recombine independently from the host mechanisms. These independent pathways are used for repairing damaged phage DNA and for exchanging DNA to increase diversity among the related phages. In Enterobacteria phage M13, high recombination frequency was observed within the origin of phage DNA replication in the *E. coli* host. There, the crossovers have occurred at the nucleotide adjacent to the nick at the replication origin, because of joining to a nucleotide at a remote site in the genome. These results implicated a breakage-and-religation mechanism of such apparently illegitimate cross-overs.

Importantly enough, many of these phage recombination mechanisms are analogous to the pathways operating in the host bacteria. For instance, Rec proteins of phages T4 and T7 are analogous to bacterial RecA, RecG, RuvC, or RecBCD proteins, while RecE pathway in the rac prophage of *E. coli* K-12 or the phage 1 red system influenced the studies of bacterial systems. A correlation of different stages of DNA recombination with transcription and DNA replication during Enterobacteria phage T4 growth cycle is shown in **Figure 1**.

Phage lambda (λ) has a recombination system that can substitute for the RecF pathway components in *E. coli*. The Enterobacteria phage λ moves its viral genome into and out of the bacterial chromosome using site-specific recombination. Based on crystal structures of the reaction intermediates, it is clear how the Enterobacteria phage λ integrase interacts with both core and regulatory DNA elements (**Figure 2**).

Recombination between viral DNA and host genes can lead to acquisition of cellular genes by DNA viruses. For instance, tRNA genes are present in Enterobacteria phage T4. Interestingly, these tRNA sequences contain introns suggesting that Enterobacteria phage T4 must have passed through a eukaryotic host during evolution. Similar viral–host recombination events were observed for retroviruses in eukaryotic cells.

Genetic recombination in DNA viruses is often studied using functional marker mutations. In single-component DNA viruses recombination occurs by exchanging DNA fragments, whereas in segmented DNA viruses, additional events rely on reassortment of the entire genome segments. This complicates the recombination behavior observed among mutants. One method of recombination analysis utilizes so-called conditional-lethal types, where the cells are infected with two variants and the recombinants are selected after application of nonpermissive conditions (two-factor crosses). This allows the mutants to be organized into complementation groups with the relative positions of mutations being placed on a linear map. Another method is called three-factor crosses. Here three

