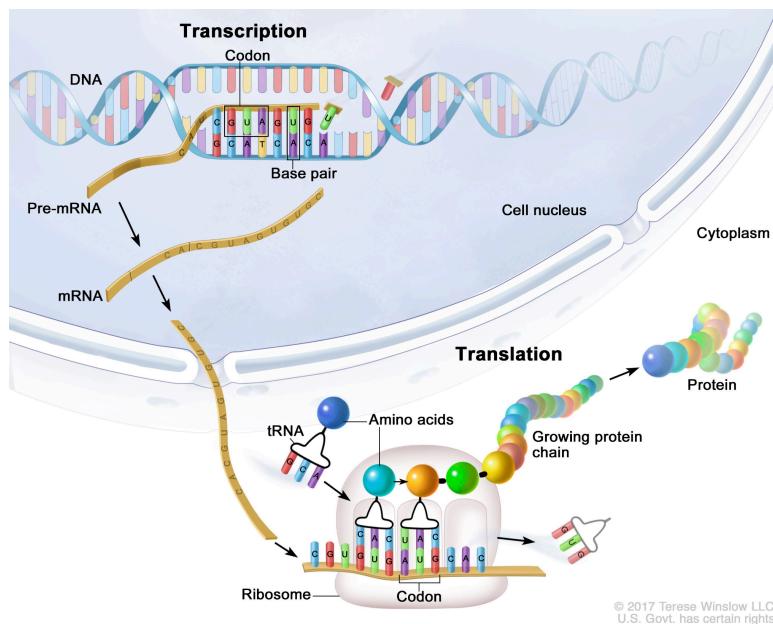
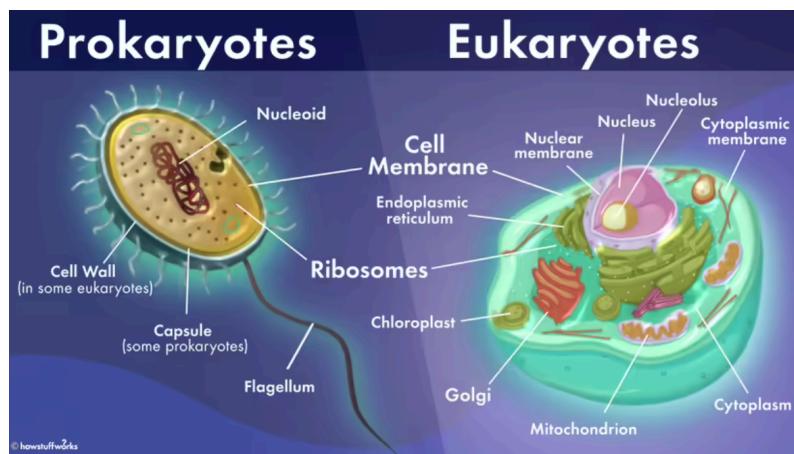


Transcription and translation are processes that turn **DNA into proteins**. DNA and RNA are made up of four building blocks (C, A, T/U, and G).

- **Transcription:** In the **cell's nucleus**, a gene in DNA is copied into mRNA.
- **Translation:** The mRNA moves to the **cytoplasm**, where ribosomes read the mRNA. tRNA brings amino acids to the ribosome, matching its sequence to the mRNA. The ribosome links the amino acids together to form a protein. Once all amino acids are joined, the protein is complete and released.

RNA, made up of nucleotides (adenine, guanine, cytosine, and uracil), mostly exists as a **single strand** and can vary in length and structure. RNA viruses use RNA instead of DNA for genetic material and can cause diseases. **Transcription is the process of making RNA from DNA, and translation is the process of making proteins from RNA.**





Prokaryotic cells are simpler and lack the eukaryote's membrane-bound organelles and nucleus, which encapsulate the cell's DNA.

Types of RNA:

- mRNA:** Carries the **genetic code from DNA for protein creation**. In eukaryotes, **mRNA is processed (introns are removed, exons joined, and caps/tails are added)** before being used in protein synthesis.
- tRNA:** Brings **amino acids to the ribosome to build proteins**, matching mRNA codons with specific amino acids.
- rRNA:** **Forms ribosomes, which assemble proteins** from amino acids.

