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Adenovirus vectors for high-efficiency gene transfer into mammalian cells

Frank L. Graham

Characteristics such as versatility, stability and high-level expression make adenovirus vectors invaluable tools for the expression of transgenes in mammalian cells for the development of recombinant viral vaccines and for delivery of therapeutic genes.

Adenoviruses are used extensively to deliver genes into mammalian cells, particularly where there is a requirement for high-level expression of transgene products in cultured cells, or for use as recombinant viral vaccines or in gene therapy (reviewed in Ref. 1). The boundaries between the latter two applications are somewhat blurred, as the use of viral vectors as vaccines (e.g. for immunotherapy of cancer) is not fundamentally different from their use in gene therapy. These viruses are particularly well suited for many applications for several reasons: their stability and ability to grow to high titres; their ease of manipulation and purification; and their ability to transduce many mammalian cell types from numerous species, including both dividing and nondividing cells *in vitro* and *in vivo*.

Vectorology

The adenovirion[2] is a nonenveloped icosahedral capsid of approximately 700 nm comprising only protein and DNA, the latter consisting of a linear double-stranded DNA of approximately 30–40 kb (Fig. 1a). DNA replication and virion assembly take place in the nucleus of infected cells, and the production of huge amounts of virions and virion products results in cell death and the release of several thousand infectious viruses per cell at the end of the replication cycle. There are many kinds of adenovirus vectors and many ways of constructing them[3]. At one extreme are the nondefective vectors that retain all essential viral genes and have inserts of foreign DNA in nonessential regions of the genome, and at the other extreme are the vectors from which all viral genes have been deleted and substituted with foreign DNA (up to 36 kb)[4].

The transcriptional organization of a typical adenovirus genome is illustrated in Fig. 1b. From the perspective of adenovectorology, the most important regions are the early regions 1 and 3 (E1 and E3). E3 is nonessential and can be deleted without interfering with the ability of the virus to replicate, and E1, although essential, can also be deleted, resulting in a defective virus that is propagated in E1-expressing cells such as 293 cells (Ad5-transformed human embryonic kidney cells).

The most commonly used vectors are those containing deletions of E1 and E3, with inserts of foreign DNA in E1. Such vectors, which are generally referred to as first-generation (FG) vectors, are defective for replication in normal cells but can efficiently transduce most cells. FG vectors are particularly useful for gene transfer into cultured cells and for gene therapy applications that require transient gene expression. FG vectors are not suitable for long-term expression because they retain most viral genes and express them at low levels, resulting in an immune response against transduced cells *in vivo*. Currently, the best available adenovirus vectors for long-term expression *in vivo* are ones from which all viral genes have been deleted[5]. These fully deleted (FD)

0167-5699/00/$ – see front matter PII: S0167-5699(00)01676-5

vectors must be propagated in the presence of a helper virus that provides all the viral functions and virion capsid proteins needed in *trans* for virus replication, and are often referred to as 'helper-dependent' vectors[4].

Applications

FG vectors are easy to engineer, propagate and purify, and have numerous uses where efficient gene delivery and high-level expression are desired. Thus, they are excellent research tools, and will be used increasingly as novel genes are discovered and their products become a subject for investigation. Because the vectors can deliver genes encoding antigens and express them at high levels *in vivo* in any mammalian species, they are excellent candidates as recombinant viral vaccines. Indeed, vectors capable of immunizing animals against rabies[6], herpes viruses[7], rotaviruses[8] and coronaviruses[9] have all been developed. FG vectors are particularly suited for use in cancer immunotherapy strategies because of the ability of the vector to tranduce most cell types, including nondividing cells, and its ability to express transgene products to high levels. In these regimens, transient expression is preferred over long-term expression, and the inflammatory response and cytotoxic T lymphocyte (CTL) activity associated with administration of FG vectors may be advantageous. Several FG vectors have been produced that express a variety of cytokines and other immunomodulatory proteins[10,11]. These have yielded encouraging results when tested in tumour models in animals and some have been used in clinical trials[12].