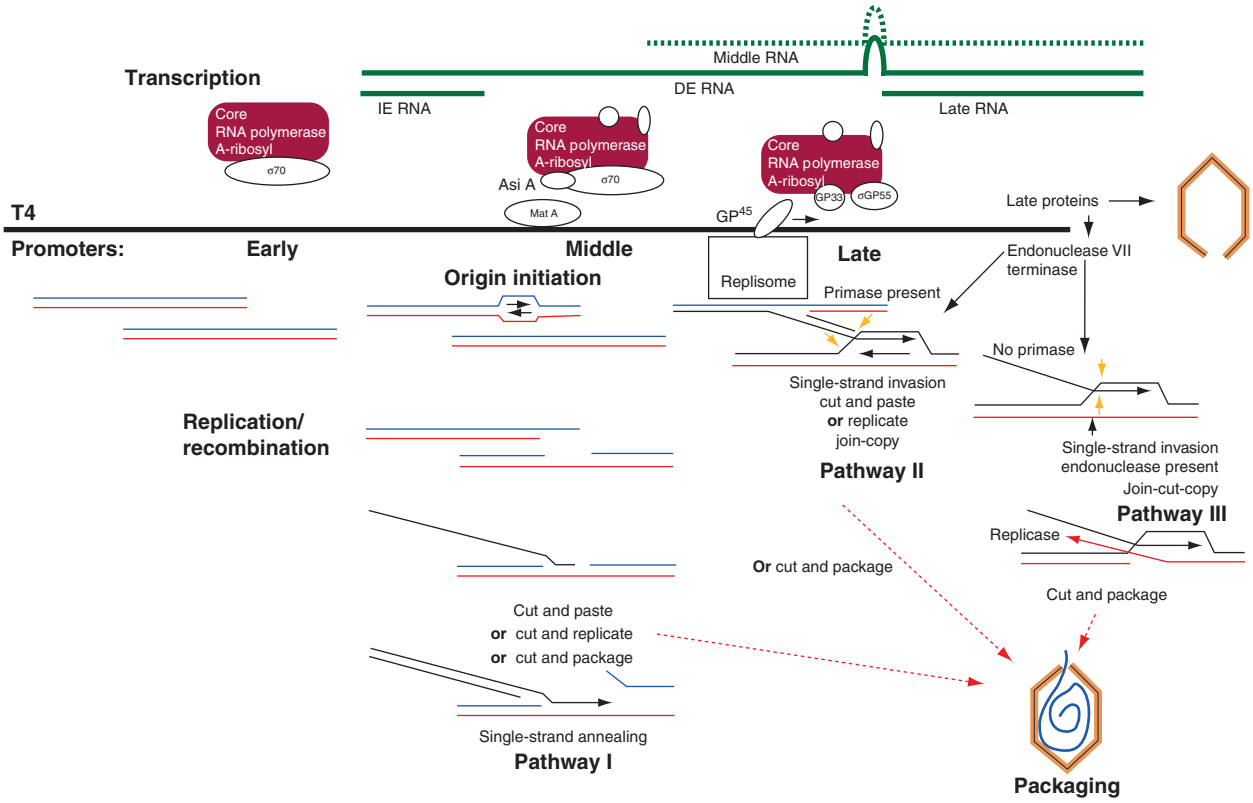

Figure 1 Diagram of the relationship between the Enterobacteria phage T4 transcriptional pattern and the different mechanisms of DNA replication and recombination. (a) Shows the transcripts initiated from early, middle, and late promoters by sequentially modified host RNA polymerase. Hairpins in several early and middle transcripts inhibit the translation of the late genes present on these mRNAs. (b) Depicts the pathways of DNA replication and recombination. Hatched lines represent strands of homologous regions of DNA, and arrows point to positions of endonuclease cuts. Reproduced from Mosig G (1998) Recombination and recombination-dependent DNA replication in enterobacteria phage T4. *Annual Review of Genetics* 32: 379–413, with permission from Annual Reviews.

mutations are employed, with crossing-over occurring between two mutations while the third mutation is not selected. This allows for determination of linkage relationships among mutants and of the order of marker mutations. Due to reassortment, both the two-factor and the three-factor crosses are of less use in segmented DNA viruses.

DNA viruses of eukaryotes also recombine their genomic material. For instance, herpes simplex virus (HSV) was found to support recombination while using pairs of temperature-sensitive mutants (two-factor crossings). In fact, a recombination-dependent mechanism of HSV-1 DNA replication has been described. The recombination frequency was proportional to the distance between mutations which suggested the lack of specific signal sequences responsible for the crossing-over. By using three-factor crossing, the HSV system involved two ts mutants and a syncytial plaque morphology as an unselectable marker. Similarly, in case of adenoviruses, the host range determined by the helper function of two mutations has been used as a third marker between ts mutants. Here, intertypic crosses between ts mutants have been identified based on segregation patterns and the restriction enzyme polymorphism.

Epstein–Barr virus (EBV) is a member of the family *Herpesviridae*, and it carries a long double-stranded genomic DNA, that shows a high-degree variation among strains. These variations include single base changes, restriction site polymorphism, insertions, or deletions. Based on tracking these mutations, it was found that some EBV strains arose due to DNA recombination.

Poxviruses represent the largest DNA viruses known (except those of algae and the mimivius). Homologous recombination was detected in the genome of vaccinia virus (VV), based on the high frequency of intertypic crossovers, the marker rescue, and the sequencing of recombinants. These processes could be both intra- and intermolecular, and they depend on the size of the DNA target. It has been suggested that either viral DNA replication itself or the activity of the viral DNA polymerase might participate in VV DNA recombination. Indeed, some VV proteins with DNA strand transfer activity have been identified.

The DNA genome of Simian virus 40 (SV40, *Papovaviridae*) was found to recombine in somatic cells. The artificially constructed recombinant circular oligomers were used to find high general recombination frequency

