MICROBIOLOGICAL REVIEWS, Dec. 1987, p. 431–438
0146-0749/87/040431-08$02.00/0

Vol. 51, No. 4

Complementation for Replication by Unrelated Animal Viruses Containing DNA Genomes

KAREN D. COCKLEY AND FRED RAPP*

Department of Microbiology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

INTRODUCTION

Viruses have proven to be of major importance in medical science both as infectious agents and as models for molecular studies. Animal viruses containing deoxyribonucleic acid (DNA) genomes, in particular, provide simple model systems that can be used to address many complex biological problems. All animal DNA viruses have small genomes, relative to the host cells they infect, that can be easily manipulated to examine the mechanisms of replication and gene regulation. Investigation of the genetic material of viruses also provides information that may help to elucidate the mechanisms associated with gene regulation in higher organisms. DNA viruses have served as a focus of intense interest to virologists for many years (79).

The study of DNA virus-virus interactions within host cells has provided much knowledge concerning both the organization and the expression of genetic information (79). A variety of interactions, both genetic and nongenetic, may occur between two viruses in culture. Several types of nongenetic interactions that may occur are (i) phenotypic mixing, (ii) genotypic mixing, (iii) interference, (iv) enhancement, and (v) complementation. The study of these different types of interactions has contributed greatly to our understanding of gene function.

Complementation, the focus of this review, involves the interaction between two viruses, one or both of which may be defective and unable to replicate in the infected host cell. This interaction results in replication of one or both viruses under conditions in which multiplication would not ordinarily occur. The progeny produced maintain the genotype and phenotype of the parental virus. Different types of complementation are possible, and the mechanisms by which they occur may vary (Table 1).

* Corresponding author.

SCOPE

As the science of virology evolved, it became apparent to investigators that many viruses had a limited host range and were not capable of completing a full replicative cycle under various conditions. Early on, investigators demonstrated the ability of genetically related viruses of the same group to aid in the replication of otherwise growth-restricted viruses (35, 78). Only gradually have virologists come to appreciate the many interactions between unrelated DNA virus genomes.

This manuscript reviews the knowledge that has accumulated to date concerning the interrelationship between otherwise structurally and genetically unrelated animal DNA viruses for replication. The complementary interactions described may occur between two defective viruses, both capable of adsorbing and penetrating host cells but incapable of completing a replicative cycle, or between an active virus, capable of replication, and a defective or otherwise inactive virus.

Only defective or inactive viruses or systems whose complementary interactions have begun to be addressed at the molecular level are discussed. Although the interrelatedness between viruses of the same family for replication has been the focus of scientific experimentation, this topic does not fall within the limits of this review. Instead, those complementary interactions occurring between divergent animal DNA viruses that result in replication of an otherwise growth-inhibited virus are addressed.

COMPLEMENTATION FOR REPLICATION BY UNRELATED DNA VIRUSES

Lytic Replication of Helper-Dependent Parvoviruses

Adeno-associated virus (AAV), originally identified as a contaminant of adenovirus stocks (2, 3, 62, 65, 89; M. D. Hoggan, Fed. Proc. 24:248, 1965), is a defective parvovirus

TABLE 1. Overview: complementation for replication by unrelated DNA viruses

Defective/inactive virus	Complementary virus	Helper function	Reference(s)
Adenovirus-SV40 hybrid	Adenovirus	Transcapsidation	Rapp et al. (80)
AAV	Adenovirus	E1A, E1B, E2A, E4	Carter et al. (20), Ostrove and Berns (71)
	HSV-1 and -2	Unknown	Buller et al. (16)
	HCMV	Unknown	McPherson et al. (63)
	Vaccinia virus	Unknown	Schlehofer (96)
Adenovirus mutant dl312	Pseudorabies virus	ICP-4	Feldman et al. (34)
	HCMV	IE genes	Tevethia and Spector (100)
Latent HSV-1 and -2	HCMV	Early genes	Colberg-Poley et al. (25, 26), Wigdahl et al. (106)
Adenovirus in simian cells[a]	SV40	-COOH terminus	Fey et al. (36)
		SV40 large T	Polvino-Bodnar and Cole (74)
	Adenovirus-SV40 hybrids		Rapp and Melnick (83)

[a] Precise mapping of the helper function occurs at 14 base pairs at 0.193 map unit on the SV40 map (104).

that exhibits absolute dependence on coinfection with a helper virus for productive replication (9, 10). In the absence of a helper virus, AAV efficiently integrates into host cell chromosomes via its inverted terminal repeats (4, 88). The ability of AAV to integrate its genome into cellular DNA is not unique and is a characteristic shared by all other known DNA viruses that infect the nucleus. The characteristics of AAV that distinguish it from other nuclear DNA viruses include the following: (i) lack of apparent phenotypic alteration in host cell chromosomes after AAV integration; (ii) lack of cellular transformation after AAV integration; and (iii) readily rescuable nature of the AAV genome after superinfection with a helper virus (see subsection, "Reactivation of Latent Parvoviruses"). Three distinct groups of DNA viruses, adenoviruses, herpesviruses, and poxviruses, complement replication of defective parvoviruses.

Complementation by adenoviruses. Adenovirus appears to be the more common natural helper of AAV (2, 4, 44, 73). Because adenovirus is far less complex than herpesviruses and poxviruses, much more is known about the interactions between AAV and adenovirus. A two-way interaction occurs between the "helper" adenovirus and the "defective" AAV. Adenovirus supplies the needed helper function for AAV replication and possibly for transcription and translation, whereas AAV inhibits lytic replication as well as the oncogenicity of adenovirus.

All of the helper functions (20) supplied by adenovirus have been mapped to the early genes. Although only six early adenovirus transcriptional regions (E1A, E1B, E2A, E2B, E3, and E4) have been identified, studies of the interaction between adenovirus and AAV have been complicated by the multiple protein products coded for by these genes. Despite this problem, evidence provided by adenovirus mutants and microinjection and transfection studies suggests that the adenovirus early region genes E1A, E1B, E2A, and E4 are required for supplying AAV helper functions (19, 48, 49, 59, 67, 86, 87) (Fig. 1A). Evidence also suggests that the adenovirus VA-1 ribonucleic acid (RNA) gene is required for AAV replication (48). The E2B region, thought to code for both the adenovirus terminal protein and DNA polymerase (97), is not essential for AAV replication. Of the adenovirus genes required for AAV help, only the E1B and E4 regions appear to directly affect AAV DNA replication.

Inhibition of adenovirus lytic infection after coinfection with defective AAV has consistently been observed (21, 91).

