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DIRECCIÓN DE NUEVAS CREACIONES

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Abogada
Luz Clemencia de Páez

ASUNTO		
	Radicación	07-80240
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	09984
	Folios	007

Patente de Invención ☐ Patente de Modelo de Utilidad ☐

Vía Nacional ☐

Vía PCT (fase nacional) ☐ Cap.I ■ Cap. II ☐

No. Sol. Internacional PCT/NL2006/000009
Pub. Internacional WO 2006/085749

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción:	Folios: 97 a 228	06/08/2007
Reivindicaciones:	Folios: 229 a 236	06/08/2007
	Folios: 273 a 276	21/02/2012
Figuras:	Folios: 238 a 247	06/08/2007
Prioridad:	EP05075042.1	07/01/2005
	EP05075725.1	29/03/2005

2. Análisis de la Prioridad

No se acepta la reivindicación de prioridad porque esta solicitud reclama dos secuencias SEQ N°1 y SEQ N°2 que no habían sido previamente divulgadas en los documentos reclamados como prioridad.

3. Objeto de la invención

Título de la solicitud: "Virus de planta designado virus torrado del tomate".

El objeto de la presente solicitud es caracterizar un virus de plantas de tomate, los métodos para detectar dicho virus, a métodos para detectar las plantas resistentes y métodos para producir plantas resistentes.

El problema planteado en esta solicitud es la necesidad de caracterizar el virus torrado del tomate (ToTV) y crear plantas resistentes a este.

Para solucionar este problema técnico la solicitud en estudio suministra la secuencia nucleótida del virus torrado del tomate, un método para detectarlo y la identificación de plantas resistentes al mismo.

El objeto de la solicitud definida anteriormente se clasificó en la IPC: C12N 7/00, A01H 3/00.

4. No es invención (*Artículo 15 D486 literal b*)

El ácido nucleico aislado del virus torrado del tomate que comprende las SEQ N° 1 ó SEQ N°2 no se considera invención (reivindicación 1), por ser parte del genoma de un organismo tal como se encuentra en la naturaleza. Lo anterior, de acuerdo con las secuencias reportadas en el NCBI que divulgan la secuencia completa del RNA del virus torrado del tomate (Genbank N° acceso DQ388880, DQ388879) y que muestran 100% de identidad con las secuencias reclamadas en la presente invención.

Igualmente, el método para producir anticuerpos contra ToTV y el anticuerpo así producido reclamado en las reivindicaciones 7, 8-10, corresponde a un anticuerpo producido en un proceso biológico natural, como parte de una respuesta inmune natural. Por tanto, esta materia no se considera invención.

De esta manera las reivindicaciones mencionadas deben eliminarse.

5. Excepción a la Patentabilidad (*Artículo 20 D 486 literal c*)

El objeto de la reivindicaciones 14 a 22 reclama procesos esencialmente biológicos y plantas. Esta materia no es patentable de acuerdo con el Art citado.

6. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de la reivindicación 28 no es patentable, de acuerdo con el Art 14.

7. De la solicitud de Patente

Descripción (*artículo 28 D486*)

A lo largo del capítulo descriptivo no se encuentra sustento sobre el vector de expresión que comprende el ToTV, puesto que no hay información sobre los vectores que se emplean, componentes del vector, segmentos de DNA del ToTV insertados ni la expresión del mismo. Además, no se encuentra sustento para los agentes antivirales reclamados, en la descripción estos agentes no son identificados ni ensayados, sino simplemente enunciados (Fls. 196 a 198). Igualmente, la planta resistente a ToTV no es obtenida ni divulgados los métodos de obtención de la misma.

Se recomienda revisar el capítulo descriptivo y restringir la materia del capítulo reivindicatorio de acuerdo con la materia sustentada en la descripción.

Reivindicaciones *(artículo 30 D 486)*

El capítulo reivindicatorio presentado carece de claridad y concisión por cuánto:

1) El ácido nucleico y el polipéptido reclamado no puede definirse a partir de porcentajes de identidad, puesto que esta definición da lugar a múltiples opciones de secuencias las cuales además no tener sustento podrían no tener la misma actividad (reivindicaciones 1, 4, 23-26);

2) El vector reclamado en la reivindicación 2 no tiene todos sus elementos definidos pues no es posible establecer de que tipo de vector se trata, que fragmento de la secuencia es insertado. Lo anterior es relevante puesto que las secuencias mencionadas en la reivindicación 1 son muy largas;