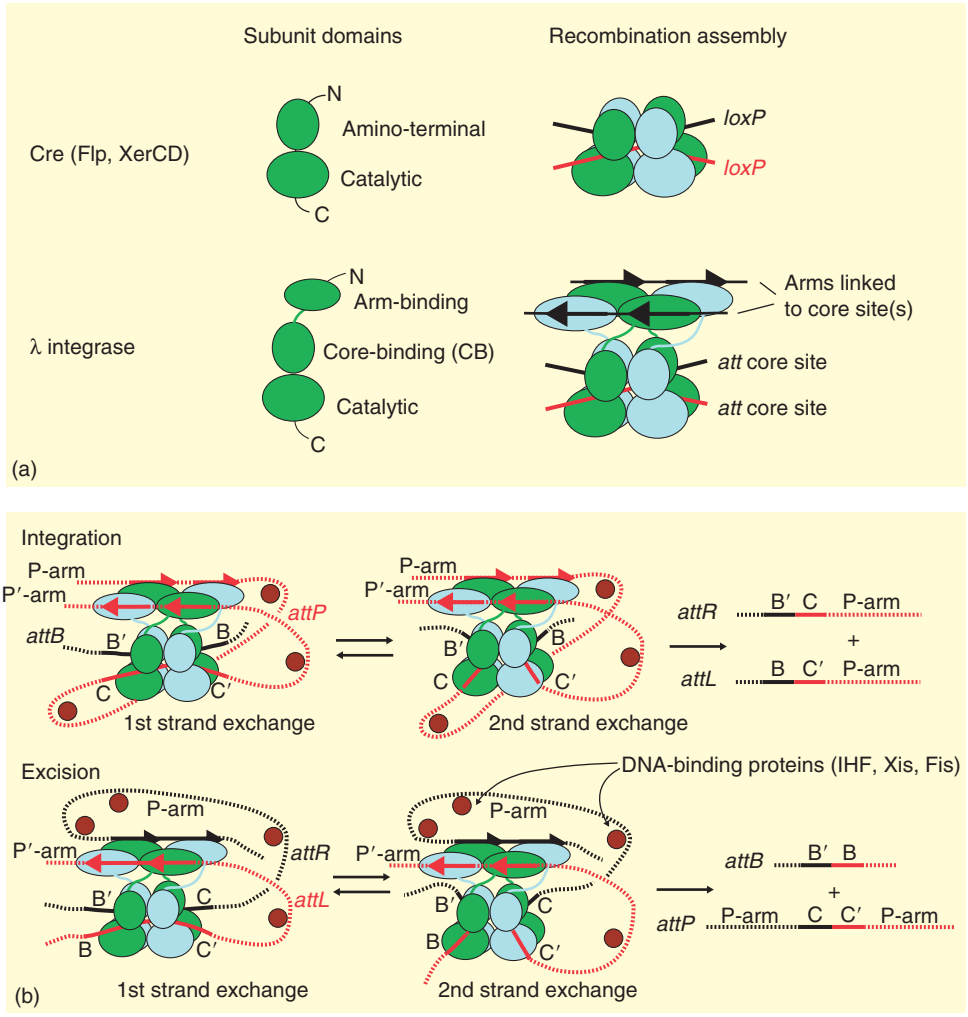

Figure 2 (a) Enterobacteria phage λ integrase compared to the simpler recombinases. Tyrosine recombinases such as Cre have two domains that bind the core recombination sites and carry out recombination on their own. Enterobacteria phage λ integrase has a third, amino-terminal 'arm binding' domain that binds to the arm region of the attachment site. The DNA complex cartoon for Enterobacteria phage λ integrase (lower right) represents the new crystal structures. (b) Integration and excision by Enterobacteria phage λ integrase. The first and second strand exchange cartoons represent the first and second halves of the recombination reaction, respectively. In the first half of integration, for example, Enterobacteria phage λ integrase brings *attP* and *attB* sites together and exchanges the first pair of strands to generate a Holliday junction intermediate. In the second half of the reaction, the Holliday intermediate has isomerized to form a distinct quaternary structure and exchange of the second pair of strands generates recombinant *attL* and *attR* products. Reproduced from Van Duyne GD (2005) Enterobacteria phage λ integrase: Armed for recombination. *Current Biology* 15: R658–R660, with permission from Elsevier.

of SV40 DNA. However, homologous recombination events were rare.

Among plant DNA viruses, genetic recombination was studied in case of geminiviruses and caulimoviruses. The geminiviruses carry a single-stranded DNA genome, composed of either one or two circular DNA molecules. Frequent intermolecular crossing-over events were observed by using mutant combinations. Homologous crossovers were detected to occur intramolecularly between tandem repeats of a geminivirus DNA using agro-infected tobacco plants. The mechanism may involve either homologous crossing-over events or copy-choice processes that rely on template switching by DNA replicase. Moreover, deletions, insertions, and more profound rearrangements have been detected in the geminivirus DNA. These are the illegitimate recombination processes that may involve aberrant breakage-and-religation events or errors in DNA replication, that could occur either inter- or intramolecularly.

Cauliflower mosaic virus (CaMV) belongs to a family of plant double-stranded (ds) DNA pararetroviruses that replicate via reverse transcription. A high recombination rate was observed during CaMV infection *in planta*. These crossovers could occur at the DNA level (thus in the nucleus) or at the RNA level (thus more likely during reverse transcription in cytoplasm). However, features

such as recombinational hot spots and mismatch repair might indicate replicative (i.e., RNA) step, whereas mismatch repair can occur due to the formation of heteroduplex intermediates and thus suggest DNA recombination. These data further suggest that CaMV has the recombination mechanisms available at both steps of its life cycle. Recombination between CaMV variants and the CaMV transgenic mRNAs has been reported and this is believed to represent the RNA–RNA recombination events that happen during reverse transcription.

Recombination in RNA Viruses

RNA is the genetic material in RNA viruses, and a high mutation rate has been observed for the viral RNA genome. This likely occurs during RNA replication by means of action of an RNA-dependent RNA polymerase enzyme due to either replication errors or because of the replicase switching among viral RNA templates. The terms of classic population genetics do not describe RNA viruses. A better description of RNA viral populations is provided with a term 'quasispecies' that has been proposed to address a distribution of RNA variants in the infected tissue.

Many of the RNA viruses limit their life cycle to cytoplasm and thus the observed recombination events among RNAs of plus-stranded RNA viruses must occur outside the nucleus. In general, the RNA crossing-over processes are categorized as being either homologous or nonhomologous, but some earlier authors proposed that there are homologous, aberrant homologous, and nonhomologous RNA recombination types. Aberrant homologous recombination involves crossovers between related RNAs, but the crosses occur at not-corresponding sites leading to sequences insertions or deletions. More recently, mechanistic models were utilized to define the following RNA–RNA recombination classes: (1) The 'similarity-essential' recombination, where substantial sequence similarity between the parental RNAs is required as the major RNA determinant; (2) The 'similarity-nonessential' recombination does not require sequence similarity between the parental RNAs, although such regions may be present; and (3) There is the 'similarity-assisted' recombination where sequence similarity can influence the frequency or the recombination sites but additional RNA determinants are also critical.

