Genetic Recombination in RNA Viruses

M. M. C. LAI

1 Introduction

Recombination in RNA viruses involves the exchange of genetic information between two nonsegmented RNA genomes, as distinct from the reassortment of RNA seen in viruses containing segmented genomes. The mechanism of RNA recombination appears to be similar to the generation of defective interfering (DI) RNA, since they both involve polymerase jumping during RNA synthesis. However, unlike the production of DI RNA, which is a relatively common phenomenon among RNA viruses, RNA recombination has so far only been demonstrated in a few RNA viruses. Homologous RNA recombination, which is defined

Howard Hughes Medical Institute and Department of Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033, USA

as the exchange of two comparable RNA regions at precise locations, was first detected in poliovirus by HIRST (1962) and LEDINKO (1963) in the early 1960s. Soon after, another member of the picornavirus family, foot-and-mouth disease virus (FMDV), an aphthovirus, also was shown to undergo recombination (PRINGLE 1965). Subsequently, a series of temperature-sensitive (ts) mutants was used to determine recombination frequencies and do obtain a linear genetic recombination map (COOPER 1968, 1977). The definitive evidence for the occurrence of RNA recombination eventually came from the biochemical analysis of protein and RNA structure of the recombinant viruses (KING et al. 1982). The second virus family to be shown to undergo RNA recombination is coronavirus (LAI et al. 1985). And more recently, several plant viruses, including brome mosaic virus and cowpea chlorotic mottle virus, also have been shown to undergo RNA recombination in rare situations (BUJARSKI and KAESBERG 1986; ALLISON et al. 1990). Thus, RNA recombination is being recognized increasingly as a general biological phenomenon among RNA viruses and probably plays an important role in viral biology and virus evolution. This chapter will deal mainly with homologous RNA recombination and will not discuss DI RNA, which is one form of nonhomologous RNA recombination.

2 RNA Recombination in Tissue Culture

2.1 Picornavirus Recombination

In poliovirus and FMDV infections, RNA recombination readily occurs between closely related strains (intratypic recombination). With more distantly related virus strains (intertypic recombination), the recombination frequency drops proportionately. A linear genetic map has been obtained, in which recombination frequencies are additive (COOPER 1968, 1977; LAKE et al. 1975). Recombination appears to occur primarily in the 3'-half of the genome, within which recombination has been demonstrated at many different sites (KING et al. 1985). However, for reasons that are not clear, recombination events have not been demonstrated in the capsid protein genes of FMDV (KING et al. 1985; KING 1988). Recombination events involve faithful and accurate crossovers, without insertions, deletions, or mismatches. Occasionally, multiple crossover events can be detected in a recombinant virus during a single growth cycle (KING et al. 1985), suggesting that the recombination frequency is quite high.

2.2 Coronavirus Recombination

Several selection markers have been used in the isolation of coronavirus recombinants, including temperature-sensitive phenotypes, monoclonal anti-

body neutralization-resistance phenotypes and differences in cytopathicity. Similar to picornaviruses, RNA recombination can occur almost anywhere on the genome. Furthermore, many recombinants contain multiple crossover events, some of which occur at sites outside the selection markers, and are therefore not enriched by selection. These findings suggest an extremely high frequency of RNA recombination in coronaviruses. In certain situations, recombinant viruses can become the predominant population among the viruses. For example, in a recombination study between a *ts A59* strain and a wild-type JHM strain of mouse hepatitis virus (MHV), the recombinant viruses accounted for more than 95% of the virus progeny, after only two passages in tissue culture (MAKINO et al. 1986). Thus, RNA recombination may provide a powerful evolutionary tool for coronaviruses. A linear genetic map based on recombination frequencies also has been obtained for murine coronaviruses, using a panel of ts mutants (BARIC et al. 1990). Similar to picornaviruses, the recombination frequencies are additive.