3) El polinucleótido reclamado en la reivindicación 3 es definido a partir del resultado a alcanzar con el mismo, lo cual no es una característica técnica fundamental del mismo;

4) El polipéptido reclamado en las reivindicaciones 4 y 5 debe ser definido a partir de su secuencia. La indicación de una secuencia nucleótida para reclamar un polipéptido no es técnicamente válida. Lo mismo aplica para el antígeno reclamado en la reivindicación 6;

5) El anticuerpo reclamado debe ser definido a partir de su estructura completa, esto es, de las secuencias de las regiones variables de la **cadena ligera y cadena pesada**. La alusión a su función no permite establecer su estructura, la cual si es una característica fundamental. La estructura de este anticuerpo sin embargo, no tiene sustento en la descripción;

6) La planta resistente a ToTV carece de sustento en la descripción;

7) El kit de la reivindicación 23, el método para la producción de una composición diagnóstica de la reivindicación 24 y la composición de diagnóstico de la reivindicación 25, son caracterizadas a partir de los mismos elementos. Sin embargo, cada una de estas reivindicaciones debería tener su propia caracterización, por ejemplo el método para la producción de la composición debería indicar las etapas fundamentales para llevarlo a cabo y la composición de diagnóstico debe indicar los componentes y excipientes con sus respectivas proporciones;

8) El agente antivírico de la reivindicación 26 no tiene sustento en la descripción y no es definido a partir de su propia estructura;

9) La reivindicación 27 se refiere a la misma materia reclamada en la reivindicación 2;

10) La forma atenuada del virus no es divulgada ni caracterizada a partir de su estructura;

De esta manera, el capítulo reivindicatorio presentado no cumple con los requisitos establecidos en el art. 30 de la Decisión 486.

7. Determinación del Estado de la Técnica

Para determinar el estado de la técnica se ha tenido en cuenta la fecha de presentación de la solicitud internacional: 09/01/2006.

Teniendo en cuenta la fecha indicada se realizó búsqueda en el estado de la técnica de documentos relacionados con la solicitud, encontrándose los siguientes documentos relevantes a la misma:

N°	Documento	Título	Fecha de prioridad
D1	EMBL, N° acceso AY829112	"Maize chlorotic dwarf virus isolate M1, complete genome"	13/12/2004
D2	EMBL, N° acceso M95497	"Rice tungro spherical virus polyprotein gene, complete cds"	18/06/1992
D3	Biopharmaceuticals, biochemistry and biotechnology. G.Walsh. Wiley Ed. Pág 468-479	"Vectors used in gene therapy"	2003

Resumen de los documentos citados:

D1: Registro de secuencia nucleótida y aminoácida del virus clorótico enano del maíz. http://www.ebi.ac.uk/ena/data/view/AY829112

D2: Registro de secuencia nucleótida y aminoácida del virus esférico del arroz. http://www.ebi.ac.uk/ena/data/view/M95497

D3: Resumen de los vectores comúnmente usados en terapia génica, incluida la transformación de plantas (Fls. 310-315).

8. Análisis de patentabilidad (*artículo 14 D486*)

Nivel inventivo (*artículo 18 D486*)

La reivindicación 2 de la solicitud reclama un vector de expresión caracterizado porque comprende un ácido nucleico de conformidad con la reivindicación 1, es decir que comprende de las secuencias N°1 ó 2 ó secuencias que tienen una homología de secuencia nucleótida de al menos 50% a la SEQ N°1 o a la SEQ N°2, y sus hebras complementarias y fragmentos específicos de ToTV de las mismas.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características **esenciales**	D1	D3
Vector de expresión	Secuencia nucleótida (ver registro)	Vectores de expresión (pág 468-479 D3)
Secuencias N°1 ó 2 ó secuencias que tienen una homología de secuencia nucleótida de al menos 50% a la SEQ N°1 o a la SEQ N°2	SEQ con 58% identidad en un fragmento de 367 nucleótidos (secuencia) de las SEQ N°1 o 2 de la solicitud	Diversos vectores empleados para terapia con ácidos nucleicos (pág 468-479, tabla 11.2 D3)

En el documento D1 se divulga la secuencia nucleótida y aminoácida del virus clorótico enano del maíz, la secuencia nucleótida tiene un fragmento de 367 pares de bases con un 58% de identidad con las secuencias SEQ N°1 ó N°2 de la solicitud.

