

## Adenovirus Homologous Recombination Does Not Require Expression of the Immediate-Early E1a Gene

LISA H. EPSTEIN AND C. S. H. YOUNG\*

*Department of Microbiology, College of Physicians and Surgeons, Columbia University,  
New York, New York 10032*

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To investigate whether early genes other than those involved directly in DNA replication are required for efficient adenovirus recombination, pairs of viruses with deletions in E1a, E1b 496R, E1b 196R, or E4 and containing differing restriction site markers were used to infect both permissive and non- or semipermissive cells. Recombination was assayed among intracellular and extracellular genomes by restriction digestion and blot hybridization. Recombination was delayed in infections of nonpermissive cells with E1a<sup>-</sup> viruses until a time consistent with the late onset of DNA replication characteristic of the cell type. This shows that E1a expression is not absolutely required for adenovirus recombination. Similar tests with deletion mutations in E1b and E4 also show that these genes are not required for efficient recombination. Taken together with earlier results showing that recombination depends on DNA replication, it is likely that adenovirus recombination is a consequence of cellular repair functions acting on the substrates produced by replication.

Initial observations on the frequency of recombination between differently marked adenoviruses showed that very high proportions of recombinants could be obtained in the yield from mixed infections (15, 29, 38). The high proportion of recombinants was in marked contrast to the very low mitotic recombination frequencies observed in mammalian cells in culture (e.g., see reference 25), and thus it was entirely plausible that the virus encoded all the functions necessary for its own recombination. Two lines of evidence have cast doubt on this simple inference. First, it was shown that functional genes could be formed efficiently by recombination between overlapping gene fragments transfected or injected into cells (13; reviewed in reference 28). In other words, provided the DNA substrates were in the correct molecular form, the cellular machinery could recombine them. Second, it became clear that the onset and extent of viral recombination were dependent upon viral DNA replication (39, 40), and this suggested that the driving force behind adenovirus recombination could be the production of the single strands produced by the strand displacement mechanism of replication, an idea first proposed by Flint et al. (11). Genetic segregation data, showing a polarity of recombination from the ends of the genome (22), were consistent with initiation from the ends, perhaps by invasion of the displaced strand. Taken together, these results suggest a simple model for adenovirus recombination in which highly recombinogenic single strands, produced by DNA replication, invade recipient duplexes (perhaps from the ends) and the recombinational intermediates are resolved by cellular nucleases and ligase. Thus, adenoviral recombination would be a by-product of replication and would not necessarily imply the production of virus-encoded proteins specific for recombination.

However, the demonstration that DNA replication is necessary for recombination does not mean that it is sufficient, as other viral products might play a role too. By analogy with bacteriophages, candidates for *rec* genes would

lie among the early and delayed-early classes (27). In this report, we examine the effects of mutations in the adenovirus E1a, E1b 196R, E1b 496R, and E4 genes upon viral recombination.

The strategy for examining the potential role of early genes in recombination is based on the use of restriction site polymorphisms, as first described by Williams et al. (37) and developed for intracellular DNA by Young and Silverstein (40). For three of the four mutations examined, pairs of viruses containing a mutation in the desired early gene, but with distinguishable restriction sites in *trans* elsewhere in the genome, were constructed (Fig. 1). Recombination in the interval between the restriction sites can be detected among the intracellular DNA from doubly infected cells by the formation of two new restriction fragments, the smaller of which is diagnostic for recombination and cannot be formed by partial digestion of the parental genomes. Essentially the same strategy was followed for E4, but the deletions used were of different origin, namely, from adenovirus type 2 (35) and adenovirus type 5 (16), and the distinguishable restriction sites were in *cis*. Table 1 lists the viruses, the method of construction, and the origin of the mutations. Because of the availability of cell lines expressing individual early genes, most experiments were set up in a fully orthogonal design, in which infections were set up with pairs of wild-type or mutant viruses in both permissive and nonpermissive hosts. This design allows corrections to be made for any recombinational phenotype not associated with the specific deletion but perhaps arising from unknown aspects of the construction.