3 Recombination In Vivo

3.1 Recombination in Experimental Animals, Plants and Humans

Viral RNA recombination has been demonstrated experimentally in animals and plants, and in natural viral infections of humans. RNA recombination in animals is best illustrated in the coronavirus system. When MHV was inoculated intracerebrally into mice, progeny virus harvested from the brain contained a large percentage of recombinants (KECK et al. 1988a). The types of recombinants obtained in vivo are similar to those obtained from mixed infections in tissue culture. Although similar studies have not been performed with picornaviruses, recombinants have been detected between vaccine strains in poliovirus vaccinees (KEW and NOTTAY 1984; MINOR et al. 1986). The recombinants have been isolated from both vaccine-associated poliomyelitis patients and healthy vaccinees. Certain types of recombinants, particularly those between type 3 and type 2 poliovirus vaccine strains, are frequently isolated. One study suggests that this recombinant could become the predominant virus population in vaccinees (MINOR et al. 1986).

Several plant viruses also have been shown to undergo RNA recombination in plants or protoplasts. In general, these studies did not involve homologous recombination between two wild-type virus strains, but rather, between a defective viral RNA and a helper wild-type virus RNA. Brome mosaic virus, cowpea chlorotic mottle virus, and turnip crinkle virus underwent this type of recombination when a defective RNA was introduced to plants together with the helper viruses. RNA recombination restored the replicating ability of the defective RNA (BUJARSKI and KAESBERG 1986; ALLISON et al. 1990; RAO and HALL

1990; CASCONE et al. 1990). The ability of bromovirus RNAs to undergo homologous recombination is variable and probably depends on the conditions of infection. Recently nonhomologous recombination involving these RNAs also has been reported (RAO and HALL 1990).

3.2 RNA Recombination in Natural Viral Evolution

The sequence analysis of RNA viruses has suggested that, in some cases, these RNA viruses might have been derived by RNA recombination with other viruses during natural viral infections. For example, western equine encephalitis virus may be a recombinant derived from Sindbis virus and eastern equine encephalitis virus as the result of a crossover event (HAHN et al. 1988). Also, based on the degree of divergence of different parts of the viral genome, it has been argued that some of the natural isolates of the avian coronavirus, infectious bronchitis virus (IBV), may have undergone recombination with each other, resulting in the exchange of portions of their genomes (KUSTERS et al. 1989). An even more dramatic example of recombination may have occurred in MHV. This virus contains a hemagglutinin-esterase (HE) gene, which closely resembles the analogous gene of influenza C virus (LUYTJES et al. 1988). This gene potential could have been acquired by MHV recombination with influenza C virus. It is not clear whether the local sequence homology between MHV and influenza C virus is sufficient to allow homologous recombination, nor is there experimental proof that these two viruses have indeed undergone recombination; however, sequence information provides a strong argument for the occurrence of RNA recombination in nature. Several plant viruses, including cowpea chlorotic mottle virus (ALLISON et al. 1989) and tobacco rattle virus (ANGENENT et al. 1990), also have sequence arrangements which suggest that recombination events have occurred during the evolution of these viruses. Furthermore, several positive-strand RNA animal and plant viruses, which have dissimilar genomic organizations, possess conserved functional domains in their genomes (HASELOFF et al. 1984). RNA recombination involving these functional modules best explains the mechanism of evolution of these viruses.

4 Recombination Frequency

Although RNA recombination generally is a rare event among RNA viruses, picornaviruses and coronaviruses show a surprisingly high frequency of recombination. Genetic mapping studies have suggested that the recombination frequency is approximately 2.2% for the entire poliovirus RNA genome (COOPER 1968, 1977; LAKE et al. 1975). Using a series of temperature-sensitive mutants of FMDV, McCAHON et al. (1977) found a recombination frequency of 0.9% for a region of approximately 3 kb. Considering the possibility that recombination probably occurs in both directions, and occurs not only between