Teniendo en cuenta que la solicitud reclama un vector que contiene una secuencia hasta con el 50% de identidad de las SEQ N°1 ó 2 ó fragmentos de estas mismas secuencias, entonces pese a que se traten de diferentes virus, estas secuencias tienen un segmento con un porcentaje de identidad como el segmento que podría estar insertado en el vector de la solicitud.

Por su parte, el documento D2 divulga la secuencia nucleótida y aminoácida del virus esférico del arroz, la secuencia nucleótida tiene un fragmento de 1685 pares de bases con un 52% de identidad con las secuencias SEQ N°1 ó N°2 de la solicitud.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características **esenciales**	D2	D3
Vector de expresión	Secuencia nucleótida (ver registro)	Vectores de expresión (pág 468-479 D3)
Secuencias N°1 ó 2 ó secuencias que tienen una homología de secuencia nucleótida de al menos 50% a la SEQ N°1 o a la SEQ N°2	SEQ con 52% identidad en un fragmento de 1685 nucleótidos (secuencia) de las SEQ N°1 ó 2 de la solicitud	Diversos vectores empleados para terapia con ácidos nucleicos (pág 468-479, tabla 11.2 D3)

Teniendo en cuenta que la solicitud reclama un vector que contiene una secuencia hasta con el 50% de identidad de las SEQ N°1 ó 2 ó fragmentos de estas mismas secuencias, entonces pese a que se traten de diferentes virus, estas secuencias tienen un segmento con un porcentaje de identidad como el segmento que podría estar insertado en el vector de la solicitud.

La diferencia entre la materia reclamada en la reivindicación 2 de la solicitud y D1 ó D2, es que estas últimas divulgan una secuencia nucleótida de un virus mientras que la solicitud reclama un vector de expresión que contiene una secuencia nucleótida de un virus.

El efecto que produce esta diferencia no resulta evidente puesto que la solicitud no presenta información sobre el desarrollo de dicho vector, no divulga el vector empleado, los elementos que conforman el mismo (promotores, segmento nucleótido específico insertado) y las pruebas de expresión realizadas.

El problema técnico objetivo resuelto por la invención es obtener un vector recombinante que contenga un fragmento de un virus que afecta plantas.

Sin embargo, las técnicas de uso y transformación de vectores para ser empleados en terapia génica o transformaciones genéticas, son conocidas para la persona del oficio normalmente versada en la materia. Así lo demuestra, el documento D3 que es un libro de texto que menciona varios de los vectores usados para terapia con ácidos nucleícos. D3 divulga varios vectores empleados y su forma de uso en la técnica (Fls. 310 a 315).

Es también conocido el uso de un vector para lograr la transformación celular, es decir para lograr que un segmento particular de DNA sea expresado en un determinado organismo, luego el desarrollo del vector a partir de la secuencia de acido nucleico ya conocida resulta obvia.

De esta manera, frente a la información divulgada en D1 y D3, ó la información divulgada en D2 y D3 la invención reclamada en la reivindicación 2 carece de nivel inventivo e incumple el artículo 18 de la D486. Teniendo en cuenta que las reivindicaciones dependientes no aportan una característica inventiva a la solicitud, todas ellas carecen de nivel inventivo e incumplen el artículo 18 de la D486.

9. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	EMBL, N° acceso AY829112	2 y dependientes	Nivel inventivo
D2	EMBL, N° acceso M95497	2 y dependientes	Nivel inventivo
D3	Biopharmaceuticals, biochemistry and biotechnology. G.Walsh. Wiley Ed. Pág 468-479	2 y dependientes	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante para dar respuesta a este requerimiento y presentarla por escrito