The E1a immediate-early gene regulates expression from all other adenovirus early promoters and specific cellular genes (reviewed in reference 5). Failure of E1a<sup>-</sup> viruses to recombine would imply either that E1a directly encoded a recombinational function or that it affected the expression of another gene(s) whose product(s) played a role(s) in recombination.

In the first experiment, KB8 cells, which constitutively express E1a, and the control KB parent KB7 were used as the permissive and nonpermissive hosts, respectively (3).

\* Corresponding author.

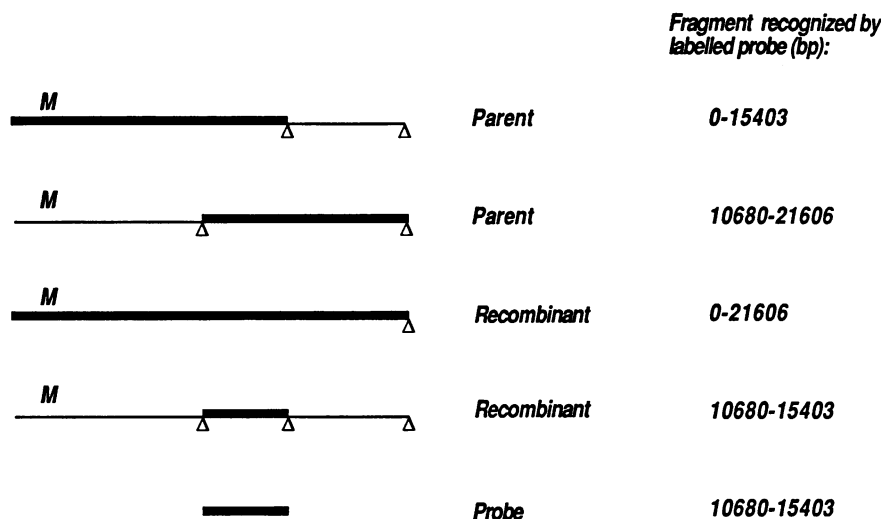


FIG. 1. General design of the recombination assay. The mutation (M) whose effects upon recombination were to be examined is present in both parents. The parents are differentially marked by a pair of restriction site alleles ( $\Delta$ ) usually in *trans*. In the design shown, these are the adenovirus type 2 *Bam*HI sites at bp 10680 and bp 15403 and their absent adenovirus type 5 counterparts. There is a site common to both parents at bp 21606. After coinfection, intracellular DNA is extracted by a modification of the Hirt technique (33) and restricted by the enzyme corresponding to the allelic sites, and the fragments are separated by agarose gel electrophoresis. Hybridization to a labelled probe containing the sequences between the restriction sites detects fragments derived from parental and recombinant genomes (thick black lines).

Cells were infected in monolayer culture at a multiplicity of infection (MOI) of 10 of either pair of parents, individual samples were harvested at intervals, and intracellular DNA was extracted, restricted with *Bam*HI, and examined by blot hybridization. The results in Fig. 2 show that both pairs of viruses can recombine efficiently in KB8 cells and that the wild-type pair replicates and recombines efficiently in the KB7 cells. As expected, the E1a<sup>-</sup> mutant pair replicates very poorly in nonpermissive KB7 cells, with DNA replication delayed to between 20 and 24 h postinfection. On the original autoradiograph, a very faint recombinant band can

be detected, suggesting that E1a is not absolutely required for recombination. However, to address this more carefully, a higher input of virus is necessary, and for this purpose strongly adherent HeLa cells are preferable. Accordingly, HeLa cells in monolayer culture were infected with a MOI of 200 of each parent. The results in Fig. 3 show that recombinant product can be detected first at 26.5 h postinfection and that the quantity increases up to 48 h. From the reconstruction lanes shown on the left of the figure, it can be estimated that the final level of recombinant product is between 3 and 10% of the parental bands. These results demonstrate that