different parents, but also between different molecules of the same virus, the authors argued that the recombination frequency for the entire picornavirus RNA could be extrapolated to between 10% and 20% (KING 1988). KIRKEGAARD and BALTIMORE (1986) reported a recombination frequency of 0.1% between two selection markers separated by only 190 nucleotides. Based on a similar argument, it was suggested that the recombination frequency for the entire poliovirus genome would be roughly 15% (KING 1988). This frequency is extraordinarily high, but has never been experimentally shown. The recombination frequency estimates should be viewed with caution, since different regions of the RNA recombine with different efficiencies and the selection methods used often favor certain types of recombinants. An example of this bias is the observation that the cross over sites in picornavirus RNA are clustered in the 3'-half of the genome (MCCAHON et al. 1977). Rarely has recombination been detected in the 5'-half of the genome. Recombination frequency was significantly lower when more distantly related picornaviruses was studied.

The recombination frequency of coronavirus has been measured directly and proved to be even higher than the figure estimated for picornaviruses. That recombinants could be detected even in the absence of any selection pressure was an indication of the extraordinarily high recombination frequency of coronaviruses (MAKINO et al. 1986). In a study in which recombinants were detected randomly without selection pressure, recombinants accounted for 10% of the progeny released from a mixed infection (MAKINO et al. 1986). Also, in several recombination studies using two selection markers, many recombinants not only had the expected crossover sites between the two selection markers, but also had crossover sites outside the selection markers (MAKINO et al. 1981; KECK et al. 1988b). The presence of the latter type of crossover sites strongly suggests that recombination occurred at such a high frequency that no selection pressure was needed for the detection of crossovers. Furthermore, many recombinants demonstrate multiple crossovers in the viral genome after only a single growth cycle (KECK et al. 1987, 1988b). Each of these findings suggests that coronavirus recombination can be detected readily. The recombination frequency for the entire coronavirus genome (31 kb) has been determined from a series of recombination studies using a large panel of temperature-sensitive mutants. These studies estimated the recombination frequency of MHV to be nearly 25% (BARIC et al. 1990). Since the recombinant viruses analyzed covered more than two-thirds of the genome, the estimated recombination frequency should be very close to the actual recombination frequency of coronavirus. These recombination frequencies are translated into roughly 1% recombination per 1300 nucleotides for coronavirus RNA and 1700 nucleotides for poliovirus RNA (BARIC et al. 1990). These frequencies compare favorably with the figures (1% per 200 bp for T4 phage, and per 1750 bp for *Escherichia coli*) for DNA recombination (HAYES 1968). Thus, the studies with picornavirus and coronavirus indicated a recombination frequency much higher than expected. No figure is available for the recombination frequency of plant RNA viruses; however, it appears to be lower.

5 Nonhomologous Recombination Between Viral RNA and Unrelated Viral or Cellular RNAs

Besides homologous recombination, some RNA viruses can incorporate either unrelated viral genes or cellular genes into the viral genome, possibly by a nonhomologous recombination mechanism. A characteristic of this type of recombination is that the cellular RNAs or individual viral RNAs involved either do not replicate by themselves or replicate by a mechanism different from that of the RNA virus in question. Therefore, the recombination cannot be explained simply by polymerase jumping from one viral RNA to a different RNA during the course of RNA synthesis. There are several examples of this kind of recombination:

1. Coronavirus MHV contains a HE gene, which was probably derived from influenza C virus by recombination (LUYTJES et al. 1988). These two viruses are unrelated, and the mechanisms of their RNA synthesis are quite different.
2. The pestivirus bovine viral diarrhea virus occasionally incorporates a cellular gene into its viral genome. One of the cellular genes frequently incorporated is the ubiquitin gene (MEYERS et al. 1991). The incorporation of cellular genes, particularly ubiquitin, appears to correlate well with the cytopathogenic potential of the virus. The acquisition of this cellular gene by the virus via a nonhomologous recombination mechanism seems most likely.
3. A mechanism of nonhomologous recombination could also account for the gene rearrangements observed in some RNA viruses. For instance, between the coronaviruses MHV and IBV, the gene order for the matrix protein and a nonstructural protein, i.e., genes 5 and 6 of MHV, respectively, is reversed (LAI 1990). This reversion of gene order could be explained by the recombination of viral RNA with a postulated RNA cassette containing an individual viral gene. Since each coronavirus gene is flanked by similar intergenic sequences (LAI 1990), each viral gene could be considered a gene cassette which can recombine and rearrange within the viral genome. RNA recombination also could explain the conservation of certain functional elements among the RNA genomes of many animal and plant viruses, which have dissimilar genomic organization (HASELOFF et al. 1984).