Enzyme	Transcription Product	Minimum Required Initiation Factors
RNA Pol I (14 subunits)	rRNA 45S	SL1*/TIF1B/core factor TIF1A/RRN3 UBF/UAF
RNA Pol II (12 subunits)	mRNA snRNA miRNA	TFIIA TFIIB TFIID* TFIIE TFIIF TFIIC
RNA Pol III (17 subunits)	tRNA rRNA 5S small RNAs	TFIIC TFIIB*

RNA types & functions



Types of RNAs	Primary Function(s)
mRNA - messenger	translation (protein synthesis) regulatory
rRNA - ribosomal	translation (protein synthesis) <catalytic>
t-RNA - transfer	translation (protein synthesis)
hnRNA - heterogeneous nuclear	precursors & intermediates of mature mRNAs & other RNAs
scRNA - small cytoplasmic	signal recognition particle (SRP) tRNA processing <catalytic>
snRNA - small nuclear snoRNA - small nucleolar	mRNA processing, poly A addition <catalytic> rRNA processing/maturation/methylation
regulatory RNAs (siRNA, miRNA, etc.)	regulation of transcription and translation,

Double-Stranded RNA (dsRNA)

Double-stranded RNA (dsRNA) is a high-molecular-weight RNA with two complementary strands held together by hydrogen bonds. It has distinct properties, such as:

1. A structure expected of an RNA duplex.
2. Lower light absorbance compared to single-stranded RNA (ssRNA).
3. Strong hypochromicity (a decrease in absorbance when forming a double helix).
4. Temperature-dependent stability.
5. High sedimentation coefficients.

Where is dsRNA Found?

- It exists in the **genomes of certain viruses** in animals, plants, fungi, and bacteria.
- It also appears as a byproduct in viral RNA replication.
- Scientists can **synthesize dsRNA** in the lab, such as poly(A)-poly(U) or poly(I)-poly(C) complexes.
- Other RNA types, like mRNA and tRNA, may form **partial double-stranded structures**, but they are irregular and incomplete.