TABLE 1. Derivation and genotype of viruses

Virus	Serotype <sup>a</sup>	Mutation <sup>b</sup>	Restriction site <sup>c</sup>	Derivation <sup>d</sup>	Reference(s) <sup>e</sup>
Ad2	2	None	<i>Eco</i> RI, bp 21338 <i>Eco</i> RI, bp 25633		
Ad5	5	None			
TLL1	5/2ND1	None	<i>Bam</i> HI, bp 10680	Tpx81(RI) × LL24(Xh)	21, 7
15.5	5/2ND1	None	<i>Bam</i> HI, bp 15403	I <sub>0</sub> 115.1(Ba) × LLX1(Pa)	22, 7
LEV1	5/2	H5dl110 (E1b 496R)	<i>Bam</i> HI, bp 10680	pd1110(Xh) × Tpx81(Cl)	2, 21
LEV3	5/2ND1	H5dl110 (E1b 496R)	<i>Bam</i> HI, bp 15403	H5dl110(Ba) × 15.5(Xh)	2 and above
LEV4	5/2ND1	H5dl111 (E1b 196R)	<i>Bam</i> HI, bp 15403	H5dl111(Ba) × 15.5(Xh)	1 and above
LEV5	5/2ND1	H5dl111 (E1b 196R)	<i>Bam</i> HI, bp 10680	H5dl111(Ba) × TLL1(Pa)	1 and above
LEV6	5/2ND1	H5dl312 (E1a <sup>-</sup> )	<i>Bam</i> HI, bp 15403	H5dl312(Ba) × 15.5(Xh)	17 and above
LEV7	5/2ND1	H5dl312 (E1a <sup>-</sup> )	<i>Bam</i> HI, bp 10680	H5dl312(Sa) × TLL1(Pa)	17 and above
H2dl808	2	H2dl808 (E4 <sup>-</sup> )	<i>Eco</i> RI, bp 21338 <i>Eco</i> RI, bp 25633		35
H5dl366	5	H5dl366 (E4 <sup>-</sup> )			16

<sup>a</sup> Viruses with hybrid serotype have the adenovirus type 5 (Ad5) hexon and the Ad2 fiber derived from Ad2<sup>+</sup>ND1. All chimeric viruses derive from P54, a recombinant between H5ts142 and Ad2<sup>+</sup>ND1ts4 (21).

<sup>b</sup> H5dl110 has a deletion of bp 2333 to 2804 (inclusive). This creates a nonfunctional fragment of the E1b 496R polypeptide. H5dl111 has a deletion from bp 1849 to 1950 (inclusive) and a 16-bp insert at the site of deletion. A 6.8K polypeptide fragment with 43 amino acids from the 196R polypeptide should be produced. H5dl312 has a deletion extending from bp 448 to 1349 (inclusive), and because the promoter is missing, no E1a products are made. H2dl808 and H5dl366 both have deletions extending throughout most of the E4 coding region.

<sup>c</sup> Restriction sites refer to the Ad2-specific site used in the recombination assay. For example, LEV1 has the *Bam*HI site at bp 10680 but is missing that at bp 15403.

<sup>d</sup> All derivations were by overlap recombination (33). Each recombination is described as the leftward fragment times the rightward fragment, with the enzyme used to create the terminus of the fragments in parentheses. Ba, *Bam*HI; Cl, *Cla*I; Pa, *Pae*R71; RI, *Eco*RI; Sa, *Sal*I; Xh, *Xho*I.

<sup>e</sup> The two references refer to each of the parents used in the overlap.