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

JOSE LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 08 JUN. 2012

Elaboró: N.Lamprea NLP.
Revisó: Consuelo Leguizamón

Expediente 07-80240 1er Art.45

BIOPHARMACEUTICALS

BIOCHEMISTRY AND BIOTECHNOLOGY

Second Edition

Gary Walsh

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WILEY

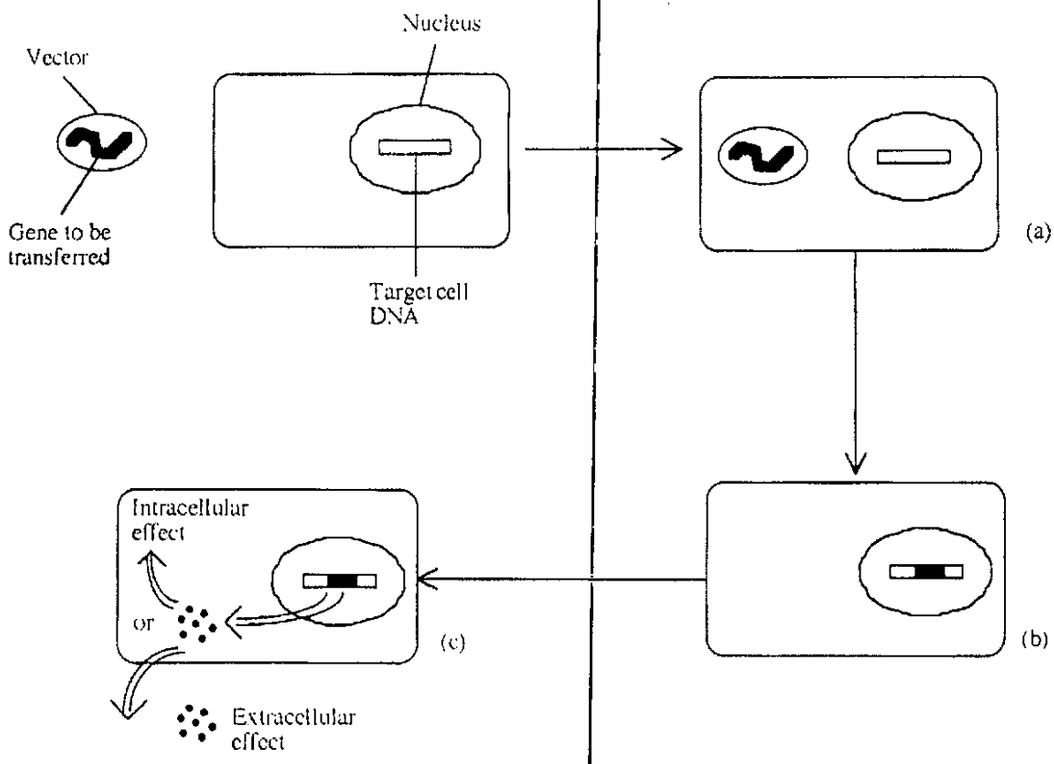


Figure 11.1. Simplified schematic representation of the basis of gene therapy. The genetic material to be transferred is firstly usually packaged into some form of vector, which serves to deliver the nucleic acid to the target cell. (a) Entry of the therapeutic nucleic acid — often still associated with its vector — into the cell cytoplasm. (b) Transfer of the nucleic acid into the nucleus of the recipient cell. This is often, although not always, followed by integration of the foreign genetic material into the cellular DNA. (c) The foreign gene (whether integrated or not) is expressed, resulting in the synthesis of the desired protein product. Regulatory elements of the nucleic acid transferred may be designed to ensure that the protein product is retained within the cell, or is exported from the cell, as necessary. Refer to the text for further details

Table 11.2. Vector systems used to deliver genes into mammalian cells. To date, the majority of clinical trials undertaken have utilized retroviral vector systems. Non-viral systems have generally been employed least often, although some, e.g. nucleic acid-containing liposomes, may be used more extensively in the future. Some of the methods tested, e.g. calcium phosphate precipitation, electroporation and particle acceleration, are unlikely to be employed to any great extent in gene therapy protocols

Viral-based vector systems	Non-viral-based vector systems
Retroviruses	Nucleic acid containing liposomes
Adenoviruses	Molecular conjugates
Adeno-associated virus	Direct injection of naked DNA
Herpes virus	$CaPO_4$ precipitation
Polio virus	Electroporation
Vaccinia virus	Particle acceleration

considerations, such as their ease of isolation and culture, their capacity to express (and excrete) the protein product, and their half-lives *in vivo*.

Several cell types, including keratinocytes, myoblasts and fibroblasts, have been studied in this regard. It has been shown, for example, that myoblasts into which the factor IX gene and the growth hormone gene have been introduced could express their protein products and secrete them into the circulation.

Vectors used in gene therapy

A list of the various vectors capable of introducing genes into recipient cells has been provided in Table 11.2. These vectors are conveniently categorized as being viral-based or non-viral-based systems. The main vector systems developed thus far are discussed in somewhat more detail below.

