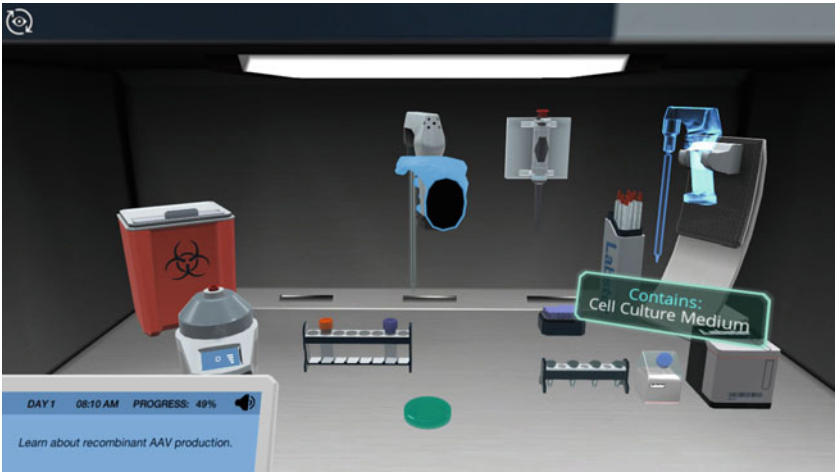
Viral Gene Therapy

4



A. Gardner et al., *Labster Virtual Lab Experiments: Genetics of Human Diseases*,
https://doi.org/10.1007/978-3-662-58744-7_4

4.1 Viral Gene Therapy Simulation

In the previous three chapters we've covered the impact our genetics can have on our health. In this final chapter we reverse this and look at the exciting field of gene therapy. In the Viral Gene Therapy simulation, you will learn about the use of modified viruses and how we can manipulate their genetic code to fix mutations inside our own bodies. Based on recent exciting research findings you'll investigate the role of genetics in heart failure. Will you be able to design a virus that can help to improve the symptoms of heart failure in patients?

Using Viruses to Treat Heart Failure
Heart failure is one of the major health issues facing Western populations. Dietary and lifestyle causes are well understood; however, a significant number of individuals are at risk based purely on their genetics. If we know which gene and mutation is linked with a disease, then we should be able to "fix" this via gene therapy, using the infectious power of viruses to improve our health (Fig. 4.1).

Design, Produce and Test Your Virus in the Lab
While gene therapy sounds simple on paper, there are lots of obstacles standing in the way of a viable treatment. The first is in identifying a suitable virus and

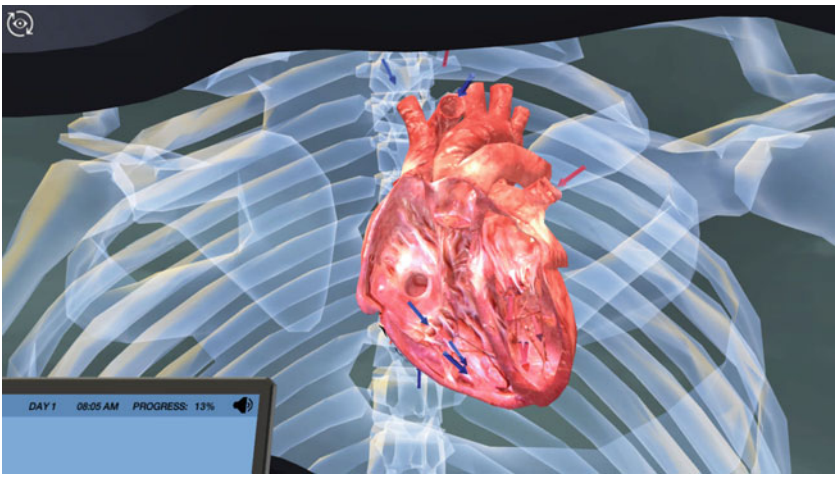


Fig. 4.1 Learn about the heart and how heart failure is a growing risk in the Viral Gene Therapy simulation