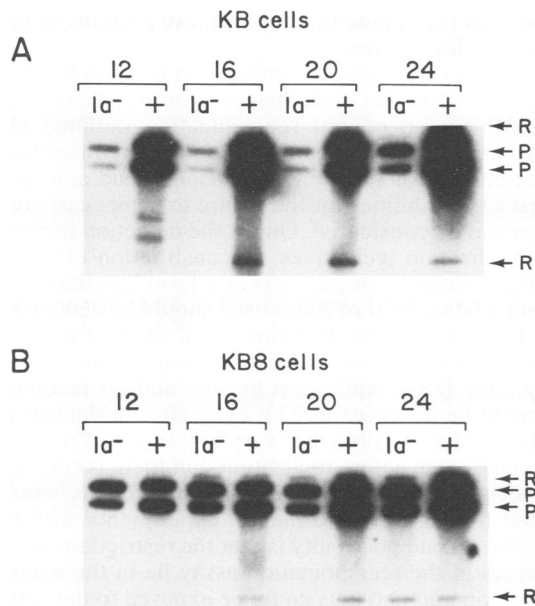


FIG. 2. Testing the effects of a deletion in E1a upon recombination in permissive and nonpermissive cells. KB (A) and KB8 (B) cells (expressing E1a), grown as monolayers in 35-mm culture dishes, were infected with LEV6 and LEV7 ( $E1a^-$ ) or TLL1 and 15.5 (wild type) at a MOI of 10 for each virus. At the hours indicated above the lanes, intracellular nucleic acid was isolated by a modification of the Hirt technique (33), and 1  $\mu$ g was restricted with *Bam*HI. The digest was examined by gel electrophoresis and blot hybridization essentially as described previously (39). P and R, fragments derived from parental and recombinant genomes, respectively.

E1a function is not required for homologous recombination. It has been shown previously that E1a functions also are dispensable for nonhomologous integration (32).

Although it is clear from Fig. 3 that E1a is not absolutely required for adenovirus recombination, the results do not eliminate the possibility that adenovirus functions downstream of E1a are required, because the block to their expression in  $E1a^-$  viruses is not absolute. At this level of input virus, the other early genes would be expressed, albeit in a delayed fashion and at reduced rates (26). Indeed, limited DNA replication is evident from the intensities of the parental fragments in the autoradiographs in Fig. 3 and, to a lesser extent, in Fig. 2, demonstrating that E2 functions are expressed. This is in accord with previous results which had strongly suggested that DNA replication was necessary for efficient recombination (39).

To examine the role in recombination of delayed early functions, other than those required for DNA replication, viruses containing deletions in E1b 196R (21K), E1b 496R (55K), and E4 were tested in both nonpermissive and the appropriate permissive cell lines. Perhaps the most interesting observation concerns E1b 21K. Deletions in this gene have pleiotropic phenotypes, but the most relevant point for recombination is that in semipermissive cell lines, both cellular and viral DNAs are degraded (18; complete references in reference 36). Although the mechanism for the degradation is not known, it can be imagined that it might stimulate or depress recombination frequencies. KB cells in monolayer culture were infected with each pair of viruses at a MOI of 10. As a control, KB18 cells which constitutively express the E1b genes (3) were infected similarly. Most of

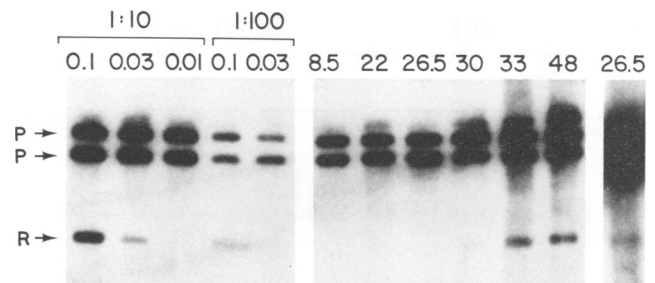


FIG. 3. Testing the effects of a deletion in E1a upon recombination in high-multiplicity infection of nonpermissive HeLa cells. HeLa cells in 35-mm culture dishes were infected with LEV6 and LEV7 at a MOI of 200 of each parent. Samples of intracellular nucleic acid were isolated at the hours indicated above the lanes in the right panel and processed as described in the legend to Fig. 2. The rightmost lane is from an exposure sevenfold longer than that of the main body of the figure. The reconstruction standards in the left panel were made by isolating intracellular nucleic acid from a 72-h time point in single infections with TLL1, 15.5, and a wild-type virus (P54 [21]) with one of the recombinant configurations of *Bam*HI sites. 1:10 and 1:100 refer to the dilution of the nucleic acid mix prior to digestion and analysis, and 0.1, 0.03, and 0.01 refer to the proportion of recombinant DNA in the total. In the original autoradiograph, a recombinant band could be detected at a proportion of 0.01 in the 1:10 dilution and at a proportion of 0.03 at the 1:100 dilution.

