

| | RNAi | CRISPR |
|-----------|--|---|
| Benefits | <ul style="list-style-type: none"> Pre-designed reagents readily available Useful for studying the effect of essential genes on phenotypes Studies where temporary loss-of-function is desired (e.g., to mimic the effect of a drug) | <ul style="list-style-type: none"> Precise gene targeting with fewer off-target effects Permanent gene disruption results in robust signal Lower risk of immune response (some formats) Flexible time frame for assay |
| Drawbacks | <ul style="list-style-type: none"> Temporary gene disruption may require a narrow assay window Incomplete silencing (knockdown) may not produce a strong signal Associated with more off-target effects Silencing of multiple transcripts possible (introducing noise) Introduced RNA may stimulate immune response Laborious analysis and verification of true hits | <ul style="list-style-type: none"> Cannot be used to study essential genes |

RNAi: The Knockdown Method

RNAi (RNA interference) was discovered in the 1990s and involves **double-stranded RNA (dsRNA)** or **small RNAs (siRNAs/miRNAs)** silencing genes. The process:

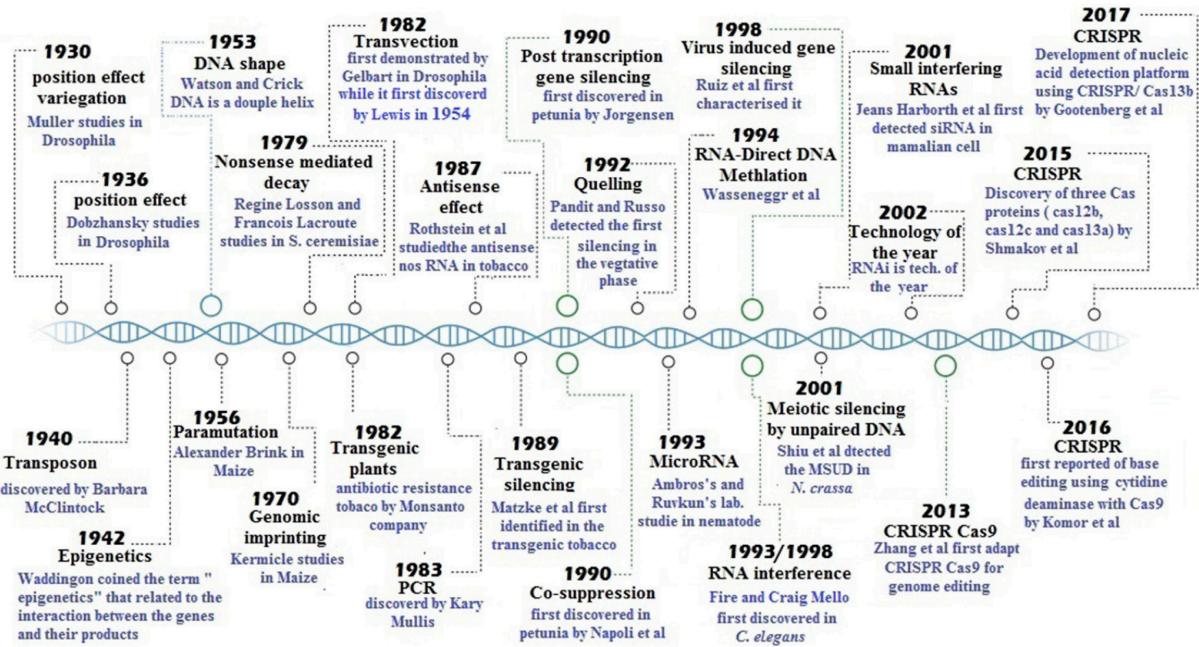
1. **dsRNA or pre-miRNA** is processed into short fragments by **Dicer**. These fragments guide the **RISC complex**, which cleaves target **mRNA** to block protein production. If there's an imperfect match, **translation** is blocked without mRNA cleavage.
<https://www.youtube.com/watch?v=U3Z4u0DKbx0>

CRISPR: The Knockout Method

CRISPR-Cas9 was developed in 2012 and involves editing DNA directly. The process:

1. **A guide RNA directs Cas9** protein to a specific **DNA sequence**.
2. Cas9 cuts the DNA, leading to **insertions or deletions** (indels) during the DNA repair process (usually via **NHEJ**).
3. This results in a **genetic knockout**, stopping protein production.

