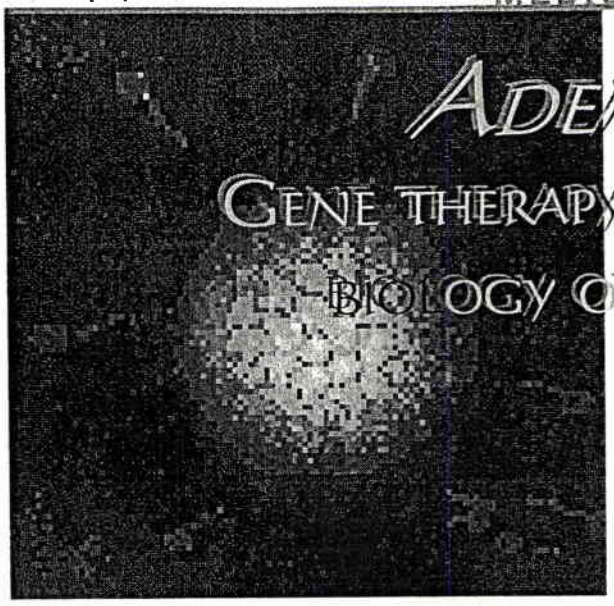

ADENOVIRIDAE

GENE THERAPY AND THE MOLECULAR BIOLOGY OF THE ADENOVIRUS

Figure 2: *Electron Micrograph of the Adenovirus*
This electron micrograph of an adenovirus shows its icosahedral capsid. Some penton fibres are also visible. They are approximately 70-90 nm in diameter and are considered to be medium-sized viruses. Courtesy of Linda Stannard, Department of Medical Microbiology, University of Cape Town (1995).

BY DEBORAH LEUNG

In the past, treatments for genetic diseases have been intended to address the consequences of a given genetic error as opposed to the cause. Due to the limitations of existing treatments, as well as advances in medical technology and genetics, new therapies are being developed that directly target the source of a disease. One way of accomplishing this is to use a vehicle called a vector to introduce a normal gene into the genome of an organism with a defective gene. Viruses are a common choice for vectors because of their ability to invade other cells and express their genetic material in the host. One virus that is frequently used in this type of application is the adenovirus. Two areas in which adenoviruses are currently being used are in the treatment of cancer and in the treatment of cystic fibrosis. Both topics will be addressed later in this article.

STRUCTURE AND CLASSIFICATION OF ADENOVIRIDAE

Adenoviruses are one of the main causes of acute respiratory disease, also known as ARD (Ryan, 1994), affecting the respiratory tracts of animals. Named after the adenoid tissue in which they were first discovered, adenoviruses consist of linear, double-stranded DNA and associated proteins, all within a non-enveloped (lacking a phospholipid bilayer), icosahedral capsid – a twenty-sided polyhedron. This capsid is made up of 252 mostly identical protein molecules, arranged to form 20 triangular faces with a glycoprotein spike at each vertex (Fig. 1) (Campbell, 1999). The glycoprotein spikes, more accurately referred to as penton fibres, consist of a slender shaft with a globular head and are involved in the process of attachment of the virus particle to the host cell (Stannard, 1995). Each subunit of the capsid – otherwise known as a capsomere – is either a penton or a hexon monomer. Penton monomers provide the base for the penton fibres. Thus, there are 12 penton monomers per viral capsid, serving as a base for each penton fibre. Hexon monomers make up the remainder of the 252 molecules – 240 hexon monomers to each adenovirus capsid. Adenoviruses are approximately 70-90 nm in diameter, with each capsomere 8-9 nm in diameter (Fig. 2).

Affecting only vertebrates, there are two main types of adenoviruses. These are the aviadenovirus, which affect avian vertebrates, and the mastadenovirus, or mammalian adenovirus. Of the almost 100 different serotypes of adenoviruses, 47 are known to affect humans (Kenneth, 1994). They are identified as Ad1 through Ad47 and are classified into six subgroups, A through F. The various serotypes differ markedly in tissue specificity and virulence (Wolff, 1994). Subgroups A and B have been known to induce tumours in newborn hamsters, and outbreaks of ARD in military recruits caused by types 4 and 7 have been documented. There have been no known cases of adenoviruses causing cancer in human cells.

