Adenoviral Vectors

Molecular biology of the adenovirus

The wild type Adenovirus is a non-enveloped virus comprising a double stranded linear 36 kb genome packaged into an icosahedral protein capsid, of approximately 90-100 nm. Each viral particle has a fiber protein that protrudes from the vertex of the icosahedron, which is attached to a knob region that mediates cell entry. The genome is flanked by two inverted terminal repeat regions of DNA (ITR) and each 5' end is attached to a 55 kDa terminal protein (TP). The genes comprising the genome can be classed into two categories; Early genes (E1 to E4) and Late genes (L1 to L5), depending on whether they are expressed before or after DNA replication (see figure 1).

Upon entry into the host cell, the knob domain of the adenoviral fiber protein binds to the coxsackievirus adenovirus receptor (CAR) (or CD46 for group B adenoviruses). Subsequently, the RGD mofif in the fiber domain interacts with αv integrin resulting in endocytosis of the viral particle and the formation of clathrin-coated pits. Once in the endosome, the pH becomes acidic allowing the capsid proteins to dissociate and the release of the virion into the cytoplasm. The microtubular network of the cell then transports the virion to the nuclear pore complex, where the viral particle disassembles and the viral DNA is able to enter the nucleus and replicate. In the early phase, the E1, E2, E3 and E4 proteins mediate the replication of the viral DNA using a combination of viral and host cell proteins. The late phase is marked by the activation of the major late promoter that drives expression of capsid proteins, resulting in viral particle assembly.

Recombinant adenoviral vectors

First Generation

Adenoviruses have the potential to be good gene transfer agents because they can be prepared to high concentrations, infect