Genetic RNA recombination has been described in many RNA virus groups. In particular, sequence data reveal RNA rearrangements reflecting RNA–RNA crossover events during RNA virus evolution. For instance, RNA rearrangements were demonstrated in the genomes of dengue virus-type I, flock house virus, hepatitis D virus, bovine viral diarrhea virus, and equine arthritis virus RNA. For plant RNA viruses, this has been demonstrated in potyviruses such as yam mosaic virus, sugarcane yellow leaf virus, and luteoviruses. Experimentally, RNA recombination has been shown to occur in picornaviruses, coronaviruses, or alphaviruses and in the following plant viruses: plum pox virus, cowpea chlorotic mottle virus, alfalfa mosaic virus, cucumber mosaic virus, tobacco mosaic virus, turnip crinkle virus (TCV), and tomato bushy stunt virus (TBSV). It has also been demonstrated in enterobacteria phage Qbeta, in negative RNA viruses, in double-stranded RNA viruses, and in retroviruses, as well as during formation of defective-interfering (DI) RNAs.

Recombination by reassortment was demonstrated for multisegmental animal RNA viruses, such as influenza virus, and in double-stranded reoviruses and orbiviruses. Specifically, the interpretation of two-factor crosses (using, e.g., ts mutants) in reoviruses turned out to be difficult due to recombination. The mutant sites cannot be ordered on a linear map and often no linkage between mutants could be detected.

Interestingly, there are examples of viral RNA recombination with host-derived sequences. These include the presence of uniquitin-coding region in bovine diarrhea virus, a sequence from 28S rRNA found in the hemagglutinin gene of influenza virus or a tRNA sequence in Sindbis virus RNA. Also, in plant viruses the host-derived sequences were found in potato leaf-roll virus isolates that carry sequences homologous to an exon of tobacco chloroplast. Chloroplast sequences were found in the actively recombining RNAs of brome mosaic virus (BMV). Several plant viruses were also confirmed to recombine with viral RNA fragments expressed in transgenic plants, including cowpea chlorotic mottle virus, red clover necrotic mottle virus, potato virus Y virus, and plum pox virus.

The existence of several RNA virus recombination systems has made possible the studies of the molecular mechanisms of RNA recombination. The majority of RNA recombination models predict copy-choice mechanisms, either due to primer extension (in flaviviruses, carmoviruses), at the subgenomic promoter regions (BMV, poliovirus), or by strand translocation (in nidoviruses). In retroviruses, there are three copy-choice mechanisms: (1) forced (strong stop) strand transfer, (2) pause-driven strand transfer, and (3) pause-independent (RNA structure-driven) strand transfer. However, in enterobacteria phage Qbeta a breakage-and-religation mechanism has been described. Details of some of these systems are discussed below.

The molecular mechanisms of the formation of both nonhomologous and homologous RNA recombinants have been studied using an efficient system of BMV. In order to increase recombination frequency, the BMV RNA3-based constructs were generated where the 3′ noncoding region was extended, while carrying partial deletions. This debilitated the replication of RNA such that the sequence got repaired by recombination with the sequences of other

BMV RNA segments. It appeared that short base-paired regions between the two parental BMV RNA molecules could target efficient nonhomologous crossovers. A proposed model predicted that the formation of local RNA–RNA heteroduplexes could function because they brought together the RNA substrates and because they slowed down the approaching replicase enzyme complexes.

These early studies also analyzed the molecular requirements of homologous recombination by inserting the BMV RNA2-derived sequences into the recombination vector. This revealed the accumulation of both precise and imprecise RNA2–RNA3 recombinants and that the recombination frequencies depended upon the composition of nucleotide sequences within the region of recombination. The crossovers tended to happen at stretches of GC-rich regions alternating with AU-rich sequences suggesting the RNA replicase switching between RNA templates. Elements capable of forming strand-specific, stem–loop structures were inserted at the modified 3′ noncoding regions of BMV RNA3 and RNA2 in either positive or negative orientations, and various combinations of parental RNAs were tested for patterns of the accumulating recombinant RNA3 components. This provided experimental evidence that homologous recombination between BMV RNAs more likely occurred during positive-rather than negative-strand synthesis.

True homologous recombination crossing-over has been observed among the RNA molecules of the same segments during BMV infections. By using nonselective marker mutations at several positions, it was demonstrated that RNA1 and RNA2 segments crossed-over at 5–10% frequency, whereas the intercistronic region in RNA3 supported an unusually high recombination frequency of 70%. The subsequent use of various deletion constructs has revealed that the high-frequency crossing-over mapped to the subgenomic promoter (sgp) region, and in particular to its internal polyA tract. Further studies have shown that sgp-mediated crossing-over has occurred at the minus-strand level (i.e., during plus-strand synthesis), most likely by discontinuous process, where the replicase complex detached from one strand and reinitiated on another strand. This process is most likely primed by a sg RNA3a intermediate, which prematurely terminates on the polyU (in minus strand) tract and re-anneals to this region on another minus RNA3 template (**Figure 3**). Also, it turned out that the frequency of crossing-over and the process of initiation of transcription of sg RNA4 were reversely linked, suggesting a competition between these two reactions.