INFECTION AND REPLICATION

Overall, only about 45% of adenovirus infections result in disease, and their most significant contribution to acute illness is in children (Kenneth, 1994). As previously mentioned, adenoviruses are one of the main causes of ARD. However, adenoviruses can also cause conjunctivitis (commonly known as "pink-eye"), gastroenteritis (inflammation of the membrane that lines the stomach and the intestines), and are known to infect lymph nodes in humans. In laboratory cell cultures, adenoviruses are able to transform, or genetically alter, cells. Adenoviruses may be transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission (National Center for Infectious Diseases, 2001).

Like all viruses, the adenovirus cannot replicate without first infecting a host cell. The multiplication cycle of adenoviruses lasts about 36 to 48 hours (Joklik, 1988), and begins with the binding of the virus to a specific host cell. The complete virus particle – commonly referred to as a virion – is then

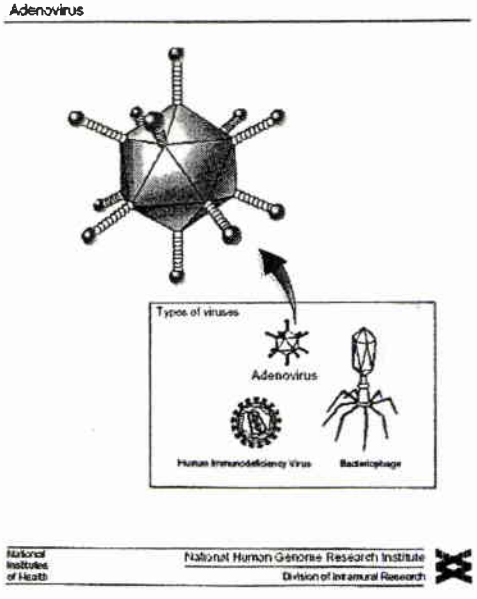

Figure 1: *Structure of the Adenovirus*
The double-stranded DNA of an adenovirus is encased within a 20-sided icosahedral capsid. Note the glycoprotein spikes at each vertex; there are 12 in total. Contrary to the Human Immunodeficiency Virus (HIV), adenoviruses do not possess a viral envelope.

introduced to the host cell through phagocytosis into a phagocytic vacuole. The toxic activity of the pentons is responsible for rupture of the phagocytic membrane and release of the particle into the cytoplasm (Cann, 1997). The icosahedral capsid is then disassembled and the core – consisting of double-stranded DNA and associated proteins – migrates to the nucleus, where the viral DNA enters through nuclear pores and is transcribed using host cell resources (Fig. 3). It is interesting to note that the idea of pre-mRNA and RNA splicing was first considered in 1977 during the study of adenoviruses.

Although studies of gene therapeutics – with adenoviruses have been promising, there are certain issues that arise when using a viral vector.

The replication cycle of the adenovirus is typically divided into three stages – immediate early, early, and late. Immediate early replication pertains to the genetic region of the viral genome referred to as E1A. This region is necessary in the promoting of the genes in the early replication stage: genes E1B, E2A, E2B, E3, and E4. Approximately 6 to 8 hours after the initial infection, late replication begins, resulting mostly in the synthesis of virion proteins. Mature virions are assembled within the nucleus, and do not immediately lead to destruction of the host cell. Accidental lysis of infected cells releases the new adenoviruses, essentially re-infecting the unsuspecting organism. This form of persistence is not referred to as "latent," but is rather known as "occult" (to cover up).

Adenoviridae in Gene Therapeutics

After having been introduced to the adenovirus itself, it is now possible to examine the various ways in which the virus plays a role in the exciting realm of gene therapeutics. Adenoviruses are ideal for use in gene therapeutics because of their substantial genome (approximately 36 kb), which can theoretically allow for the transfer of large fragments of DNA. Also, vectors based on human adenoviruses can transfer recombinant genes efficiently into a wide variety of dividing and non-dividing cells (Evans, 1996), whereas other vectors, such as retroviruses, may be limited to dividing cells only.

In cancer therapy, trials are being conducted using a modified adenovirus lacking the E1A/E1B coding region. The E1B region produces a protein that binds to a gene called *p53* found within the host cell. A tumour suppressor, p53, is responsible for blocking cell division or causing the cell to self-destruct in response to DNA damage or viral DNA infection (Evans and Lahoti, 2000). With an active *p53* gene, the virus cannot survive because the host cell will destroy itself in the event of infection. When the E1B protein binds to the *p53* protein, the virus can successfully usurp the host cell. Modified viruses can be used as a vector to deliver gene therapy or as cancer therapy by itself (Heise et al., 1997) In a cell with a defective form of the *p53* gene, as is the case with many cancerous cells, an E1-lacking adenovirus can invade and destroy the cell.