6 The Mechanism of RNA Recombination

6.1 The Copy-Choice Mechanism

There are two possible mechanisms of RNA recombination. In one case, the recombination would occur on the double-stranded RNA replicative intermediates, possibly by a breakage and reunion mechanism similar to that described for DNA recombination. The second case would be a copy-choice mechanism

involving polymerase jumping from one template to a different template during RNA replication. Thus, RNA recombination would be concomitant with RNA replication in this model. The majority of evidence currently available favors the latter mechanism. The most definitive evidence came from studies by KIRKEGAARD and BALTIMORE (1986), who examined poliovirus RNA recombination under conditions in which the replication of one parental virus was selectively blocked before superinfecting with a second virus. The results showed that recombination was dependent on RNA replication, and thus involved polymerase jumping from one template to another during RNA synthesis. The detailed mechanism of copy-choice recombination is still not known. Presumably, RNA synthesis proceeds by a discontinuous process, pausing at various sites of strong secondary structure. This transcriptional pausing would allow RNA polymerase, together with the incomplete RNA products, to be dissociated from the original RNA template and subsequently bind to the same or a different RNA template and continue transcription. When this polymerase complex binds to a different template, the transcriptional product would be a recombinant RNA. This RNA recombination model is consistent with the following observations:

1. Transcriptional pausing has been demonstrated in many experimental systems, including RNA-dependent RNA synthesis in RNA phages and DNA-dependent RNA transcription in bacteria (KASSAVETIS and CHAMBERLIN 1981; MILLS et al. 1978). It appears that both DNA-dependent and RNA-dependent RNA synthesis is inherently discontinuous. Conceivably, some viral RNA-dependent RNA polymerases may be nonprocessive, thus allowing for pausing RNA products to be dissociated from templates.
2. In coronavirus MHV-infected cells, pausing RNA transcripts of various sizes, all of which initiated from the 5'-end of the genome, have been detected (BARIC et al. 1987). The sizes of these RNA intermediates suggest that they result from polymerase pausing at sites of strong secondary structure. In addition, some of these RNA species were separated from the template RNA (BARIC et al. 1987), and potentially could be the precursor RNAs for recombination. Direct biochemical evidence that these RNA intermediates actually participate in the reinitiation of RNA synthesis and RNA recombination has not yet been obtained.

6.2 Does RNA Recombination Occur During Negative- or Positive-Strand RNA Synthesis?

The study by KIRKEGAARD and BALTIMORE (1986), using conditions where the RNA replication of one of the parental viruses was blocked suggested that RNA recombination occurs primarily during the negative-strand RNA synthesis of poliovirus. However, it is possible that RNA recombination also could occur during positive-strand RNA synthesis, since more positive-strand RNA is synthesized, allowing more ample opportunities for RNA recombination. In a

study of coronavirus RNA recombination involving transfected RNA fragments, it was shown that only the positive-strand RNA fragment could recombine with the viral genome (C.L. LIAO and M.M.C. LAI, unpublished observation). It is likely that RNA recombination can take place during both positive- and negative-strand RNA synthesis; this issue remains to be investigated.