The role of replicase proteins in BMV RNA recombination has been studied by using well-characterized 1a and 2a protein mutants. A ts mutation in protein 1a 5′ shifted the crossover sites indicating the participation of helicase domain of 1a. Likewise, mutations at several regions of 2a affected the frequency of nonhomologous recombination. The relationship between replication and recombination was studied by using BMV variants that

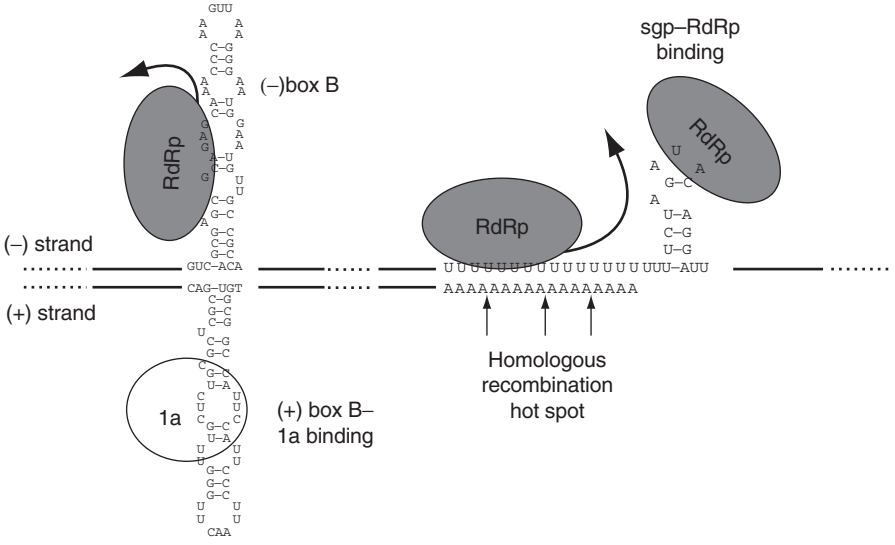

Figure 3 Model illustrating the synthesis of sg RNA3a in view of multiple functions of the intergenic region in (–) RNA3. The BMV RdRp enzyme complex (represented by gray ovals) migrates alongside the (–)-strand RNA template and pauses (represented by curved arrows) at the secondary structure or, most notably, at the oligoU tract, leading to the formation of subgenomic sgRNA3a. Yet another molecule of the RdRp enzyme binds to the sgp and initiates the *de novo* synthesis of sgRNA4. Also, the rehybridization of the sgRNA3a oligoA tail to the RNA3 (–) template can resume full-length copying which primes the observed RNA3–RNA3 recombination (5, 69). The (+) and (–) RNA strands are represented by thick lines and both the oligoU tract in the (–) strand template and the oligoA 3′ termini are exposed. The stem-and-loop structures adopted by the (+) and the (–) strands upstream to their oligoU (A) tracts are shown. The binding region to protein 1a via the box B of the stem–loop structure in (+) strands is shown. Reproduced from Wierzchoslawski R, Urbanowicz A, Dzianott A, Figlerowicz M, and Bujarski JJ (2006) Characterization of a novel 5′ subgenomic RNA3a derived from RNA3 of brome mosaic bromovirus. *Journal of Virology* 80: 12357–12366, with permission from American Society for Microbiology.

carried mutations in 1a and 2a genes. This revealed that the 1a helicase and the 2a N-terminal or core domains were functionally linked during both processes *in vivo* and *in vitro*. Also, it was shown that the characteristics of homologous and nonhomologous crossovers could be modified separately by mutations at different protein sites. All these studies confirmed the involvement of replicase proteins in recombination and supported the template-switching model.

More recently, the role of host factors in BMV recombination was addressed by using both yeast and Arabidopsis systems. In yeast, transient co-expression of two derivatives of BMV genomic RNA3 supported intermolecular homologous recombination at the RNA level but only when parental RNAs carried the *cis*-acting replication signals. The results implied that recombination occurred during RNA replication. In *Arabidopsis*, the use of gene-knock-out mutations in the RNA interference pathway revealed that BMV can recombine according to both the copy-choice template-switching and to the breakage-and-religation mechanisms.

The role of replicase proteins in RNA recombination has also been studied in other RNA viruses. For TCV, a small single-stranded RNA virus, a high-frequency recombination was observed between satellite RNA D and a chimeric subviral RNA C. The crossing-over most likely relied on viral replicase enzyme switching templates during plus-strand synthesis of RNA D which reinitiated RNA elongation on the acceptor minus-strand RNA C template. The participation of replicase proteins was demonstrated *in vitro*, where a chimeric RNA template containing the *in vivo* hot-spot region from RNA D joined to the hot-spot region from RNA C. Structural elements such as a priming stem in RNA C and the replicase binding hairpin, also from RNA C, turned to play key roles during recombination, probably reflecting late steps of RNA recombination such as strand transfer and primer elongation. The host factors related to the host-mediated viral RNA turnover have been found to participate in tombusvirus RNA recombination. The screening of essential yeast genes mutants identified host genes that affected the accumulation of TBSV recombinants, including genes for RNA transcription/metabolism, and for protein metabolism/transport. Suppression of TBSV RNA recombination was observed by the yeast Xrn1p 5′–3′ exoribonuclease, likely due to rapid removal of the 5′ truncated RNAs, the substrates of recombination. These 5′ truncated viral RNAs are generated by host endoribonucleases, such as the Ngl2p endoribonuclease.