Other applications of adenoviruses in gene therapy include the in-vivo treatment of cystic fibrosis (CF), an autosomal-recessive disease affecting exocrine glands. The in-vivo approach involves introduction of the corrected gene directly into body cells that lack said gene (Tolstoshev, 1993). Because some of the most debilitating effects of the disease occur in the epithelial cells of the lung, adenoviruses are an ideal choice of vector. A mutation in the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR) leads to the manufacture of defective CFTR proteins that are the basis of the symptoms associated with the disorder. Adenovirus vectors used in the treatment of CF have both E1A and E1B coding regions deleted, with a "normal" version of the CFTR gene inserted in their place. Treatments may be administered via aerosol directly to lung epithelia, but must be re-administered periodically because the gene correction is targeted to non-dividing cells and thus must be repeated as the cells die and are replaced.

Although studies of gene therapeutics with adenoviruses have been promising, there are certain issues that arise when using a viral vector. One complication is the possibility of a natural immune response to the modified virus. Infection of the target cell by a viral vector ideally results in expression of the desired gene, but can also lead to the expression of various viral antigens. These antigens evoke an immune response, and the target cell – along with the potential treatment – is destroyed. The body's response also includes the manufacture of antibodies against the vector, rendering subsequent doses of the treatment less effective.

Adenoviruses have many more applications within the field of gene therapeutics, but it is hoped that the inquisitive reader will take up the quest of seeking out more information, as it is well worth the time to become familiar with the various facets of the scientific world. Gene therapy is a new and fascinating field with many prospects, many of which have yet to emerge.

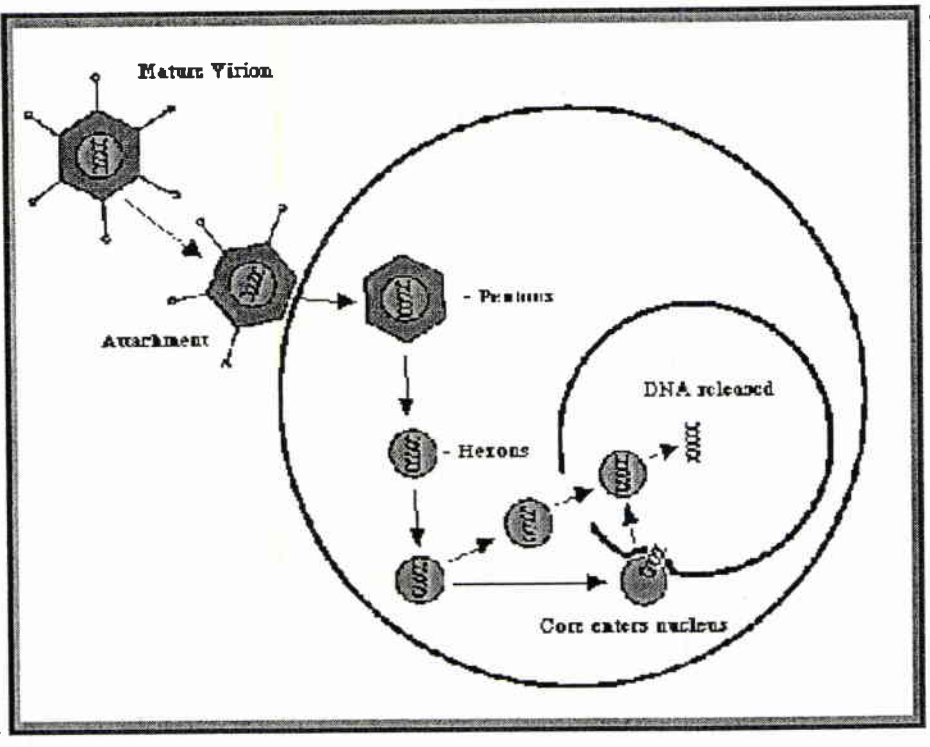

Figure 3: Penetration of a Host Cell

The adenovirus enters the cell through phagocytosis after binding to specific receptor sites on the host cell membrane. Activity of pentons in the capsid breaks the phagocytic membrane, releasing the virion into the cytoplasm. First the pentons of the capsid are removed, followed by the removal of the hexons. Finally, the core (made up of DNA and associated proteins) migrates to the nucleus, where viral DNA enters through nuclear pores and transcription can occur.

Image courtesy of Alan Cann, 1997. University of Leicester. 4 Jan 2002. <http://www.tulane.edu/~dmsander/WWW/335/Adenoviruses.html>