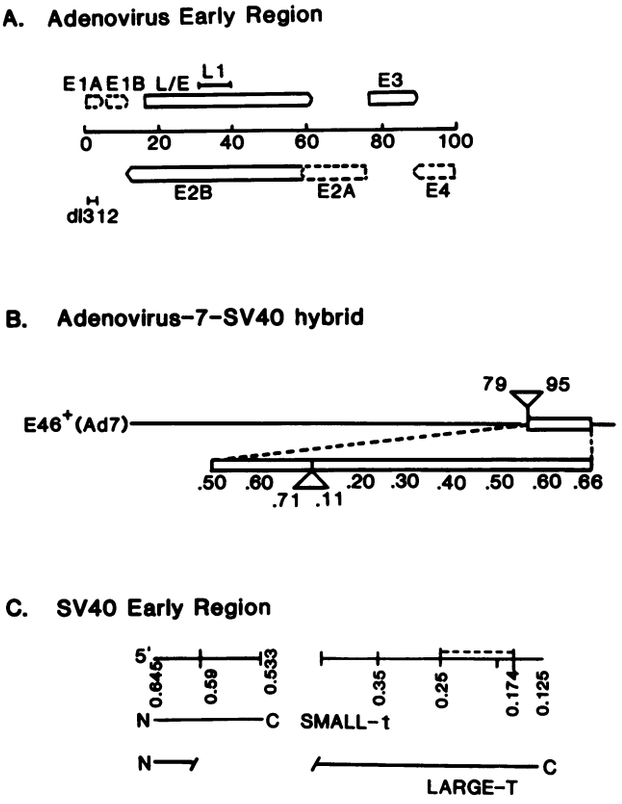

FIG. 1. Genomic maps of the (A) adenovirus early region transcription units (34), (B) adenovirus type 7-SV40 hybrid virus E46$^+$ (53), and (C) SV40 early region. The dashed arrow indicates Adenovirus early region genes necessary for replication of AAV. Only E1B and E4 appear to have a direct effect on AAV (A). E46$^+$ (B) contains both adenovirus type 7 sequence (solid line) and SV40 DNA (boxes). The SV40 segment (box) below the map represents the portion of SV40 inserted into hybrid virus. Triangles above and below the map represent deletions in adenovirus type 7 and SV40 portions of hybrid virus, respectively. The SV40 early region (C) encodes both small and large T antigens. Only a portion of the COOH terminus of large T antigen (dashed line), including specifically 14 base pairs at 0.193 map unit (104), is required for growth of adenovirus in simian cells.

DNA replication is the earliest part of the adenovirus replication cycle that appears to be inhibited. This inhibitory activity is multiplicity dependent; at very high multiplicities of AAV infection, even AAV replication becomes inhibited (21). If AAV infection is delayed until 6 or 7 h after adenovirus infection, no inhibition is observed (20), suggesting a competition between AAV and adenovirus for one or more adenovirus early gene products or for a limited cellular product. During the inhibition of adenovirus replication, it is not yet understood whether most cells are producing many AAV virions and only a few adenovirions or whether the majority of cells are producing AAV, with only a few producing adenovirus. In addition, the question still remains as to whether this strong in vitro inhibition has any relevance in vivo.

The induction of sarcomas in newborn Syrian hamsters by adenovirus also is inhibited by coinfection with AAV. Upon coinfection with AAV and adenovirus, induction of tumors is delayed and the number of tumors is significantly reduced (23, 56, 61). Coinfection is strictly required to produce this effect (61), although DNA can substitute for intact virions (29), and, in fact, only the terminal sequences of the genome are absolutely required to restrict oncogenicity. AAV infection of hamster embryo cells transformed by adenovirus also significantly inhibits oncogenicity, including inhibition of several properties related to transformation in culture, such as decreased saturation density and decreased ability to clone in methylcellulose (72).

The inhibition of adenovirus oncogenicity by AAV superinfection also has been investigated at the molecular level (72). The most significant finding to date is the decrease (80%) in the amount of detectable tumor antigen produced (72). Correlated with this decrease in tumor antigen is the observation that the inhibitory effect can be overcome in vivo; increasing the number of cells by fivefold increases the amount of tumor antigen and concomitantly decreases the period of tumor induction and the time available for the animal to mount an immune response.

Our current knowledge therefore suggests that the adenovirus helper functions required to support replication of AAV include the E1A, E1B, E2A, E4, and VA-1 RNA genes. Of these essential adenovirus genes, only E1B and E4 appear to directly affect replication of AAV. After coinfection, AAV has a demonstrated capacity to inhibit not only adenovirus DNA replication in a multiplicity-dependent fashion but also the oncogenicity of adenovirus. Only the terminal sequences of the AAV genome are essential to restrict adenovirus oncogenicity, which appears to be associated with a decrease in detectable adenovirus tumor antigen.

Complementation by herpesviruses. In addition to adenoviruses, herpesviruses are known to provide helper activity for AAV multiplication (1, 16, 63). The herpesvirus helper function initially was thought to be incomplete (1). AAV DNA (15, 90), RNA (22, 90), and protein (1, 12, 13) syntheses originally were reported to be induced by herpesviruses, although AAV infectivity could not be demonstrated (1, 12, 13, 90). However, more recent studies by Buller and co-workers (16) demonstrated that herpes simplex virus (HSV) is capable of providing complete help for AAV replication. Both HSV types 1 and 2 (HSV-1 and HSV-2) induce complete and relatively efficient multiplication of AAV in several human cell lines, including KB, HeLa, Hep-2, and human embryonic kidney (16). Only one obvious helper-related difference in AAV synthesis is observed when HSV instead of adenovirus help is provided. Multiplication of AAV via HSV-induced help results in the earlier appearance and peaking of AAV DNA, RNA, and protein syntheses (16), undoubtedly reflective of the short replication cycle of HSV. The increased rate of AAV precursor synthesis is not associated with any marked difference in AAV yield.

Coinfection with AAV and human cytomegalovirus (HCMV) strain Towne in human embryonic fibroblasts also has demonstrated accumulation of AAV capsid antigen and production of infectious AAV (63). There is a 24-h lag in production of AAV when helper activity is provided by HCMV as opposed to adenovirus (63). In addition, McPherson et al. (63) observed an HCMV AAV-associated synergistic effect on cellular cytopathology. The success of these investigators in demonstrating complementation for AAV replication by HCMV, in contrast to others (1), is attributed to the use of high-titer HCMV stocks ($>10^7$ plaque-forming units/ml) as well as to the use of the entire infected cell culture as opposed to just the supernatant for assays (63). It appears that newly replicated AAV produced during HCMV coinfection remains cell associated for several days, in contrast to AAV produced during adenovirus coinfection which is quickly released into the supernatant medium.

