HUMAN GENE THERAPY 15:1022–1033 (November 2004)
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Review

Biology of Adenovirus and Its Use as a Vector for Gene Therapy

MICHAEL J. McCONNELL and MICHAEL J. IMPERIALE

INTRODUCTION

Adenoviruses were first discovered half a century ago by Rowe and colleagues, who were trying to culture adenoid tissue in the laboratory (Rowe *et al*., 1953). Since that time, nonhuman adenoviruses have been isolated from a number of species including chimpanzees, pig, mouse, and dog, as well as other mammalian and avian species (Shenk, 1996). Although human adenoviruses cause significant levels of respiratory, ocular, and gastrointestinal disease, they have been the object of intense study over the years mainly as a model system for basic eukaryotic cellular processes such as transcription, RNA processing, DNA replication, translation, and oncogenesis. Early on, it was shown that the virus could recombine during growth in culture (Lewis *et al*., 1966a,b; Pierce *et al*., 1968; Lewis and Rowe, 1970), ultimately setting the stage for the use of the virus as a vector for gene delivery into cells, animals, and humans. Many features of adenovirus make it well suited for gene delivery, including the ability to grow recombinant viruses to high titers, a relatively high capacity for transgene insertion, and efficient transduction of both quiescent and actively dividing cells, usually without incorporation of viral DNA into the host cell genome. These characteristics, as well as the development of many methods for manipulating the viral genome, have made adenovirus a popular choice as a gene delivery vehicle. This is evidenced by the fact that adenovirus is currently being used in roughly one-quarter of all gene therapy clinical trials, making it second only to the use of retroviral vectors (*Journal of Genetic Medicine* Website, www.wiley.co.uk/genmed/clinical). In this review, we summarize our current understanding of the life cycle of the human adenoviruses and then discuss how the structure and function of viral genes impact the use of the virus as a gene delivery vehicle. Readers desiring a more comprehensive coverage of the biology of the virus are directed to the volumes edited by Doerfler and Böhm (2003, 2004).

VIRUS STRUCTURE

The adenovirus virion is a nonenveloped icosahedral particle about 70–90 nm in size with an outer protein shell surrounding an inner nucleoprotein core (Fig. 1). The facets of the virus capsid are composed primarily of trimers of the hexon protein, as well as a number of other minor components including protein IIIa (pIIIa), pVI, pVIII, and pIX. The capsid vertices consist of the penton base, which acts to anchor the fiber protein, the moiety responsible for primary attachment of virions to the cell surface. Adenovirus cores contain the viral DNA as well as pV, mu, and the histone-like protein pVII. The genome itself is a linear, double-stranded DNA that is approximately 36 kb long. Each end of the genome has an inverted terminal repeat (ITR) of 100–140 bp to which the terminal protein is covalently linked. Genes are encoded on both strands of the DNA in a series of overlapping transcription units (Fig. 2). Virions also contain approximately 10 copies of the adenovirus protease, a cysteine endopeptidase that cleaves many of the structural preproteins into their mature form at the final stage of viral assembly.

THE VIRAL LIFE CYCLE

Binding and entry

The 51 distinct serotypes of human adenovirus have been classified into six groups (A–F) based on sequence homology and their ability to agglutinate red blood cells (Shenk, 1996). Most studies have been carried out on adenovirus serotype 2 (Ad2) and Ad5 and, unless otherwise stated, it should be assumed that the information below refers to work done on these serotypes. For all groups, except group B adenoviruses, initial attachment of virion particles to the cell surface occurs through binding of the fiber knob to the coxsackievirus B and adeno-

Department of Microbiology and Immunology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109.

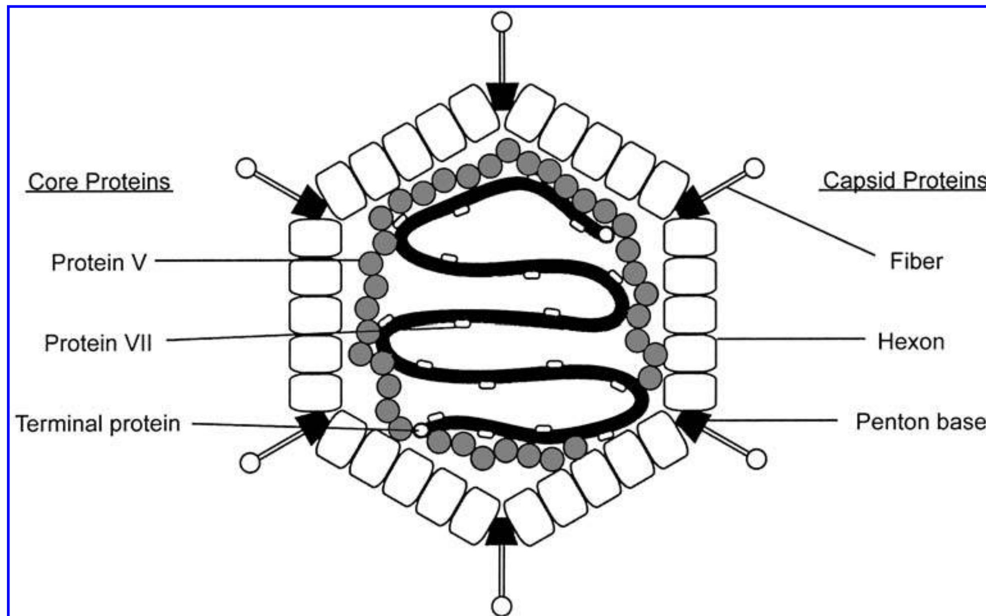

FIG. 1. Schematic of the adenovirus particle, showing major components of the capsid and the core. Adapted from Shenk (1996).

virus receptor (CAR). CAR is a type 1 transmembrane protein in the immunoglobulin superfamily and is present in many human tissues including heart, lung, liver, and brain (reviewed by Howitt *et al*., 2003). CAR normally functions as a cell-to-cell adhesion molecule on the basolateral surface of epithelial cells (Honda *et al*., 2000). Work has suggested that excess fiber produced during infection may act to disrupt epithelial barriers by blocking CAR function, thus promoting efficient spread of virus within and between tissues (Walters *et al*., 2002). The CD46 molecule, a complement-regulatory protein, has been identified as a cellular receptor for group B adenoviruses (Gaggar *et al*., 2003; Segerman *et al*., 2003; Sirena *et al*., 2004). Group B adenoviruses have therefore received considerable attention because of their ability to transduce cells, such as hematopoietic stem cells, dendritic cells, and malignant tumor cells, which can be resistant to infection by adenovirus groups that use CAR as the primary attachment receptor (Shayakhmetov *et al*., 2000; Rea *et al*., 2001; Havenga *et al*., 2002). Strategies that take advantage of the various cell tropisms between adenovirus groups have been developed, including the construction of chimeric viruses carrying fiber genes from alternative serotypes (Mastrangeli *et al*., 1996; Balamotis *et al*., 2004; Slager *et al*., 2004).

After initial attachment to the cell surface, an exposed RGD motif on the penton base interacts with members of the α_v integrin family, triggering virus internalization by clathrin-dependent, receptor-mediated endocytosis (Stewart *et al*., 1997; Meier *et al*., 2002). For Ad2 and Ad5, the acidic environment of the endosome induces escape of virions into the cytoplasm, although the mechanisms underlying this process are poorly understood. Once in the cytoplasm, dynein mediates trafficking of virions along microtubules toward the nucleus, where they subsequently dock with the nuclear pore complex (NPC) (Trotman *et al*., 2001; Kelkar *et al*., 2004). Disassembly of the cap-

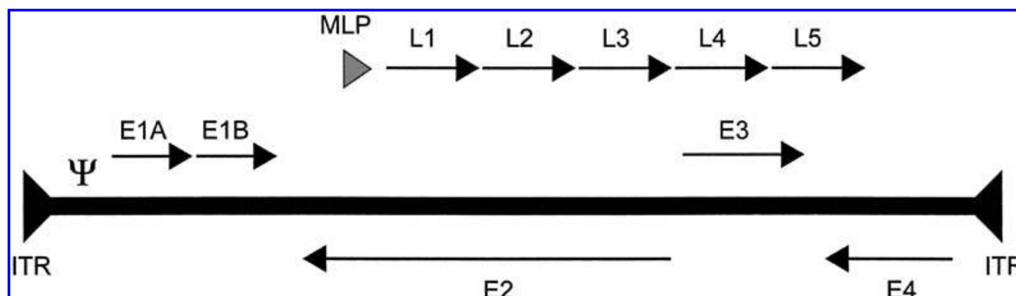

FIG. 2. Map of the adenovirus genome and transcription units. The central, solid line represents the viral genome. Positions of the left and right ITRs, the packaging sequence (ψ), the early transcription units (E1A, E1B, E2, E3, and E4), and the major late transcription unit (major late promoter [MLP], L1–L5) are shown. Arrows indicate the direction of transcription.

sid at the NPC allows for import of the viral genome and commencement of the viral transcriptional program.

Early genes and DNA replication

The first viral transcription unit to be expressed is E1A. As with almost all adenovirus transcription units, E1A produces multiple mRNA and protein products by way of differential mRNA processing. Two E1A transcripts are produced during early infection: a 13S mRNA encoding the 289R (where R stands for amino acid residues) protein (in Ad5) and a 12S mRNA encoding the 243R protein. These proteins can immortalize primary cells in culture and, when expressed in conjunction with E1B proteins, cause tumors in rodents (reviewed by Ben-Israel and Kleinberger, 2002). Despite their transforming potential, the E1A and E1B genes, referred to collectively as the E1 region, are not associated with human cancers. During infection, the E1A proteins function to *trans*-activate the other adenovirus early transcription units (E1B, E2, E3, and E4) and to induce the cell to enter S phase in order to create an environment optimal for virus replication (Berk, 1986). The primary mechanism by which E1A forces quiescent cells to actively cycle is by interfering with proteins of the retinoblastoma (Rb) pathway (Harlow *et al*., 1986; Moran, 1993). Rb acts as a tumor suppressor by inhibiting cell cycle progression via binding to E2F, a transcriptional activator that promotes expression of genes necessary for driving the cell into S phase (Nevins, 1995). The 289R and 243R products are able to sequester Rb and release repression of E2F, allowing it to activate its target genes. E1A proteins have also been shown to modulate the activity of p107 and p130, two members of the Rb family that are also involved in regulating cell cycle progression (Parreno *et al*., 2001).

The E1A proteins have been shown to use a variety of mechanisms to subvert cell cycle checkpoints in addition to interfering with proteins of the Rb family. E1A can directly bind and inhibit components involved in cell cycle control such as the cyclin-dependent kinase inhibitor p21 (Chattopadhyay *et al*., 2001). Furthermore, E1A can interact with a number of host factors involved in mediating chromatin structure including p400 (Fuchs *et al*., 2001) and the histone acetyltransferases (HATs) p300/CBP, pCAF, and TRRAP/GCN5 (Chakravarti *et al*., 1999; Hamamori *et al*., 1999; Lang and Hearing, 2003). Chromatin remodeling is thought to increase the accessibility of DNA to the transcriptional machinery, suggesting that interactions between E1A and HATs may act to promote the expression of genes necessary for induction of S phase. It has also been shown that the 289R protein can mediate expression of target genes by binding directly to cellular transcriptional machinery such as the transcription factor CBF1, a component of the Notch signaling pathway, and the mammalian mediator subunit, Sur2 (Ansieau *et al*., 2001; Stevens *et al*., 2002).