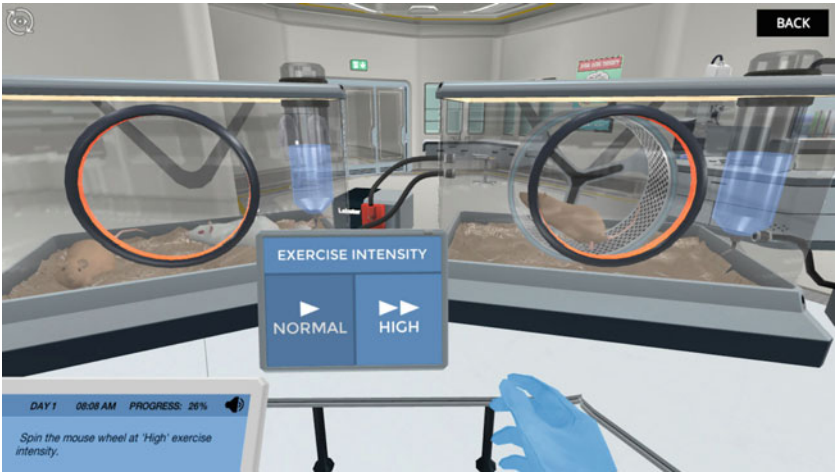


Fig. 4.2 Design and test your virus to treat heart failure in a suitable model in the Viral Gene Therapy simulation

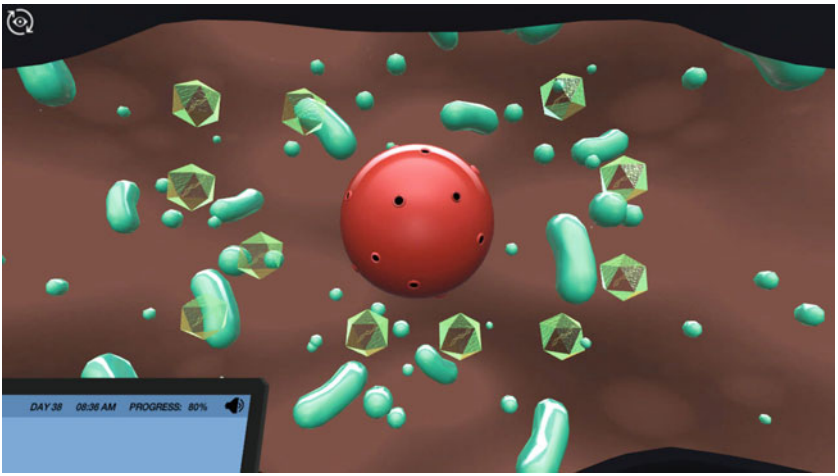


Fig. 4.3 Observe your virus in action in the Viral Gene Therapy simulation

deciding on the best way to insert the genetic material within. Then, the efficiency of the produced viruses needs to be tested in a suitable model system, in this case, a mouse model (Fig. 4.2). Since this is a virtual lab, no real animals will be harmed in this experiment, and results are available immediately instead of the usual five weeks!

Test the Efficacy of Your Gene Therapy Treatment
Once you have designed your gene therapy treatment and produced your viruses, the next step is to see if it has a beneficial effect (Fig. 4.3). Will your virus cure a mouse model of heart failure and do you think it would make a suitable therapeutic treatment for humans?

4.2 Viral Gene Therapy Theory Content

Does using viruses as a form of therapy sound like science fiction to you? You'd be surprised, but it's an increasingly popular technique used to deliver functional genes into patients. In this lab, you will learn about the use of modified viruses and how to equip them with therapeutic genes. The content below covers all the related theory needed to complete the Viral Gene Therapy simulation successfully.

Gene Therapy

Gene therapy is a technique for treating disease by altering the patient's genetic material. Most often, gene therapy works by introducing a healthy copy of a defective gene (also called therapeutic gene) into the patient's cells. Different vectors exist that carry and deliver the therapeutic gene into the patient's cells. Viral vectors are most commonly used, but also non-viral methods exist, such as injection of naked DNA or DNA complexes.