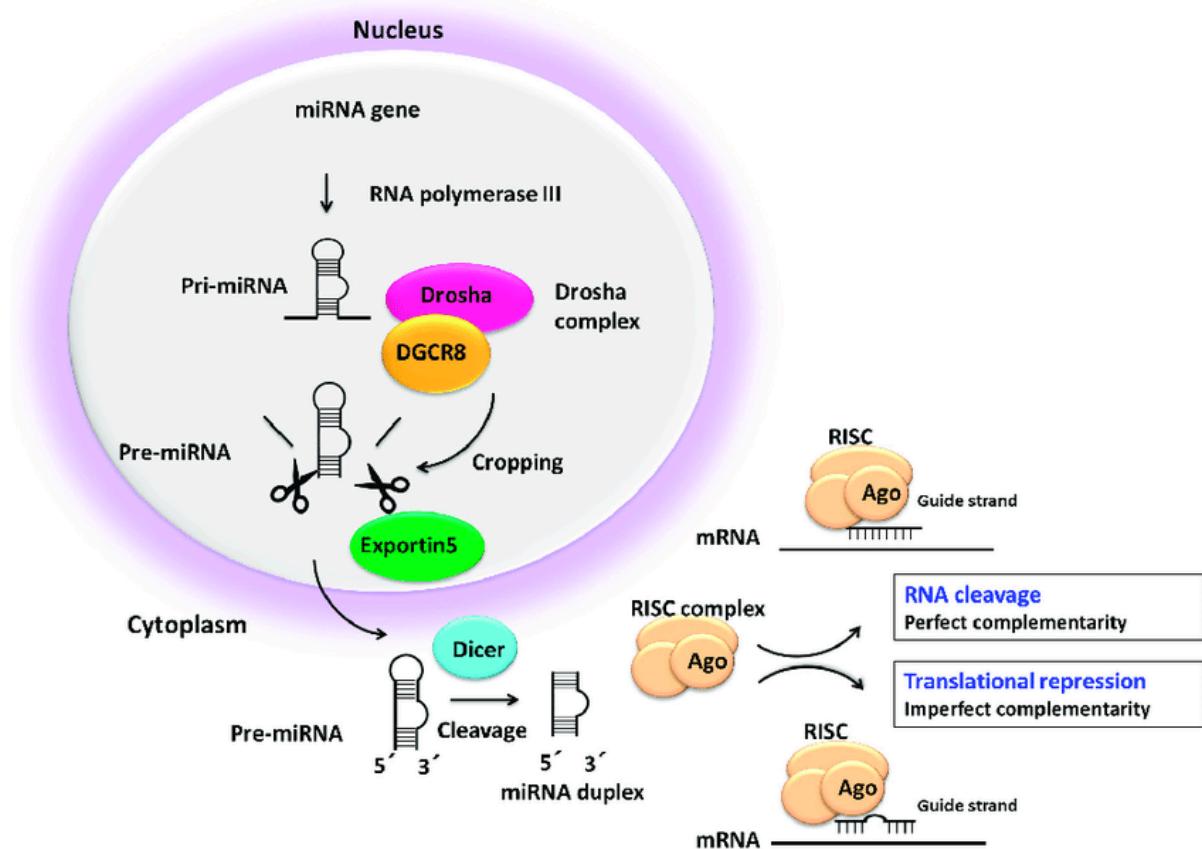
Applications in Genetic Engineering

- **dsRNA in Pest Resistance:** It is used to protect genetically modified crops by silencing harmful genes in pests.
- **Nanotechnology for dsRNA Delivery:** Scientists use nanomaterials like **layered double hydroxide (LDH) clay nanosheets** and **single-walled nanotubes (SWNTs)** to transport dsRNA into plants efficiently, helping in **crop protection and sustainable agriculture**.

RNA Silencing and Gene Regulation

- Enzymes like **Dicer** cut dsRNA into **small interfering RNA (siRNA)**.
- The **RNA-induced silencing complex (RISC)** uses siRNA to target and destroy matching messenger RNA (mRNA), preventing harmful gene expression.
- This **gene-silencing mechanism** is widely used in plant biotechnology and medical research.

dsRNA plays a crucial role in **genetic regulation, pest resistance, and biotechnology**. Advances in **nanotechnology** have made dsRNA delivery more efficient, paving the way for innovative applications in **agriculture and medicine**.



Simplified Explanation:

In *Agrobacterium tumefaciens*, **vir genes** are essential for transferring DNA (T-DNA) into plant cells, causing tumor formation (crown gall disease). These genes are located on the **Ti plasmid** and are activated by plant signals, especially **phenolic compounds** like acetosyringone.

What are vir genes?

- "Vir" stands for **virulence**—these genes help the bacterium infect plants.
- They are found on the **Ti (tumor-inducing) plasmid** and enable DNA transfer to plant cells.

- The proteins they produce process and transfer **T-DNA**, which integrates into the plant genome.

How are vir genes activated?

- Plant wounds** release signals (phenolic compounds) that trigger vir gene activation.
- A **two-component system** controls this:
 - VirA** (sensor) detects plant signals.
 - VirG** (activator) switches on other vir genes.