The exact helper functions provided by HSV and HCMV have not yet been defined. However, the success of investigators (16, 63) in demonstrating complementation of AAV by HSV and HCMV provides a tool which may prove useful in probing the early regulatory functions of the herpesviruses. Although AAV infection has not been associated with any human disease to date, the report of its interaction with the herpesviruses permits speculation on the role of AAV in human viral pathogenesis via AAV persistence or integration into human cells or both. The demonstrated ability of herpesviruses to complement AAV replication in human cells poses many questions which can now begin to be investigated in vitro at the molecular level as well as in vivo with animal models.

Complementation by poxviruses. In addition to adenoviruses and herpesviruses, a third virus group, the poxviruses, has recently been demonstrated to function as helper viruses for replication of defective parvoviruses (96). Poxviruses represent a large virus family composed of six genera. All poxviruses share a common nucleoprotein antigen and contain several virion-encoded enzymes, including a DNA-dependent RNA polymerase. The virion-encoded DNA polymerase may be correlated with its cytoplasmic site of replication. However, DNA polymerases with homology to the poxvirus DNA polymerase (31) are encoded by members of at least two other virus families, including the herpesviruses and the adenoviruses.

Vaccinia virus, a member of the *Orthopoxvirus* genus, is the prototype member of the poxvirus family (66). During examination of amplification of specific DNA sequences by vaccinia virus, Schlehofer (96) demonstrated that preinfection of simian virus 40 (SV40)-transformed human kidney (NB-E) cells with AAV type 5 (AAV-5) inhibited vaccinia virus-induced SV40-specific amplification. In addition, he observed that, during coinfection, vaccinia virus complemented AAV for replication of AAV DNA and for synthesis of AAV structural antigens (96). Analysis of AAV/vaccinia virus-coinfected cells was accomplished by dispersed cell assays, using ^{32}P-labeled AAV-5 DNA, and by immunofluorescence assays with monoclonal antibodies directed against AAV-5 capsid proteins. The effect of AAV-5 on replication of vaccinia virus in NB-E cells, the effect of AAV-5 on transformation by vaccinia virus (57), and the specific poxvirus helper functions essential for replication of defective parvoviruses have not yet been defined.

Although all three virus groups shown to support replication of defective parvoviruses possess a homologous, virus-encoded DNA polymerase (31), involvement of this enzyme in providing the helper function(s) necessary to support replication of defective parvoviruses is questionable, based on evidence that adenovirus E2B, which encodes the DNA polymerase gene, is not essential for providing AAV help (97). Future investigations into the nature of defective parvovirus replication are necessary if the essential helper functions provided by adenoviruses, herpesviruses, and poxviruses are to be clearly defined.

Reactivation of Latent Parvovirus by Adenoviruses

The rescue of integrated latent AAV by adenovirus represents a unique situation compared with complementation of AAV by adenovirus following coinfection. Latent AAV infection, which is an integral part of the replicative cycle of this virus, was discovered by Berns et al. (11). Berns and colleagues (11) demonstrated that when African green monkey kidney cells, found uniformly negative for AAV antigens and infectivity, were challenged with an AAV-free adenovirus stock, AAV was produced in >20% of the cultures.

Several systems have been developed to study the mechanism of AAV latency and reactivation (8, 11, 24, 28, 38, 40, 45, 71). Data derived from these studies are consistent with the integration of AAV DNA as a linear tandem repeat into cellular DNA. The integration process appears to be specific for the terminal sequence of the viral genome but random with respect to cell DNA sequences. Results of these studies also indicate that another adenovirus helper function, in addition to those necessary for provision of help during coinfection, may be required for rescue of latent AAV. Adenovirus hr6, a host-range mutant mapped to early region E1B and deficient in adenovirus T-antigen synthesis (38, 40), is capable of supporting AAV DNA as well as wild-type virus replication (71). However, superinfection with hr6 is incapable of rescuing latent AAV as measured by AAV DNA synthesis (71). Although the results of Ostrove and Berns (71) appear to define the 58,000-molecular-weight (58K) adenovirus T antigen as a specific requirement for rescue of latent AAV, Cheung et al. (24) challenged this claim when they reported that continued passage of latently infected cells results in excision of free AAV copies in the absence of adenovirus. To date, controversy still exists concerning the strict definition of the adenovirus functions required for rescue of latent AAV.

Herpesviruses

Herpesviruses represent one of the more diverse groups of DNA-containing viruses. There are at least five distinct human herpesviruses, HSV-1, HSV-2, varicella-zoster virus, HCMV, and Epstein-Barr virus, which vary considerably with respect to host range and biological properties. Characteristic of the herpesvirus family are a linear double-stranded DNA genome, ranging in molecular weight from 80 $\times 10^6$ to 150 $\times 10^6$; an icosahedral nucleocapsid containing 162 capsomers; and a membranous envelope acquired as the nucleocapsid buds through the cell membrane. Also characteristic of human herpesvirus infections are the three principal phases of gene expression (immediate-early [IE], early [E], and late [L]), which are regulated in a cascade fashion. Although the intimate details associated with replication do vary, infection with any of the human herpesviruses results in one of the following biological effects: (i) lytic growth; (ii) latent or persistent infection; or (iii) transformation.

Interaction of adenovirus and the herpesviruses. Both adeno- and herpesviruses encode transactive transcriptional factors which are responsible for transactivation of certain other genes (7, 30, 47, 51, 68, 75, 105). The E1A gene of adenovirus and the ICP-4 gene of herpesvirus activate transcription of the early region of each virus, respectively. Although transactivation of transcription by E1A and ICP-4 cannot be defined strictly as complementation, it does provide a well-characterized system in which to study the mechanisms of viral gene regulation.

HCMV, another member of the herpesvirus family, has been demonstrated not only to functionally replace E1A for adenovirus activation, but also to complement the adenovirus type 5 immediate early ($E1A^-$) mutant dl312 for lytic growth. The measurement of adenovirus production is possible because, unlike pseudorabies virus, a swine herpesvirus (6, 52), HCMV does not shut off host protein synthesis (98) within the time required for completion of the adenovirus lytic cycle, nor does it produce apparent cytopathology in the cell line used to titrate adenovirus. Although the HCMV gene products that mediate complementation of adenovirus have not yet been identified, IE genes have been implicated based on the shutoff of host macromolecular synthesis within 24 h after adenovirus infection (39), a time known to precede accumulation of late HCMV products (98, 99).