FD vectors are technically more difficult to engineer, propagate and purify than FG vectors but have a much higher therapeutic index and give much longer expression *in vivo*[5]. Thus, FD vectors may find use in 'classical' gene therapy such as enzyme replacement, where the desired outcome is permanent expression of the transgene product.

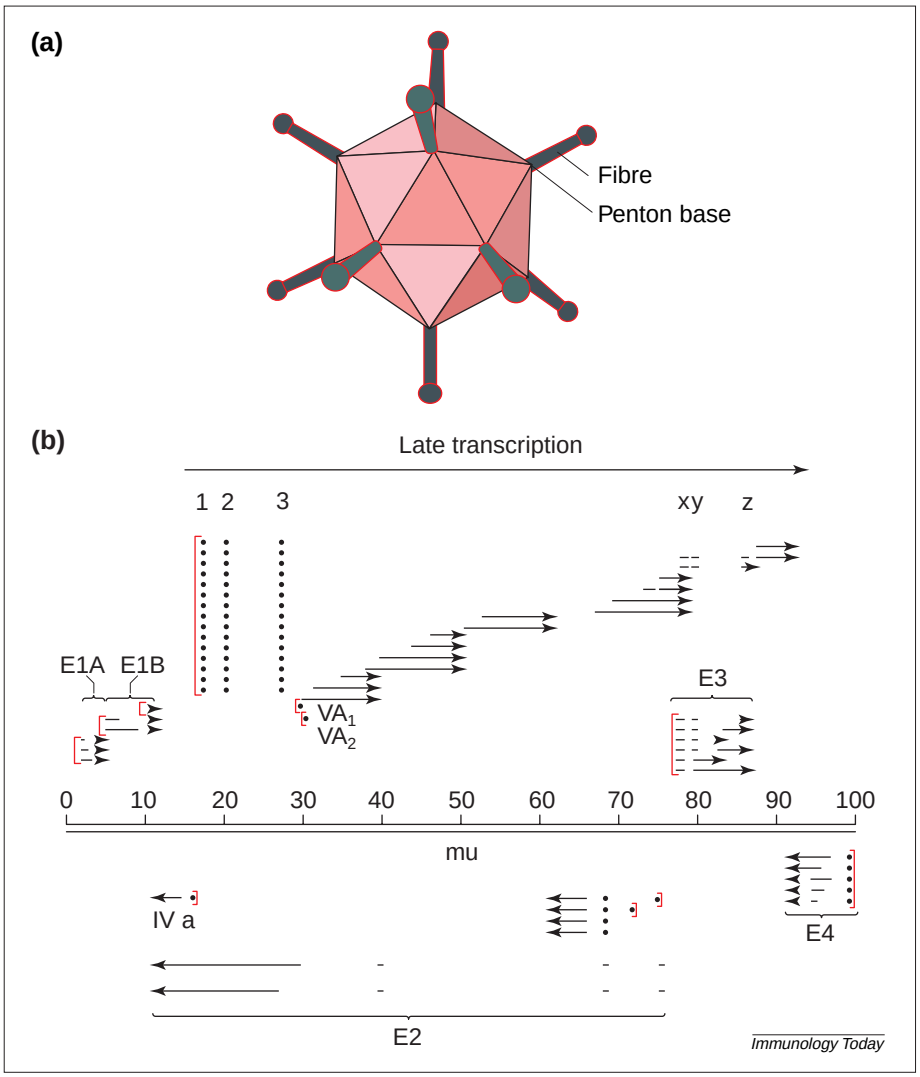

Fig. 1. *(a) The adenovirus virion is an icosahedron with protrusions, called fibres, attached to a penton base at each of the 12 vertices. The capsid protein that forms the major component of the 20 facets is called hexon. A dozen or so additional proteins make up the capsid and core of the virion. Approximately 20% of the molecular mass of the particle comprises DNA packaged as a linear double-stranded molecule. (b) Organization of the viral genome [100 map units (mu) = 36 kb]. Promoters are shown as [(in red). Transcription from the major late promoter at 16 mu generates a single long transcript that is spliced into late mRNAs as indicated. 1, 2, 3 and x, y, z represent leader RNAs attached to various late messages. The viral genome has four regions that are transcribed early in the replication cycle, of which only E3 is not essential for virus replication; E3 is primarily involved in regulating the host immune response to viral infection. E1 encodes essential functions but can also be deleted to produce viruses that are severely attenuated and must be propagated in cells, such as 293, that express E1. The DNA-packaging capacity of the virion is limited to approximately 105% of the wild-type genome but deletion of E1 and E3 sequences increases the packaging capacity of adenovirus vectors to as much as 8 kb. The only sequences needed in* cis *for viral DNA replication and packaging of DNA into virions are the inverted terminal repeats (ITRs) of approximately 100 bp and a packaging signal (ψ) located adjacent to the left ITR and spanning approximately 200 bp. Thus, if all necessary gene products are provided in* trans, *virtually the entire genome can be deleted and substituted with as much as 36 kb of foreign DNA. This is the basis for development of fully deleted (FD) vectors, also called 'helper-dependent' vectors because the only currently available technology for propagating these vectors is in cells coinfected with a helper virus.*

Concluding remarks

In summary, adenovirus vectors come in many forms and have great versatility and high efficacy when designed and used appropriately. They will play an increasingly important role as agents for gene transfer into mammalian cells.

This work was supported by grants from the National Institutes of Health, Medical Research Council of Canada (MRC) and the National Cancer

Institute of Canada (NCIC), and by Merck Research Laboratories.