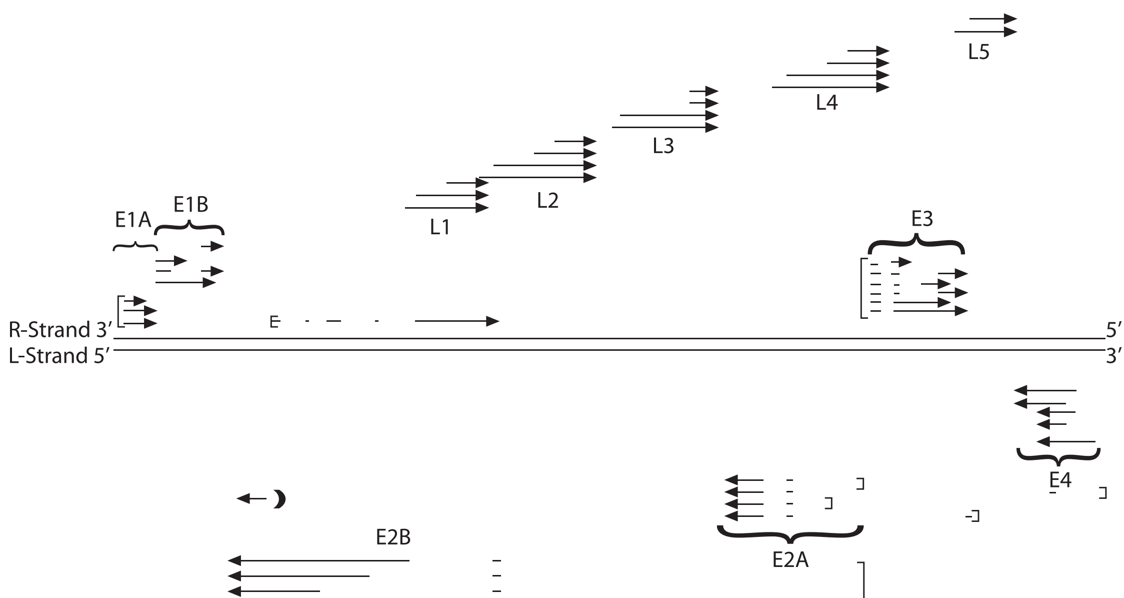

figure 1 adenoviral genome

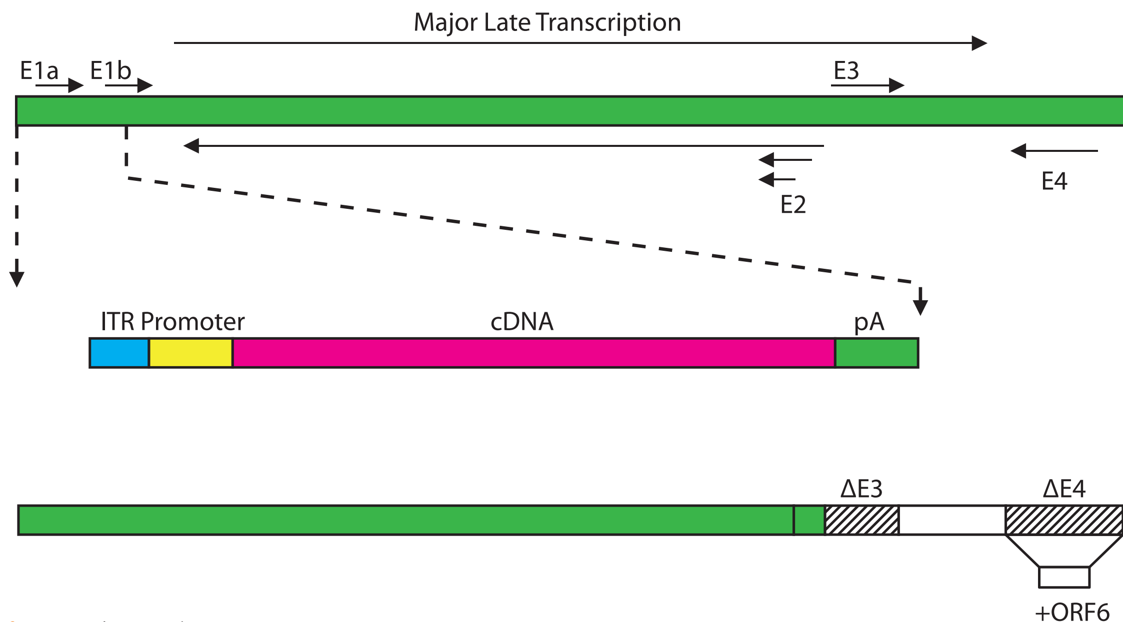

figure 2 adenoviral vectors

a broad range of cells and are non-tumourigenic, as their DNA does not integrate into the host genome. Adenoviruses can be made into replication-defective vectors by removing the E1 region located in the left side of the viral DNA, this early transcript encodes for proteins that activate the transcription of all other viral proteins. Typically, an expression cassette encoding the therapeutic gene is inserted into the deleted E1 region and the resultant recombinant viral vector is propagated in a cell line that expresses the adenoviral E1 genes (see figure 2).

Over the past three decades a number of these complementing cell lines have been made, e.g. HEK 293, HER 911 and PER. C6. The first generation of adenoviral vectors contained deletions in both the essential E1 and non-essential E3 regions, the result was a vector that could contain up to 7.5 kb of exogenous DNA. However, after administration into animals, the expression of transgene was found to be transient, due to an intensive CD8+ T-cell response mediated against both the virus and therapeutic protein.

Second generation

To further limit the leaky expression of viral proteins and expand the capacity of adenoviral vectors, further deletions have been made in the adenoviral genome. Viruses with additional deletions in the E2 or E4 regions have been made by a number of researchers, creating recombinant vectors that have to be propagated in special complementing cell lines, expressing the E2 or E4 regions, e.g. 293-C7 (E2), 293-ORF6 (E4) or 911-E4. These vectors can encode up to 10.5 kb of exogenous DNA and have the capacity to mediate transgene expression over a longer period of time, however, they still elicit a significant immune response that hampers their use as

long-term gene expression vectors.

Third generation – Gutted

The entire adenoviral genome, with exception of the essential cis elements (5' and 3' ITRs and packaging signal) can be removed to generate recombinant gutted (or gutless) adenoviral vectors. These vectors are considered the safest adenoviral vectors due to their lack of viral proteins, and they elicit a very limited innate immune response after administration to animals. They also have a large capacity for exogenous DNA, being able to package up to 36 kb of transgene. However, their production is a tricky process, given that they can only be propagated in the presence of a helper adenovirus. The major issue in production of gutted adenoviruses is the removal of the helper virus, often a first generation viral vector. Due to the difference in the length of the viral genome between the vector and the helper, the viruses can be separated on a CsCl density gradient by ultra-centrifugation. However, vectors still contain up to 1% contaminating helper virus after this procedure and require further purification. The phenomenon of site-specific recombination can be effectively employed in order to reduce the contaminating levels of helper virus to less than 0.1%. In this approach the packaging signal of the helper virus is flanked by two recombination signals (e.g. loxP sites). The virus is then propagated in a special cell line that expresses the recombination protein that recognises these signals (e.g. CRE recombinase). The packaging signal is thereby excised from the helper virus and its genome can no longer be packaged into the capsid proteins, thus allowing for efficient packaging and production of the vector genome only. The gutted adenoviral vectors perhaps represent the best hope for an effective, large capacity vector based on the adenoviruses, however, until the efficiency of vector production is significantly improved, it is unlikely that these vectors will be widely applied in the clinic.

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