Cell cycle deregulation by E1A results in accumulation of the tumor suppressor p53. During the response to stressful stimuli, activation of the p53 pathway can result in apoptosis, preventing the survival of potentially damaged cells. In infected cells, the adenovirus E1B-55K protein acts to block p53-dependent apoptosis by directly binding p53 and inhibiting its ability to induce expression of proapoptotic genes (Sarnow *et al*., 1982; Ben-Israel and Kleinberger, 2002). Work has shown that the E4 region product E4orf6 cooperates with E1B-55K to carry out these functions (Querido *et al*., 2001a,b). Expression of E1A during infection also promotes apoptosis by sensitizing cells to the tumor necrosis factor α (TNF-α)- and TRAIL (TNF-related apoptosis-inducing ligand)-mediated death receptor pathways (Shao *et al*., 1999; Routes *et al*., 2000). The second product of the E1B transcription unit, the 19K protein, is able to block downstream mediators of these pathways and inhibit programmed cell death (Perez and White, 2000; Tollefson *et al*., 2001). In the case of TNF-α-mediated apoptosis, the E1B-19K protein can bind directly to the proapoptotic proteins Bak and Bax to prevent mitochondria-mediated apoptosis (Sundararajan *et al*., 2001). It is thought that these mechanisms for inhibiting apoptosis keep the cell alive as long as possible in order to maximize viral yields (Rao *et al*., 1992). In addition to its antiapoptotic functions, the E1B-55K protein facilitates the transport of viral mRNAs to the cytoplasm during the late stages of infection (Pilder *et al*., 1986).

The E2 region encodes proteins necessary for replication of the viral genome: DNA polymerase, preterminal protein, and the 72-kDa single-stranded DNA-binding protein (reviewed by de Jong *et al*., 2003). Even though adenovirus replicates in the nucleus, it requires its own enzymatic machinery because of its chromosomal structure. It does not have telomeres, so the integrity of the DNA ends is ensured by the use of a viral protein, preterminal protein (pTP). The preterminal protein is covalently linked to the 5′ end of each genome strand and acts as a primer for the viral DNA polymerase. Initiation of polymerization begins by covalently attaching the first nucleotide of the growing chain to the pTP. Genome replication occurs via a strand displacement method mediated by the 72-kDa protein and the DNA polymerase.

Products of the viral E3 region function to subvert the host immune response and allow persistence of infected cells. The immune system has evolved a number of mechanisms for destroying virus-infected cells, including cell lysis by cytotoxic T lymphocytes and activation of receptor-mediated apoptotic pathways by chemokines. The E3-gp19K protein acts in two ways to prevent the presentation of viral antigens by the MHC class I pathway and subsequent cell lysis by cytotoxic T cells. E3-gp19K was first found to prevent translocation of MHC class I molecules to the cell surface by sequestering them in the endoplasmic reticulum (ER; Burgert *et al*., 1987). More recently, it has been shown that E3-gp19K can bind to TAP (transporter associated with antigen processing), an ER protein responsible for transporting cytosolic antigens into the lumen, suggesting that the E3-gp19K protein may directly interfere with the loading of peptides onto MHC class I molecules (Bennett *et al*., 1999). The E3-10.4K, 14.5K, and 14.7K proteins have all been shown to inhibit the induction of apoptosis by the chemokines TNF-α, Fas ligand (FasL), and TRAIL (Gooding *et al*., 1991; Shisler *et al*., 1997; Chen *et al*., 1998; Li *et al*., 1998; Benedict *et al*., 2001). In the case of E3-10.4K and 14.5K proteins, this inhibition occurs by inducing the clearance of chemokine receptors from the cell surface and targeting them to the lysosome for degradation, hence their designation as the receptor internalization and degradation (RID) complex. The E3-10.4K and 14.5K proteins have also been shown to block

TNF-α-induced secretion of the inflammatory mediator arachidonic acid, possibly via their inhibition of phospholipase A_2 translocation to the cell membrane (Krajcsi *et al*., 1996; Dimitrov *et al*., 1997).

The E4 transcription unit encodes a number of proteins that have been known to play a role in cell cycle control and transformation; however, the mechanisms underlying these functions have remained unclear until recently. Early studies on the E4orf1 protein of Ad9 demonstrated that it is able to induce estrogen-dependent mammary tumors in mice (Javier *et al*., 1991). Interestingly, Ad9 E4orf1 can bind the candidate tumor suppressor ZO (zonula occludens) protein whereas the non-oncogenic Ad5 E4orf1 cannot (Glaunsinger *et al*., 2001). Furthermore, the transforming ability of Ad9 E4orf1 is dependent on its ability to stimulate membrane-associated phosphatidylinositol 3-kinase (Frese *et al*., 2003). These functions suggest that transformation by E4orf1 of Ad9 occurs via mechanisms distinct from those employed by E1 region proteins. In Ad2 and Ad5, E4orf3 and E4orf6 encode gene products with a number of diverse functions. Both proteins have been shown to increase the ability of E1 genes to transform primary rodent cells, increase the expression of viral late genes, and inhibit genome concatemerization by cellular DNA repair enzymes (reviewed by Tauber and Dobner, 2001; Stracker *et al*., 2002). In the case of E4orf6, enhanced transformation is thought to occur via its ability to block p53-mediated *trans*-activation by inhibiting the binding of p53 to cellular transcription factors (Dobner *et al*., 1996). E4orf6 has also been shown to cooperate with the E1B-55K protein in targeting p53 for degradation by cullin-containing ubiquitin ligases (Querido *et al*., 2001a). In addition to the functions listed, the E4orf3 protein also mediates the organization of nuclear structures termed PML oncogenic domains (PODs; Carvalho *et al*., 1995). Although the function of these domains is not clear, they have been shown to play a role in transformation, transcription, and apoptosis in infected cells (reviewed by Maul, 1998). Interestingly, an E4orf3 point mutant unable to facilitate the rearrangement of PODs was severely defective in viral DNA replication (Evans and Hearing, 2003). The E4orf6/7 transcript is produced by fusion of orf7 and a 5′ portion of orf6. The functions of this gene product appear to complement those of the E1A proteins in activating E2F-dependent promoters. Whereas E1A sequesters Rb, releasing repression of E2F, the E4orf6/7 protein binds directly to E2F and promotes its activation of viral and cellular promoters (Hardy *et al*., 1989; Huang and Hearing, 1989; Schaley *et al*., 2000). Most products of the E4 region have antiapoptotic effects; however, the E4orf4 protein interacts with protein phosphatase 2A to stimulate p53-independent apoptosis (Shtrichman *et al*., 1999). It is speculated that induction of programmed cell death pathways by E4orf4 may facilitate the release of progeny virions during the late stages of infection.

Late gene expression and viral assembly

Most adenovirus late genes are expressed from five regions, L1–L5, and are transcribed from one promoter, the major late promoter (MLP). The major late transcription unit (MLTU) encodes approximately 15 to 20 different mRNAs, all of which are derived from a single pre-mRNA by differential splicing and polyadenylation. These transcripts primarily encode structural proteins of the virus and other proteins involved in virion assembly. In addition, the 100K protein, a product of the L4 region, carries out a number of functions during the late phase of infection including acting as a chaperone for hexon trimerization and mediating recruitment of ribosomes to viral mRNAs (Cepko and Sharp, 1982; Xi *et al*., 2004). After the onset of DNA replication, transcription from the MLP is induced to high levels, ensuring the production of adequate amounts of structural proteins for the assembly of progeny virions. Manipulation of late genes encoding the structural components of the capsid has been explored as a strategy for changing the tropism of gene therapy vectors. Alterations of hexon, penton, fiber, and pIX have been shown to successfully mediate retargeting (Wickham *et al*., 1995; Dmitriev *et al*., 1998; Vigne *et al*., 1999; Vellinga *et al*., 2004).

Although virion assembly takes place in the nucleus, hexon trimerization begins in the cytoplasm, where the 100K protein associates with hexon soon after translation (Cepko and Sharp, 1983). Hexon trimers are then translocated to the nucleus in a process involving pVI (Wodrich *et al*., 2003). Once there, they associate with penton and minor capsid components to form the protein shell. Early pulse–chase experiments and the analysis of temperature-sensitive mutants suggested that genome encapsidation occurs via insertion of viral DNA into these preformed capsids (Edvardsson *et al*., 1976; D'Halluin *et al*., 1978a,b). Furthermore, characterization of viruses with mutations in the L1-52/55K gene indicates that this protein is required for the encapsidation process as infection with these mutants produces only empty or partially filled capsids (Hasson *et al*., 1989; Gustin and Imperiale, 1998). However, an alternative model has been suggested in which genome-containing cores nucleate assembly of the protein coat. Findings that the viral IVa2 protein is required for the formation of capsids and binds to the adenovirus packaging sequence, a region on the virus chromosome required for efficient genome packaging, support this model of assembly (Zhang and Imperiale, 2000, 2003; Zhang *et al*., 2001). Interestingly, the IVa2 protein was shown to bind the L1-52/55K protein in infected cells, although the relevance of this interaction remains unclear (Gustin *et al*., 1996). The L4-33K protein also appears to play a role in virus assembly as mutants carrying complete or partial deletions of this gene are defective in capsid formation (Fessler and Young, 1999; Finnen *et al*., 2001).

The packaging sequence itself is a series of seven repeats (A1–A7) at the left end of the genome (Hearing *et al*., 1987). Although each of the repeats fits a consensus motif, they are not functionally equivalent as A1, A2, A5, and A6 have been shown to be most important for genome encapsidation (Grable and Hearing, 1990). Once assembly and DNA encapsidation have occurred, the adenovirus protease cleaves a subset of the structural proteins into their mature form to produce fully infectious virions (reviewed by Mangel *et al*., 2003). Cell lysis and release of progeny virions occur approximately 30 hr postinfection in a process involving the E3-11.6K protein, also called the adenovirus death protein (ADP; Tollefson *et al*., 1996a). Unlike other products of the E3 region, ADP is produced only during the late phase of infection and is transcribed from the MLP rather than the E3 promoter (Tollefson *et al*., 1996b).

USE OF ADENOVIRUS VECTORS

For any gene therapy vector to attain utility in patients it must deliver genes to the intended target and provide expression for an appropriate length of time to achieve a therapeutic effect. Ideally, a vector should be administered systemically and infect only tissues in which gene delivery is desired, thus limiting toxicity to surrounding tissues and organs. Targeting adenovirus vectors to specific tissues has proven to be problematic, however, because of high-level expression of adenovirus receptors on many cell types. Many strategies have been developed that show promise in altering the tropism of adenovirus vectors (Curiel, 1999; Wickham, 2000). Much progress has also been made in achieving prolonged, robust expression of transgenes after vector administration. Improvements along these lines have been obtained mostly through deletion of multiple adenovirus genes, thereby reducing vector replication and the subsequent immune destruction of transduced cells. The construction and biology of these vectors are discussed below. A schematic showing the genome structure of commonly used vectors is presented in Fig. 3.