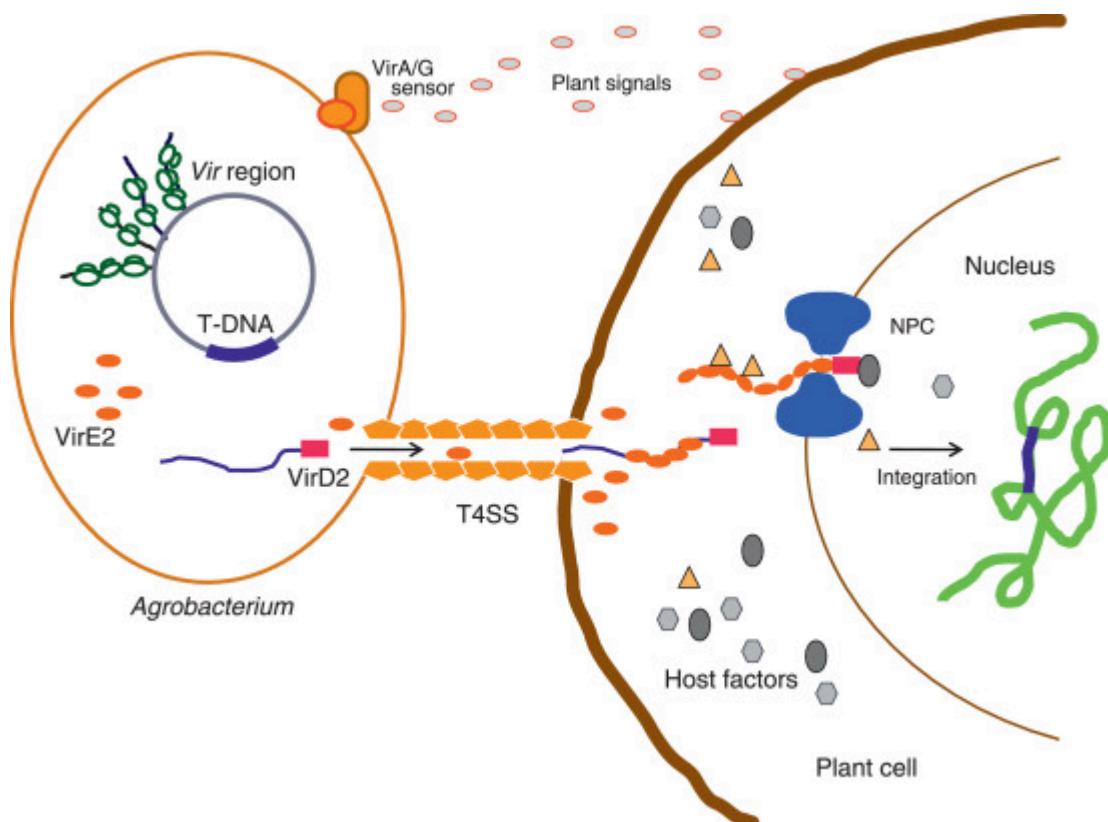
Key vir genes and their roles:

- VirA & VirG** – Detect plant signals and activate vir genes.
- VirB, VirC, VirD, VirE** – Help move T-DNA into the plant cell.
- VirD2 & VirD1** – Cut the T-DNA at specific points for transfer.
- VirD4** – Assists in T-DNA transport.

Other factors affecting vir gene activation:

- Sugars & acidic pH** also help induce vir genes.
- ChvE protein** senses sugars and boosts vir gene activation.
- The specific **plant species** and **bacterial strain** can affect the process.

These **vir genes** play a crucial role in genetic engineering, as scientists use *Agrobacterium* to introduce desired genes into plants.



Recombinant DNA Technology:

Recombinant DNA technology allows scientists to alter DNA by combining genes from different species or creating new genes. This is done using tools like enzymes to cut DNA, and then the modified DNA is copied in bacteria or yeast. In 1972, scientists first created recombinant DNA by joining DNA from different species using special enzymes. This breakthrough led to the development of many biotech companies.

Simplified Explanation with Genes Included:

Recombinant DNA (rDNA) technology has helped develop crops resistant to pests, diseases, and environmental stresses. Examples include **Bt cotton** (insect-resistant) and **Roundup Ready soybeans** (herbicide-tolerant).

1. Insect Resistance

- **Bt Crops:** Contain the **cry** genes from *Bacillus thuringiensis* (Bt), which produce proteins toxic to insect pests.
 - **Examples:** Bt cotton, Bt corn, Bt potato.
 - **Genes Involved:** **cry1Ac, cry2Ab, cry3Bb1** (kill specific insect pests).
 - **Benefits:** Reduces insecticide use, increases crop yield, and lowers environmental impact.
- **Other Insect-Resistant Crops:** Engineered with insecticidal proteins from bacteria.
 - **Examples:** Crops resistant to aphids, bollworms, and caterpillars.