Reversal of HSV latency. The study of reactivation of HSV-1 and HSV-2 from a latent state in vitro demonstrated an interaction between two human herpesviruses which may be of importance during herpesvirus latency in vivo. Results revealed that superinfection with HCMV induces replication of HSV-1 and HSV-2 in latently infected human cell cultures (25, 26, 106). HSV latency was established in human embryonic lung cell cultures by treatment with 1-β-D-arabinofuranosylcytosine (25 μg/ml) at 37°C followed by inhibitor removal and temperature increase to 39.5 to 40°C (25, 106). Reactivation of latent HSV was evoked by either temperature shift down to 37°C or HCMV (strain AD169 or Towne) superinfection of latently infected cultures at 39.5 to 40°C (25, 26, 106). When latently infected cell cultures superinfected with low levels of HCMV were maintained at 39.5 to 40°C, harvested at various intervals, and titrated for HSV and HCMV, HSV was detected as early as 24 h after HCMV superinfection (26). Inactivation of HCMV by various means prior to superinfection abrogated its positive regulatory effects on latent HSV-1 and HSV-2. Reactivation of latent HSV after superinfection with four HCMV temperature-sensitive mutants (107, 108), from four different complementation groups, all unable to synthesize virus DNA at the nonpermissive temperature, implicates an early HCMV-specific function in the reactivation of latent HSV (26).

Interaction of Adenoviruses and Papovaviruses

The papovaviruses are a group of small DNA-containing tumor viruses with genomes ranging in molecular weight from 2×10^6 to 5×10^6. The papovavirus family, composed of papillomavirus, polyomavirus, and SV40, was originally classified by Melnick in 1962 (64). As a result of their relatively small size, papovaviruses are good candidates for viral genetic studies. Failure to propagate papillomaviruses in cell culture has impeded progress, although polyomavirus (murine papovavirus) and SV40 (simian papovavirus) have both been studied extensively. Due to recent advances in the study of complementation by SV40, the molecular mechanism associated with complementation by this papovavirus

has become well understood and therefore is used as a model for discussion in this review.

The result of infection of a susceptible cell by SV40 depends on the cell type infected. Lytic infection occurs after infection of monkey cells by SV40, although infection is abortive in many other cell types. During abortive SV40 infection, viral genetic information is expressed to a limited extent and can be retained and passed on to infected cell progeny, providing the potential for transformation. Abortive SV40 infection also can occur in monkey cells as a result of infection by defective SV40 virions. Superinfecting these cells with SV40 can cause lytic SV40 infection. SV40 also has been shown to complement other unrelated abortive DNA viruses for productive infection in simian cells (76).

Adenovirus-SV40 hybrids. The complementary interaction between nonproductive adenovirus and the simian papovavirus SV40 was recognized during the study of abortive adenovirus infection of simian cells (69, 79). As a result of the continued passage of adenovirus in simian cells (which often are contaminated with SV40) for the purpose of vaccine development, a unique defective form of SV40, the adenovirus-SV40 hybrid, was discovered (94). The hybrid virus was demonstrated to be dependent on adenovirus for replication (46, 84, 93).

The adenovirus-SV40 hybrids or PARA-adenoviruses (particle aiding the replication of adenovirus), as they are sometimes referred to, represent a unique strain of defective viruses that has been carefully characterized. This mutant virus strain, unable to produce SV40 capsid proteins, contains two types of viral particles: wild-type adenovirus particles and virus particles containing hybrid genomes composed of both adenovirus and SV40 enclosed in adenoviral capsids (94). The original hybrid genome ($E46^+$) (Fig. 1B) was demonstrated by heteroduplex analysis to contain a deletion of approximately 16% of the adenoviral type 7 DNA and an insertion corresponding to 75% of the complete SV40 DNA (54). Fine-mapping studies revealed a left boundary for the SV40 insertion at map position 0.50 on the SV40 map, which extends in a clockwise direction (relative to the SV40 map) with a right endpoint at map position 0.66 (53). In addition, the SV40 sequences between 0.71 and 0.11 map units are deleted and the sequences between 0.49 and 0.66 map units are repeated in tandem. The adenoviral genomic sequences deleted map to the sequences between positions 79 and 95 on the adenovirus type 7 physical map (53). The $E46^+$ hybrid contains almost exclusively SV40 early sequences and upon infection produces SV40 T and U antigens but not SV40 V antigen or capsid proteins (93). Inoculation of hamsters with the serially passaged adenovirus type 7 stock induces SV40-like tumors in vivo (46) and synthesis of SV40 tumor antigens in vitro (84, 93). Despite repeated passage of the adenovirus-SV40 defective viruses in culture, the SV40 genomic component is not lost because it is required for replication of adenovirus in green monkey kidney cells (83).

African green monkey kidney cells represent the cell culture of choice for growth of the PARA-adenoviruses; both hybrid virus and wild-type adenovirus replicate if cells are coinfected with both genomes (14, 95). Based on studies of plaque kinetics, investigators have demonstrated the requirement for adenovirus help in replication of the PARA particles in simian cells. Helper adenovirus infection of simian cells assures replication of the adenovirus-SV40 hybrids and results in enhanced titers of hybrid virus as well as conversion of plaque kinetics from 2 hits to 1 hit (17, 77). Human embryonic kidney cells also support replication of both components of the hybrid population. However, like replication in simian cells, the hybrids grown in human embryonic kidney cells require adenovirus for replication, whereas adenovirus can replicate in the absence of the coinfecting hybrid virus (18, 94).

The phenomenon of transcapsidation, originally observed by Rapp and co-workers (80), demonstrated that a large number of adenovirus serotypes can substitute for adenovirus type 7 in complementation of the hybrid genome. The defective DNA of PARA-adenoviruses replicates but no adenovirus capsids are synthesized (80, 92). Consequently, the PARA-adenovirus progeny become encased in an adenovirus capsid. Transcapsidation between PARA and adenovirus types 1, 2, 5, 6, and 12 has been reported (81, 92). In addition to adenovirus complementation of the PARA hybrid viruses, reciprocal complementation provided by the SV40 component of the hybrid genome can enhance replication of wild-type adenovirus (76, 83) (see below). As demonstrated, the adenovirus-SV40 hybrids provide a unique example of dual complementation in which active adenovirus complements a defective mutant genome for replication and the defective genome reciprocates by enhancing replication of the active adenovirus virion.

Complementation of adenovirus in simian cells. Infection of simian cells with adenovirus results in establishment of a nonpermissive viral infection in contrast to similarly infected human cells (50, 70, 82). The yield of adenovirus in simian cells can be enhanced to levels comparable to those in human cells by complementation after coinfection with SV40 (69, 76, 79). Although the exact nature of the block in adenovirus replication in monkey cells is not understood, it is clear that the early events of adenovirus multiplication, including synthesis of DNA, are not affected (32, 33, 37, 43, 85). However, some late virion proteins are synthesized in greatly reduced amounts (5, 10, 37, 43, 60, 78), which may be correlated with reduced messenger RNA levels (58).