First-generation vectors

As described above, genes in the E1 region are necessary for activation of viral promoters and expression of both early and late genes. Thus, removal of the E1 coding sequence results in viruses that are severely impaired in their ability to replicate. Furthermore, the E1 region encodes the oncogenic transforming functions of the virus. For these reasons, replacement of the E1 region with transgenes was the initial strategy used in the construction of adenovirus vectors, giving rise to the so-called first-generation vectors. The ability to delete the E1 region is made possible by the existence of cell lines that provide these functions *in trans*. The classic cell line for this purpose is the 293 cell line, a human embryonic kidney-derived line that has been transformed by the adenovirus E1 region (Graham *et al.*, 1977). Production of E1-deleted vectors was initially carried out by homologous recombination in mammalian cells between constructs carrying the left and right ends of the genome (Chinnadurai *et al.*, 1979). However, this method proved to be inefficient and has prompted the development of techniques relying on standard cloning in bacteria and subsequent transfection of recombinant chromosomes into mammalian cells for virus production (reviewed by Danthinne and Imperiale, 2000). Removal of the E1 region alone allows approximately 5.1 kb for insertion of therapeutic genes because adenovirus can package up to 38 kb without affecting viral titer and growth rate (Bett *et al.*, 1993).

Many of the first-generation vectors also contain a deletion in the E3 region, mainly for practical reasons. To optimize the yield of vectors in early experiments using overlap recombination, investigators used the Ad type 5 mutant *dl*309 or its derivatives, which contain in the E1 region two unique restriction sites due to partial deletion of E3 (Jones and Shenk, 1978). Thus, the likelihood of regeneration of the starting wild-type virus, which could arise as a result either of incomplete restriction digestion or religation of viral DNA in the cell, was minimized. Furthermore, E3 genes are entirely dispensable for virus growth *in vitro* and their removal, together with deletion of E1 genes, allows up to 8.2 kb for transgene insertion. Data have suggested that expression of E3 genes from vectors may be beneficial *in vivo* because of their ability to dampen many host immune processes. It has been reported that expression of the entire E3 region or the E3-gp19K product alone can increase persistence of transgene expression in some rodent models (Bruder *et al.*, 1997; Ilan *et al.*, 1997). However, conflicting data have shown that expression of the E3-gp19K protein has no effect on the length of transgene expression (Schowalter *et al.*, 1997). These discrepancies may be due in part to differences in the nature of the transgene or the tissue type that was analyzed. Nevertheless, the inclusion of E3 genes in vectors remains an area of active investigation.

Although first-generation vectors have proven to be highly promising as vehicles for gene delivery, problems do exist. The first drawback associated with these vectors becomes apparent during vector production. Recombination between the E1 region sequences in the complementing cell line and the recombinant virus can give rise to viral progeny with functional E1 genes that are replication competent (Lochmuller *et al.*, 1994). Thus, recombinant virus stocks must be assayed for the pres-

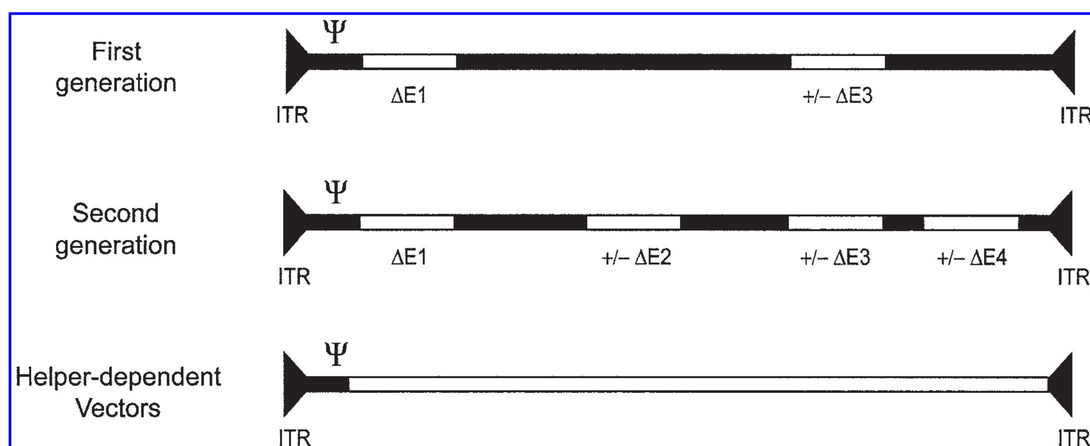

FIG. 3. Genome structure of first-generation, second-generation, and helper-dependent vectors. Regions that have been deleted are indicated by open boxes.

ence of replication-competent viruses. Helper cell lines such as PER.C6 and 911, in which the overlap between E1 sequences in the cell and those commonly present on recombinant virus chromosomes is reduced, have been constructed in order to minimize this occurrence (Fallaux *et al*., 1996, 1998). The second and more troublesome problem associated with the use of first-generation vectors is their stimulation of a cellular immune response, resulting in the destruction of transduced cells that are expressing therapeutic transgenes. Indeed, a number of early studies showed that administration of E1-deleted vectors to immune-competent animals results in only transient transgene expression (Yang *et al*., 1994a, 1995; Dai *et al*., 1995). It is theorized that the immune response is stimulated by low levels of replication that can occur even in the absence of the E1 genes. This idea is supported by findings that genome replication and late gene expression can occur from E1-deleted vectors *in vivo* (Yang *et al*., 1994a,b). Although stimulation of a robust immune response may preclude the use of first-generation vectors in some settings, they still remain promising for applications requiring short-term gene expression such as cancer therapy and vaccination.

Second-generation vectors

To prevent the immune response generated by low-level replication of E1-deleted viruses, vectors deleted for multiple genes have been created to inhibit viral gene expression more effectively. These second-generation vectors have been constructed primarily by the removal of E2 and E4 coding sequences, also providing the benefit of a larger capacity for transgene insertion. The major drawback encountered during construction of these multiply deleted viruses is the need for isolation of cell lines expressing the missing functions *in trans*. Although this can be a time-consuming process, vectors propagated in these cells are less likely to undergo recombination to give replication-competent viruses. In the case of E2 genes, cell lines have been produced that stably express the single-stranded DNA-binding protein, preterminal protein, the viral DNA polymerase, or a combination of the three (Schaack *et al*., 1995; Amalfitano *et al*., 1996; Gorziglia *et al*., 1996; Amalfitano and Chamberlain, 1997). Vectors containing deletions in these genes are incapable of genome replication, and in the case of polymerase-deficient vectors, no replication occurs even in the presence of high levels of E1A (Amalfitano *et al*., 1998). Furthermore, experiments in immune-competent mice demonstrate that transgene expression from vectors lacking the DNA polymerase gene was sustained much longer than from vectors without the deletion (Hu *et al*., 1999). Results from experiments in which all or part of the E4 region has been deleted are less clear. As described above, the E4 region encodes products involved in many aspects of viral replication. It was thus theorized that removal of all or part of the E4 transcription unit would impair viral replication and gene expression such that an immune response would not be triggered. Rodent models have suggested that the deletion of some or all E4 proteins may affect the length and level of transgene expression; however, this regulation appears to be both tissue and promoter specific (Armentano *et al*., 1997; Brough *et al*., 1997; Dedieu *et al*., 1997; Wang *et al*., 1997; Lusky *et al*., 1998; Grave *et al*., 2000).

Helper-dependent vectors

The approach that holds perhaps the most promise for long-term gene expression in the absence of complicating effects due to the presence of viral genes is that of gutted, or helper-dependent, adenovirus vectors (Clemens *et al*., 1996; Chen *et al*., 1997). In this strategy, all of the viral structural genes are deleted from the viral chromosome, leaving just the two ITRs and the packaging signal. Such a chromosome can accommodate up to 37 kb of transgene sequences. To propagate the helper-dependent genome, the presence of a helper virus that provides the functions required for replication and assembly is required, as production of a complementing cell line has not been possible because of the need for high levels of some virion components and the toxicity of some of these proteins to the cell. The main problem to date is the inability to completely separate virions containing the helper-dependent chromosome from those containing the helper virus genome (Steinwaerder *et al*., 1999; Sandig *et al*., 2000). Early strategies that were pursued to reduce helper virus contamination included the use of a helper virus carrying a mutated packaging signal, and minimizing the size of the helper-dependent chromosome compared with that of the helper virus with the hope that the two types of virions could be separated on the basis of their different densities. Even using these techniques, however, helper-dependent virus preparations contained significant levels of contaminating helper virus. More recently, helper viruses in which the packaging sequence is flanked by *loxP* or *frt* sites have been constructed (Parks *et al*., 1996; Umana *et al*., 2001). When these viruses are used to propagate helper-dependent vectors in cells expressing Cre and Flp, respectively, the packaging sequence on the helper virus is excised, resulting in a significantly lower percentage of contaminating helper virus. Indeed, by deriving improved helper cell lines and culture conditions, helper virus levels can be reduced to below 0.01% (Palmer and Ng, 2003). However, recombination between helper-dependent and helper chromosomes, leading to helper chromosomes that can be packaged, is still encountered during virus propagation. A novel method using baculovirus to provide helper functions was reported to allow for production of helper-dependent vectors without contamination by helper virions, although attempts to use this process for large-scale preparations also resulted in the formation of replication-competent viruses (Cheshenko *et al*., 2001). Nevertheless, *in vivo* studies using helper-dependent vectors have produced promising results (DelloRusso *et al*., 2002; Ehrhardt and Kay, 2002; Mian *et al*., 2004; Muruve *et al*., 2004; Pastore *et al*., 2004).

Oncolytic vectors

Gene correction strategies require that vectors deliver therapeutic genes to target tissues and allow for the persistence of transduced cells. Adenovirus vectors that aim to selectively kill target tissues, termed oncolytic or conditionally replicating adenoviruses (CRAds), have been developed for the treatment of cancer. Malignant cells often have mutations in tumor suppressor genes required for regulating cell cycle progression, such as the *p53* and *Rb1* genes. Selective replication of oncolytic adenoviruses lies in their ability to replicate only in cells in which these cell cycle checkpoints have been disrupted. As

described above, the E1B-55K protein normally inhibits the ability of p53 to induce apoptosis in infected cells. Thus, it was posited that a vector deleted for the E1B-55K gene should be able to replicate in and lyse only a cell in which p53 function has been lost (Bischoff *et al*., 1996). Indeed, vectors deleted for the E1B-55K gene have been tested in animal models and clinical trials and show promise in selectively destroying malignant tissues (Heise *et al*., 1997; McCormick, 2003). Interestingly, the dependence of these viruses on mutations in p53 to replicate is unclear as they seem able to replicate in tumor cells regardless of p53 status, all the while sparing normal cells from lysis (Goodrum and Ornelles, 1998; Hall *et al*., 1998; Rothmann *et al*., 1998; Harada and Berk, 1999). Cancer cells lacking Rb function have been targeted in a similar way, using viruses in which the Rb-binding domains, but not the domains necessary for promoter activation, have been deleted from the E1A proteins (Fueyo *et al*., 2000). An alternative approach for achieving conditional replication is to place the E1A and/or E2 and E4 transcription units under the control of a promoter active only in malignant tissues. One example of this strategy is placement of E1A under the control of the human telomerase reverse transcriptase (hTERT) promoter, a promoter active in many cancer cells but not in normal tissues (Zou *et al*., 2004). Most recently, oncolytic adenoviruses carrying "suicide genes" have been used, thereby increasing the toxicity of vectors to target tissues (Hawkins and Hermiston, 2001; Hawkins *et al*., 2001).