the cells from each infection were processed for the isolation of purified virus-encapsidated DNA, and a small sample was used for the extraction of intracellular DNA. Results with purified viral DNA show that recombination can occur in the semipermissive cells (Fig. 4; compare lanes 5 and 6). Although the absolute amount of recombinant product is smaller than in the permissive infection or with the wild-type pair of viruses in either cell type (lanes 7 and 8), the parental viral DNA yield is similarly reduced. This was to be expected from the fact that the yield of infectious virus was lowered some 30-fold (data not shown). Quantitation of the radioactivity contained in recombinant and parental bands showed no significant decrease in the recombinant fraction in the 21K $^-$  virus infections in the semipermissive versus permissive infections (lanes 5 and 6). These results also demonstrate the degradation of viral DNA seen in the intracellular DNA isolated from cells infected with 21K $^-$  viruses (lane 1), where the recombinant band can be detected with difficulty in the background of degraded DNA. The results from intracellular DNAs isolated from the other infections (lanes 2 to 4) are similar to those obtained from the purified viral DNA (lanes 5 to 8). We can conclude that the E1b mutation has no substantial effect on recombinant fraction and thus that the E1b 21K protein is unlikely to play a role in adenovirus recombination.

Similar experiments were carried out with viruses containing mutations in the E1b 496R gene, again with KB18 and KB7 or KB cells as the permissive and semipermissive host cells, respectively. No difference could be detected in the recombinant fraction observed in infections with any cell type and with both mutant and wild-type virus pairs (data not shown). To test the role of E4, crosses were performed with deletion mutants from adenovirus type 2 and adenovirus type 5 that are unable to express any of the E4 polypeptides (16, 35). The permissive cells were from W162, a Vero cell line expressing E4 functions (34), and KB cells were used as the nonpermissive host. Although the recombinant fraction

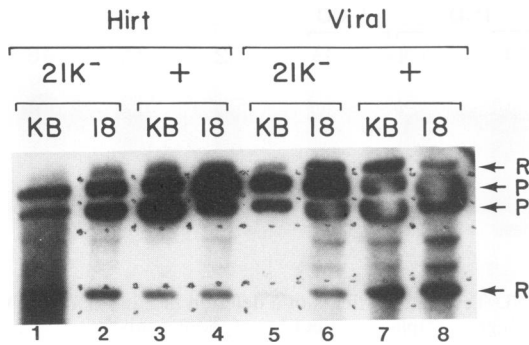


FIG. 4. Testing the effects of a mutation in the E1b 196R (21K) polypeptide upon recombination in permissive and semipermissive cells. KB and KB18 cells (expressing E1b) in monolayer culture in 175-cm<sup>2</sup> flasks were infected with LEV4 and LEV5 (E1b 21K<sup>-</sup>) or TLL1 and 15.5 (wild type) at a MOI of 10 of each parent. At 48 h postinfection, cells were removed from the flasks and the majority were processed for the isolation of purified virus. Viral DNA was isolated from CsCl-banded virions. A small sample of infected cells was processed for the isolation of intracellular nucleic acid. All samples were subsequently examined as described in the legend to Fig. 2. The headings (top to bottom) refer to the route of isolation of the DNA, the genotype of the parents, and the cell type infected. Parental (P) and recombinant (R) fragments are indicated. The anomalously migrating fragments seen in several of the samples were produced by "star" activity of the particular *Bam*HI digestion. The small marks to the sides of the lanes are an alignment grid for excision of the recombinant and parental fragments for quantitation of recombination.