Enhancement of adenovirus growth in simian cells has been attributed to an early function of SV40 (37, 55). Only a portion of the SV40 genome, specifically the carboxyl-terminal 113 amino acids of SV40 large T antigen (residues 596 through 708, encoded by 0.25 through 0.174 map units), is required to provide the adenovirus helper function (36, 74) (Fig. 1C). Study of the SV40-associated adenovirus helper function, using either adenovirus-SV40 hybrid mutants (36, 41, 42) or SV40 mutants (27, 74), implicates the 3′ end of the early region of SV40 in the provision of helper activity. In addition, Tijan et al. (101) demonstrated that microinjection of a 23K adenovirus type 2-SV40 hybrid fusion protein produced in nondefective adenovirus type 2-SV40 hybrid virus-infected cells containing a portion of the SV40 *A* gene provides the adenovirus helper function. Grodzicker and colleagues (42) reported that the presence in nondefective adenovirus type 2-SV40 hybrid virus-infected cells of a 30K hybrid protein, containing a carboxyl-terminal amino acid sequence encoded by the 3′-terminal region of the SV40 *A* gene, is responsible for mediation of adenovirus helper activity. Although the requirement for the COOH-terminal sequences of SV40 large T antigen for adenovirus replication in simian cells has been demonstrated (37, 55, 74), a direct role for the SV40 T antigen in mediation of this helper function has only been implied (104).

Recently, however, the helper function of SV40 large T antigen has been identified as an independent functional domain of SV40 (104). dlA2459 is a nonviable SV40 mutant (103) known to lack those large T-antigen residues required for adenovirus helper function (14 base pairs at 0.193 map

unit) (102). The block in productive infection by dlA2459 has recently been shown to occur at the same stage of infection as that in productive adenovirus infection in simian cells (104), suggesting a requirement for the SV40 adenovirus helper function during lytic infection by SV40. In addition to dlA2459, Tornow et al. (104) prepared other mutants affecting the COOH-terminal portion of large T antigen. One mutant, inv 2408, contains an inversion of the DNA between *Bam*H and *Bcl*I sites (0.144 to 0.189 map units) which results in transposition of the COOH-terminal 18 amino acids of VP-1 (104). The VP-1 T-antigen fusion protein produced by inv 2408 can overcome the defect of dlA2459, indicating that the helper function information of large T antigen represents a separate and separable SV40 functional domain (104).

These investigations have clearly defined the helper function provided by SV40 for complementation of adenovirus replication in simian cells at the molecular level. The helper function, which appears to be an independent functional domain of SV40 (104) maps within the COOH-terminal 113 amino acids of SV40 large T-antigen residues 596 to 708 (0.25 to 0.174 map units) (36, 74). Precise mapping of the function, which appears to be essential for not only complementation of adenovirus but also efficient SV40 capsid protein synthesis, has now been targeted at 14 base pairs at 0.193 map unit on the SV40 map (104) (Fig. 1C).

CONCLUDING REMARKS

The utility of animal DNA viruses as models for studying the mechanisms of viral infections is well known. Although substantial advances have been made in our understanding of viral gene function, continued efforts are necessary to dissect the molecular mechanisms underlying virus replication and the effect on host cells leading to disease. This review attempts to summarize the subset of events that enable viruses restricted for growth to replicate in the presence of genetically unrelated viruses. Such interactions at the molecular level will clearly shed light on virus gene functions in systems with limited genetic information and lower complexity than mammalian cells.

ACKNOWLEDGMENTS

We thank Elaine Neidigh for secretarial support and Melissa Clement for editorial assistance.

Work in this laboratory is supported in part by Public Health Service grants CA 09124, CA 27503, and CA 34479 awarded by the National Cancer Institute.