THE IMMUNE RESPONSE TO VECTORS

After systemic administration of adenovirus, a large proportion of vector is sequestered by the liver. Subsequent uptake by resident macrophages results in the rapid release of large quantities of inflammatory cytokines (Muruve *et al*., 1999). Thus, in addition to the adaptive immune response brought about by low-level expression of viral genes, a substantial innate immune response is triggered on virus administration. Activation of the innate immune system is stimulated by the virus particle and therefore is not dependent on transcription from viral DNA. This response can cause inflammation of both target and surrounding tissues, resulting in considerable loss of transduced cells, and can also lead to severe systemic toxicities (reviewed by Liu and Muruve, 2003). Furthermore, high titers of antibodies against capsid proteins, either preexisting because of previous exposure to natural virus or generated as a result of vector administration, may inhibit subsequent dosing with the same vector. A number of strategies are being developed that aim to avoid antibody-mediated vector neutralization in order to allow for repeated dosing. These include the use of vectors derived from serotypes that do not cross-react, the use of adenoviruses from other species (e.g., chimpanzee), and coadministration of vectors with immunosuppressive drugs (Mastrangeli *et al*., 1996; Mack *et al*., 1997; Fitzgerald *et al*., 2003; Reyes-Sandoval *et al*., 2004). Another strategy that has also shown promise is to coat vectors with inert chemicals such as polyethylene glycol (Croyle *et al*., 2001, 2002). In addition to problems associated with the production of neutralizing antibodies, loss of transgene expression can occur via the actions of antiviral cytokines. The mechanisms by which transcription is turned off are not well understood, although there is evidence that cytokines can interfere with the function of some commonly used promoters (Qin *et al*., 1997).

SUMMARY AND FUTURE DIRECTIONS

In the early days of gene therapy, adenovirus vectors were looked on with great excitement as potential tools for almost any therapeutic application. The potency of the immune response to first-generation vectors, in retrospect, might have been anticipated because it was known that the requirement of the E1 region for replication was not absolute (Gaynor and Berk, 1983; Imperiale *et al*., 1984). Nevertheless, the utility of adenovirus for future gene correction strategies may rebound if advances in the development of helper-dependent vectors continue at their present rate, resulting in increased removal of helper virus contamination. Similarly, vectors carrying mutations in E2 genes show great promise. In the meantime, adenovirus is arguably the vector of choice for treatment of localized cancer, as the results of ongoing clinical trials are encouraging. Additional modifications to the tropism of the virus will be required, however, before adenovirus will be useful for systemic treatment of metastatic disease. Perhaps the greatest obstacle to both systemic delivery and long-term gene correction will be the innate immune response, to which even helper-dependent vectors are susceptible. Although the previous 50 years have provided considerable knowledge of adenovirus biology, we clearly need to continue our study of this intriguing virus and how it interfaces with the host in order to make it a more generally useful tool for gene therapy.

REFERENCES

AMALFITANO, A., and CHAMBERLAIN, J.S. (1997). Isolation and characterization of packaging cell lines that coexpress the adenovirus E1, DNA polymerase, and preterminal proteins: Implications for gene therapy. Gene Ther. **4,** 258–263.

AMALFITANO, A., BEGY, C.R., and CHAMBERLAIN, J.S. (1996). Improved adenovirus packaging cell lines to support the growth of replication-defective gene-delivery vectors. Proc. Natl. Acad. Sci. U.S.A. **93,** 3352–3356.

AMALFITANO, A., HAUSER, M.A., HU, H., SERRA, D., BEGY, C.R., and CHAMBERLAIN, J.S. (1998). Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. J. Virol. **72,** 926–933.

ANSIEAU, S., STROBL, L.J., and LEUTZ, A. (2001). Activation of the Notch-regulated transcription factor CBF1/RBP-Jκ through the 13SE1A oncoprotein. Genes Dev. **15,** 380–385.

ARMENTANO, D., ZABNER, J., SACKS, C., SOOKDEO, C.C., SMITH, M.P., ST. GEORGE, J.A., WADSWORTH, S.C., SMITH, A.E., and GREGORY, R.J. (1997). Effect of the E4 region on the persistence of transgene expression from adenovirus vectors. J. Virol. **71,** 2408–2416.

BALAMOTIS, M.A., HUANG, K., and MITANI, K. (2004). Efficient delivery and stable gene expression in a hematopoietic cell line using a chimeric serotype 35 fiber pseudotyped helper-dependent adenoviral vector. Virology **324,** 229–237.

BENEDICT, C.A., NORRIS, P.S., PRIGOZY, T.I., BODMER, J.L., MAHR, J.A., GARNETT, C.T., MARTINON, F., TSCHOPP, J.,

GOODING, L.R., and WARE, C.F. (2001). Three adenovirus E3 proteins cooperate to evade apoptosis by tumor necrosis factor-related apoptosis-inducing ligand receptor-1 and -2. J. Biol. Chem. **276,** 3270–3278.

BEN-ISRAEL, H., and KLEINBERGER, T. (2002). Adenovirus and cell cycle control. Front. Biosci. **7,** dl369–d1395.

BENNETT, E.M., BENNINK, J.R., YEWDELL, J.W., and BRODSKY, F.M. (1999). Cutting edge: Adenovirus E19 has two mechanisms for affecting class I MHC expression. J. Immunol. **162,** 5049–5052.

BERK, A.J. (1986). Adenovirus promoters and E1A transactivation. Annu. Rev. Genet. **20,** 45–79.

BETT, A.J., PREVEC, L., and GRAHAM, F.L. (1993). Packaging capacity and stability of human adenovirus type 5 vectors. J. Virol. **67,** 5911–5921.

BISCHOFF, J.R., KIRN, D.H., WILLIAMS, A., HEISE, C., HORN, S., MUNA, M., NG, L., NYE, J.A., SAMPSON-JOHANNES, A., FATTAEY, A., and MCCORMICK, F. (1996). An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science **274,** 373–376.

BROUGH, D.E., HSU, C., KULESA, V.A., LEE, G.M., CANTOLUPO, L.J., LIZONOVA, A., and KOVESDI, I. (1997). Activation of transgene expression by early region 4 is responsible for a high level of persistent transgene expression from adenovirus vectors *in vivo*. J. Virol. **71,** 9206–9213.

BRUDER, J.T., JIE, T., MCVEY, D.L., and KOVESDI, I. (1997). Expression of gp19K increases the persistence of transgene expression from an adenovirus vector in the mouse lung and liver. J. Virol. **71,** 7623–7628.

BURGERT, H.G., MARYANSKI, J.L., and KVIST, S. (1987). “E3/19K” protein of adenovirus type 2 inhibits lysis of cytolytic T lymphocytes by blocking cell-surface expression of histocompatibility class I antigens. Proc. Natl. Acad. Sci. U.S.A. **84,** 1356–1360.

CARVALHO, T., SEELER, J.S., OHMAN, K., JORDAN, P., PETTERSSON, U., AKUSJARVI, G., CARMO-FONSECA, M., and DEJEAN, A. (1995). Targeting of adenovirus E1A and E4-ORF3 proteins to nuclear matrix-associated PML bodies. J. Cell Biol. **131,** 45–56.

CEPKO, C.L., and SHARP, P.A. (1982). Assembly of adenovirus major capsid protein is mediated by a nonvirion protein. Cell **31,** 407–415.

CEPKO, C.L., and SHARP, P.A. (1983). Analysis of Ad5 hexon and 100K *ts* mutants using conformation-specific monoclonal antibodies. Virology **129,** 137–154.

CHAKRAVARTI, D., OGRYZKO, V., KAO, H.Y., NASH, A., CHEN, H., NAKATANI, Y., and EVANS, R.M. (1999). A viral mechanism for inhibition of p300 and PCAF acetyltransferase activity. Cell **96,** 393–403.

CHATTOPADHYAY, D., GHOSH, M.K., MAL, A., and HARTER, M.L. (2001). Inactivation of p21 by E1A leads to the induction of apoptosis in DNA-damaged cells. J. Virol. **75,** 9844–9856.

CHEN, H.H., MACK, L.M., KELLY, R., ONTELL, M., KOCHANEK, S., and CLEMENS, P.R. (1997). Persistence in muscle of an adenoviral vector that lacks all viral genes. Proc. Natl. Acad. Sci. U.S.A. **94,** 1645–1650.

CHEN, P., TIAN, J., KOVESDI, I., and BRUDER, J.T. (1998). Interaction of the adenovirus 14.7-kDa protein with FLICE inhibits Fas ligand-induced apoptosis. J. Biol. Chem. **273,** 5815–5820.

CHESHENKO, N., KROUGLIAK, N., EISENSMITH, R.C., and KROUGLIAK, V.A. (2001). A novel system for the production of fully deleted adenovirus vectors that does not require helper adenovirus. Gene Ther. **8,** 846–854.

CHINNADURAI, G., CHINNADURAI, S., and BRUSCA, J. (1979). Physical mapping of a large-plaque mutation of adenovirus type 2. J. Virol. **32,** 623–628.

CLEMENS, P.R., KOCHANEK, S., SUNADA, Y., CHAN, S., CHEN, H.H., CAMPBELL, K.P., and CASKEY, C.T. (1996). *In vivo* muscle gene transfer of full-length dystrophin with an adenoviral vector that lacks all viral genes. Gene Ther. **3,** 965–972.

CROYLE, M.A., CHIRMULE, N., ZHANG, Y., and WILSON, J.M. (2001). “Stealth” adenoviruses blunt cell-mediated and humoral immune responses against the virus and allow for significant gene expression upon readministration in the lung. J. Virol. **75,** 4792–4801.

CROYLE, M.A., CHIRMULE, N., ZHANG, Y., and WILSON, J.M. (2002). PEGylation of E1-deleted adenovirus vectors allows significant gene expression on readministration to liver. Hum. Gene Ther. **13,** 1887–1900.

CURIEL, D.T. (1999). Strategies to adapt adenoviral vectors for targeted delivery. Ann. N.Y. Acad. Sci. **886,** 158–171.