at 24 h postinfection was lower in the KB cells than in the WI62 cells (data not shown), this was commensurate with the lower extent of DNA replication expected in nonpermissive cells (16, 35). We can conclude that E4 functions do not play a major role in adenovirus recombination. Furthermore, because late protein production is severely diminished in the E4 deletion mutants (16, 35), it is very unlikely that a late gene function is involved in recombination. Although no formal tests for recombination proficiency were conducted with deletions in E3, many common strains used in the laboratory contain such deletions, and no effects on recombination have been noted.

Taken together with earlier results (39), it seems likely that none of the currently defined early gene functions, with the exception of those involved in DNA replication, is essential for adenovirus recombination. In the E1a<sup>-</sup> crosses, the results support previous suggestions (39, 40) that the onset of recombination is closely tied to the onset of DNA replication, as recombination was not observed until the delayed time of replication characteristic of E1a<sup>-</sup> mutations in nonpermissive cells. However, extensive replication may not be necessary for high levels of recombination. This is seen clearly in Fig. 3, where the increase in DNA from the input level detected at 8.5 h to the final time point at 48 h is less than fivefold, yet the recombinant fraction increases from undetectable levels at 22 h to some 3 to 10% of the total at 48 h postinfection. It is important to stress that these and previous results do not rule out the possibility that the viral proteins involved in replication also have a separate role in recombination which might be uncovered by appropriate mutagenesis. Detailed examinations of the potential domains of the DNA-binding protein, DNA polymerase, and terminal protein are being conducted (9, 14, 20, 23, 24), and it may be

necessary to reexamine the role of these proteins in light of subsequent discoveries.

The inability to detect recombination prior to DNA replication, while it is one of the bases upon which we propose the model for adenoviral recombination outlined above, poses a paradox in that it has been shown that unreplicable transfected DNA is recombined efficiently and quickly (12). Two trivial possibilities for the failure to detect early recombination can be considered. One is the detection limit of our blot hybridization techniques. Reconstruction experiments show that recombinant products at a level less than 0.3% of the input when a MOI of 200 is used should be detected (Fig. 3 and data not shown). The kinetics of the E1a cross show the appearance of about 1% recombinant product at 26.5 h, shortly after DNA replication begins, and no recombinant band could be observed at 22 h even after prolonged exposure. It remains possible that very low levels of recombination do take place before replication, but there is certainly no rapid accumulation of recombinant product, as would be expected from the transformation experiments with naked DNA. The second possibility is that the restriction sites used as markers in the recombination assay lie in the major late transcription unit and thus could be exposed to the action of the recombination machinery only at late times. There is now abundant evidence from eukaryotic systems that transcription can stimulate recombination (reviewed in references 6 and 30). However, tests with restriction sites that lie in early regions E1b and E2b reveal no earlier onset of recombination (data not shown). Thus, we conclude that adenoviral genomes, unlike transfected DNA, are refractory to recombination or are otherwise protected from cellular recombination functions at early times. The basis for this is not known but could reflect aspects of the nucleoprotein structure of the adenovirus genome prior to replication.

All of the eukaryotic DNA-containing viruses exhibit high rates of recombination, yet so far, viral recombination functions have been demonstrated only in the poxviruses (4, 10, 19), although their identity has not been determined. Because poxvirus recombination takes place in the cytoplasm, recombination would be expected to require the expression of viral genes. On the other hand, in the nuclear DNA-containing viruses, recombination may be a consequence of cellular repair enzymes acting on the novel substrates presented by viral DNA replication. With the development of completely defined *in vitro* DNA replication systems for both adenovirus and simian virus 40 (reviewed in references 8 and 31), it may be possible to exploit them to study the molecular mechanisms involved in cellular DNA repair.