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



| | RNAi | TALE Repression | TALEN | Cas9 Nuclease | CRISPRi | CRISPRa |
|--------------------------------------|--------------------------------------|---------------------------------|------------------------------------|-----------------------------------|---------------------------------|---------------------------------|
| Loss-of-function mechanism | Post-transcriptional RNA degradation | Repression of transcription | Frame shift DNA mutation | Frame shift DNA mutation | Repression of transcription | Activation of transcription |
| Result | Reversible knockdown | Reversible knockdown | Permanent knockout | Permanent knockout | Reversible knockdown | Reversible activation |
| Transgenes | si/shRNA | TALE-KRAB | TALEN | Cas9 nuclease | dCas9-KRAB | dCas9-VP64 |
| Guiding sequence | si/shRNA | DBD | DBD | sgRNA | sgRNA | sgRNA |
| Required sequence information | Transcriptome | Annotated TSS | Transcriptome | Transcriptome | Annotated TSS | Annotated TSS |
| Off-target space | Transcriptome | Window around TSS | Genome; requires FokI dimerization | Genome; cuts as monomer | Window around TSS | Window around TSS |
| Transcript variants | All variants via conserved region | Only variants from the same TSS | All variants via conserved region | All variants via conserved region | Only variants from the same TSS | Only variants from the same TSS |

Genes are the instructions that control the functions of an organism. In most organisms, genes are made of DNA, which is transcribed into RNA, and then translated into proteins that perform various functions in the body.

Gene Knockout

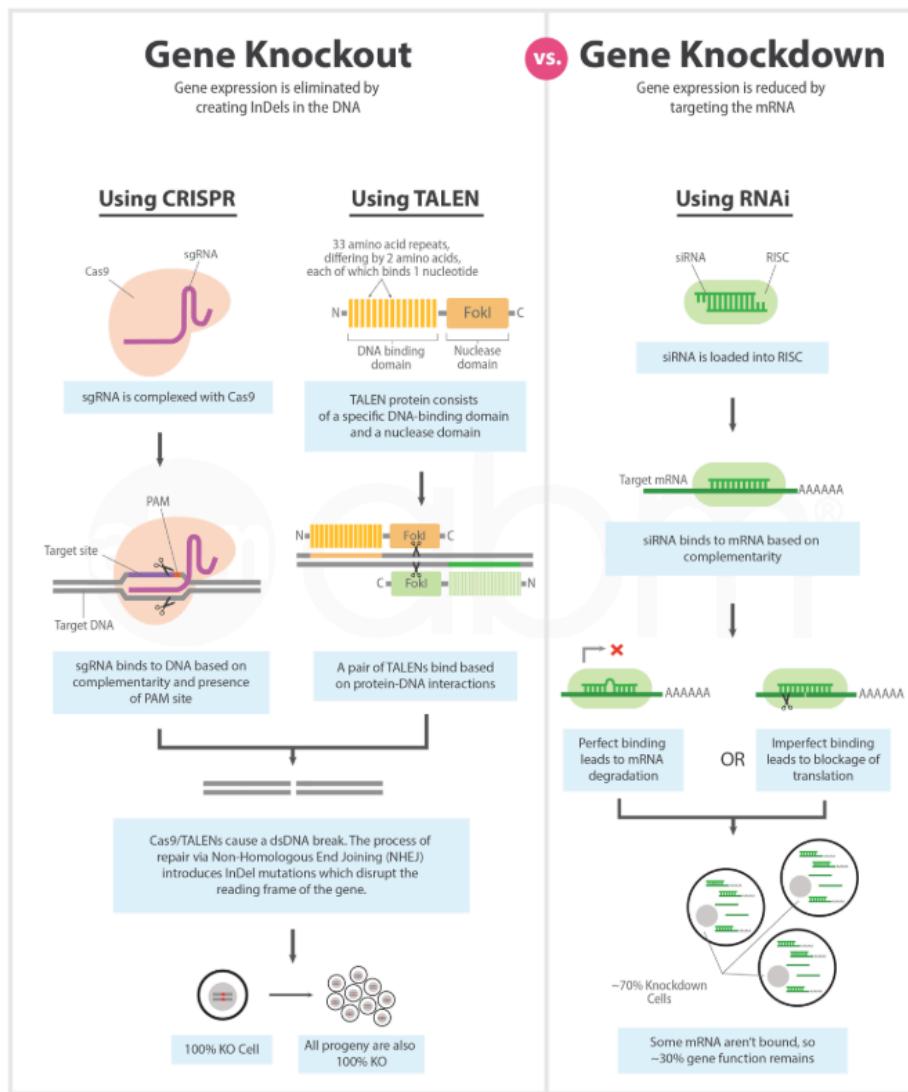
Gene knockout refers to permanently removing or deactivating a gene through genetic engineering. This method was first tested in *E. coli* and later adapted for other organisms, like mice, to study gene effects, particularly for human health. For example, knockout mice have been used to study Xip proteins and their role in heart diseases like Sudden Unexplained Nocturnal Death Syndrome (SUNDS) and Brugada Syndrome.