Retroviral vectors

In the region of 80% of gene therapy clinical trials undertaken to date have employed retroviral vectors as gene delivery systems. Retroviruses are enveloped viruses. Their genome consists of single-stranded RNA (ssRNA) of approximately 5–8 kb. Upon entry into sensitive cells, the viral RNA is reverse-transcribed and eventually yields double-stranded DNA. This subsequently integrates into the host cell genome (Box 11.1). The basic retroviral genome contains a minimum of three structural genes; *gag* (codes for core viral protein), *pol* (codes for reverse transcriptase) and *env* (codes for the viral envelope proteins). At either end of the viral genome are the long terminal repeats (LTRs), which harbour powerful promoter and enhancer regions and sequences required to promote integration into the host DNA. Also present, immediately adjacent to the 5′ LTR, is the packing sequence (ψ). This is required to promote viral RNA packaging.

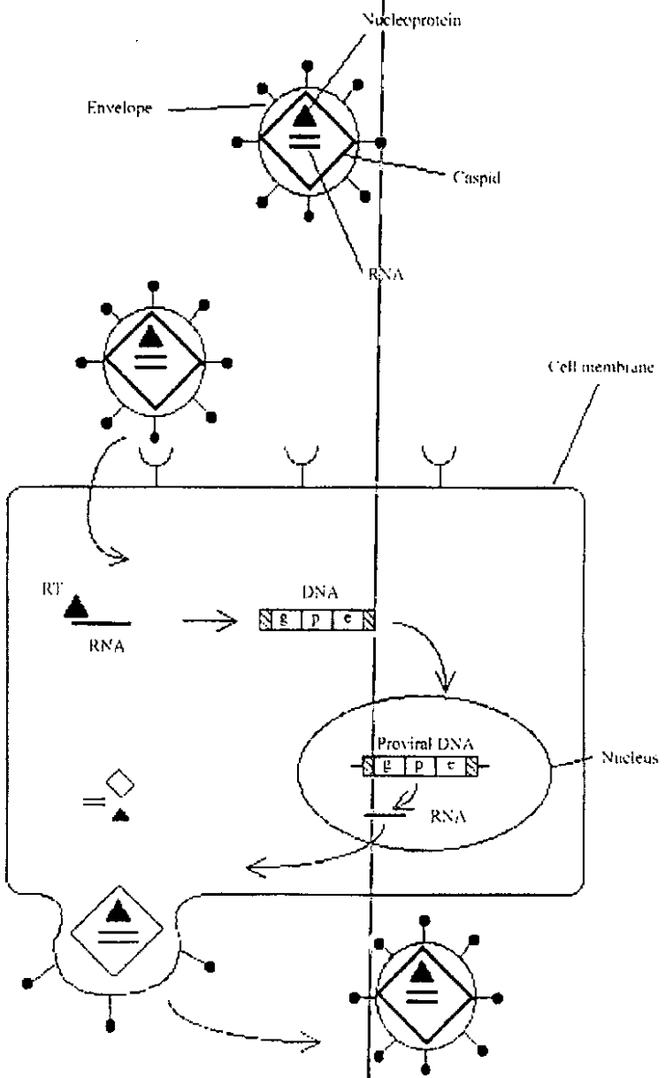
The ability of such retroviruses to (a) effectively enter various cell types and (b) integrate their genome into the host cell genome in a stable, long-term fashion, made them obvious potential vectors for gene therapy.

The construction of retroviruses to function as gene vectors entails replacing the endogenous viral genes, required for normal viral replication, with the exogenous gene of interest (Figure 11.3a). Removal of the viral structural genes means that the resulting vector cannot itself replicate. In order to generate mature virion particles harbouring the vector nucleic acid (Figure 11.3b), this genetic material must be introduced into a 'packing cell'. These are recombinant cells that have previously been engineered to contain the *gag*, *pol* and *env* structural genes (Figure 11.4). In this way, packing cells are capable of producing mature but replication-deficient viral particles, harbouring the gene to be transferred (see later section on Manufacture of viral vectors). These viral particles function as so-called 'one-time, single-hit' gene transfer systems.

More recently, various modifications have been introduced to this basic retroviral system. The inclusion of the 5′ end of the gag gene is shown to enhance levels of vector production by up to 200-fold. Additionally, specific promoters have been introduced in order to attempt to control expression of the inserted gene. Most work has focused upon the use of tissue-specific promoters in an effort to limit expression of the desired gene to a specific tissue type. The most commonly employed (recombinant-deficient) retrovirus used in this regard has been derived from the Maloney murine leukaemia virus (MoMuLV).