DAI, Y., SCHWARZ, E.M., GU, D., ZHANG, W.W., SARVETNICK, N., and VERMA, I.M. (1995). Cellular and humoral immune responses to adenoviral vectors containing factor IX gene: Tolerization of factor IX and vector antigens allows for long-term expression. Proc. Natl. Acad. Sci. U.S.A. **92,** 1401–1405.

DANTHINNE, X., and IMPERIALE, M.J. (2000). Production of first generation adenovirus vectors: A review. Gene Ther. **7,** 1707–1714.

DEDIEU, J.F., VIGNE, E., TORRENT, C., JULLIEN, C., MAHFOUZ, I., CAILLAUD, J.M., AUBAILLY, N., ORSINI, C., GUILLAUME, J.M., OPOLON, P., DELAERE, P., PERRICAUDET, M., and YEH, P. (1997). Long-term gene delivery into the livers of immunocompetent mice with E1/E4-defective adenoviruses. J. Virol. **71,** 4626–4637.

DE JONG, R.N., VAN DER VLIET, P.C., and BRENKMAN, A.B. (2003). Adenovirus DNA replication: Protein priming, jumping back and the role of the DNA binding protein DBP. Curr. Top. Microbiol. Immunol. **272,** 187–211.

DELLORUSSO, C., SCOTT, J.M., HARTIGAN-O’CONNOR, D., SALVATORI, G., BARJOT, C., ROBINSON, A.S., CRAWFORD, R.W., BROOKS, S.V., and CHAMBERLAIN, J.S. (2002). Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. Proc. Natl. Acad. Sci. U.S.A. **99,** 12979–12984.

D’HALLUIN, J.C., MARTIN, G.R., TORPIER, G., and BOULANGER, P.A. (1978a). Adenovirus type 2 assembly analyzed by reversible cross-linking of labile intermediates. J. Virol. **26,** 357–363.

D’HALLUIN, J.C., MILLEVILLE, M., BOULANGER, P.A., and MARTIN, G.R. (1978b). Temperature-sensitive mutant of adenovirus type 2 blocked in virion assembly: Accumulation of light intermediate particles. J. Virol. **26,** 344–356.

DIMITROV, T., KRAJCSI, P., HERMISTON, T.W., TOLLEFSON, A.E., HANNINK, M., and WOLD, W.S. (1997). Adenovirus E3-10.4K/14.5K protein complex inhibits tumor necrosis factor-induced translocation of cytosolic phospholipase A2 to membranes. J. Virol. **71,** 2830–2837.

DMITRIEV, I., KRASNYKH, V., MILLER, C.R., WANG, M., KASHENTSEVA, E., MIKHEEVA, G., BELOUSOVA, N., and CURIEL, D.T. (1998). An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. J. Virol. **72,** 9706–9713.

DOBNER, T., HORIKOSHI, N., RUBENWOLF, S., and SHENK, T. (1996). Blockage by adenovirus E4orf6 of transcriptional activation by the p53 tumor suppressor. Science **272,** 1470–1473.

DOERFLER, W., and BÖHM, P., eds. (2003; 2004). Current Topics in Microbiology and Immunology, Vols. 272 and 273: *Adenoviruses: Model and Vectors in Virus–Host Interactions*. (Springer-Verlag, Berlin).

EDVARDSSON, B., EVERITT, E., JORNVALL, H., PRAGE, L., and PHILIPSON, L. (1976). Intermediates in adenovirus assembly. J. Virol. **19,** 533–547.

EHRHARDT, A., and KAY, M.A. (2002). A new adenoviral helper-

dependent vector results in long-term therapeutic levels of human coagulation factor IX at low doses in vivo. Blood **99,** 3923–3930.
EVANS, J.D., and HEARING, P. (2003). Distinct roles of the adenovirus E4 ORF3 protein in viral DNA replication and inhibition of genome concatenation. J. Virol. **77,** 5295–5304.
FALLAUX, F.J., KRANENBURG, O., CRAMER, S.J., HOUWELING, A., VAN ORMONDT, H., HOEBEN, R.C., and VAN DER EB, A.J. (1996). Characterization of 911: A new helper cell line for the titration and propagation of early region 1-deleted adenoviral vectors. Hum. Gene Ther. **7,** 215–222.
FALLAUX, F.J., BOUT, A., VAN DER VELDE, I., VAN DEN WOLLENBERG, D.J., HEHIR, K.M., KEEGAN, J., AUGER, C., CRAMER, S.J., VAN ORMONDT, H., VAN DER EB, A.J., VALERIO, D., and HOEBEN, R.C. (1998). New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses. Hum. Gene Ther. **9,** 1909–1917.
FESSLER, S.P., and YOUNG, C.S. (1999). The role of the L4 33K gene in adenovirus infection. Virology **263,** 507–516.
FINNEN, R.L., BIDDLE, J.F., and FLINT, J. (2001). Truncation of the human adenovirus type 5 L4 33-kDa protein: Evidence for an essential role of the carboxy-terminus in the viral infectious cycle. Virology **289,** 388–399.
FITZGERALD, J.C., GAO, G.P., REYES-SANDOVAL, A., PAVLAKIS, G.N., XIANG, Z.Q., WLAZLO, A.P., GILES-DAVIS, W., WILSON, J.M., and ERTL, H.C. (2003). A simian replication-defective adenoviral recombinant vaccine to HIV-1 Gag. J. Immunol. **170,** 1416–1422.
FRESE, K.K., LEE, S.S., THOMAS, D.L., LATORRE, I.J., WEISS, R.S., GLAUNSINGER, B.A., and JAVIER, R.T. (2003). Selective PDZ protein-dependent stimulation of phosphatidylinositol 3-kinase by the adenovirus E4-ORF1 oncoprotein. Oncogene **22,** 710–721.
FUCHS, M., GERBER, J., DRAPKIN, R., SIF, S., IKURA, T., OGRYZKO, V., LANE, W.S., NAKATANI, Y., and LIVINGSTON, D.M. (2001). The p400 complex is an essential E1A transformation target. Cell **106,** 297–307.
FUEYO, J., GOMEZ-MANZANO, C., ALEMANY, R., LEE, P.S., MCDONNELL, T.J., MITLIANGA, P., SHI, Y.X., LEVIN, V.A., YUNG, W.K., and KYRITSIS, A.P. (2000). A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect *in vivo*. Oncogene **19,** 2–12.
GAGGAR, A., SHAYAKHMETOV, D.M., and LIEBER, A. (2003). CD46 is a cellular receptor for group B adenoviruses. Nat. Med. **9,** 1408–1412.
GAYNOR, R.B., and BERK, A.J. (1983). Cis-acting induction of adenovirus transcription. Cell **33,** 683–693.
GLAUNSINGER, B.A., WEISS, R.S., LEE, S.S., and JAVIER, R. (2001). Link of the unique oncogenic properties of adenovirus type 9 E4-ORF1 to a select interaction with the candidate tumor suppressor protein ZO-2. EMBO J. **20,** 5578–5586.
GOODING, L.R., RANHEIM, T.S., TOLLEFSON, A.E., AQUINO, L., DUERKSEN-HUGHES, P., HORTON, T.M., and WOLD, W.S. (1991). The 10,400- and 14,500-dalton proteins encoded by region E3 of adenovirus function together to protect many but not all mouse cell lines against lysis by tumor necrosis factor. J. Virol. **65,** 4114–4123.
GOODRUM, F.D., and ORNELLES, D.A. (1998). p53 status does not determine outcome of E1B 55-kilodalton mutant adenovirus lytic infection. J. Virol. **72,** 9479–9490.
GORZIGLIA, M.I., KADAN, M.J., YEI, S., LIM, J., LEE, G.M., LUTHRA, R., and TRAPNELL, B.C. (1996). Elimination of both E1 and E2 from adenovirus vectors further improves prospects for *in vivo* human gene therapy. J. Virol. **70,** 4173–4178.
GRABLE, M., and HEARING, P. (1990). Adenovirus type 5 packaging domain is composed of a repeated element that is functionally redundant. J. Virol. **64,** 2047–2056.
GRAHAM, F.L., SMILEY, J., RUSSELL, W.C., and NAIRN, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36,** 59–74.
GRAVE, L., DREYER, D., DIETERLE, A., LEROY, P., MICHOU, A.I., DODERER, C., PAVIRANI, A., LUSKY, M., and MEHTALI, M. (2000). Differential influence of the E4 adenoviral genes on viral and cellular promoters. J. Gene Med. **2,** 433–443.
GUSTIN, K.E., and IMPERIALE, M.J. (1998). Encapsidation of viral DNA requires the adenovirus L1 52/55-kilodalton protein. J. Virol. **72,** 7860–7870.
GUSTIN, K.E., LUTZ, P., and IMPERIALE, M.J. (1996). Interaction of the adenovirus L1 52/55-kilodalton protein with the IVa2 gene product during infection. J. Virol. **70,** 6463–6467.
HALL, A.R., DIX, B.R., O'CARROLL, S.J., and BRAITHWAITE, A.W. (1998). p53-dependent cell death/apoptosis is required for a productive adenovirus infection. Nat. Med. **4,** 1068–1072.
HAMAMORI, Y., SARTORELLI, V., OGRYZKO, V., PURI, P.L., WU, H.Y., WANG, J.Y., NAKATANI, Y., and KEDES, L. (1999). Regulation of histone acetyltransferases p300 and PCAF by the bHLH protein twist and adenoviral oncoprotein E1A. Cell **96,** 405–413.
HARADA, J.N., and BERK, A.J. (1999). p53-Independent and -dependent requirements for E1B-55K in adenovirus type 5 replication. J. Virol. **73,** 5333–5344.
HARDY, S., ENGEL, D.A., and SHENK, T. (1989). An adenovirus early region 4 gene product is required for induction of the infection-specific form of cellular E2F activity. Genes Dev. **3,** 1062–1074.
HARLOW, E., WHYTE, P., FRANZA, B.R., JR., and SCHLEY, C. (1986). Association of adenovirus early-region 1A proteins with cellular polypeptides. Mol. Cell. Biol. **6,** 1579–1589.
HASSON, T.B., SOLOWAY, P.D., ORNELLES, D.A., DOERFLER, W., and SHENK, T. (1989). Adenovirus L1 52- and 55-kilodalton proteins are required for assembly of virions. J. Virol. **63,** 3612–3621.
HAVENGA, M.J., LEMCKERT, A.A., OPHORST, O.J., VAN MEIJER, M., GERMERAAD, W.T., GRIMBERGEN, J., VAN DEN DOEL, M.A., VOGELS, R., VAN DEUTEKOM, J., JANSON, A.A., DE BRUIJN, J.D., UYTDEHAAG, F., QUAX, P.H., LOGTENBERG, T., MEHTALI, M., and BOUT, A. (2002). Exploiting the natural diversity in adenovirus tropism for therapy and prevention of disease. J. Virol. **76,** 4612–4620.
HAWKINS, L.K., and HERMISTON, T. (2001). Gene delivery from the E3 region of replicating human adenovirus: Evaluation of the E3B region. Gene Ther. **8,** 1142–1148.
HAWKINS, L.K., JOHNSON, L., BAUZON, M., NYE, J.A., CASTRO, D., KITZES, G.A., YOUNG, M.D., HOLT, J.K., TROWN, P., and HERMISTON, T.W. (2001). Gene delivery from the E3 region of replicating human adenovirus: Evaluation of the 6.7K/gp19K region. Gene Ther. **8,** 1123–1131.
HEARING, P., SAMULSKI, R.J., WISHART, W.L., and SHENK, T. (1987). Identification of a repeated sequence element required for efficient encapsidation of the adenovirus type 5 chromosome. J. Virol. **61,** 2555–2558.
HEISE, C., SAMPSON-JOHANNES, A., WILLIAMS, A., MCCORMICK, F., VON HOFF, D.D., and KIRN, D.H. (1997). ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat. Med. **3,** 639–645.
HONDA, T., SAITOH, H., MASUKO, M., KATAGIRI-ABE, T., TOMINAGA, K., KOZAKAI, I., KOBAYASHI, K., KUMANISHI, T., WATANABE, Y.G., ODANI, S., and KUWANO, R. (2000). The coxsackievirus–adenovirus receptor protein as a cell adhesion molecule in the developing mouse brain. Brain Res. Mol. Brain Res. **77,** 19–28.
HOWITT, J., ANDERSON, C.W., and FREIMUTH, P. (2003). Adenovirus interaction with its cellular receptor CAR. Curr. Top. Microbiol. Immunol. **272,** 331–364.