6.3 Are There Specific Sequence Requirements for RNA Recombination and Are There Recombination "Hot Spots"?

If RNA recombination proceeds by a copy-choice mechanism whereby pausing RNA products dissociate and rebind to a different RNA template, one would predict that RNA recombination should occur at sites of strong sequence homology between the two parental viruses and also occur more readily between closely related viral RNAs (McCAHON et al. 1977, 1985; KING 1988). In picornaviruses, recombination frequency is indeed higher between the more closely related strains than distantly related ones. Furthermore, sequence analysis of some recombinants suggested that recombination occurred more frequently at sites of strong secondary structure (TOLSKAYA et al. 1987). In addition, there is evidence for recombinational hot spots in MHV (BANNER et al. 1990). One of the putative recombinational hot spots corresponds to a hypervariable region in which frequent deletions occur after virus passage in tissue culture or animals. Thus, it appears that the same secondary structure of RNA is responsible for deletions and recombination. All of these studies suggest that RNA recombination occurs at sites corresponding to the presence of RNA secondary structure or similar nucleotide sequences between the two parental RNAs. However, the study by KIRKEGAARD and BALTIMORE (1986) suggested that recombination could occur practically anywhere within a 190-nucleotide stretch of poliovirus RNA, and there was no requirement for sequence homology between the parental viruses, suggesting that there were no preferred recombination sites. Nevertheless, this study did not rule out the possibility that, when the entire RNA genome is concerned, there may be preferred recombination sites. In the case of MHV, no common sequences are observed among the individual recombination sites, although there is clustering of recombination sites (BANNER et al. 1990).

All the recombination studies reported so far involved the selection of viable recombinant viruses. Thus, the types of recombinants obtained may not reflect the actual mechanism of RNA recombination, but result from selection pressures. A recent study examining MHV RNA recombination in the absence of artificial selection pressure (BANNER and LAI, 1991) supported this possibility. In this study, two MHVs were coinfected into a susceptible cell line, and the intracellular RNA and viruses released were screened for recombinants using the polymerase chain reaction (PCR) without selection pressure. It was found that recombination sites were distributed almost evenly throughout the region encompassed by the two primers used for PCR. However, when these viruses were passaged further in tissue culture, the crossover sites of most surviving recom-

binants were clustered in a particular region of the genome (BANNER and LAI, 1991). These data suggest that while recombination events are random, certain types of recombinants confer selective advantages. Thus, the types of recombinants isolated in any recombination study are likely to represent only those which have selective advantages under the conditions used. Nevertheless, it cannot be ruled out that certain RNA structures may indeed favor RNA recombination. In particular, the clustering of recombination sites in the noncoding regions of viral RNA may reflect actual recombinational hot spots in mechanistic terms.

Many questions remain unanswered concerning the mechanism of RNA recombination. For instance, what are the enzymatic requirements for RNA recombination? And are there any particular properties associated with enzymes which allow RNA recombination, in contrast to enzymes which do not, e.g., vesicular stomatitis virus (VSV) RNA polymerase?

7 The Biological Significance of RNA Recombination

Why have picornaviruses and coronaviruses acquired the ability to undergo a high frequency of homologous RNA recombination, in contrast to other RNA viruses? The answers may lie in the properties of their RNA polymerases. The RNA polymerases of these viruses may have nonprocessive properties, thus allowing the pausing RNA transcription products to be dissociated from the RNA template during RNA replication. The dissociated, incomplete RNA products may then participate in RNA recombination. Obviously, RNA recombination provides evolutionary advantages for these viruses, which may have special needs because of the properties of their RNA genomes:

1. Recombination may be a mechanism to eliminate errors in RNA synthesis. In the VSV system, it has been shown that RNA polymerase has an enormously high error frequency, in the order of 10^{-4} (STEINHAUER and HOLLAND 1986). For most RNA viruses, deleterious mutations can be partially overcome by genetic complementation. Since picornaviruses synthesize a polyprotein, most of their gene products function *in cis* and cannot be complemented easily. Thus, RNA recombination provides an escape mechanism in lieu of genetic complementation. In the case of coronaviruses, the need for RNA recombination is dictated by the extremely large size of its RNA genome, which ranges from 27 to 31 kb (LEE et al. 1991). In the absence of a proofreading mechanism, many RNA molecules would likely be nonfunctional due to the accumulation of multiple errors during RNA synthesis. RNA recombination may allow the virus to eliminate the defective segments and thereby retain the biological activities of its RNA. It is not clear whether picornavirus and coronavirus polymerases differ from the polymerases of other RNA viruses. It is known that coronavirus RNA transcription involves a discontinuous process (LAI 1990); thus, coronavirus RNA polymerase is quite adept at

jumping or switching templates during RNA synthesis. This inherent poly-
merase property may account for the extremely high frequency of RNA
recombination observed in coronaviruses.