Box 11.1. The retroviral life cycle

The retroviral life cycle begins with the entry of the enveloped virus into the cell. The viral reverse transcriptase enzyme then copies the viral RNA genome into a single (minus) DNA strand and, using this as a template, generates double-stranded (ds) DNA. The dsDNA is then randomly integrated into the host cell genome (the proviral DNA). Transcription of the proviral genes host cell's transcription machinery yields mRNA that directs synthesis of mature virion particles. The viral particles bud out from the cell's plasma membrane, picking up a membrane-derived outer coat as they do so.



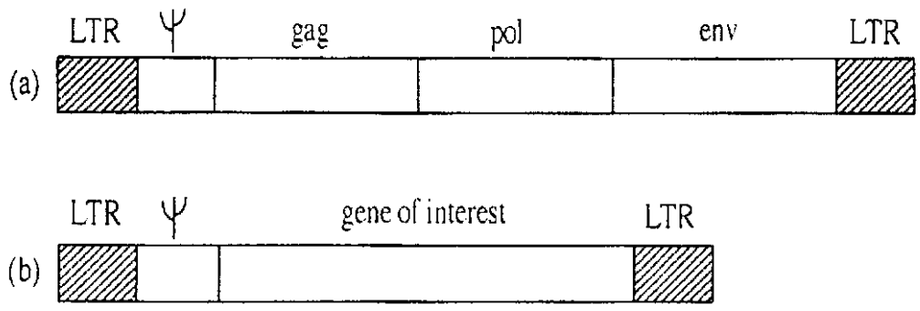


Figure 11.3. Schematic representation of (a) the proviral genome of a basic retrovirus and (b) the genome of a basic engineered retroviral vector carrying the gene of interest. Refer to text for further details

Retroviruses display a number of properties/characteristics that influences their potential as vectors in gene therapy protocols. These may be summarized as follows:

- Retroviruses as a group have been studied in detail and their biochemistry and molecular biology is well understood.
- Most retroviruses can integrate their proviral DNA only into actively replicating cells.
- The efficiency of gene transfer to most sensitive cell types is very high, often approaching 100%.
- Integrated DNA can be subject to long-term, relatively high-level expression.
- Proviral DNA integrates randomly into the host chromosomes.
- Retroviruses are promiscuous in that they infect a variety of dividing cell types.
- Complete copies of the proviral DNA are passed on to daughter cells if the original recipient cell divides.
- Good, high-level titre stocks of replication-incompetent retroviral particles can be produced.
- Safety studies using retroviral vectors have already been carried out on various animal species.

The fact that they have been well studied, display almost 100% transduction efficacy in sensitive cells and that the transferred genes are usually subject to long-term, fairly high-level expression renders retroviruses powerful potential vectors. These advantages form the basis of their widespread use in this regard.

However, many of the other characteristics listed serve to curtail the application of retroviruses as gene therapy vectors. In most instances, their ability to infect only dividing cells clearly restricts their use. Their lack of selectivity in terms of the dividing cell types they infect is also a disadvantage. They will not infect all dividing cell types—the entry of any specific retrovirus being dependent upon the existence of an appropriate viral receptor on the surface of a target cell. As the identity of most retroviral receptors is still unknown, it remains difficult to predict the entire range of cell types any retrovirus is likely to infect during a gene therapy protocol. Integration and expression of the exogenous gene in cells other than target cells could result in physiological complications.

An additional drawback with regard to retroviral-based vectors is the propensity of the transferred gene to integrate randomly into the chromosomes of the recipient cells. Integration of the transferred DNA in the middle of a gene whose product plays a critical role in the cell