Coronavirus RNAs were found to recombine between the genomic and DI RNA molecules. It was postulated that recombination has occurred due to the nonprocessive nature of the coronavirus RNA polymerase enzyme (**Figure 4**) and an efficient protocol for targeted recombination has been developed.

Similarly, in nodaviruses, the two-partite RNA viruses, recombination processes were found to occur between RNA segments at a site that potentially could secure base pairing between the nascent strand and the acceptor template. The recombination sites might have been chosen based on factors such as the similarity to the origin of replication or special secondary structures. A postulated model implies the polymerase to interact directly with the acceptor nodavirus RNA template.

A double-stranded RNA Pseudomonas phage Phi6 was hypothesized to recombine its RNA based on a copy-choice template switching mechanism, where the crossovers would have occurred inside the virus capsid structures at regions with almost no sequence similarity. Interestingly, the frequency of recombination was enhanced by conditions that prevented the minus-strand synthesis. Experiments were designed to reveal the effects of drift on existing genetic

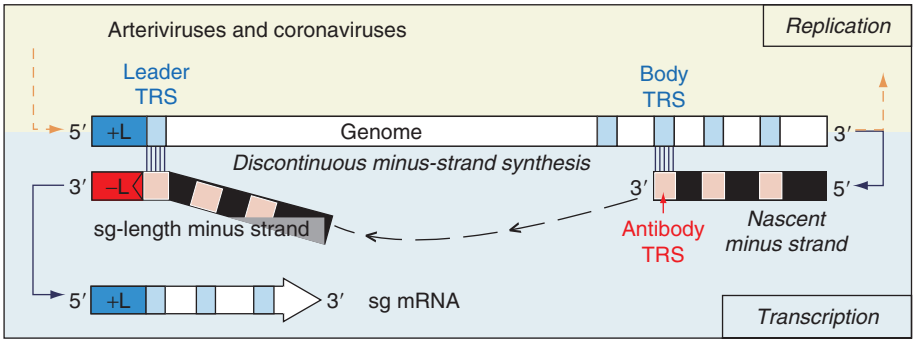

Figure 4 Models for discontinuous transcription from minus-strand sg-length templates in arteriviruses and coronaviruses. These viruses have a common 59 leader sequence on all viral mRNAs. Discontinuous extension of minus-strand RNA synthesis has been proposed as the mechanism to produce sg-length minus-strand templates for transcription. The replicase/transcriptase can attenuate at one of the body TRSs in the 39-proximal part of the genome, after which the nascent minus strand extends with the anti-leader ('L') sequence. Next, the completed sg-length minus strands serve as templates for transcription. Reproduced from Pasternak AO, Spaan WJM, and Snijder EJ (2006) Nidovirus transcription: How to make sense...? *Journal of General Virology* 87: 1403–1421, with permission from Society for General Microbiology.

variation by minimizing the influence of variation on beneficial mutation rate. The segmented genome of the pseudomonas phage Phi6 has allowed to present the first empirical evidence that the advantage of sex during adaptation increases with the intensity of drift.

The enterobacteria phage Qbeta, a small single-stranded RNA virus, could recombine both *in vivo* and *in vitro*. Here, the mechanism of recombination was not based on a template-switching by the replicase, but rather via a replicase-mediated splicing-type religation of RNA fragments. The system produced nonhomologous recombinants, whereas the frequency of homologous crossovers was low. These data suggested an RNA trans-esterification reaction catalyzed by a conformation acquired by enterobacteria phage Qbeta replicase during RNA synthesis. In summary, the results on various plus-strand RNA virus systems demonstrate the availability of a variety of template-switch mechanisms, the mutual-primer-extension on two overlapping RNA strands, the primer-extension on one full-length RNA strand, as well as both replicative and nonreplicative trans-esterification mechanisms where a piece of another RNA is added to the 3′ terminus of an RNA either by viral RdRp or by other enzymes (e.g., RNA ligase), respectively.

The recombination events in retroviruses contribute significantly to genetic variability of these viruses. The crossovers do occur by reverse transcriptase jumpings between the two genomic RNA molecules inside virion capsids. Apparently, the virally encoded reverse transcriptase enzymes have been evolutionarily selected to prone the jumpings between templates during reverse transcription. It turns out that the recombinant jumpings between RNA templates are responsible for both inter- and intramolecular template switchings and also for the formation of defective retroviral genomes. It has been found that the most stable interactions between two copies of retrovirus RNAs were within the 5′ nucleotides 1–754. There is experimental evidence demonstrating that the template 'kissing' interactions effectively promote recombination within the HIV-I 5′ untranslated region. The possibilities of recombination in retroviruses at the DNA level (of the integrated provirus sequences) were discussed earlier in this article.