2. RNA recombination may be a mechanism for virus evolution. RNA recombi-
nation may allow viruses to adapt quickly to a change in environment. For
example, in children receiving poliovirus vaccines, recombination of the virus
populations in the gastrointestinal tract occurs very quickly. The recombi-
nants, which commonly involve type 2 and type 3 vaccine strains (MINOR et al.
1986), apparently have a selective advantage under these conditions and
become the predominant population in a few days after vaccination. Even in
tissue culture cells, recombinants may outgrow parental viruses under certain
conditions, as demonstrated by coronavirus recombination.

3. Although recombination may lead to the divergence and heterogeneity of
RNA viruses, it potentially may result in the convergence of viral sequences,
particularly in viruses such as coronavirus which undergo a high frequency of
recombination. Frequent recombination among poliovirus serotypes could
have resulted in the disappearance of serotype barriers. Therefore, the
maintenance of such serotype differences must require some selective
pressure.

4. Nonhomologous recombination generates DI RNA, gene rearrangement, or
insertion of cellular genes, all of which have significant biological conse-
quences on viral biology.

RNA recombination also can have deleterious effects. Recombination
between viruses of low virulence may yield recombinant viruses of unexpected
virulence. This has been demonstrated by the emergence of neurotropic
poliovirus as a result of recombination between poliovirus vaccine strains (KEW
and NOTTAY 1984). Similar results have been shown with several DNA virus
recombinants. For example, two avirulent herpes simplex virus strains can
recombine in vivo to yield a highly virulent virus (JAVIER et al. 1986). Similar
observations also have been made with pseudorabies virus (KATZ et al. 1990).
Thus, RNA recombination could pose a potentially serious problem in the
administration of live, attenuated vaccines.

8 Epilogue

Although RNA recombination so far has been demonstrated only in a few
viruses, the list of RNA viruses able to generate recombinants is growing. Many
can undergo either homologous or nonhomologous recombination. Thus, RNA
recombination plays an important role in the biology and evolution of RNA
viruses. From certain points of view, RNA recombination is similar to the
recombination seen in retroviruses, which also have a very high frequency of
recombination and a nonprocessive polymerase (reverse transcriptase) (HU and

TEMIN 1990). Although the enzymes involved in retrovirus and RNA virus recombination are different, they are similar from the mechanistic point of view. It seems likely that most RNA viruses have an ability to recombine, as implied from their ability to undergo RNA rearrangement and to generate DI RNA. The failure to detect homologous recombination in them may be due simply to inappropriate selection pressures. It will be a challenge to demonstrate the possible occurrence of genetic recombination in other RNA viruses.

References

Allison RF, Janda M, Ahlquist P (1989) Sequence of cowpea chlorotic mottle virus RNAs 2 and 3 and evidence of a recombination event during bromovirus evolution. Virology 172: 321–330

Allison RF, Thompson C, Ahlquist P (1990) Regeneration of a functional RNA virus genome by recombination between deletion mutants and requirement for cowpea chlorotic mottle virus 3a and coat genes for systemic infection. Proc Natl Acad Sci USA 87: 1820–1824

Angenent GC, Posthumus E, Brederode FT, Bol JF (1990) Genome structure of tobacco rattle virus strain PLB: further evidence on the occurrence of RNA recombination among tobraviruses. Virology 171: 271–274

Banner LR, Keck JG, Lai MMC (1990) A clustering of RNA recombination sites adjacent to a hypervariable region of the peplomer gene of murine coronavirus. Virology 175: 548–555