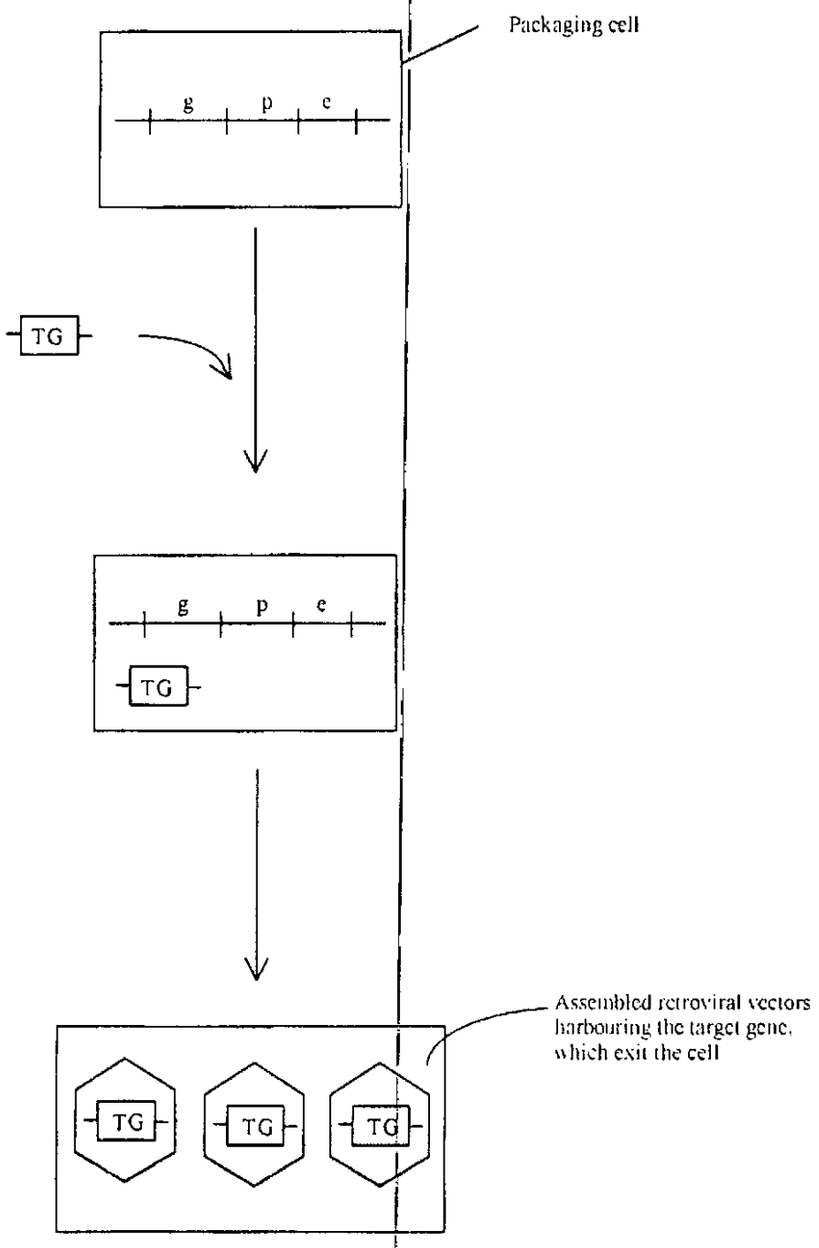


Figure 11.4. The use of packaging cells to generate replication-deficient retroviral vectors. The packaging cell is an engineered animal cell into which the retroviral *gag* (g), *pol* (p) and *env* (e) genes have been introduced. The cell line chosen must be one which the (replication deficient) virus can infect. The engineered retroviral vector genome (which is carrying the target gene: TG) is then incubated with the packaging cell. This results in the generation and assembly of mature replication-deficient retroviral vector particles. These exit the cell and will replicate by entering other packaging cells. By completing a number of such replication cycles, large quantities of the desired retroviral vectors are produced

could irrevocably damage cellular function, e.g. disruption of a central metabolic enzyme could cause cell death, while disruption of a tumour suppresser gene could give rise to cellular transformation; in addition, integration of the proviral nucleic acid to sites adjacent to quiescent cellular proto-oncogenes could result in their activation. Another impediment to routine use of retroviral vectors is the relatively labile nature of these particles. Thus, while retroviruses are relatively easy to propagate, they are often damaged by subsequent purification and concentration—steps essential for their clinical use.

Additional viral-based vectors

A number of additional viral types may also prove useful as vectors in the practice of gene therapy. Chief amongst these are the adenoviruses. Adeno-associated virus, the herpes virus and a number of other viruses are also being considered (Table 11.2).

Adenoviruses are relatively large, non-enveloped structures, housing double-stranded DNA as their genetic material. Their genome is much larger (approximately 35 kb), and more complex than those of retroviruses. In most instances, only a small fraction of this genome is removed when constructing an adenovirus-based vector. Upon cellular infection, adenoviral DNA becomes localized in the nucleus, but does not integrate into the host cell DNA. Usually, infection by wild-type adenoviruses is associated with, at most, mild clinical symptoms in humans.

As potential vectors for gene therapy, adenoviruses display a number of both advantages and disadvantages (Table 11.3). Their major advantage relates to their ability to efficiently infect non-dividing cells and the usually observed expression of large quantities of the desired gene products. However, the failure of the adenoviral-based DNA to integrate into the host cell generally means that its survival, and hence the duration of gene expression, is limited. Adenovirus-based vectors carrying various marker genes (i.e. a gene whose expression product is easily detected) have been administered to animals. Marker gene expression has been subsequently noted in various tissues, including heart, liver, muscle, bone marrow, CNS and endothelial cells. Duration of marker gene expression ranged from 2–3 weeks to several months.