HU, H., SERRA, D., and AMALFITANO, A. (1999). Persistence of an [E1–, polymerase–] adenovirus vector despite transduction of a neoantigen into immune-competent mice. Hum. Gene Ther. **10,** 355–364.

HUANG, M.M., and HEARING, P. (1989). The adenovirus early region 4 open reading frame 6/7 protein regulates the DNA binding activity of the cellular transcription factor, E2F, through a direct complex. Genes Dev. **3,** 1699–1710.

ILAN, Y., DROGUETT, G., CHOWDHURY, N.R., LI, Y., SENGUPTA, K., THUMMALA, N.R., DAVIDSON, A., CHOWDHURY, J.R., and HORWITZ, M.S. (1997). Insertion of the adenoviral E3 region into a recombinant viral vector prevents antiviral humoral and cellular immune responses and permits long-term gene expression. Proc. Natl. Acad. Sci. U.S.A. **94,** 2587–2592.

IMPERIALE, M.J., KAO, H.T., FELDMAN, L.T., NEVINS, J.R., and STRICKLAND, S. (1984). Common control of the heat shock gene and early adenovirus genes: Evidence for a cellular E1A-like activity. Mol. Cell. Biol. **4,** 867–874.

JAVIER, R., RASKA, K., Jr., MACDONALD, G.J., and SHENK, T. (1991). Human adenovirus type 9-induced rat mammary tumors. J. Virol. **65,** 3192–3202.

JONES, N., and SHENK, T. (1978). Isolation of deletion and substitution mutants of adenovirus type 5. Cell **13,** 181–188.

KELKAR, S.A., PFISTER, K.K., CRYSTAL, R.G., and LEOPOLD, P.L. (2004). Cytoplasmic dynein mediates adenovirus binding to microtubules. J. Virol. **78,** 10122–10132.

KRAJCSI, P., DIMITROV, T., HERMISTON, T.W., TOLLEFSON, A.E., RANHEIM, T.S., VANDE POL, S.B., STEPHENSON, A.H., and WOLD, W.S. (1996). The adenovirus E3-14.7K protein and the E3-10.4K/14.5K complex of proteins, which independently inhibit tumor necrosis factor (TNF)-induced apoptosis, also independently inhibit TNF-induced release of arachidonic acid. J. Virol. **70,** 4904–4913.

LANG, S.E., and HEARING, P. (2003). The adenovirus E1A oncoprotein recruits the cellular TRRAP/GCN5 histone acetyltransferase complex. Oncogene **22,** 2836–2841.

LEWIS, A.M., JR., and ROWE, W.P. (1970). Isolation of two plaque variants from the adenovirus type 2–simian virus 40 hybrid population which differ in their efficiency in yielding simian virus 40. J. Virol. **5,** 413–420.

LEWIS, A.M., JR., BAUM, S.G., PRIGGE, K.O., and ROWE, W.P. (1966a). Occurrence of adenovirus–SV40 hybrids among monkey kidney cell adapted strains of adenovirus. Proc. Soc. Exp. Biol. Med. **122,** 214–218.

LEWIS, A.M., JR., PRIGGE, K.O., and ROWE, W.P. (1966b). Studies of adenovirus–SV40 hybrid viruses. IV. An adenovirus type 2 strain carrying the infectious SV40 genome. Proc. Natl. Acad. Sci. U.S.A. **55,** 526–531.

LI, Y., KANG, J., and HORWITZ, M.S. (1998). Interaction of an adenovirus E3 14.7-kilodalton protein with a novel tumor necrosis factor α-inducible cellular protein containing leucine zipper domains. Mol. Cell. Biol. **18,** 1601–1610.

LIU, Q., and MURUVE, D.A. (2003). Molecular basis of the inflammatory response to adenovirus vectors. Gene Ther. **10,** 935–940.

LOCHMULLER, H., JANI, A., HUARD, J., PRESCOTT, S., SIMONEAU, M., MASSIE, B., KARPATI, G., and ACSADI, G. (1994). Emergence of early region 1-containing replication-competent adenovirus in stocks of replication-defective adenovirus recombinants (ΔE1 + ΔE3) during multiple passages in 293 cells. Hum. Gene Ther. **5,** 1485–1491.

LUSKY, M., CHRIST, M., RITTNER, K., DIETERLE, A., DREYER, D., MOUROT, B., SCHULTZ, H., STOECKEL, F., PAVIRANI, A., and MEHTALI, M. (1998). *In vitro* and *in vivo* biology of recombinant adenovirus vectors with E1, E1/E2A, or E1/E4 deleted. J. Virol. **72,** 2022–2032.

MACK, C.A., SONG, W.R., CARPENTER, H., WICKHAM, T.J., KOVESDI, I., HARVEY, B.G., MAGOVERN, C.J., ISOM, O.W., ROSENGART, T., FALCK-PEDERSEN, E., HACKETT, N.R., CRYSTAL, R.G., and MASTRANGELI, A. (1997). Circumvention of anti-adenovirus neutralizing immunity by administration of an adenoviral vector of an alternate serotype. Hum. Gene Ther. **8,** 99–109.

MANGEL, W.F., BANIECKI, M.L., and McGRATH, W.J. (2003). Specific interactions of the adenovirus proteinase with the viral DNA, an 11-amino-acid viral peptide, and the cellular protein actin. Cell. Mol. Life Sci. **60,** 2347–2355.

MASTRANGELI, A., HARVEY, B.G., YAO, J., WOLFF, G., KOVESDI, I., CRYSTAL, R.G., and FALCK-PEDERSEN, E. (1996). "Sero-switch" adenovirus-mediated *in vivo* gene transfer: Circumvention of anti-adenovirus humoral immune defenses against repeat adenovirus vector administration by changing the adenovirus serotype. Hum. Gene Ther. **7,** 79–87.

MAUL, G.G. (1998). Nuclear domain 10, the site of DNA virus transcription and replication. Bioessays **20,** 660–667.

MCCORMICK, F. (2003). Cancer-specific viruses and the development of ONYX-015. Cancer Biol. Ther. **2,** S157–S160.

MEIER, O., BOUCKE, K., HAMMER, S.V., KELLER, S., STIDWILL, R.P., HEMMI, S., and GREBER, U.F. (2002). Adenovirus triggers macropinocytosis and endosomal leakage together with its clathrin-mediated uptake. J. Cell Biol. **158,** 1119–1131.

MIAN, A., MCCORMACK, W.M., JR., MANE, V., KLEPPE, S., NG, P., FINEGOLD, M., O'BRIEN, W.E., RODGERS, J.R., BEAUDET, A.L., and LEE, B. (2004). Long-term correction of ornithine transcarbamylase deficiency by WPRE-mediated overexpression using a helper-dependent adenovirus. Mol. Ther. **10,** 492–499.

MORAN, E. (1993). DNA tumor virus transforming proteins and the cell cycle. Curr. Opin. Genet. Dev. **3,** 63–70.

MURUVE, D.A., BARNES, M.J., STILLMAN, I.E., and LIBERMANN, T.A. (1999). Adenoviral gene therapy leads to rapid induction of multiple chemokines and acute neutrophil-dependent hepatic injury *in vivo*. Hum. Gene Ther. **10,** 965–976.

MURUVE, D.A., COTTER, M.J., ZAISS, A.K., WHITE, L.R., LIU, Q., CHAN, T., CLARK, S.A., ROSS, P.J., MEULENBROEK, R.A., MAELANDSMO, G.M., and PARKS, R.J. (2004). Helper-dependent adenovirus vectors elicit intact innate but attenuated adaptive host immune responses in vivo. J. Virol. **78,** 5966–5972.

NEVINS, J.R. (1995). Adenovirus E1A: Transcription regulation and alteration of cell growth control. Curr. Top. Microbiol. Immunol. **199,** 25–32.

PALMER, D., and NG, P. (2003). Improved system for helper-dependent adenoviral vector production. Mol. Ther. **8,** 846–852.

PARKS, R.J., CHEN, L., ANTON, M., SANKAR, U., RUDNICKI, M.A., and GRAHAM, F.L. (1996). A helper-dependent adenovirus vector system: Removal of helper virus by Cre-mediated excision of the viral packaging signal. Proc. Natl. Acad. Sci. U.S.A. **93,** 13565–13570.

PARRENO, M., GARRIGA, J., LIMON, A., ALBRECHT, J.H., and GRANA, X. (2001). E1A modulates phosphorylation of p130 and p107 by differentially regulating the activity of G_1/S cyclin/CDK complexes. Oncogene **20,** 4793–4806.

PASTORE, L., BELALCAZAR, L.M., OKA, K., CELA, R., LEE, B., CHAN, L., and BEAUDET, A.L. (2004). Helper-dependent adenoviral vector-mediated long-term expression of human apolipoprotein A-I reduces atherosclerosis in apo E-deficient mice. Gene **327,** 153–160.

PEREZ, D., and WHITE, E. (2000). TNF-α signals apoptosis through a bid-dependent conformational change in Bax that is inhibited by E1B 19K. Mol. Cell **6,** 53–63.

PIERCE, W.E., ROSENBAUM, M.J., EDWARDS, E.A., PECKINPAUGH, R.O., and JACKSON, G.G. (1968). Live and inactivated adenovirus vaccines for the prevention of acute respiratory illness in naval recruits. Am. J. Epidemiol. **87,** 237–246.

PILDER, S., MOORE, M., LOGAN, J., and SHENK, T. (1986). The

adenovirus E1B-55K transforming polypeptide modulates transport or cytoplasmic stabilization of viral and host cell mRNAs. Mol. Cell. Biol. **6,** 470–476.