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#### REFERENCES

1. Babiss, L. E., P. B. Fisher, and H. S. Ginsberg. 1984. Effect on transformation of mutations in the early region 1b-encoded 21- and 55-kilodalton proteins of adenovirus 5. *J. Virol.* **52**:389-395.
2. Babiss, L. E., and H. S. Ginsberg. 1984. Adenovirus type 5 early region 1b gene product is required for efficient shutoff of host protein synthesis. *J. Virol.* **50**:202-212.
3. Babiss, L. E., C. S. H. Young, P. B. Fisher, and H. S. Ginsberg. 1983. Expression of adenovirus E1a and E1b gene products and the *Escherichia coli* XGPRT gene in KB cells. *J. Virol.* **46**:454-465.

4. **Ball, L. A.** 1987. High-frequency homologous recombination in vaccinia virus. *J. Virol.* **61**:1788–1795.
5. **Berk, A. J.** 1986. Adenovirus promoters and Ela transactivation. *Annu. Rev. Genet.* **20**:45–79.
6. **Blackwell, T. K., and F. W. Alt.** 1991. Mechanism and developmental program of immunoglobulin gene rearrangement in mammals. *Annu. Rev. Genet.* **23**:605–636.
7. **Brunet, L. J., L. E. Babiss, C. S. H. Young, and D. R. Mills.** 1987. Mutations in the adenovirus major late promoter: effects upon viability and transcription during infection. *Mol. Cell. Biol.* **7**:1091–1100.
8. **Challberg, M. D., and T. J. Kelly, Jr.** 1989. Animal virus DNA replication. *Annu. Rev. Biochem.* **58**:671–717.
9. **Chen, M., and M. S. Horwitz.** 1989. Dissection of functional domains of adenovirus DNA polymerase by linker-insertion mutagenesis. *Proc. Natl. Acad. Sci. USA* **86**:6116–6120.
10. **Evans, D. H., D. Stuart, and G. McFadden.** 1988. High levels of genetic recombination among cotransfected plasmid DNAs in poxvirus-infected mammalian cells. *J. Virol.* **62**:367–375.
11. **Flint, S. J., S. M. Berget, and P. A. Sharp.** 1976. Characterization of single-stranded viral DNA sequences present during replication of adenovirus types 2 and 5. *Cell* **9**:559–571.
12. **Folger, K. R., K. Thomas, and M. R. Capecchi.** 1985. Nonreciprocal exchanges of information between DNA duplexes cojected into mammalian cell nuclei. *Mol. Cell. Biol.* **5**:59–69.
13. **Folger, K. R., E. A. Wong, G. Wahl, and M. R. Capecchi.** 1982. Patterns of integration of DNA microinjected into cultured mammalian cells: evidence for homologous recombination between injected plasmid DNA molecules. *Mol. Cell. Biol.* **2**:1372–1387.
14. **Freimuth, P. I., and H. S. Ginsberg.** 1986. Codon insertion mutants of the adenovirus terminal protein. *Proc. Natl. Acad. Sci. USA* **83**:7816–7820.
15. **Ginsberg, H. S., and C. S. H. Young.** 1977. The genetics of adenoviruses, p. 27–88. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), *Comprehensive virology*, vol. 9. Plenum Publishing Corp., New York.
16. **Halbert, D. N., J. R. Cutt, and T. Shenk.** 1986. Adenovirus early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. *J. Virol.* **56**:250–257.
17. **Jones, N., and T. Shenk.** 1979. Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. *Cell* **17**:683–689.
18. **Lai Fatt, R. B., and S. Mak.** 1982. Mapping of an adenovirus function involved in the inhibition of DNA degradation. *J. Virol.* **42**:969–977.
19. **Merchlinsky, M.** 1989. Intramolecular homologous recombination in cells infected with temperature-sensitive mutants of vaccinia virus. *J. Virol.* **63**:2030–2035.
20. **Morin, N., C. Delsert, and D. F. Klessig.** 1989. Mutations that affect phosphorylation of the adenovirus DNA-binding protein alter its ability to enhance its own synthesis. *J. Virol.* **63**:5228–5237.