Therapeutic Gene

Therapeutic genes are used in gene therapy as functional copies of disease-causing genes. They are delivered as nucleic acid polymers (RNA or DNA) that replace the mutated or lost gene in the patient treated.

Table 4.1 Baltimore classification. Viruses can be classified into one of seven groups based on the structure of their genetic material and their method of replication. Ds = double-strand, ss = single-strand, (+) = positive-sense, (−) = negative-sense

Group	Name	Description	Example
I	dsDNA	Double-stranded DNA	Herpesviruses
II	ssDNA	Single-stranded DNA	Parvoviruses
III	dsRNA	Double-stranded RNA	Reoviruses
IV	(+)ssRNA	Single-stranded RNA, positive sense strand	Flaviviruses
V	(−)ssRNA	Single-stranded RNA, negative sense strand	Orthomyxoviruses
VI	ssRNA-RT	Single-stranded RNA, positive sense strand, DNA as replication intermediate	Retroviruses
VII	dsDNA-RT	Double-stranded DNA, RNA as replication intermediate	Hepadnaviruses

Viruses

Viruses are small infectious agents that can only replicate inside the living cells of other host organisms. When not replicating in a host cell viruses exist as stable particles also known as virions. The size of most viruses vary from 20 to 300 nm and hence can only be seen using an electron microscope. Viruses are built from a protein coat known as the capsid which protects the genetic material carried within. Some viruses, such as retroviruses, carry a lipid envelope around the capsid. The type of genetic material found within viruses can vary widely and is often used to classify them into one of seven groups as for example in the Baltimore classification, named after David Baltimore (Table 4.1).

Viral Vectors in Gene Therapy

In gene therapy, viral and non-viral vectors are used to deliver a therapeutic gene to a patient's cells.

Viruses are the perfect tool for nucleic acid delivery as they naturally evolved to insert their genetic material into cells to replicate. Some viruses, such as retroviruses, even insert their genes into the host cell's genome. In order to ensure the safety of the viral vectors, either the whole viral genome is replaced by the therapeutic gene or the parts that are disease-causing are deleted from the viral vector (Fig. 4.4).

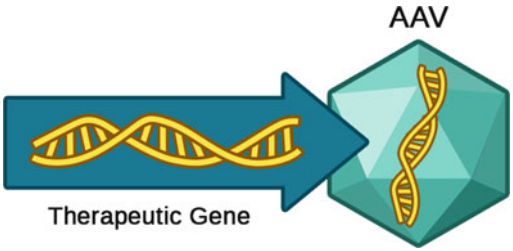


Fig. 4.4 Incorporation of a therapeutic gene into the viral vector adeno-associated virus (AAV). By incorporating a therapeutic gene into a vector, in this case AAV, it is then possible to deliver this gene to the body, or specific organ or tissue of an affected individual

Most commonly used viral vectors are:

- Adenoviruses
- Adeno-associated virus (AAV)
- Retrovirus
- Herpes simplex virus
- Vaccinia virus

Adeno-associated Virus (AAV)

The adeno-associated viruses (AAV) are small, single-stranded DNA viruses that belong to the family of *Parvoviridae*.

AAV infect humans and other primates; however, they are not known to cause any disease. The lack of pathogenicity makes AAV attractive as viral vectors for gene therapy.

AAV are characterized by an icosahedral capsid and a genome size of 4.7 kb. The genome contains two open reading frames, encoding for *rep* and *cap*. *Rep* is composed of four genes encoding for viral proteins required for replication. *Cap* contains VP1, VP2, and VP3, which together build the virus capsid.

AAV in Gene Therapy

The desired therapeutic gene cDNA is inserted between two inverted terminal repeats (ITRs) that aid the packaging of the viral genome into the virus capsid.

Once infected with a recombinant AAV, the introduced therapeutic gene will not integrate into the host genome but remain as episomal concatemers in the cell nucleus. Consequently, AAV DNA is lost upon cell division as the episomal DNA is not replicated. Since AAV doesn't integrate into the human genome, it does not present the risk of random insertion and mutagenesis, which makes AAV a more predictable viral vector for gene therapy compared to retroviral vectors.