Banner LR, LAI MMC (1991) Random nature of coronavirus RNA recombination in the absence of selection pressure. Virology 185: 441–445

Baric RS, Shieh C-K, Stohlman SA, Lai MMC (1987) Analysis of intracellular small RNAs of mouse hepatitis virus: evidence for discontinuous transcription. Virology 156: 342–354

Baric RS, Fu K, Schaad MC, Stohlman SA (1990) Establishing a genetic recombination map for murine coronavirus strain A59 complementation groups. Virology 177: 646–656

Bujarski JJ, Kaesberg P (1986) Genetic recombination between RNA components of a multipartite plant virus. Nature 321: 528–531

Cascone PJ, Carpenter CD, Li XH, Simon AE (1990) Recombination between satellite RNAs of turnip crinkle virus. EMBO J. 9: 1709–1715

Cooper PD (1968) A genetic map of poliovirus temperature-sensitive mutants. Virology 35: 584–596

Cooper PD (1977) Genetics of picornaviruses. In: Fraenkel-Conrat H, Wagner RR (eds) Comprehensive virology, vol 9. Plenum, New York, pp 133–208

Hahn CS, Lustin S, Strauss EG, Strauss JH (1988) Western equine encephalitis virus is a recombinant virus. Proc Natl Acad Sci USA 85: 5997–6001

Haseloff J, Goelet P, Zimmern D, Ahlquist P, Dasgupta R, Kaesberg P (1984) Striking similarities in amino acid sequence among nonstructural proteins encoded by RNA viruses that have dissimilar genomic organization. Proc Natl Acad Sci USA 81: 4358–4362

Hayes W (1968) In: The genetics of bacteria and their viruses, 2nd edn. Blackwell, Oxford

Hirst GK (1962) Genetic recombination with Newcastle disease virus, polioviruses and influenza virus. Cold Spring Harbor Symp Quant Biol 27: 303–309

Hu W-S, Temin HM (1990) Genetic consequences of packaging two RNA genomes in one retroviral particle: pseudodiploidy and high rate of genetic recombination. Proc Natl Acad Sci USA 87: 1556–1560

Javier RT, Sedarati F, Stevens JG (1986) Two avirulent herpes simplex viruses generate lethal recombinants in vivo. Science 234: 746–749

Kassavetis GA, Chamberlin MJ (1981) Pausing and termination of transcription within the early region of bacteriophage T7 DNA in vitro. J Biol Chem 256: 2777–2786

Katz JB, Henderson LM, Erickson GA (1990) Recombination in vivo of pseudorabies vaccine strains to produce new virus strains. Vaccine 8: 286–288

Keck JG, Stohlman SA, Soe LH, Makino S, Lai MMC (1987) Multiple recombination sites at the 5'-end of murine coronavirus RNA. Virology 156: 331–341

Keck JG, Matsushima GK, Makino S, Fleming JO, Vannier DM, Stohlman SA, Lai MMC (1988a) In vivo RNA–RNA recombination of coronavirus in mouse brain. J Virol 62: 1810–1813

Keck JG, Soe LH, Makino S, Stohlman SA, Lai MMC (1988b) RNA recombination of murine coronaviruses: recombination between fusion-positive MHV-A59 and fusion-negative MHV-2. J Virol 62: 1989–1998

Kew OM, Nottay BK (1984) Evolution of the oral polio vaccine strains in humans occurs by both mutation and intramolecular recombination. In: Chanock RM, Lerner RA (eds) Modern approaches to vaccines: molecular and chemical basis of virus virulence and immunogenicity. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 357–362

King AMQ (1988) Recombination in positive strand RNA viruses. In: Domingo E, Holland JJ, Ahlquist P (eds) RNA genetics, vol 2. CRC, Boca Raton, FL, pp 149–165

King AMQ, McCahon D, Slade WR, Newman JWI (1982) Recombination in RNA. Cell 29: 921–928