21. **Munz, P. L., C. Young, and C. S. H. Young.** 1983. The genetic analysis of adenovirus recombination in triparental and superinfection crosses. *Virology* **126**:576–586.
22. **Munz, P. L., and C. S. H. Young.** 1984. Polarity in adenovirus recombination. *Virology* **135**:503–514.
23. **Neale, G. A. M., and G. R. Kitchingman.** 1990. Conserved region 3 of the adenovirus type 5 DNA-binding protein is important for interaction with single-stranded DNA. *J. Virol.* **64**:630–638.
24. **Roovers, D. J., P. F. Overman, X.-Q. Chen, and J. S. Sussenbach.** 1991. Linker mutation scanning of the genes encoding the adenovirus type 5 terminal protein precursor and DNA polymerase. *Virology* **180**:273–284.
25. **Rosenstrauss, M. J., and L. A. Chasin.** 1978. Separation of linked markers in Chinese hamster cell hybrids: mitotic recombination is not involved. *Genetics* **90**:735–760.
26. **Shenk, T., N. Jones, W. Colby, and D. Fowlkes.** 1979. Functional analysis of adenovirus-5 host range deletion mutants defective for transformation of rat embryo cells. *Cold Spring Harbor Symp. Quant. Biol.* **44**:367–375.
27. **Stahl, F.** 1979. Genetic recombination. Thinking about it in phage and fungi. W. H. Freeman & Co., San Francisco.
28. **Subramani, S., and B. L. Seaton.** 1988. Homologous recombination in mitotically dividing mammalian cells, p. 549–573. *In* R. Kucherlapati and G. R. Smith (ed.), *Genetic recombination*. American Society for Microbiology, Washington, D.C.
29. **Takemori, N.** 1972. Genetic studies with tumorigenic adenoviruses. III. Recombination in adenovirus type 12. *Virology* **47**:157–167.
30. **Thomas, B. J., and R. Rothstein.** 1991. Sex, maps, and imprinting. *Cell* **64**:1–3.
31. **Tsurimoto, T., T. Melendy, and B. Stillman.** 1990. Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin. *Nature (London)* **346**:534–539.
32. **Van Doren, K., D. Hanahan, and Y. Gluzman.** 1984. Infection of eucaryotic cells by helper-independent recombinant adenoviruses: early region 1 is not obligatory for integration of viral DNA. *J. Virol.* **50**:606–614.
33. **Volkert, F. C., and C. S. H. Young.** 1983. The genetic analysis of recombination using adenovirus overlapping terminal DNA fragments. *Virology* **125**:175–193.
34. **Weinberg, D. H., and G. Ketner.** 1983. A cell line that supports the growth of a defective early region 4 deletion mutant of human adenovirus type 2. *Proc. Natl. Acad. Sci. USA* **80**:5383–5386.
35. **Weinberg, D. H., and G. Ketner.** 1986. Adenoviral early region 4 is required for efficient viral DNA replication and for late gene expression. *J. Virol.* **57**:833–838.
36. **White, E., and B. Stillman.** 1987. Expression of adenovirus E1b mutant phenotypes is dependent on the host cell and on synthesis of E1a proteins. *J. Virol.* **61**:426–435.
37. **Williams, J., T. Grodzicker, P. Sharp, and S. Sambrook.** 1975. Adenovirus recombination: physical mapping of crossover events. *Cell* **4**:113–119.
38. **Williams, J. F., and S. Ustaçelebi.** 1971. Complementation and recombination with temperature-sensitive mutants of adenovirus type 5. *J. Gen. Virol.* **13**:345–348.
39. **Young, C. S. H., G. Cachianes, P. Munz, and S. Silverstein.** 1984. Replication and recombination in adenovirus-infected cells are temporally and functionally related. *J. Virol.* **51**:571–577.
40. **Young, C. S. H., and S. J. Silverstein.** 1980. The kinetics of adenovirus recombination in homotypic and heterotypic genetic crosses. *Virology* **101**:503–515.