In addition, AAV shows a great relative selectivity for heart muscle cells and is therefore the preferred gene delivery system for targeting the heart, as for example in the case of treating heart failure patients (more details in the following sections of this chapter).

Compared to other viral vectors, AAV has a rather small cloning capacity and requires the complete replacement of the 4.7 kb genome. This allows for the incorporation of only small therapeutic genes.

The application of AAV as a viral vector for gene therapy is limited by the fact that some patients were unnoticedly infected with AAV and therefore produced neutralizing antibodies against the virus, which impairs the efficiency of the treatment.

Retroviral Vectors

Retroviruses are lipid-enveloped viruses comprised of linear single-stranded RNA genomes of 7 to 11 kb. The main features of retroviral vectors are the reverse transcription of the viral RNA genome into DNA, and stable integration into the host DNA. Lentivirus is a genus of retroviruses. Example of lentiviruses is human immunodeficiency virus (HIV).

At the end of all retroviral genomes there are two long terminal repeat (LTR) sequences. The LTR sequences become the border of the *gag*, *pol* and *env* genes. Lentiviruses also encode for *tat* and *rev*. The function of each gene is as follows:

- ***gag:*** structural protein
- ***pol:*** nucleic acid polymerase/integrases
- ***env:*** surface glycoprotein
- ***tat:*** regulatory protein for gene expression
- ***rev:*** regulatory protein accessory genes

Retroviral Vectors in Gene Therapy

Among virus types that are generally used in clinical gene therapy, the retrovirus is the leading delivery system due to its efficiency.

In order to increase vector biosafety, the retroviral vector gene is modified by removing up to six genes essential for HIV replication and pathogenesis. The overall principle of lentiviral vector gene transfer is described as follows:

- The genes that encode the structural proteins (*gag*, *pol*, and *env*) are deleted from the virus vector (plasmid).
- The viral vector carries the gene of interest and psi packaging. The virus vector is co-transfected with packaging plasmids that carry the viral structural genes to the packaging cell line. The recombinant lentivirus is then produced in the cell line before being extracted.
- The recombinant lentivirus particle is transduced into target cells, where reverse transcription, foreign gene integration, and foreign gene ectopic expression take place.

The advantages of retroviral vectors include:

- Stable integration into the host genome
- High-level expression
- Capability to infect a broad variety of target cell types
- Ability to carry foreign genes up to 8 kb

However, one disadvantage of using retroviruses as vectors in gene therapy is the potential for insertional mutagenesis by random viral integration into the host DNA. When this occurs in tumor suppressor genes it can lead to the development of cancer.

Targeting in Gene Therapy

One challenge in the development of the gene therapy approach using viral vectors is the specific targeting of the vector to a tissue inside the body, since an untargeted vector can potentially lead to serious side-effects.

One possibility to prevent those side-effects is the engineering of the viral surface proteins so that they specifically bind to cell receptors that are only expressed in the tissue to be targeted. This approach is also called pseudo-typing.

In addition, some viral vectors are known to naturally infect specific tissues, such as adeno-associated virus (AAV) 2 that presents natural tropism towards skeletal muscles, neurons, vascular smooth muscles, and liver cells.

Finally, the gene therapy drug can be injected or given intravenously to ensure that a specific tissue in the body is targeted.

Another layer of specificity can further be achieved by expressing the therapeutic gene from a tissue-specific promoter.

The Heart

The heart is a fist-sized organ which sits in the middle of the chest, and slightly to the left. Forming part of the circulatory system it functions to pump blood around the body by "beating", or more accurately by undergoing a controlled series of contractions known as the cardiac cycle. Comprised of four chambers, the upper left and right atria and the lower left and right ventricles, through which blood flows in a single direction.

The Cardiac Cycle

The cardiac cycle is the period of time that begins with contraction of the atria and ends with ventricular relaxation. The cardiac cycle includes all events associated with the blood flow through the heart during one complete heartbeat.