QIN, L., DING, Y., PAHUD, D.R., CHANG, E., IMPERIALE, M.J., and BROMBERG, J.S. (1997). Promoter attenuation in gene therapy: Interferon-γ and tumor necrosis factor-α inhibit transgene expression. Hum. Gene Ther. **8,** 2019–2029.

QUERIDO, E., BLANCHETTE, P., YAN, Q., KAMURA, T., MORRISON, M., BOIVIN, D., KAELIN, W.G., CONAWAY, R.C., CONAWAY, J.W., and BRANTON, P.E. (2001a). Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. Genes Dev. **15,** 3104–3117.

QUERIDO, E., MORRISON, M.R., CHU-PHAM-DANG, H., THIRLWELL, S.W., BOIVIN, D., BRANTON, P.E., and MORISSON, M.R. (2001b). Identification of three functions of the adenovirus e4orf6 protein that mediate p53 degradation by the E4orf6–E1B55K complex. J. Virol. **75,** 699–709.

RAO, L., DEBBAS, M., SABBATINI, P., HOCKENBERY, D., KORSMEYER, S., and WHITE, E. (1992). The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. Proc. Natl. Acad. Sci. U.S.A. **89,** 7742–7746.

REA, D., HAVENGA, M.J., VAN DEN ASSEM, M., SUTMULLER, R.P., LEMCKERT, A., HOEBEN, R.C., BOUT, A., MELIEF, C.J., and OFFRINGA, R. (2001). Highly efficient transduction of human monocyte-derived dendritic cells with subgroup B fiber-modified adenovirus vectors enhances transgene-encoded antigen presentation to cytotoxic T cells. J. Immunol. **166,** 5236–5244.

REYES-SANDOVAL, A., FITZGERALD, J.C., GRANT, R., ROY, S., XIANG, Z.Q., LI, Y., GAO, G.P., WILSON, J.M., and ERTL, H.C. (2004). Human immunodeficiency virus type 1-specific immune responses in primates upon sequential immunization with adenoviral vaccine carriers of human and simian serotypes. J. Virol. **78,** 7392–7399.

ROTHMANN, T., HENGSTERMANN, A., WHITAKER, N.J., SCHEFFNER, M., and ZUR HAUSEN, H. (1998). Replication of ONYX-015, a potential anticancer adenovirus, is independent of p53 status in tumor cells. J. Virol. **72,** 9470–9478.

ROUTES, J.M., RYAN, S., CLASE, A., MIURA, T., KUHL, A., POTTER, T.A., and COOK, J.L. (2000). Adenovirus E1A oncogene expression in tumor cells enhances killing by TNF-related apoptosis-inducing ligand (TRAIL). J. Immunol. **165,** 4522–4527.

ROWE, W.P., HUEBNER, R.J., GILMORE, L.K., PARROTT, R.H., and WARD, T.G. (1953). Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exp. Biol. Med. **84,** 570–573.

SANDIG, V., YOUIL, R., BETT, A.J., FRANLIN, L.L., OSHIMA, M., MAIONE, D., WANG, F., METZKER, M.L., SAVINO, R., and CASKEY, C.T. (2000). Optimization of the helper-dependent adenovirus system for production and potency *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **97,** 1002–1007.

SARNOW, P., HO, Y.S., WILLIAMS, J., and LEVINE, A.J. (1982). Adenovirus E1b-58kd tumor antigen and SV40 large tumor antigen are physically associated with the same 54 kd cellular protein in transformed cells. Cell **28,** 387–394.

SCHAACK, J., GUO, X., HO, W.Y., KARLOK, M., CHEN, C., and ORNELLES, D. (1995). Adenovirus type 5 precursor terminal protein-expressing 293 and HeLa cell lines. J. Virol. **69,** 4079–4085.

SCHALEY, J., O'CONNOR, R.J., TAYLOR, L.J., BAR-SAGI, D., and HEARING, P. (2000). Induction of the cellular E2F-1 promoter by the adenovirus E4-6/7 protein. J. Virol. **74,** 2084–2093.

SCHOWALTER, D.B., TUBB, J.C., LIU, M., WILSON, C.B., and KAY, M.A. (1997). Heterologous expression of adenovirus E3-gp19K in an E1a-deleted adenovirus vector inhibits MHC I expression *in vitro*, but does not prolong transgene expression *in vivo*. Gene Ther. **4,** 351–360.

SEGERMAN, A., ATKINSON, J.P., MARTTILA, M., DENNERQUIST, V., WADELL, G., and ARNBERG, N. (2003). Adenovirus type 11 uses CD46 as a cellular receptor. J. Virol. **77,** 9183–9191.

SHAO, R., HU, M.C., ZHOU, B.P., LIN, S.Y., CHIAO, P.J., VON LINDERN, R.H., SPOHN, B., and HUNG, M.C. (1999). E1A sensitizes cells to tumor necrosis factor-induced apoptosis through inhibition of IκB kinases and nuclear factor κB activities. J. Biol. Chem. **274,** 21495–21498.

SHAYAKHMETOV, D.M., PAPAYANNOPOULOU, T., STAMATOYANNOPOULOS, G., and LIEBER, A. (2000). Efficient gene transfer into human CD34$^+$ cells by a retargeted adenovirus vector. J. Virol. **74,** 2567–2583.

SHENK, T. (1996). Adenoviridae: The viruses and their replication. In *Virology*. B.N. Fields, D.M. Knipe, and P.M. Howley, eds. (Lippincott-Raven, New York) pp. 2111–2148.

SHISLER, J., YANG, C., WALTER, B., WARE, C.F., and GOODING, L.R. (1997). The adenovirus E3-10.4K/14.5K complex mediates loss of cell surface Fas (CD95) and resistance to Fas-induced apoptosis. J. Virol. **71,** 8299–8306.

SHTRICHMAN, R., SHARF, R., BARR, H., DOBNER, T., and KLEINBERGER, T. (1999). Induction of apoptosis by adenovirus E4orf4 protein is specific to transformed cells and requires an interaction with protein phosphatase 2A. Proc. Natl. Acad. Sci. U.S.A. **96,** 10080–10085.

SIRENA, D., LILIENFELD, B., EISENHUT, M., KALIN, S., BOUCKE, K., BEERLI, R.R., VOGT, L., RUEDL, C., BACHMANN, M.F., GREBER, U.F., and HEMMI, S. (2004). The human membrane cofactor CD46 is a receptor for species B adenovirus serotype 3. J. Virol. **78,** 4454–4462.

SLAGER, E.H., VAN DER MINNE, C.E., GOUDSMIT, J., VAN OERS, J.M., KOSTENSE, S., HAVENGA, M.J., OSANTO, S., and GRIFFIOEN, M. (2004). Induction of CAMEL/NY-ESO-ORF2-specific CD8$^+$ T cells upon stimulation with dendritic cells infected with a modified Ad5 vector expressing a chimeric Ad5/35 fiber. Cancer Gene Ther. **11,** 227–236.

STEINWAERDER, D.S., CARLSON, C.A., and LIEBER, A. (1999). Generation of adenovirus vectors devoid of all viral genes by recombination between inverted repeats. J. Virol. **73,** 9303–9313.

STEVENS, J.L., CANTIN, G.T., WANG, G., SHEVCHENKO, A., and BERK, A.J. (2002). Transcription control by E1A and MAP kinase pathway via Sur2 mediator subunit. Science **296,** 755–758.

STEWART, P.L., CHIU, C.Y., HUANG, S., MUIR, T., ZHAO, Y., CHAIT, B., MATHIAS, P., and NEMEROW, G.R. (1997). Cryo-EM visualization of an exposed RGD epitope on adenovirus that escapes antibody neutralization. EMBO J. **16,** 1189–1198.

STRACKER, T.H., CARSON, C.T., and WEITZMAN, M.D. (2002). Adenovirus oncoproteins inactivate the Mre11–Rad50–NBS1 DNA repair complex. Nature **418,** 348–352.

SUNDARARAJAN, R., CUCONATI, A., NELSON, D., and WHITE, E. (2001). Tumor necrosis factor-α induces Bax–Bak interaction and apoptosis, which is inhibited by adenovirus E1B 19K. J. Biol. Chem. **276,** 45120–45127.

TAUBER, B., and DOBNER, T. (2001). Adenovirus early E4 genes in viral oncogenesis. Oncogene **20,** 7847–7854.

TOLLEFSON, A.E., RYERSE, J.S., SCARIA, A., HERMISTON, T.W., and WOLD, W.S. (1996a). The E3-11.6-kDa adenovirus death protein (ADP) is required for efficient cell death: Characterization of cells infected with *adp* mutants. Virology **220,** 152–162.

TOLLEFSON, A.E., SCARIA, A., HERMISTON, T.W., RYERSE, J.S., WOLD, L.J., and WOLD, W.S. (1996b). The adenovirus death protein (E3-11.6K) is required at very late stages of infection for efficient cell lysis and release of adenovirus from infected cells. J. Virol. **70,** 2296–2306.

TOLLEFSON, A.E., TOTH, K., DORONIN, K., KUPPUSWAMY, M.,

DORONINA, O.A., LICHTENSTEIN, D.L., HERMISTON, T.W., SMITH, C.A., and WOLD, W.S. (2001). Inhibition of TRAIL-induced apoptosis and forced internalization of TRAIL receptor 1 by adenovirus proteins. J. Virol. **75,** 8875–8887.

TROTMAN, L.C., MOSBERGER, N., FORNEROD, M., STIDWILL, R.P., and GREBER, U.F. (2001). Import of adenovirus DNA involves the nuclear pore complex receptor CAN/Nup214 and histone H1. Nat. Cell Biol. **3,** 1092–1100.

UMANA, P., GERDES, C.A., STONE, D., DAVIS, J.R., WARD, D., CASTRO, M.G., and LOWENSTEIN, P.R. (2001). Efficient FLPe recombinase enables scalable production of helper-dependent adenoviral vectors with negligible helper-virus contamination. Nat. Biotechnol. **19,** 582–585.

VELLINGA, J., RABELINK, M.J., CRAMER, S.J., VAN DEN WOLLENBERG, D.J., VAN DER MEULEN, H., LEPPARD, K.N., FALLAUX, F.J., and HOEBEN, R.C. (2004). Spacers increase the accessibility of peptide ligands linked to the carboxyl terminus of adenovirus minor capsid protein IX. J. Virol. **78,** 3470–3479.

VIGNE, E., MAHFOUZ, I., DEDIEU, J.F., BRIE, A., PERRICAUDET, M., and YEH, P. (1999). RGD inclusion in the hexon monomer provides adenovirus type 5-based vectors with a fiber knob-independent pathway for infection. J. Virol. **73,** 5156–5161.

WALTERS, R.W., FREIMUTH, P., MONINGER, T.O., GANSKE, I., ZABNER, J., and WELSH, M.J. (2002). Adenovirus fiber disrupts CAR-mediated intercellular adhesion allowing virus escape. Cell **110,** 789–799.