King AMQ, McCahon D, Saunders K, Newman JWI, Slade WR (1985) Multiple sites of recombination within the RNA genome of foot-and-mouth disease virus. Virus Res 3: 373–384

Kirkegaard K, Baltimore D (1986) The mechanism of RNA recombination in poliovirus. Cell 47: 433–443

Kusters JG, Niesters HGM, Lenstra JA, Horzinek MC, van der Zeijst BAM (1989) Phylogeny of antigenic variants of avian coronavirus IBV. Virology 169: 217–221

Lai MMC (1990) Coronavirus: organization, replication and expression of genome. Annu Rev Microbiol 44: 303–333

Lai MMC, Baric RS, Makino S, Keck JG, Egbert J, Leibowitz JL, Stohlman SA (1985) Recombination between nonsegmented RNA genomes of murine coronaviruses. J Virol 56: 449–456

Lake JR, Priston RAJ, Slade WR (1975) A genetic recombination map of foot- and-mouth disease virus. J Gen Virol 27: 355–367

Ledinko N (1963) Genetic recombination with poliovirus type 1: studies of crosses between a normal horse serum-resistant mutant and several guanidine-resistant mutants of the same strain. Virology 20: 107–119

Lee HJ, Shieh C-K, Gorbalenya AE, Koonin EV, La Monica N, Tuler J, Bagdzhadzhyan A, Lai MMC (1991) The complete sequence (22 kilobases) of murine coronavirus gene 1 encoding the putative proteases and RNA polymerases. Virology 180: 567–582

Luytjes W, Bredenbeek PJ, Noten AFH, Horzinek MC, Spaan WJM (1988) Sequence of mouse hepatitis virus A59 mRNA 2: indications for RNA-recombination between coronavirus and influenza C virus. Virology 166: 415–422

Makino S, Keck JG, Stohlman SA, Lai MMC (1986) High-frequency RNA recombination of murine coronaviruses. J Virol 57: 729–737

Makino S, Fleming JO, Keck JG, Stohlman SA, Lai MMC (1987) RNA recombination of coronaviruses: localization of neutralizing epitopes and neuropathogenic determinants on the carboxyl terminus of peplomers. Proc Natl Acad Sci USA 84: 6567–6571

McCahon D, King AMQ, Roe DS, Slade WR, Newman JWI, Cleary AM (1985) Isolation and biochemical characterization of intertypic recombinants of foot- and-mouth disease virus. Virus Res 3: 87–100

McCahon D, Slade WR, Priston RA, Lake JR (1971) An extended genetic recombination map of foot-and-mouth disease virus. J Gen Virol 35: 555–565

Meyers G, Tautz N, Dubovi EJ, Thiel H-J (1991) Viral cytopathogenicity correlated with integration of ubiquitin-coding sequences. Virology 180: 602–616

Mills DR, Dobkin C, Kramer FR (1978) Template-determined, variable rate of RNA chain elongation. Cell 15: 541–550

Minor PD, John A, Ferguson M, Icenogle JP (1986) Antigenic and molecular evolution of the vaccine strain of type 3 poliovirus during the period of excretion by a primary vaccine. J Gen Virol 67: 693–706

Pringle CR (1965) Evidence of genetic recombination in foot- and-mouth disease virus. Virology 25: 48–54

Rao ALN, Hall TC (1990) Requirement for a viral trans-acting factor encoded by brome mosaic virus RNA-2 provides strong selection in vivo for functional recombinants. J Virol 64: 2437–2441

Steinhauer DA, Holland JJ (1986) Direct method for quantitation of extreme polymerase error frequencies at selected single base sites in viral RNA. J Virol 57: 219–228

Tolskaya EA, Romanova LI, Blinov VM, Victorova EG, Sinyakov AN, Kolesnikova MS, Agol VI (1987) Studies on the recombination between RNA genomes of poliovirus: the primary structure and nonrandom distribution of crossover regions in the genomes of intertypic poliovirus recombinants. Virology 161: 54–61