Deoxygenated blood flows into the right atrium and is pumped into the right ventricle, before it is in turn pumped into the pulmonary capillaries of the lung for oxygenation. Oxygenated blood returns from the lungs into the right atrium before passing into the right ventricle and then into the main circulatory system. When a chamber is actively contracting it is said to be in systole, whereas a relaxing chamber is said to be in diastole.

A single cardiac cycle therefore begins with atrial systole and continues to ventricular systole, atrial diastole, and ventricular diastole. Interruption to the cardiac cycle can be fatal and is a major form of heart failure.

Ejection Fraction (EF)

The ejection fraction (EF) describes the amount of blood that is pumped out of the ventricles with each contraction of the heart (Fig. 4.5). The resulting percentage

$$\frac{\text{Amount of blood pumped out of the ventricle}}{\text{Total amount of blood in ventricle}} = \textbf{Ejection fraction (\%)}$$

Fig. 4.5 Calculation of the ejection fraction (EF). The EF is calculated by dividing the amount of blood pumped out of a ventricle, by the total amount of blood in the ventricle. If a large proportion of blood remains in the ventricle, the EF will be low and this indicative of poor heart health

indicates heart performance and helps to diagnose heart failure or other types of heart disease. Normal EF values for a healthy heart are 50 to 75%.

Heart Failure

Heart failure, sometimes also called congestive heart failure, describes a condition in which the heart is not able to pump sufficient blood to cover the body's needs. In particular, the heart wall, or myocardium, is dilated in heart failure patients.

Heart failure is a fatal condition and the most common reason for hospitalization of people above the age of 65. The causes of heart failure include coronary artery disease, high blood pressure, heart attacks, or conditions that overwork the heart.

Typical symptoms of heart failure are shortness of breath, fluid retention (e.g. swollen ankles, legs, abdomen), reduced ability to exercise, weakness and fatigue, rapid or irregular heartbeats. Heart failure can be triggered for example during physical exercise when the heart is under strenuous effort.

Treatment of Heart Failure

Conventional treatments of heart failure include ACE-inhibitors, beta-blocker, diuretic, and Angiotensin II Receptor Blockers (ARB's). However, most of the commonly available drugs have high side effects, and many patients still have a poor life expectancy despite treatment. Consequently, new types of treatments are needed.

Viral gene therapy using AAV as a vector and *SERCA2a* as a therapeutic gene demonstrates a promising new therapeutic approach in the field of heart failure (Fig. 4.6).

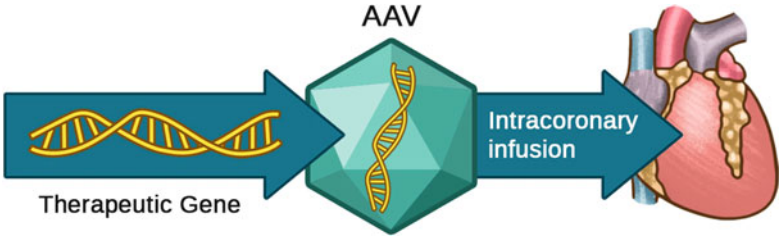


Fig. 4.6 **In the case of gene therapy treatment of heart failure, the therapeutic gene is directly targeted to the heart, for example by intracoronary infusion**

SERCA

Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), is a calcium ATPase-type P-ATPase. It resides in the sarcoplasmic reticulum (SR) in myocytes and regulates re-uptake of Ca^{2+} into the SR. It thereby regulates the contractile properties of muscle cells in the heart and thus the cardiac cycle. Three major paralogs exist:

- SERCA1
- SERCA2
- SERCA3

SERCA2a was identified to specifically regulate the Ca^{2+} cycling in cardiomyocytes, particularly Ca^{2+} removal, which triggers myocardial relaxation. SERCA2a is therefore considered a critical factor in the progression of heart failure (Fig. 4.7).

Recombinant AAV Production for Gene Therapy

Several systems exist to produce recombinant AAV. The most common system is based on a co-transfection of HEK293 cells (as described below) as the packaging cell line (Fig. 4.8 and 4.9). Following lipid-based co-transfection, cells are harvested by lysis and the virus is purified for infection of target cells.