Gene Knockdown

Gene knockdown involves reducing or suppressing the activity of a gene without permanently removing it. This can be done using oligonucleotides to target the gene's RNA and temporarily decrease its expression. Gene silencing (like RNA interference) and gene editing are forms of gene knockdown. It can be complete or partial, depending on how much the gene's activity is reduced. This method is often used to study the roles of genes in diseases like cancer and neurological disorders.

Conditional Knockout

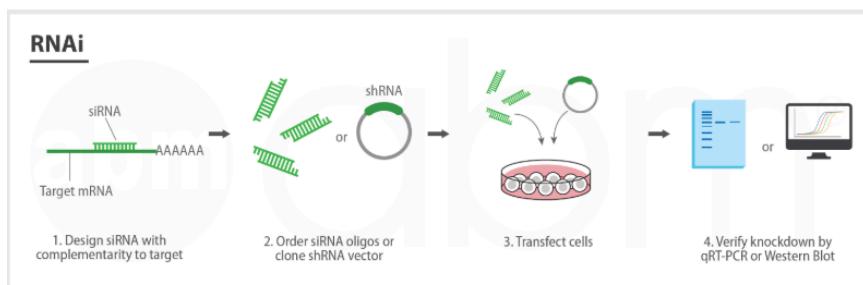
Conditional knockout is a method used to deactivate genes that would be lethal if completely removed. It uses specific tools like Cre recombinase or newer gene editing technologies (such as CRISPR) to control when and where a gene is deactivated. This approach allows researchers to study genes that are vital to survival but also crucial for understanding gene function in specific conditions.



RNAi Off-Target Effects

RNA interference (RNAi) is used to silence genes, but it can accidentally affect other genes with similar sequences, especially in the 3' UTR of mRNA. This can cause unintended gene silencing and interfere with normal cellular processes, especially if too much siRNA is used.