WANG, Q., GREENBURG, G., BUNCH, D., FARSON, D., and FINER, M.H. (1997). Persistent transgene expression in mouse liver following *in vivo* gene transfer with a ΔE1/ΔE4 adenovirus vector. Gene Ther. **4,** 393–400.

WICKHAM, T.J. (2000). Targeting adenovirus. Gene Ther. **7,** 110–114.

WICKHAM, T.J., CARRION, M.E., and KOVESDI, I. (1995). Targeting of adenovirus penton base to new receptors through replacement of its RGD motif with other receptor-specific peptide motifs. Gene Ther. **2,** 750–756.

WODRICH, H., GUAN, T., CINGOLANI, G., VON SEGGERN, D., NEMEROW, G., and GERACE, L. (2003). Switch from capsid protein import to adenovirus assembly by cleavage of nuclear transport signals. EMBO J. **22,** 6245–6255.

XI, Q., CUESTA, R., and SCHNEIDER, R.J. (2004). Tethering of eIF4G to adenoviral mRNAs by viral 100k protein drives ribosome shunting. Genes Dev. **18,** 1997–2009.

YANG, Y., NUNES, F.A., BERENCSI, K., FURTH, E.E., GÖNCZÖL, E., and WILSON, J.M. (1994a). Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. Proc. Natl. Acad. Sci. U.S.A. **91,** 4407–4411.

YANG, Y., NUNES, F.A., BERENCSI, K., GONCZOL, E., ENGELHARDT, J.F., and WILSON, J.M. (1994b). Inactivation of E2a in recombinant adenoviruses improves the prospect for gene therapy in cystic fibrosis. Nat. Genet. **7,** 362–369.

YANG, Y., LI, Q., ERTL, H.C., and WILSON, J.M. (1995). Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. J. Virol. **69,** 2004–2015.

ZHANG, W., and IMPERIALE, M.J. (2000). Interaction of the adenovirus IVa2 protein with viral packaging sequences. J. Virol. **74,** 2687–2693.

ZHANG, W., and IMPERIALE, M.J. (2003). Requirement of the adenovirus IVa2 protein for virus assembly. J. Virol. **77,** 3586–3594.

ZHANG, W., LOW, J.A., CHRISTENSEN, J.B., and IMPERIALE, M.J. (2001). Role for the adenovirus IVa2 protein in packaging of viral DNA. J. Virol. **75,** 10446–10454.

ZOU, W., LUO, C., ZHANG, Z., LIU, J., GU, J., PEI, Z., QIAN, C., and LIU, X. (2004). A novel oncolytic adenovirus targeting to telomerase activity in tumor cells with potent. Oncogene **23,** 457–464.

Address reprint requests to:
Dr. Michael J. Imperiale
Department of Microbiology and Immunology and
Comprehensive Cancer Center
University of Michigan Medical School
1500 E. Medical Center Drive
Ann Arbor, MI 48109-0942

E-mail: imperial@umich.edu

Received for publication September 22; accepted after revision October 5, 2004.

Published online: October 29, 2004.

This article has been cited by:

1. W. C. Adams, E. Bond, M. J. E. Havenga, L. Holterman, J. Goudsmit, G. B. Karlsson Hedestam, R. A. Koup, K. Lore. 2009. Adenovirus serotype 5 infects human dendritic cells via a coxsackievirus-adenovirus receptor-independent receptor pathway mediated by lactoferrin and DC-SIGN. *Journal of General Virology* **90**:7, 1600-1610. [CrossRef]
2. Alejandra M. Camino, Catalina Atorrasagasti, Daniela Maccio, Federico Prada, Edgardo Salvatierra, Miguel Rizzo, Laura Alaniz, Jorge B. Aquino, Osvaldo. L. Podhajcer, Marcelo Silva, Guillermo Mazzolini. 2008. Adenovirus-mediated inhibition of SPARC attenuates liver fibrosis in rats. *The Journal of Gene Medicine* **10**:9, 993-1004. [CrossRef]
3. Massimo Conese, Sante Di Gioia, Stefano Castellani. 2008. Gene therapy for cystic fibrosis. *Expert Opinion on Therapeutic Patents* **18**:8, 929-943. [CrossRef]
4. Steven J Gray, R Jude Samulski. 2008. Optimizing gene delivery vectors for the treatment of heart disease. *Expert Opinion on Biological Therapy* **8**:7, 911-922. [CrossRef]
5. D M Appledorn, A Kiang, A McBride, H Jiang, S Seregin, J M Scott, R Stringer, Y Kousa, M Hoban, M M Frank, A Amalfitano. 2008. Wild-type adenoviruses from groups A–F evoke unique innate immune responses, of which HAd3 and SAd23 are partially complement dependent. *Gene Therapy* **15**:12, 885-901. [CrossRef]
6. Dirk M. Nettelbeck. 2008. Cellular genetic tools to control oncolytic adenoviruses for virotherapy of cancer. *Journal of Molecular Medicine* **86**:4, 363-377. [CrossRef]
7. Ami Patel, Yi Zhang, Maria Croyle, Kaylie Tran, Michael Gray, Jim Strong, Heinz Feldmann, James M. Wilson, Gary P. Kobinger. 2007. Mucosal Delivery of Adenovirus-Based Vaccine Protects against Ebola Virus Infection in Mice. *The Journal of Infectious Diseases* **196**:S2, S413-S420. [CrossRef]
8. Stefania Lamartina, Monica Cimino, Giuseppe Roscilli, Ernesta Dammassa, Domenico Lazzaro, Rossella Rota, Gennaro Ciliberto, Carlo Toniatti. 2007. Helper-dependent adenovirus for the gene therapy of proliferative retinopathies: stable gene transfer, regulated gene expression and therapeutic efficacy. *The Journal of Gene Medicine* **9**:10, 862-874. [CrossRef]
9. Mirjana Urosevic, Kazuyasu Fujii, Bastien Calmels, Elisabeth Laine, Nikita Kobert, Bruce Acres, Reinhard Dummer. 2007. Type I IFN innate immune response to adenovirus-mediated IFN-γ gene transfer contributes to the regression of cutaneous lymphomas. *Journal of Clinical Investigation* **117**:10, 2834-2846. [CrossRef]
10. Jinyong Luo, Zhong-Liang Deng, Xiaoji Luo, Ni Tang, Wen-Xin Song, Jin Chen, Katie A Sharff, Hue H Luu, Rex C Haydon, Kenneth W Kinzler, Bert Vogelstein, Tong-Chuan He. 2007. A protocol for rapid generation of recombinant adenoviruses using the AdEasy system. *Nature Protocols* **2**:5, 1236-1247. [CrossRef]
11. Gang Niu, Zhengming Xiong, Zhen Cheng, Weibo Cai, Sanjiv S. Gambhir, Lei Xing, Xiaoyuan Chen. 2007. In Vivo Bioluminescence Tumor Imaging of RGD Peptide-modified Adenoviral Vector Encoding Firefly Luciferase Reporter Gene. *Molecular Imaging and Biology* **9**:3, 126-134. [CrossRef]
12. Michael P Seiler, Stephen Gottschalk, Vincenzo Cerullo, Maheshika Ratnayake, Viraj P Mane, Christian Clarke, Donna J Palmer, Philip Ng, Cliona M Rooney, Brendan Lee. 2007. Dendritic Cell Function After Gene Transfer with Adenovirus-calcium Phosphate Co-precipitates. *Molecular Therapy* **15**:2, 386-392. [CrossRef]
13. Hiroyuki Mizuguchi, Naoko Funakoshi, Tetsuji Hosono, Fuminori Sakurai, Kenji Kawabata, Teruhide Yamaguchi, Takao Hayakawa. 2007. Rapid Construction of Small Interfering RNA-Expressing Adenoviral Vectors on the Basis of Direct Cloning of Short Hairpin RNA-Coding DNAs. *Human Gene Therapy*, ahead of print061222104941001. [CrossRef]
14. Hiroyuki Mizuguchi , Naoko Funakoshi , Tetsuji Hosono , Fuminori Sakurai , Kenji Kawabata , Teruhide Yamaguchi , Takao Hayakawa . 2007. Rapid Construction of Small Interfering RNA-Expressing Adenoviral Vectors on the Basis of Direct Cloning of Short Hairpin RNA-Coding DNAsRapid Construction of Small Interfering RNA-Expressing Adenoviral Vectors on the Basis of Direct Cloning of Short Hairpin RNA-Coding DNAs. *Human Gene Therapy* **18**:1, 74-80. [Abstract] [PDF] [PDF Plus]
15. Qingfu Xu, Maria T. Arevalo, Michael E. Pichichero, Mingtao Zeng. 2006. A new complementing cell line for replication-incompetent E1–deleted adenovirus propagation. *Cytotechnology* **51**:3, 133-140. [CrossRef]
16. Chloe Zubieta, Laurent Blanchoin, Stephen Cusack. 2006. Structural and biochemical characterization of a human adenovirus 2/12 penton base chimera. *FEBS Journal* **273**:18, 4336-4345. [CrossRef]
17. Ying Wu , Zheng-Yu Li , Dr. Xia Zhao , Bing Kan , Yu-Quan Wei . 2006. Inhibition of Ovarian Tumor Growth by Gene Therapy with Recombinant Soluble Vascular Endothelial Growth Factor Receptor 2Inhibition of Ovarian Tumor Growth by Gene Therapy with Recombinant Soluble Vascular Endothelial Growth Factor Receptor 2. *Human Gene Therapy* **17**:9, 941-948. [Abstract] [PDF] [PDF Plus]
18. Adam M. Sonabend, Ilya V. Ulasov, Maciej S. Lesniak. 2006. Conditionally replicative adenoviral vectors for malignant glioma. *Reviews in Medical Virology* **16**:2, 99-115. [CrossRef]

19. Valeria Gonzalez-Nicolini, Martin Fussenegger. 2006. A novel binary adenovirus-based dual-regulated expression system for independent transcription control of two different transgenes. *The Journal of Gene Medicine* **7**:12, 1573-1585. [CrossRef]

20. Robert Longley, Laurie Radzniak, Marc Santoro, Yung-Shyeng Tsao, Russell G. G. Condon, Peggy Lio, Marcio Voloch, Zhong Liu. 2005. Development of a Serum-free Suspension Process for the Production of a Conditionally Replicating Adenovirus using A549 Cells. *Cytotechnology* **49**:2-3, 161-171. [CrossRef]

21. Dr. Monika Lusky . 2005. Good Manufacturing Practice Production of Adenoviral Vectors for Clinical TrialsGood Manufacturing Practice Production of Adenoviral Vectors for Clinical Trials. *Human Gene Therapy* **16**:3, 281-291. [Abstract] [PDF] [PDF Plus]

22. Dr. Dan H. Barouch , Gary J. Nabel . 2005. Adenovirus Vector-Based Vaccines for Human Immunodeficiency Virus Type 1Adenovirus Vector-Based Vaccines for Human Immunodeficiency Virus Type 1. *Human Gene Therapy* **16**:2, 149-156. [Abstract] [PDF] [PDF Plus]