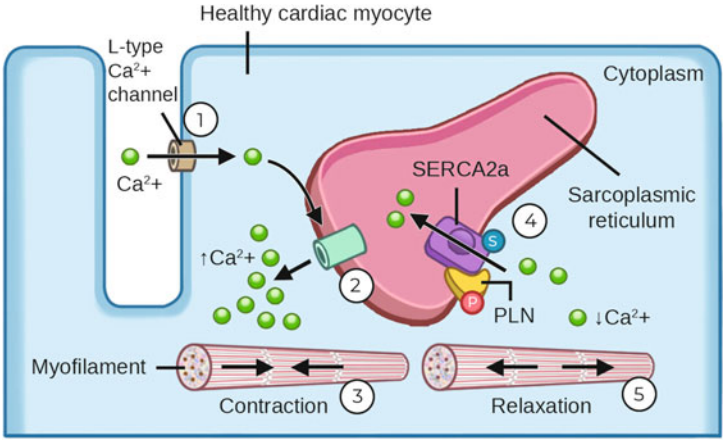


Fig. 4.7 Schematic representation of the function of SERCA2a in healthy cardiac myocytes. During diastole, Ca^{2+} enters the cell (1) and triggers the release of large amounts of Ca^{2+} from the sarcoplasmic reticulum (SR) (2). This causes the myofilaments to contract (3). At the same time, SERCA2a is released from its inhibitor PLN and shuttles Ca^{2+} back into the SR (4), which allows the myofilaments to relax (5)

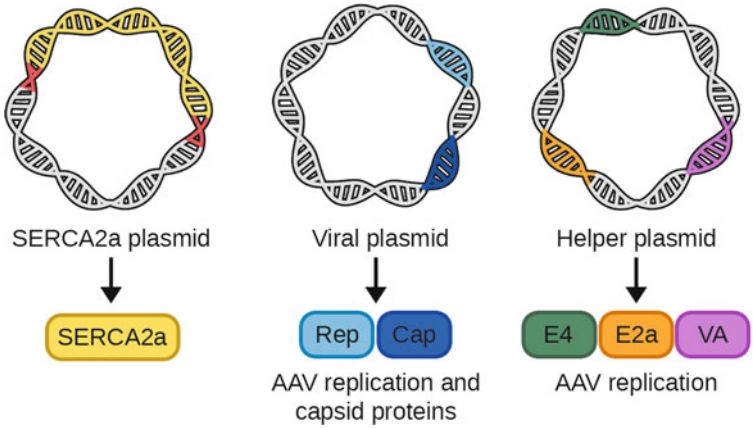


Fig. 4.8 Plasmids required for AAV production. The viral plasmid encodes for the replication (Rep) and Capsid (Cap) proteins. The helper plasmid encodes for the essential factors E4, E2a, and VA. Without those factors, no replication would occur. Finally, a third plasmid is co-transfected the therapeutic gene cDNA (shown here in yellow)