Gene Knockdown Workflow

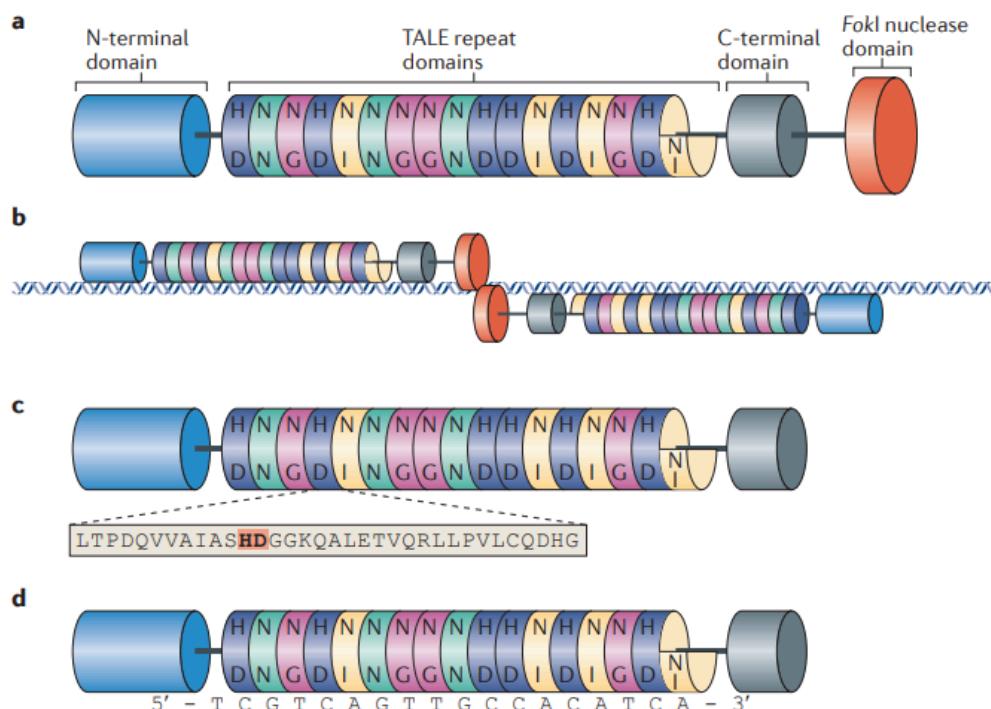


TALENs (Transcription Activator-Like Effector Nucleases) are engineered enzymes used to cut DNA. They consist of a TAL effector DNA-binding domain and a FokI nuclease domain. The TAL domain binds to DNA through repeats of amino acids, with two specific amino acids (called RVDs) determining which DNA sequence it targets.

For the FokI enzyme to cut DNA, two TALENs are needed—one for each DNA strand, spaced 12-25 base pairs apart. The FokI cut causes a DNA break, which the cell repairs through an error-prone method (NHEJ), often leading to mutations that can disable a gene.

a: A diagram of a TALEN (Transcription Activator-Like Effector Nuclease). It shows colored discs representing the TALE repeats. The two variable residues (important for DNA binding) are inside each repeat. The ends of the TALE also have domains that help with DNA binding. The red part represents the FokI nuclease domain, which cuts the DNA.

b: TALENs work as pairs (dimers) on the target DNA. The TALE domains help bind the DNA, and the FokI nuclease cuts the DNA at a specific “spacer” sequence between the two bound regions.



c: A zoomed-in view of a single TALE repeat. It shows the amino acids in the repeat, with the variable residues highlighted. These residues are key to determining which DNA bases are bound.

d: The TALE DNA-binding domain aligns with its target DNA sequence. Each TALE repeat binds to a specific base in the DNA according to the TALE code, and there's a thymine before the first base in the sequence.