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A high-resolution view of NK-cell receptors: structure and function

Marco Colonna, Alessandro Moretta, Frédéric Vély and Eric Vivier

A recent conference brought together scientists from 18 countries to discuss advances in our understanding of development, target-cell recognition, signal transduction, and effector mechanisms of natural killer (NK) cells.*

In recent years, there has been an increasing interest in the role of natural killer (NK) cells in primary host defense and their connection with adaptive immunity. NK-cell recognition of pathogen-infected cells and tumors is based on the expression of multiple cell-surface receptors that bind either major histocompatibility complex (MHC) class I or non-MHC ligands and transduce either inhibitory or activating signals. MHC class I receptors normally inhibit NK-cell activation when engaged by self-MHC, while allowing effector responses to occur when class I molecules are downregulated by viruses or transformation. Other receptors recognize ligands that have yet to be defined and trigger lysis and cytokine production. The balance of all of these signals controls NK-cell activation.

Receptors for MHC class I molecules

Studies over the past ten years have led to the discovery of two families of MHC class I receptors. Immunoglobulin (Ig) superfamily (Ig SF) receptors include the human killer cell Ig-like receptor (KIR) and the human Ig-like transcript 2 (ILT2)/leukocyte inhibitory receptor 1 (LIR-1). KIR and ILT/LIR recognize groups of class I allotypes rather than individual MHC class I–peptide complexes. In particular, KIRs with two Ig-like domains (KIR2D) recognize groups of HLA-C allotypes, which differ at positions 77–80 of the α1 domain. ILT2/LIR-1 has a very broad specificity, binding to classical and non-classical class I allotypes as well as the viral-encoded class I-like molecule UL-18. All the genes encoding KIR and ILT/LIR receptors are clustered in a ~1 Mbp leukocyte receptor complex (LRC) on human chromosome 19 (19q13.4), which has been entirely sequenced (Michael J. Wilson and John Trowsdale, Cambridge University, Cambridge, UK). Genomic analysis of the LRC in two distinct haplotypes, as well as analysis of KIR genes in the chimpanzee (Peter Parham, Stanford, CA, USA), indicate a rapid evolution of KIR in primates. Such evolution can only be partly explained by the functional link with the MHC, the products of which are ligands for some of the KIRs and the ILT molecules.

The interaction of KIRs with MHC class I

In a new and exciting development in the study of the interaction of KIRs with class I, Peter D. Sun (Rockville, MD, USA) presented the first crystal structure of a KIR (KIR2DL2) together with its ligand (HLA-Cw3) at 3Å

PII: S0167-5699(00)01697-2

*The 18th International Workshop on Natural Killer Cells was held in Giens, France, on 5–9 May 2000.