LITERATURE CITED

1. **Atchison, R. W.** 1970. The role of herpesviruses in adenovirus-associated virus replication in vitro. Virology **42**:155–162.
2. **Atchison, R. W., B. C. Casto, and W. M. Hammon.** 1965. Adenovirus-associated defective virus particles. Science **149**:754–756.
3. **Atchison, R. W., B. C. Casto, and W. M. Hammon.** 1966. Electron microscopy of adenovirus-associated virus (AAV) in cell cultures. Virology **29**:353–357.
4. **Bachmann, P. A., M. D. Hoggan, E. Kurstak, J. L. Melnick, H. G. Pereira, P. Tattersall, and C. Vago.** 1979. Parvoviridae: second report. Intervirology **11**:248–254.
5. **Baum, S. G., M. S. Horowitz, and J. V. Maizel.** 1972. Studies of the mechanism of enhancement of human adenovirus infection in monkey cells by simian virus 40. J. Virol. **10**:211–219.
6. **Ben-Porat, T., T. Rakusonova, and A. S. Kaplan.** 1971. Early functions of the genome of herpesvirus. II. Inhibition of the formation of cell-specific polysomes. Virology **46**:890–899.
7. **Berk, A. J., F. Lee, T. Harrison, J. Williams, and P. A. Sharp.** 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNA. Cell **17**:935–944.
8. **Berns, K. I., A. K. M. Cheung, J. M. Ostrove, and M. Lewis.** 1982. Adeno-associated virus latent infection, p. 249–265. *In* B. W. J. Mahy, A. C. Minson, and G. K. Darby (ed.), Virus persistence. Cambridge University Press, London.
9. **Berns, K. I., and W. W. Hauswirth.** 1979. Adeno-associated viruses. Adv. Virus Res. **25**:407–449.
10. **Berns, K. I., and T. J. Kelly, Jr.** 1974. Visualization of the inverted terminal repetition in adeno-associated virus DNA. J. Mol. Biol. **82**:267–271.
11. **Berns, K. I., T. C. Pinkerton, G. F. Thomas, and M. D. Hoggan.** 1975. Detection of adeno-associated virus (AAV)-specific nucleotide sequences in DNA isolated from latently infected Detroit 6 cells. Virology **68**:556–560.
12. **Blacklow, N. R., R. Dolin, and M. D. Hoggan.** 1971. Studies of the enhancement of an adenovirus-associated virus by herpes simplex virus. J. Gen. Virol. **10**:29–36.
13. **Blacklow, N. R., M. D. Hoggan, and M. S. McClanahan.** 1970. Adenovirus-associated viruses: enhancement by human herpesviruses. Proc. Soc. Exp. Biol. Med. **134**:952–954.
14. **Boeyé, A., J. L. Melnick, and F. Rapp.** 1966. SV40-adenovirus 'hybrids': presence of two genotypes and the requirement of their complementation for viral replication. Virology **28**:56–70.
15. **Boucher, D. W., J. L. Melnick, and H. D. Mayor.** 1971. Nonencapsidated infectious DNA of adeno-satellite virus in cells coinfected with herpesvirus. Science **173**:1293–1295.
16. **Buller, R. M. L., J. E. Jonik, E. D. Sebring, and J. A. Rose.** 1981. Herpes simplex virus types 1 and 2 help adeno-associated virus replication. J. Virol. **40**:241–247.
17. **Butel, J. S., and F. Rapp.** 1966. Detection of biologically active adenovirus unable to plaque in human cells. J. Bacteriol. **92**:433–438.
18. **Butel, J. S., and F. Rapp.** 1968. Replication of defective simian virus 40 and a monkey cell adapting component in human kidney cells. J. Virol. **2**:541–542.
19. **Carter, B., C. Marcus, C. Laughlin, and G. Ketner.** 1983. Properties of an adenovirus type 2 mutant, Ad 2d/807, having a deletion near the right-hand genome terminus: failure to help AAV replication. Virology **136**:505–516.
20. **Carter, B. J., F. J. Koczot, J. Garrison, J. Rose, and J. A. Polin.** 1973. Separate functions provided by adenovirus for adeno-associated virus multiplication. Nature (London) New Biol. **244**:71–73.
21. **Carter, B. J., C. A. Laughlin, L. M. De La Maza, and M. Myers.** 1979. Adeno-associated virus autointerference. Virology **92**:449–462.
22. **Carter, B. J., and J. A. Rose.** 1972. Adenovirus-associated virus multiplication. VIII. Analysis of in vivo transcription induced by complete or partial helper viruses. J. Virol. **10**:9–16.
23. **Casto, B. C., and C. R. Goodheart.** 1972. Inhibition of adenovirus transformation *in vitro* by AAV-1. Proc. Soc. Exp. Biol. Med. **140**:72–78.
24. **Cheung, A. K. M., M. D. Hoggan, W. W. Hauswirth, and K. I. Berns.** 1980. Integration of the adeno-associated virus genome into cellular DNA in latently infected human Detroit 6 cells. J. Virol. **33**:739–748.
25. **Colberg-Poley, A. M., H. C. Isom, and F. Rapp.** 1979. Reactivation of herpes simplex virus type 2 from a quiescent state by human cytomegalovirus. Proc. Natl. Acad. Sci. USA **76**: 5948–5951.
26. **Colberg-Poley, A. M., H. C. Isom, and F. Rapp.** 1981. Involvement of an early human cytomegalovirus function in reactivation of quiescent herpes simplex virus type 2. J. Virol. **37**:1051–1059.
27. **Cole, C. N., L. V. Crawford, and P. Berg.** 1979. Simian virus 40 mutants with deletions at the 3′ end of the early region and defective in adenovirus helper function. J. Virol. **30**:683–691.
28. **Cukor, G., N. R. Blacklow, M. D. Hoggan, and K. I. Berns.** 1984. Biology of adeno-associated virus, p. 33–66. *In* K. I. Berns (ed.), The parvoviruses. Plenum Publishing Corp., New York.
29. **De La Maza, L. M., and B. J. Carter.** 1981. Inhibition of adenovirus oncogenicity by adeno-associated virus DNA. J.