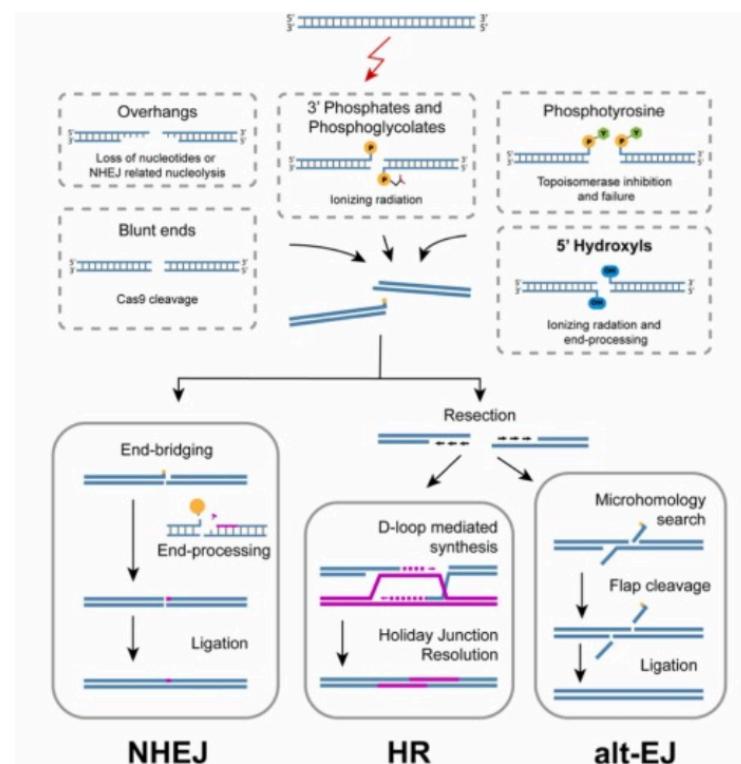
In essence, TALENs use a series of repeats to bind to DNA, and FokI cuts it at a specific spot between two repeats.

Off-Target Effects in TALENs

TALENs cause fewer off-target effects compared to other methods because FokI requires two TALENs to work. However, TALE transcriptional repressors can still cause some off-target effects as they act alone.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a gene editing tool that is simple to design and very flexible.

RNA interference (RNAi) silences genes by targeting **mRNA**, while **CRISPR (clustered regularly interspaced short palindromic repeats)** edits **DNA**, leading to permanent gene disruption or modification.



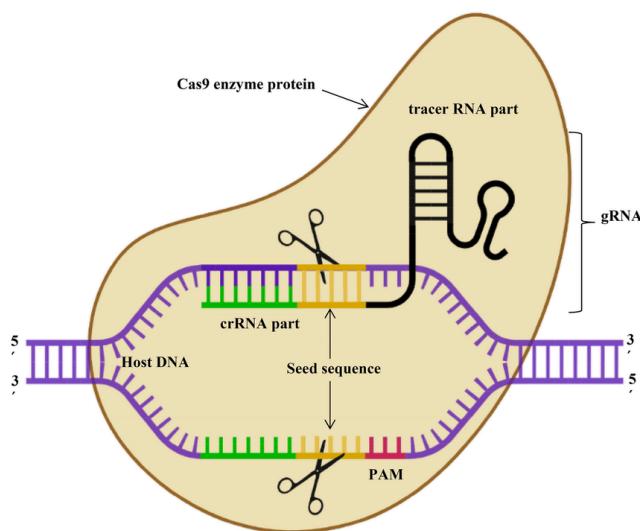
DNA double-strand breaks (deDSBs) are harmful because they can cause chromosomal rearrangements. These breaks are repaired by two main methods: **homologous recombination (HR)** and **non-homologous end joining (NHEJ)**. HR requires a matching DNA sequence and begins by trimming the broken DNA ends to expose single-stranded DNA (ssDNA). On the other hand, NHEJ repairs the breaks by directly joining the broken DNA ends with little or no trimming, and involves factors like KU, 53BP1, and RIF1, which prevent the trimming process.

When there's minimal trimming of DNA ends, an error-prone repair method called **alternative end-joining (Alt-EJ)** may take place, using microhomologies to join the ends. During DNA

replication, HR is typically used for error-free repair, but NHEJ is also active in certain cases to support cell division, especially under replication stress. NHEJ factors are found at replication forks, helping to repair DNA breaks or restart stalled replication.

In situations where replication and transcription collide, NHEJ can help repair damaged replication forks and prevent harmful mutations. However, the process depends on factors like KU, which help stabilize the replication fork by limiting excessive DNA end degradation. In some cases, when NHEJ is defective, the repair relies on Alt-EJ and cooperation between NHEJ and HR.

NHEJ also plays a role in protecting replication forks from damage during stress by limiting degradation, which is important for maintaining genome stability. Different NHEJ factors contribute to this protection in various ways, depending on the organism and context.