Defective-Interfering RNAs

There is a variety of subviral RNA molecules that are linked to viral infections. Those derived from the viral genomic RNAs and interfering with the helper virus accumulation or symptom formation are called as DI RNAs. First reports (in 1954) about DI RNAs coexisting with viral infection was provided by Paul von Magnus with influenza virus. Thereafter, numerous both animal and plant viruses were found to generate DI RNAs. Naturally occurring DI RNAs have been identified in coronavirus infections. These molecules appear to arise by a polymerase strand-switching mechanism. The leader sequence of the DI RNAs was found to switch to the helper-virus derived leader sequence, indicating that helper virus-derived leader was efficiently utilized during DI RNA synthesis. Also, the leader switching likely occurred during positive-strand DI RNA synthesis, and the helper-virus positive-strand RNA synthesis tended to recognize double-stranded RNA structures to produce positive-strand DI RNAs. The parts of the coronavirus RNA required for replication and packaging of the defective RNAs were investigated, with both the 5′- and the 3′-terminal sequences being necessary and sufficient. The coronavirus DI RNAs have been utilized to study the mechanism of site-specific RNA recombination. This process relies on the acquisition of a 5′ leader that is normally used for production of numerous coronavirus sg RNAs. Also, these DI RNAs have been used as vehicles for the generation of designed recombinants from the parental coronavirus genome.

In case of plant viruses, tombusviruses and carmoviruses were found to accumulate DI RNAs, which maintain a consistent pattern of rearranged genomic sequences flanked by the 5′ and 3′ unchanged replication signals. In some cases, the base pairing between a partial nascent strand and the acceptor template can lead to the appearance of the rearranged regions in DI RNAs.

In addition to rearranged DI RNAs, some RNA viruses accumulate defective RNAs due to a single internal deletion in the genomic RNA of the helper virus. Such examples include beet necrotic wheat mosaic furovirus, peanut clump furovirus, clover yellow mosaic potexvirus, sonchus yellow net rhabdovirus, and tomato spotted wilt tospovirus. Features such as the ability to translate or the magnitude of the defective RNA seem to affect the selection of the best-fit sizes of DI RNAs during infection.

Another type of single-deletion DI RNAs are produced during broad bean mottle bromovirus (BBMV) infections from the RNA2 segment. A model has been proposed where local complementary regions bring together the remote parts of RNA2 which then facilitates the crossover events. Similar RNA2-derived DI RNAs have been reported to accumulate during the cucumovirus infections.

The closteroviruses, the largest known plant RNA viruses, form multiple species of defective RNAs, including the citrus tristeza virus defective RNAs that arise from the recombination of a subgenomic RNA with distant 5′ portion of the virus genomic RNA (**Figure 5**). Apparently, closteroviruses can utilize sg RNAs for the rearrangement of their genomes.

Negative-strand RNA viruses also form DI RNAs. For instance, in vesicular stomatitis virus (VSV), a rhabdovirus, the *cis*-acting RNA replication terminal elements participate in the formation of the 5′-copy-back DI RNAs, reflecting likely communication between distant portions of the VSV genome.