2. Disease Resistance

- **Viral Resistance:** Viral coat protein genes help plants resist viral infections.
 - **Examples:** Papayas resistant to **papaya ringspot virus (PRSV)**, potatoes resistant to **potato leafroll virus**.
 - **Genes Involved:** **PRSV-CP (coat protein), PLRV-CP (potato leafroll virus coat protein)**.
- **Fungal Resistance:** Genes that break down fungal toxins protect plants.
 - **Example:** Wheat with the **HvUGT (UDP-glucosyltransferase)** gene from barley to fight *Fusarium* fungus.
- **Bacterial Resistance:** Genes from bacteria help plants fight bacterial diseases.
 - **Example:** Bananas resistant to bacterial wilt caused by *Xanthomonas campestris*.
 - **Gene Involved:** **Rxo1 (resistance gene from rice)**.

3. Herbicide Tolerance

- **Roundup Ready Crops:** Engineered to tolerate glyphosate, allowing better weed control.
 - **Examples:** Roundup Ready soybeans, corn.
 - **Gene Involved:** **epsps (5-enolpyruvylshikimate-3-phosphate synthase)** from *Agrobacterium sp.* strain CP4, which makes plants resistant to glyphosate.
 - **Benefits:** Improved weed management, higher yields, and reduced environmental impact.

4. Environmental Stress Tolerance

- **Drought Resistance:** Genes that regulate water stress help plants survive drought.
 - **Genes Involved:** DREB1 (dehydration-responsive element-binding protein), NCED3 (ABA biosynthesis gene).
- **Salinity Tolerance:** Salt-resistant genes help plants grow in saline soils.
 - **Genes Involved:** NHX1 (sodium-proton antiporter), HKT1 (high-affinity K⁺ transporter).
- **Arsenic Tolerance:** Plants engineered to resist arsenic contamination.
 - **Example:** *Arabidopsis* expressing PvACR3, an arsenic transporter gene from *Pteris vittata* (a fern).

These genetically modified crops help farmers produce stronger, healthier plants while reducing chemical use and environmental impact.

Recombinant DNA Technology Process:

1. Isolation of Genetic Material:

DNA containing the desired gene is extracted from the organism.

2. Cutting DNA with Restriction Enzymes:

Special enzymes act like molecular scissors, cutting DNA and vectors at specific points.

3. Joining DNA Fragments:

The pieces of DNA are joined together using an enzyme called DNA ligase to create recombinant DNA.

4. Introducing DNA into Host Cells:

The recombinant DNA is introduced into host cells (like bacteria or yeast) using methods like transformation or transduction.

5. Selection and Screening:

Cells that successfully take up the recombinant DNA are selected and tested to find those expressing the desired gene.

Gene Transfer Methods:

● Vector-Mediated Gene Transfer:

Vectors like plasmids or viruses carry the gene into the host cell.

● Direct Gene Transfer:

Methods like microinjection, gene guns, or electroporation directly deliver DNA into cells.

Gene Editing:

Gene editing is a method to modify an organism's DNA to achieve a specific goal, like

changing a protein or fixing a problematic gene. It's like editing a document, where parts of the DNA are cut, added, or changed.

Gene Editing vs. Genetic Engineering:

Gene editing is a form of genetic modification, but genetic engineering involves adding DNA from another organism, creating recombinant DNA. Gene editing usually doesn't involve foreign DNA, while genetic engineering does. Gene-edited products may need less testing compared to genetically engineered ones.

Types of Genetic Modifications:

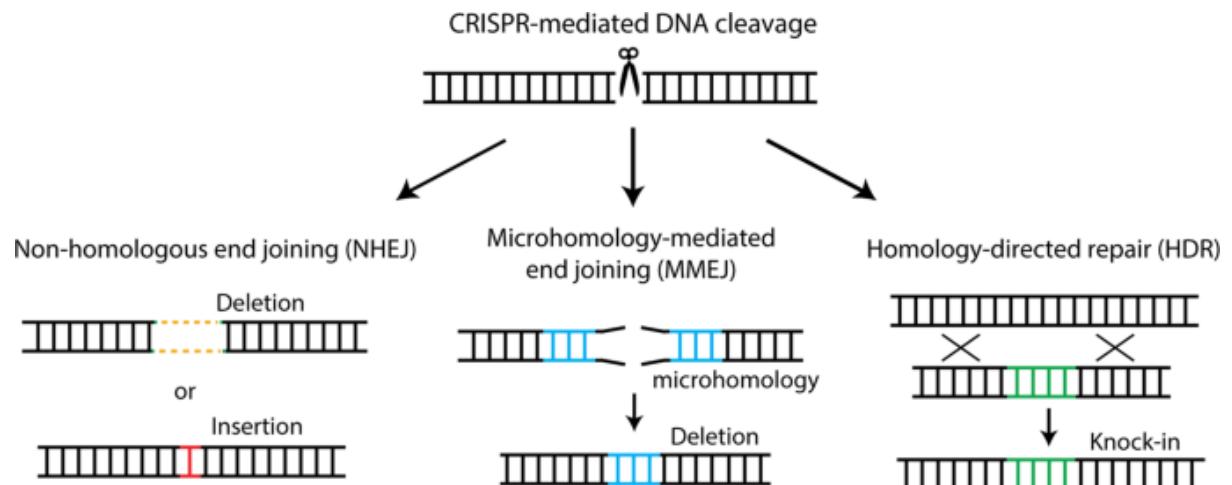
- **Gene Knockout:** Removing or turning off a gene.
- **Deletion Mutation:** Removing parts of a gene.
- **Insertion Mutation:** Adding extra DNA into a gene.
- **Substitution Mutation:** Replacing parts of a gene.
- **Point Mutation:** Changing a single base in the DNA sequence.
- **Gene Knock-In:** Adding or replacing parts of a gene.

Mutations can cause issues like stopping protein production early or changing the protein's structure.

<https://www.youtube.com/watch?v=U3Z4u0DKbx0>

DNA Repair Mechanisms: DNA breaks during gene editing and must be repaired. There are two main repair methods:

1. **Nonhomologous End Joining (NHEJ):** This repair method can cause errors by adding or deleting bases.
2. **Homology Directed Repair (HDR):** This repair method uses a matching DNA template for more accurate repairs.



Gene Editing Tools:

- **Zinc Finger Nucleases (ZFNs):** Tools that cut DNA at specific spots, but with some challenges in assembling them.
- **TALENs:** Similar to ZFNs but more accurate, though harder to make.

- **Meganucleases:** Enzymes that recognize longer DNA sequences and are less toxic.
- **CRISPR-Cas9:** A powerful, flexible, and simple tool for making precise gene edits. It's widely used but can sometimes cause unintended changes.

Applications of Genome Editing:

- **Crop Engineering:** Improving crops for better yield, disease resistance, and nutritional value.
- **GM Animals for Food Production:** Modifying animals for faster growth, disease resistance, and reduced environmental impact.
- **Vaccine Development:** Using gene editing to create safer and more effective vaccines.
- **Gene Therapy:** Treating genetic diseases by adding or fixing genes.
- **Biomanufacturing:** Using genetically modified organisms to produce products like insulin and vaccines.
- **Research into Gene Function:** Studying genes by deactivating them and observing the effects, which helps understand their roles.

Gene editing is a versatile tool with wide applications in agriculture, medicine, and research.

RNA-Induced Silencing Complex (RISC) and Gene Silencing:

- Gene silencing is a process used to stop or reduce the expression of specific genes. It's used naturally in cells and can be harnessed in research and medicine to control gene activity.
- RISC is a group of proteins that help silence genes. It works by using small RNA molecules (like siRNA or miRNA) that guide the complex to a specific RNA in the cell. Once it finds the target, RISC can silence the gene by:
 1. Blocking protein production (translation).
 2. Degrading mRNA (breaking down the messenger RNA).
 3. Affecting DNA (e.g., forming heterochromatin to block gene expression).
- Argonaute proteins, found in RISC, play a key role in recognizing and cutting target RNA or bringing in other proteins to silence the gene.