The main difference between gRNA and sgRNA is that gRNA refers to the general guide RNA used in CRISPR, which includes both the crRNA (target-specific sequence) and tracrRNA (scaffold for Cas9). sgRNA, or single guide RNA, is a simplified version where both the crRNA and tracrRNA are fused into one RNA molecule. sgRNA has become the most common format for CRISPR experiments.

CRISPR-Cas9 works with two key components: a guide RNA (gRNA) and the Cas9 nuclease. The gRNA directs Cas9 to a specific DNA target, where Cas9 makes a

double-strand break. The gRNA consists of a crRNA (which matches the target DNA) and tracrRNA (which helps bind Cas9). The Cas9 nuclease, typically from *Streptococcus pyogenes*, cuts the DNA at the target site, leading to gene knockout or insertion depending on how the cell repairs the break.

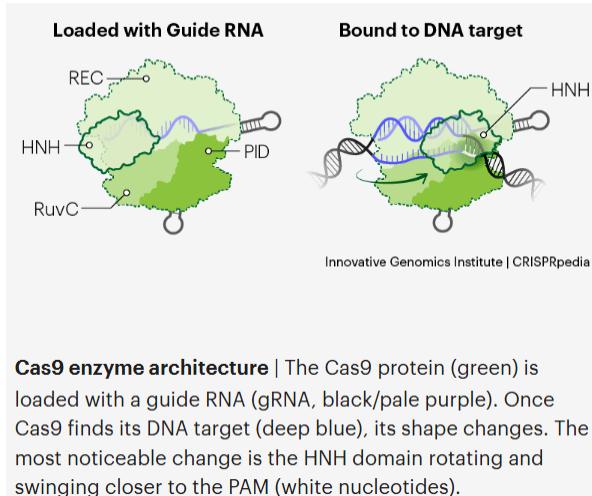
Once Cas9 binds to the DNA, it makes a double-strand break. The cell repairs the break using an **error-prone process (NHEJ)**, which often leads to small mutations (indels). These mutations can knock out the gene, but you need to screen cells to find the ones with the desired mutations that change the protein function.

Delivery of CRISPR-Cas9

To use CRISPR-Cas9 for gene editing in organisms like plants and humans, researchers must first deliver the system into cells since it doesn't naturally exist in them. Methods include plasmid transformation (for bacteria), electroporation, chemical stimulation, viral delivery (for human cells), Agrobacterium (for plants), or microinjecting embryos (for animals like mice).

Guide RNA Composition

In nature, Cas9 uses two RNA molecules: CRISPR RNA (crRNA) and tracrRNA. Researchers combined them into one single-guide RNA (sgRNA), simplifying the system and making it easier to edit DNA with just one RNA molecule.