While short-term, high-level gene expression may be appropriate for some gene therapy applications, it would be of less use for the treatment of, for example, genetic diseases, where long-term gene expression would be required. This could be achieved in theory by repeat administration of the adenoviral vector. However, adenoviruses prompt a strong immune response, which limits the efficacy of repeat administration.

Table 11.3. Some characteristic advantages and disadvantages of adenoviruses as potential vectors for gene therapy. Refer to text for further details

Advantages	Disadvantages
Adenoviruses are capable of gene transfer to non-dividing cells	Adenoviruses are highly immunogenic in man
They are easy to propagate in large quantities	The duration of expression of transferred genes can vary, and is usually transient
High levels of gene expression are usually recorded	Infection of permissive cells with wild-type adenovirus usually results in cell lysis
They are relatively stable viruses	Adenoviruses display a broad selectivity in the cell types they can infect

Additional viruses that may prove of some use as future viral vectors include adeno-associated virus and herpes virus. Adeno-associated virus is a very small, single-stranded DNA (ssDNA) virus — its genome consists of only two genes. It does not have the ability to replicate autonomously and can do so only in the presence of a co-infecting adenovirus (or other selected viruses).

Although it is found in the human population, it does not appear to be associated with any known diseases. Not surprisingly, only relatively small genes can be introduced into adeno-associated viral vector systems. Such systems, however, do provide a mechanism of gene transfer into non-dividing cells. It also seems to facilitate long-term expression of the transferred genetic material. In contrast to adenoviruses, nucleic acid transferred by adeno-associated viruses appears to be integrated into the recipient cell genome.

The herpes simplex virus (HSV) represents another potential vector system which is receiving increased attention. Because HSV is a neurotrophic virus, it may prove to be particularly useful in delivering genes to neurons of the peripheral and central nervous system. Upon infection, HSV usually remains latent in non-dividing neurons — with its genome remaining in an unintegrated form. Thus far, it has proved difficult to generate a replication-incompetent, but yet viable, herpes simplex particle. Moreover, some of the replication-incompetent viruses generated still retain an ability to damage/destroy the cells they infect. While herpes-based vector systems may one day prove useful in gene therapy, suitable and safe vector variants of HSV must first be generated and tested.

An additional virus, which has more recently gained some attention as a possible vector, is that of the sindbis virus. A member of the alphavirus family, this ssRNA virus can infect a broad range of both insect and vertebrate cells. The mature virion particles consist of the RNA genome complexed with a capsid protein C. This, in turn, is enveloped by a lipid bilayer in which two additional viral proteins, E1 and E2, are embedded. The E2 polypeptide appears to mediate viral binding to the surface receptors of susceptible cells (the major mammalian cell surface receptor it targets appears to be the highly conserved, widely distributed laminin receptor).

The sindbis virus is simple, robust, capable of infecting non-dividing cells and generally supports high levels of gene expression. However, it does display a broad host range and, hence, lacks the inherent targeting specificity characteristic of an idealized viral vector.

Recently a novel recombinant sindbis virus displaying altered host cell specificity has been generated. Scientists inserted a nucleotide sequence coding for the IgG-binding domain of *Staphyloccus aureus* into the E2 viral gene. Disruption of the E2 gene renders its protein product incapable of binding laminin (hence destroying the natural viral tropism). However, the protein A domain allows the chimeric E2 product to bind monoclonal antibodies. This altered virus may prove to be a useful generic or 'null' vector, potentially capable of being specifically targeted to any desired cell type. This would simply necessitate pre-incubation of the virus with monoclonal antibodies raised against a surface antigen unique to the proposed target cell population (Figure 11.5). Binding of the monoclonal antibody to the protein A domain would ensue and the immobilized monoclonal antibody would dictate the cell type targeted.

Initial studies using this system have proved encouraging. The altered virus (without associated monoclonal antibody) failed to infect a wide variety of human cell lines. By initially incubating with monoclonal antibody of the appropriate specificity, however, the viral particles were capable of efficiently transducing cells expressing surface receptors such as CD4, CD33 and human leukocyte antigen (HLA).

A number of other issues must now be addressed, including determining whether the IgG–protein A affinity is sufficiently high to keep the antibody associated with the virus *in vivo*. The