How Gene Silencing Works:

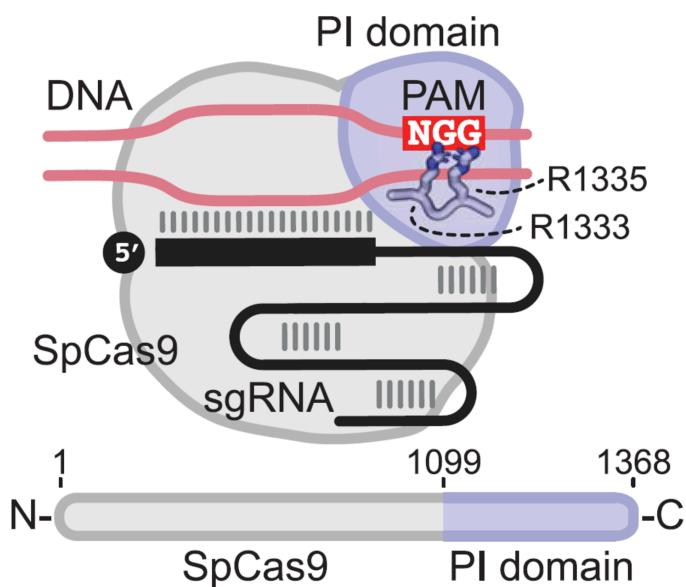
- Gene silencing can be achieved through various methods, such as RNA interference (RNAi), CRISPR-Cas9, or antisense RNA techniques.
- RNAi is triggered by double-stranded RNA (dsRNA), which is processed into small interfering RNAs (siRNA). These guide the RISC complex to target and degrade matching RNA, effectively silencing specific genes.
- CRISPR-Cas9 can also be adapted for gene silencing by using a modified version of Cas9 that binds to DNA without cutting it, preventing transcription.

Applications of Gene Silencing:

- In research, gene silencing helps scientists study the function of genes by "turning them off."
- In medicine, it has the potential to treat diseases like cancer, HIV, and genetic disorders by silencing harmful genes.
- In agriculture, gene silencing can be used to create genetically modified organisms (GMOs) with desired traits or to reduce harmful substances in plants.

Gene Silencing Techniques:

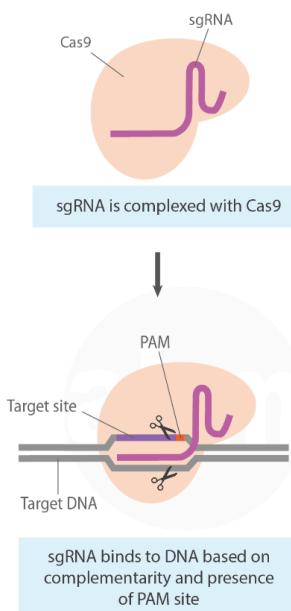
1. RNA interference (RNAi): Uses small RNAs like siRNA to target mRNA and stop protein production.
2. CRISPR-Cas9: Involves modifying genes to silence them without cutting them.
3. Antisense oligonucleotides: Bind to mRNA to prevent translation or degrade it.