Natl. Cancer Inst. 67:1323–1326.
30. **Dixon, R. A. F., and P. A. Schaffer.** 1980. Fine structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein VP175. J. Virol. 36:109–203.
31. **Earl, P. L., E. V. Jones, and B. Moss.** 1986. Homology between DNA polymerases of poxviruses, herpesviruses, and adenoviruses: nucleotide sequence of the vaccinia virus DNA polymerase gene. Proc. Natl. Acad. Sci. USA 83:3659–3663.
32. **Eron, L., H. Westphal, and G. Khoury.** 1975. Posttranscriptional restriction of human adenovirus expression in monkey cells. J. Virol. 15:1256–1261.
33. **Feldman, L. A., J. S. Butel, and F. Rapp.** 1966. Interaction of a simian papovavirus and adenovirus. I. Induction of adenovirus tumor antigen during abortive infection of simian cells. J. Bacteriol. 91:813–819.
34. **Feldman, L. T., M. J. Imperiale, and J. R. Nevins.** 1982. Activation of early adenovirus transcription by the herpesvirus immediate-early gene: evidence for a common cellular control factor. Proc. Natl. Acad. Sci. USA 79:4952–4956.
35. **Fenner, F., and J. F. Sambrook.** 1964. The genetics of animal viruses. Annu. Rev. Microbiol. 18:47–94.
36. **Fey, G., J. B. Lewis, T. Grodzicker, and A. Bothwell.** 1979. Characterization of a fused protein specified by the adenovirus type 2-simian virus 40 hybrid AD2$^+$ND1 dp 2. J. Virol. 30:201–217.
37. **Friedman, M. P., M. J. Lyons, and H. S. Ginsberg.** 1970. Biochemical consequences of type 2 adenovirus and simian virus 40 double infections of African green monkey kidney cells. J. Virol. 5:586–597.
38. **Galos, R. S., J. Williams, T. Shenk, and N. Jones.** 1980. Physical location of host-range mutants of adenovirus: deletion and marker rescue. Virology 104:510–513.
39. **Ginsberg, H. J., L. J. Bello, and A. J. Levine.** 1967. Control of biosynthesis of host macromolecules in cells infected with adenovirus, p. 547–572. *In* J. S. Colter and W. Paranchych (ed.), The molecular biology of viruses. Academic Press, Inc., New York.
40. **Graham, F. L., T. Harrison, and J. Williams.** 1978. Defective transforming capacity of adenovirus type 5 host-range mutants. Virology 86:10–21.
41. **Grodzicker, T., C. Anderson, P. A. Sharp, and J. Sambrook.** 1974. Conditional lethal mutants of adenovirus 2-simian virus 40 hybrids. I. Host-range mutants of Ad2$^+$ND1. J. Virol. 13:1237–1244.
42. **Grodzicker, T., J. B. Lewis, and C. W. Anderson.** 1976. Conditional lethal mutants of adenovirus 2-simian virus 40 hybrids. II. Ad2$^+$ND1 host-range mutants that synthesize fragments of the Ad2$^+$ND1 30K protein. J. Virol. 19:559–571.
43. **Hashimoto, K., K. Nakajima, K. Oda, and H. Shimoto.** 1973. Complementation of translational defect for growth of human adenovirus type 2 in simian cells by a simian virus 40-induced factor. J. Mol. Biol. 81:207–223.
44. **Hoggan, M. D., N. R. Blacklow, and W. P. Rowe.** 1966. Studies of small DNA viruses found in various adenovirus preparations: physical, biological and immunological characteristics. Proc. Natl. Acad. Sci. USA 55:1467–1474.
45. **Honda, H., K. Shiroki, and H. Shimojo.** 1977. Establishment and characterization of KB cell lines latently infected with adeno-associated virus type 1. Virology 82:84–92.
46. **Huebner, R. J., R. M. Chanock, B. A. Rubin, and M. J. Casey.** 1964. Induction by adenovirus type 7 of tumors in hamsters having the antigenic characteristics of SV40 virus. Proc. Natl. Acad. Sci. USA 52:1333–1340.
47. **Imperiale, M. J., L. T. Feldman, and J. R. Nevins.** 1983. Activation of gene expression by adenovirus and herpesvirus regulatory genes acting in *trans* and by a *cis*-acting adenovirus enhancer element. Cell 35:127–136.
48. **Jarik, J. E., M. M. Huston, K. Cho, and J. A. Rose.** 1982. Requirement of adenovirus DNA binding protein and VA-1 RNA for production of adeno-associated virus polypeptides. J. Cell Biochem. Suppl. 6:209.
49. **Jarik, J. E., M. M. Huston, and J. A. Rose.** 1981. Locations of adenovirus genes required for the replication of adeno-associated virus. Proc. Natl. Acad. Sci. USA 78:1925–1929.
50. **Jerkofsky, M. A., and F. Rapp.** 1969. Replication and complementation of human adenoviruses and simian papovavirus. Proc. Soc. Exp. Biol. Med. 132:987–992.
51. **Jones, N., and T. Shenk.** 1979. An adenovirus type 5 early gene function regulates expression of other early viral genes. Proc. Natl. Acad. Sci. USA 76:3665–3669.
52. **Kaplan, A. S., H. Shimono, and T. Ben-Porat.** 1970. Synthesis of proteins in cells infected with herpesvirus. III. Relative amino acid content of various proteins formed after infection. Virology 40:90–101.
53. **Kelly, T. J., Jr.** 1975. Structure of the DNA of the adenovirus 7-simian virus 40 hybrid, E46$^+$, by electron microscopy. J. Virol. 15:1267–1272.
54. **Kelly, T. J., Jr., and J. A. Rose.** 1971. Simian virus 40 integration site in an adenovirus 7-SV40 hybrid DNA molecule. Proc. Natl. Acad. Sci. USA 68:1037–1041.
55. **Kimura, G.** 1974. Genetic evidence for SV40 gene function in enhancement of replication of human adenovirus in simian cells. Nature (London) 248:590–592.
56. **Kirschstein, R. C., K. O. Smith, and E. A. Peters.** 1968. Inhibition of adenovirus 12 oncogenicity by adeno-associated virus. Proc. Soc. Exp. Biol. Med. 128:670–673.
57. **Klessing, D. F., and C. W. Anderson.** 1975. Block to multiplication of adenovirus serotype 2 in monkey cells. J. Virol. 16:1656–1668.
58. **Koziorowska, J., K. Wlodarski, and N. Mazurowa.** 1971. Transformation of mouse embryo cells by vaccina virus. J. Natl. Cancer Inst. 46:225–241.
59. **Laughlin, C. A., N. Jones, and B. J. Carter.** 1982. Effect of deletions in adenovirus early region I genes upon replication of adeno-associated virus. J. Virol. 41:868–876.
60. **Lucas, J. J., and H. Ginsberg.** 1972. Transcription and transport of virus-specific ribonucleic acid in African green monkey kidney cells abortively infected with type 2 adenovirus. J. Virol. 10:1109–1117.
61. **Mayor, H. D., G. S. Houlditch, and D. M. Mumford.** 1973. Influence of adeno-associated satellite virus on adenovirus-induced tumors in hamsters. Nature (London) New Biol. 241:44–46.
62. **Mayor, H. D., R. M. Jamison, L. E. Jordon, and J. L. Melnick.** 1965. Structure and composition of a small particle prepared from a simian adenovirus. J. Bacteriol. 90:735–742.
63. **McPherson, R. A., L. J. Rosenthal, and J. A. Rose.** 1985. Human cytomegalovirus completely helps adeno-associated virus replication. Virology 147:217–222.
64. **Melnick, J. L.** 1962. Papova virus group. Science 135:1128–1130.
65. **Melnick, J. L., H. D. Mayor, K. O. Smith, and F. Rapp.** 1965. Association of 20-millimicron particles with adenovirus. J. Bacteriol. 90:271–274.
66. **Moss, B.** 1985. Replication of poxviruses, p. 685–704. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, J. Melnick, B. Roizman, and R. Shope (ed.), Virology. Raven Press, New York.
67. **Myers, M. W., C. A. Laughlin, F. T. Jay, and B. J. Carter.** 1980. Adenovirus helper function for growth of adeno-associated virus: effect of temperature-sensitive mutations in adenovirus early gene region 2. J. Virol. 35:65–75.
68. **Nevins, J. R.** 1981. Mechanisms of activation of early viral transcription by the adenovirus E1A gene product. Cell 26:213–220.
69. **O'Connor, G. T., A. S. Rabson, I. K. Berezesky, and F. J. Paul.** 1963. Mixed infection with simian virus 40 and adenovirus 12. J. Natl. Cancer. Inst. 31:903–917.
70. **O'Connor, G. T., A. S. Rabson, R. A. Malmgren, I. K. Berezesky, and F. J. Paul.** 1965. Morphologic observations of green monkey kidney cells after single and double infection with adenovirus 12 and simian virus 40. J. Natl. Cancer Inst. 34:679–693.
71. **Ostrove, J., and K. I. Berns.** 1980. Adenovirus early region 1b gene function required for rescue of latent adeno-associated virus. Virology 104:502–505.
72. **Ostrove, J. M., D. H. Duckworth, and K. I. Berns.** 1981.