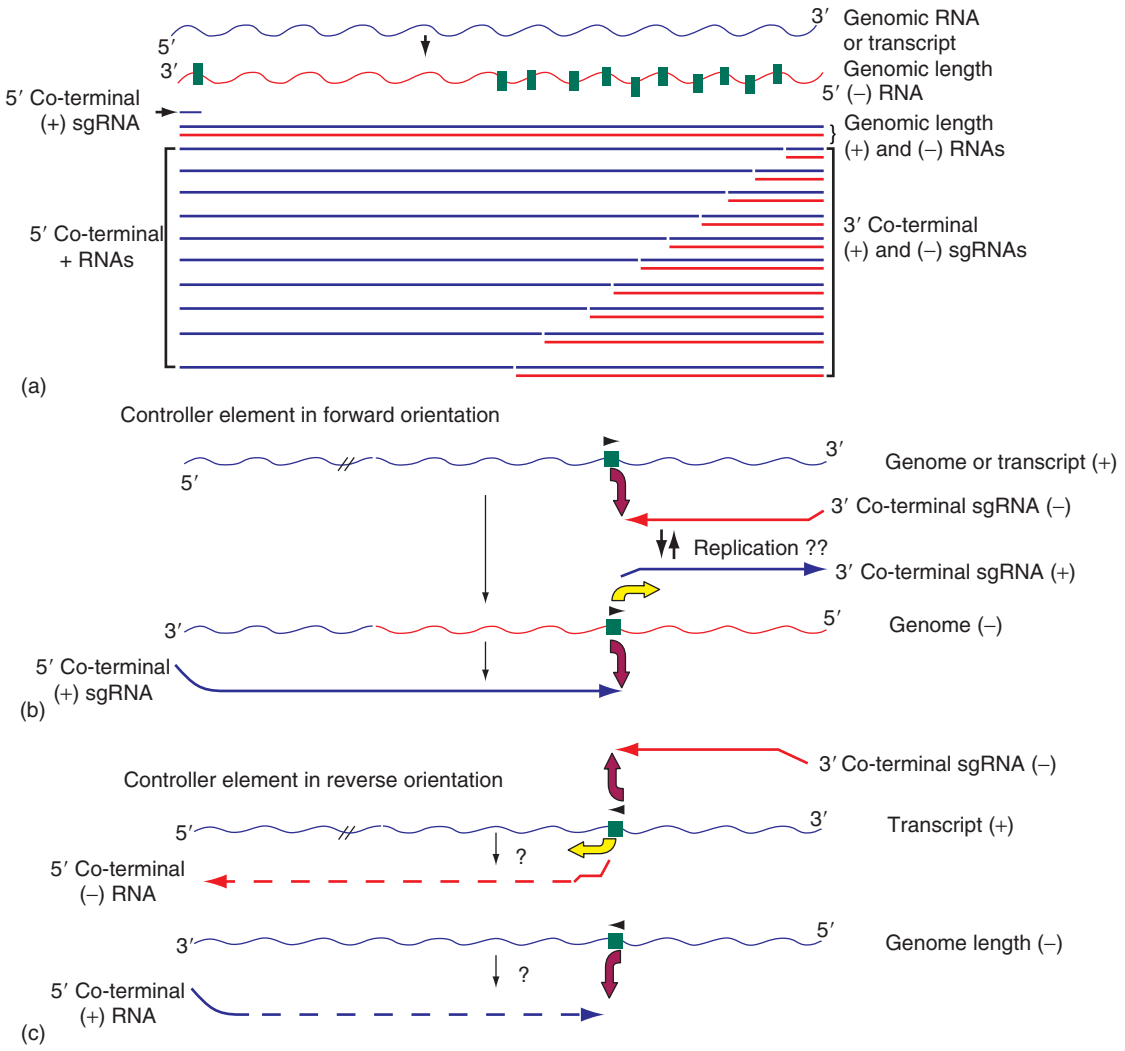

Figure 5 (a) The outline of different species of genomic RNA and 59 and 39 terminal sgRNAs potentially produced in CTV-infected cells. The positive-sense RNAs are shown in blue and the negative-sense RNAs are shown in red. The wavy line represents the genomic RNA or the plus-sense transcript (blue) and the genomic length minus-sense RNA (red) produced from the plus-sense RNA. The solid green boxes on the genomic negative-stranded RNA represent the sgRNA controller elements. The solid lines represent the full array of plus- and minus-stranded genomic and 39- and 59-terminal sgRNAs potentially produced during replication of CTV. (b, c) Models predicting the generation of 59- and 39-terminal positive- and negative-sense sgRNAs with the controller element present in normal and reverse orientation. One control region is shown for clarity. The wavy blue line represents the transcript (blue) containing the control region (green box) in normal and reverse orientation (the direction of the arrowheads above the controller element indicates the orientation of the controller element). The thick curved arrows represent the transcription termination (vertical direction, red) or promotion (horizontal direction, yellow). The solid blue lines with arrowheads represent the positive-sense 39- and 59-terminal sgRNAs and the solid red line with arrowhead indicates the 39-terminal negative-sense sgRNA. The dashed lines with arrowheads indicate the potential 59 terminal positive (blue)- and negative (red)-sense sgRNAs. Reproduced from Gowda S, Satyanarayana T, Ayllon MA, *et al*. (2001) Characterization of the *cis*-acting elements controlling subgenomic mRNAs of *Citrus tristeza virus:* Production of positive- and negative-stranded 39-terminal and positive-stranded 59 terminal RNAs. *Virology* 286: 134–151, with permission from Elsevier.

Summary and Conclusions

Genetic recombination is a common phenomenon among both DNA and RNA viruses. The recombination events have been observed based on natural rearrangements of the sequenced viral genomes. Also, experimental systems demonstrate the occurrence of recombination events that play important roles in securing the genetic diversity during viral infection. Different molecular mechanisms are involved in DNA versus RNA viruses. Many DNA viruses utilize host cellular machinery of general homologous recombination (such as meiotic crossing-over), whereas some encode their own proteins that are responsible for recombination. In addition, certain groups of DNA viruses support site-specific (nonhomologous) recombination events. In general, the virus DNA recombination mechanisms seem to involve post-DNA replication molecular events.

For RNA viruses the majority of known homologous and nonhomologous RNA recombination events appear to be integrally linked to RNA replication machinery. Various types of copy-choice (template-switching) mechanisms were proposed to describe the easy formation of RNA recombinants in numerous RNA virus systems. The roles of both special RNA signal sequences and viral proteins have been elucidated, reflecting the variety of the recombination strategies used by RNA virus groups. The involvement of host cell genes in RNA virus recombination has begun to get elucidated in several RNA viruses. Besides replicational copy-choice mechanisms, some RNA viruses use the breakage-and-religation mechanism where viral RNA gets regenerated by religation from RNA fragments, as shown experimentally for Enterobacteria phage Qbeta. New venues of RNA recombination research just emerge including our better understanding of the involvement of RNA *cis*-acting signals, the role of RNA replication, and the importance of cellular host genes such as RNA ribonucleases or RNA interference.

See also: African Cassava Mosaic Disease; Bean Golden Mosaic Virus; Brome Mosaic Virus; Cotton Leaf Curl Disease; Evolution of Viruses; Tomato Yellow Leaf Curl Virus.

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Reoviruses: General Features

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Glossary

Caspase 3−/− mice Mice that do not express caspase 3.
CTL (cytotoxic T lymphocyte) A lymphocyte capable of inducing the death of infected somatic or tumor cells. CTLs express T-cell receptors (TcRs) that can recognize a specific antigenic peptide bound to class I MHC molecules, present on all nucleated cells, and a glycoprotein called CD8.
EHBA (extrahepatic biliary atresia) A progressive congenital disorder that destroys the external bile duct structure of the liver, impairing normal bile flow (cholestasis).
SCID mice Mice with severe combined immunodeficiency, that is, cannot make T or B lymphocytes.