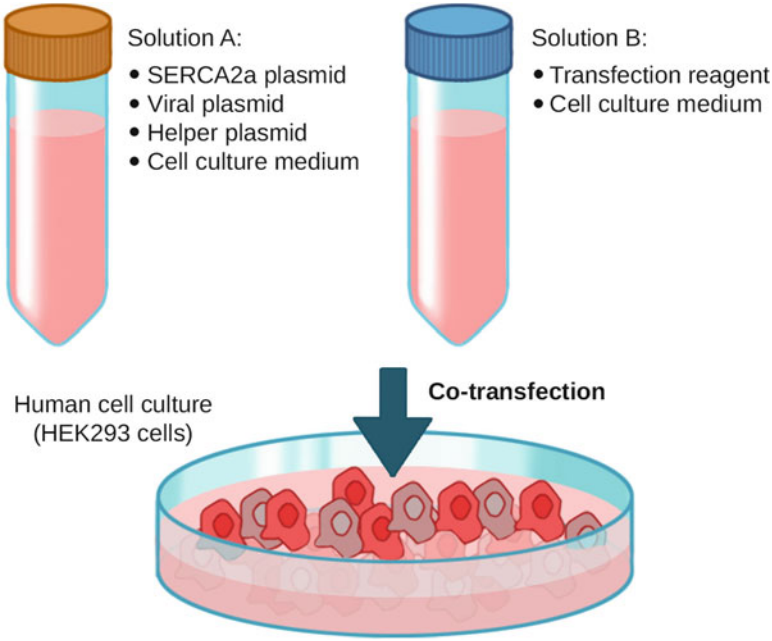


Fig. 4.9 **The co-transfection procedure.** The three plasmids described in Fig. 4.9 are then mixed with a transfection reagent such as a lipid, and then transfected into a suitable cell line, such as HEK293 cells. The cells are lysed and the final virus is purified for future use

Lipid-based Transfection

Transfection is a process that introduces nucleic acids into eukaryotic cells to modify gene expression, for the study of gene function and gene products. This technology was developed to enable nucleic acids such as DNA to enter the cells. Due to their negative net charge, which is the same as that in the cell membrane, nucleic acids cannot enter without external aid. Therefore, researchers need chemical reagents or physical stimuli to allow the nucleic acids to enter the cells. Currently, the most broadly used method is the lipid-based transfection method.

Lipid-based transfection uses cationic lipids which then combine with nucleic acids to form a "transfection complex" which will enter the cell (Fig. 4.10). Although the molecular mechanism is not fully understood yet, it is known that the transfection complex electrostatically interacts with the cell membrane, entering via endocytosis to subsequently free the nucleic acid inside the cell.

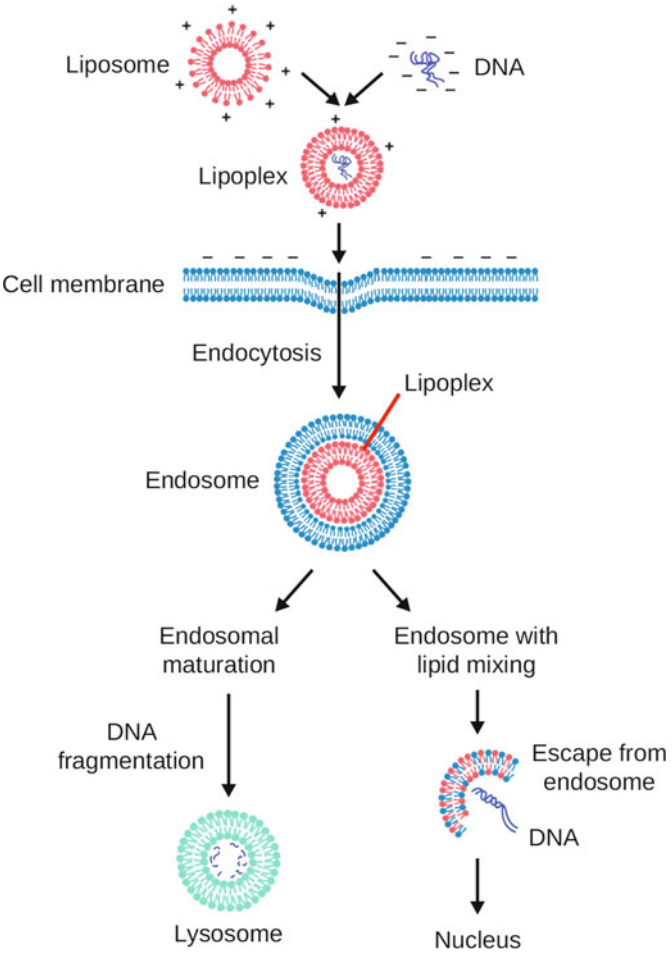


Fig. 4.10 **Complexing of DNA with a transfection reagent.** Lipids can be used to efficiently deliver genetic material such as DNA through the cell membrane. DNA is complexed with a liposome, a spherical structure comprised of a specific lipid. This lipid masks the negative charge of the genetic material thus allowing it to pass through the cell membrane. Once inside the cell the lipoplex can either be degraded by the cells lysosomal machinery, or the DNA can be released inside the cell. The cells usual transcriptional and translational machinery can then produce any proteins encoded for by the DNA molecule

This method is fast and uses easy protocols which do not require media changes. It also achieves high efficiency and expression performance and is applicable to a broad range of cell lines.