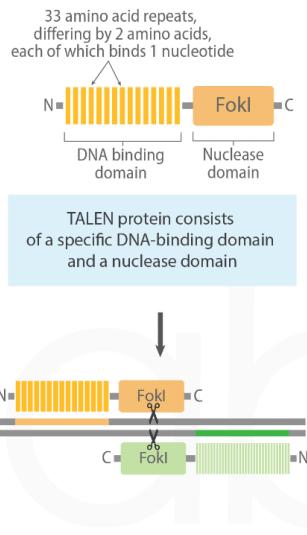
Gene Knockout

Gene expression is eliminated by creating InDels in the DNA

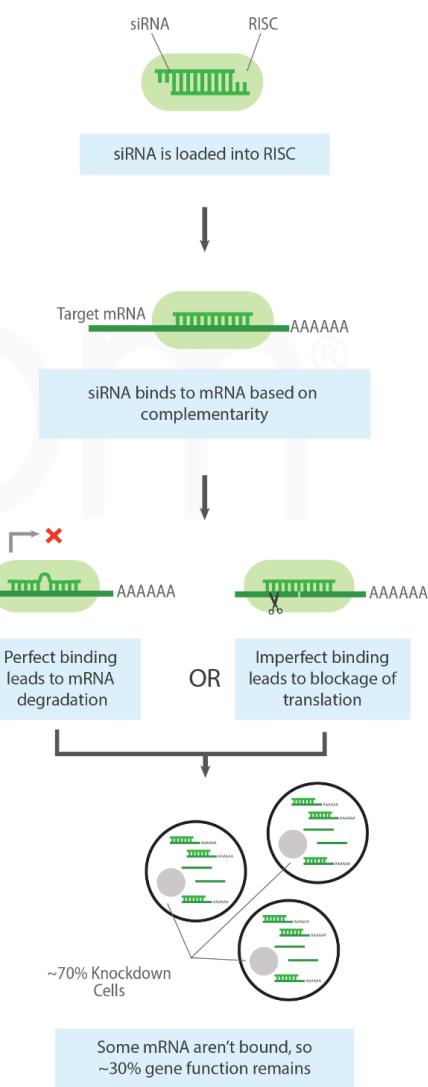
Using CRISPR



Using TALEN



Using RNAi



<https://sg.idtdna.com/pages/community/blog/post/expression-vectors-the-what-why-and-how>

<https://www.thermofisher.com/in/en/home/references/ambion-tech-support/rnai-sirna/technologies/sirna-expression-vectors--with-selectable-markers.html>

<https://vigs.solgenomics.net/>

Viral Vectors for shRNA Delivery

Due to limitations of plasmid vectors (low efficiency in primary cells), viral vectors have been developed for more effective shRNA delivery. These viral vectors can deliver shRNA to various cells, including primary cells, and can be used in animal models. However, safety issues like toxicity and immune responses remain, similar to gene therapy concerns. Over time, viral vector design improvements have made them more efficient, safe, and specific.

There are five main types of viral vectors for shRNA delivery:

1. **Retrovirus and Lentivirus:** These integrate into the host genome.
2. **AAV, Adenovirus, and HSV-1:** These remain as episomes (not integrated into the genome).

These vectors must replicate, be purified at high concentrations, target specific tissues, and express shRNA safely.

Adenoviral Vectors Adenoviral vectors are commonly used because they effectively deliver shRNA to both dividing and non-dividing cells without integrating viral DNA into the host genome. They can carry large amounts of foreign DNA and be produced in high quantities.

- **First-generation adenoviral vectors:** Deleted E1 and E3 regions.
- **Second and third generations:** Additional deletions for better safety.
- **Fourth-generation (gutless):** Only essential elements and can carry more foreign DNA (up to 37 kb).

Adenoviral vectors have been used in various cells, including cancer cells. **Oncolytic adenoviruses**, which target and kill tumor cells, are being explored for cancer therapy. However, more efficient delivery of siRNA to target cells is still needed.

Retroviral Vectors Retroviral vectors are used in gene therapy, with most of their structural genes removed and replaced with the gene of interest. Once inside the cell, the virus integrates its RNA into the host's genome. Retroviral vectors are effective in cancer cells and can be designed for inducible gene suppression (turning gene silencing on or off).

Challenges include low efficiency in non-dividing cells and gene expression loss over time. Improvements are being made to increase efficiency and reduce side effects.

Lentiviral Vectors Lentiviral vectors, derived from HIV, are particularly useful for transducing non-dividing cells and primary cells, including stem cells. They can deliver multiple shRNAs, targeting both viral and host cell genes, making them promising for gene therapy. These vectors can be inducible, offering greater control over gene silencing.

RNAi Perspectives RNA interference (RNAi) has become a key tool in gene function studies and therapy. It is easy to design, effective, and only requires low doses of siRNA. The main challenge is delivering siRNA to the correct tissue. Although non-viral methods are being developed, viral vectors are still the most efficient delivery method.

There are challenges with immune responses and targeting the right cells. Hybrid viral-non-viral vectors and inducible RNAi (with oncolytic viruses for cancer therapy) show

promise. Tissue-specific siRNA expression is also a key area for improving control over RNAi.

RNAi can also help screen for new drug targets in areas like cancer, cardiovascular diseases, and infections. However, more research is needed on the long-term effects, mechanisms of RNAi, and its use in clinical practice. For instance, AAV/shRNA vectors can cause liver damage, and more studies are needed before RNAi can be widely applied in medicine.

Gene Expression Tools and Databases:

<https://www.ncbi.nlm.nih.gov/geo/>

<https://www.ncbi.nlm.nih.gov/sra>

<https://www.ebi.ac.uk/biostudies/arrayexpress>

<https://www.ebi.ac.uk/gxa/home>