Inhibition of adenovirus oncogenicity by adeno-associated virus. Virology **113**:521–533.
73. **Parks, W. P., J. L. Melnick, R. Rongery, and H. D. Mayor.** 1967. Physical assay and growth cycle studies of a defective adeno-satellite virus. J. Virol. **1**:171–180.
74. **Polvino-Bodnar, M., and C. N. Cole.** 1982. Construction and characterization of viable deletion mutants of simian virus 40 lacking sequences near the 3′ end of the early region. J. Virol. **43**:489–502.
75. **Preston, C. M.** 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or temperature-sensitive mutant *tsK*. J. Virol. **29**:275–284.
76. **Rabson, A. S., G. T. O'Conner, I. K. Berezosky, and F. J. Paul.** 1964. Enhancement of adenovirus growth in African green monkey kidney cell cultures by SV40. Proc. Soc. Exp. Biol. Med. **116**:187–190.
77. **Rapp, F.** 1967. Complementation of deoxyribonuclease acid-containing viruses, p. 311–325. *In* Y. Ito (ed.), First International Symposium on Tumor Viruses. Subviral carcinogenesis. Research Institute, Aichi Cancer Center, Chikusa-ku, Nagoya, Japan.
78. **Rapp, F.** 1968. Dependence and complementation among animal viruses containing deoxyribonucleic acid, p. 273–293. *In* L. V. Crawford and M. G. P. Stoker (ed.), The molecular biology of viruses. Cambridge University Press, London.
79. **Rapp, F.** 1969. Defective DNA animal viruses. Annu. Rev. Microbiol. **23**:293–316.
80. **Rapp, F., J. S. Butel, and J. L. Melnick.** 1965. SV40-adenovirus 'hybrid' populations: transfer of SV40 determinants from one type of adenovirus to another. Proc. Natl. Acad. Sci. USA **54**:717–724.
81. **Rapp, F., M. A. Jerkofsky, J. L. Melnick, and B. Levy.** 1968. Variation in the oncogenic potential of human adenoviruses carrying a defective SV40 genome (PARA). J. Exp. Med. **127**:77–90.
82. **Rapp, F., M. Jerkofsky, and D. Vanderslice.** 1967. Characterization of defectiveness of human adenoviruses in green monkey kidney cells. Proc. Soc. Exp. Biol. Med. **126**:782–786.
83. **Rapp, F., and J. L. Melnick.** 1966. Papovavirus SV40, adenovirus and their hybrids: transformation, complementation, and transcapsidation. Prog. Med. Virol. **8**:349–399.
84. **Rapp, F., J. L. Melnick, J. S. Butel, and T. Kitahara.** 1964. The incorporation of SV40 genetic material into adenovirus 7 as measured by intranuclear synthesis of SV40 tumor antigen. Proc. Natl. Acad. Sci. USA **52**:1348–1352.
85. **Reich, P. R., S. G. Baum, J. A. Rose, W. P. Rowe, and S. M. Weissman.** 1966. Nucleic acid homology studies of adenovirus type 7-SV40 interactions. Proc. Natl. Acad. Sci. USA **55**: 336–341.
86. **Richardson, W. D., B. J. Carter, and H. Westphal.** 1980. Vero cells injected with adenovirus type 2 mRNA produce authentic viral polypeptide patterns: early mRNA promotes growth of adenovirus-associated virus. Proc. Natl. Acad. Sci. USA **77**:931–935.
87. **Richardson, W. D., and H. Westphal.** 1981. A cascade of adenovirus early function is required for expression of adeno-associated virus. Cell **27**:133–141.
88. **Roizman, B.** 1979. The structure and isomerization of herpes simplex virus genomes. Cell **16**:481–494.
89. **Rose, J. A.** 1974. Parvovirus reproduction, p. 1–61. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 3. Plenum Publishing Corp., New York.
90. **Rose, J. A., and B. J. Carter.** 1972. Adenovirus-associated virus multiplication. VIII. Analysis of in vivo transcription induced by complete or partial helper virus. J. Virol. **10**:9–16.
91. **Rose, J. A., and F. Koczot.** 1972. Adenovirus-associated virus multiplication. VII. Helper requirement for viral deoxyribonucleic acid and ribonucleic acid synthesis. J. Virol. **10**:1–8.
92. **Rowe, W. P.** 1965. Studies of adenovirus-SV40 hybrid viruses. III. Transfer of SV40 genes between adenovirus types. Proc. Natl. Acad. Sci. USA **54**:711–717.
93. **Rowe, W. P., and S. G. Baum.** 1964. Evidence for a possible genetic hybrid between adenovirus type 7 and SV40 viruses. Proc. Natl. Acad. Sci. USA **52**:1340–1347.
94. **Rowe, W. P., and S. G. Baum.** 1965. Studies of adenovirus-SV40 hybrid viruses. III. Defectiveness of the hybrid particles. J. Exp. Med. **122**:955–966.
95. **Rowe, W. P., S. G. Baum, W. E. Pugh, and M. D. Hoggan.** 1965. Studies of adenovirus SV40 hybrid viruses. I. Assay system and function evidence for hybridization. J. Exp. Med. **122**:943–954.
96. **Schlehofer, J. R.** 1986. Vaccina virus, herpes simplex virus, and carcinogens induce DNA amplification in a human cell line and support replication of a herpesvirus dependent parvovirus. Virology **152**:110–117.
97. **Stillman, B. W., J. B. Lewis, L. T. Chow, M. B. Matthews, and J. E. Smart.** 1981. Identification of the gene for the adenovirus terminal protein precursor. Cell **23**:497–508.
98. **Stinski, M. F.** 1977. Synthesis of proteins and glycoproteins in cells infected with human cytomegalovirus. J. Virol. **23**:751–767.
99. **Stinski, M. F.** 1978. Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. J. Virol. **26**:686–701.
100. **Tevethia, M J., and D. J. Spector.** 1984. Complementation of an adenovirus 5 immediate early mutant by human cytomegalovirus. Virology **137**:428–431.
101. **Tijan, R., G. Frey, and A. Graessman.** 1978. Biological activity of purified simian virus 40 T antigen proteins. Proc. Natl. Acad. Sci. USA **75**:1279–1283.
102. **Tornow, J., and C. N. Cole.** 1983. Nonviable mutants of simian virus 40 with deletions near the 3′ end of gene A define a function for large T antigen required after onset of viral DNA replication. J. Virol. **47**:487–494.
103. **Tornow, J., and C. N. Cole.** 1983. Intracistronic complementation in the simian virus 40 A gene. Proc. Natl. Acad. Sci. USA **80**:6312–6316.
104. **Tornow, J., M. Polvino-Bodnar, G. Santangelo, and C. N. Cole.** 1985. Two separable functional domains of simian virus 40 large T antigen: carboxyl-terminal region of simian virus 40 large T antigen is required for efficient capsid protein synthesis. J. Virol. **53**:415–424.
105. **Watson, R. J., and J. B. Clements.** 1980. A herpes simplex virus type 1 function required for early and late virus RNA synthesis. Nature (London) **285**:329–330.
106. **Wigdahl, B. L., H. C. Isom, and F. Rapp.** 1981. Repression and activation of the genome of herpes simplex viruses in human cells. Proc. Natl. Acad. Sci. USA **78**:6522–6526.
107. **Yamanishi, K., and F. Rapp.** 1977. Temperature-sensitive mutants of human cytomegalovirus. J. Virol. **24**:416–418.
108. **Yamanishi, K., and F. Rapp.** 1979. Induction of host DNA synthesis and DNA polymerase by DNA-negative temperature-sensitive mutants of human cytomegalovirus. Virology **94**:237–241.