Use of Animal Models in Research

Animal models are used in research to study diseases and to test new treatments before given to humans as part of clinical trials.

The criterion used to select the animal model depends on the objective of the study, but one of the most important considerations is the degree of conservation of the studied process in humans. Thus the similarity of the animal model is very important when aiming to apply the conclusions of the experiment to humans.

The most common animal models utilized in research are mice and rats. The genetic and physiological similarities between mice and humans coupled with short generation times and low maintenance costs make it the ideal model organism.

There is a whole catalog of mouse strains that were genetically modified to study certain diseases that would naturally not occur in mice. The data obtained from these studies is an additional advantage of mice over other mammals.

Knock-out Mice

A knock-out mouse is a laboratory mouse in which researchers have inactivated, or "knocked out" an existing gene by replacing it or disrupting it with an artificial piece of DNA. The loss of gene activity often causes changes in a mouse's phenotype, which includes appearance, behavior and other observable physical and biochemical characteristics.

Knocking out the activity of a gene provides valuable clues about what that gene normally does. Observing the characteristics of knock-out mice gives researchers information that can be used to better understand how a similar gene may cause or contribute to disease in humans. Examples of research in which knockout mice have been useful include studying and modeling different kinds of cancer, obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging and Parkinson disease. Knock-out mice also offer a biological context in which drugs and other therapies can be developed and tested.

Transmission Electron Microscopy

Electron microscopy (EM) has been proven to be an invaluable tool in the discovery and characterization of viruses because it is the only method to visualize the ultrastructure of viral particles.

In Transmission electron microscopy (TEM), a specific EM technique, the electrons pass through the sample like light through a shadow puppet screen. Dense structures absorb a lot of electrons and create a dark spot on the resulting image, just like the shadow of a puppet blocking the light. A TEM image is always black and white; staining techniques only allow increasing the density of certain structures and thereby making them appear darker. To bundle the electrons, the TEM contains strong magnets that are analogous to lenses in the light microscope. To efficiently illuminate the specimen with an electron beam, the sample slice needs to be very thin, and the body of the TEM has to be evacuated.

4.3 Let's Get Started

Wow! What an adventure, you've learned so much about genetic diseases but we want to end on a positive note. Let's use the infectious power of viruses for good and use them to "fix" disease-causing mutations in our genetic code. The field of gene therapy is incredibly exciting and represents a revolution in healthcare, will you be able to contribute to its development and help design a therapy targeting heart failure?

Techniques Used in the Lab

- Co-transfection of mammalian cells
- Viral vector production
- Electron microscopy

Learning Objectives

At the end of this simulation, you will be able to . . .

- Explain the use of gene therapy for the treatment of heart failure
- Explain the causes of heart failure

- Design a viral-mediated gene therapy approach
- Define "therapeutic gene"
- Describe the anatomy and function of the heart from a healthy person vs. a heart failure patient
- Produce replication defective recombinant adeno-associated virus

ACCESS THE VIRTUAL LAB SIMULATION HERE www.labster.com/springer **BY USING THE UNIQUE CODE AT THE END OF THE PRINTED BOOK. IF YOU USE THE E-BOOK YOU CAN PURCHASE ACCESS TO THE SIMULATIONS THROUGH THE SAME LINK.**

Further reading

Alberts B et al (2015) The molecular biology of the cell, 6th edn. Garland Science, Abingdon
Flint SJ et al (2015) Principles of Virology, 4th edn. Taylor & Francis Inc, Enfield
Hartwell L et al (2015) Genetics: from genes to genomes, 5th edn. McGraw-Hill, Boston
OpenStax (2018) Biology. OpenStax CNX. http://cnx.org/contents/185cbf87. Accessed 1 June 2018
Shareef MA, Anwer LA, Poizat C (2014) Cardiac SERCA2A/B: Therapeutic targets for heart failure. Eur J Pharmacol 724:1–8