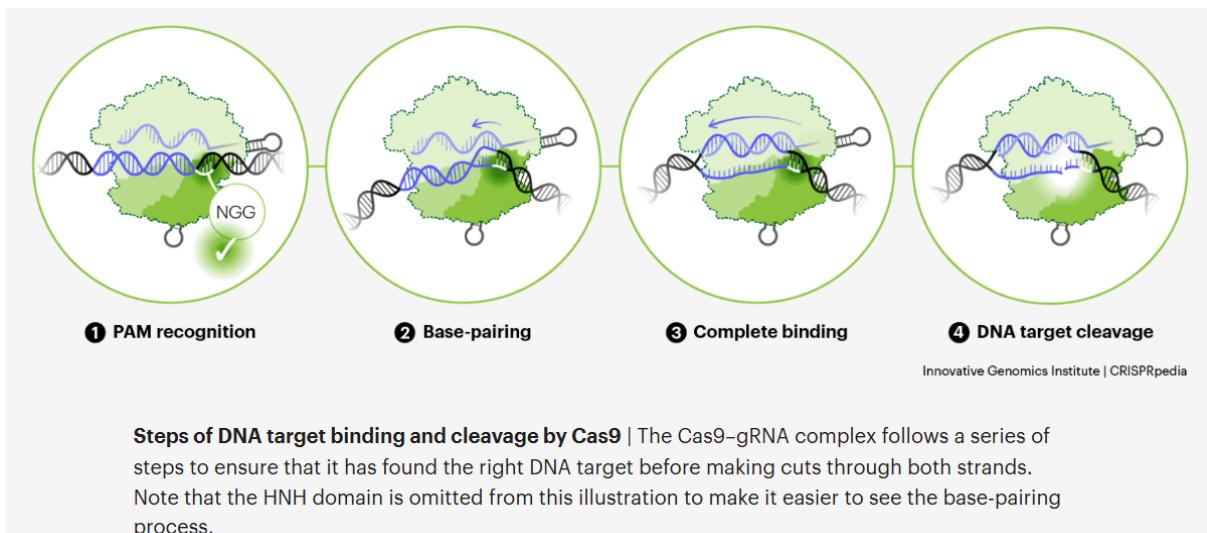


Cas Protein Variants

While Cas9 is widely used, it's not the only option. Researchers have developed more precise versions, other Cas enzymes for different DNA sequences, and systems that can do more than just cut DNA, expanding the CRISPR toolkit.

Prokaryotic vs. Eukaryotic Cells

In eukaryotes (plants, animals, etc.), CRISPR must enter the nucleus, so Cas9 is tagged with a nuclear localization signal (NLS) to help it reach the DNA. In contrast, prokaryotes (bacteria) don't have great DNA repair systems, making traditional editing difficult. Tools like base editors or CRISPR interference (CRISPRi) can avoid double-strand breaks, allowing for more precise editing.



Cas9 Structure and Function

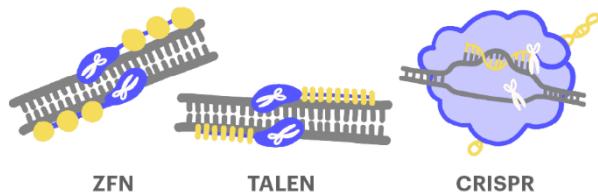
Cas9 is a two-lobed protein: the recognition lobe (REC) holds the guide RNA, while the nuclease lobe (NUC) contains two parts that cut the DNA. The protein changes shape when it binds to DNA, with the HNH domain swinging toward the target. This allows Cas9 to cut the DNA at the right place, guided by the RNA.

The Cutting Process

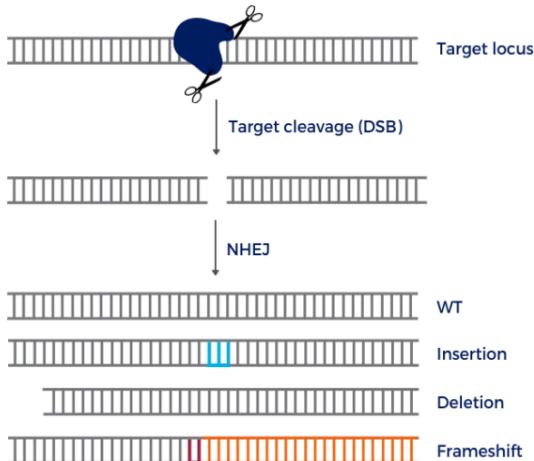
When Cas9 finds a match, it starts cutting. It needs the full 20-nucleotide guide RNA to pair with the target DNA, but sometimes it can cut even with a few mismatches (off-target cuts). Once the guide RNA is mostly paired with the DNA, the HNH domain cuts the target DNA strand, and the RuvC domain cuts the non-target strand. The cuts happen three nucleotides away from the PAM and usually leave blunt ends, though the RuvC domain might create small overhangs. After cutting, the cell's repair machinery fixes the DNA. Cas9's job is done, and the DNA is ready for editing.

Different Cas9 versions need different PAM sequences (e.g., spCas9 needs "NGG," while saCas9 needs "NNGRRT").

The simplicity of CRISPR



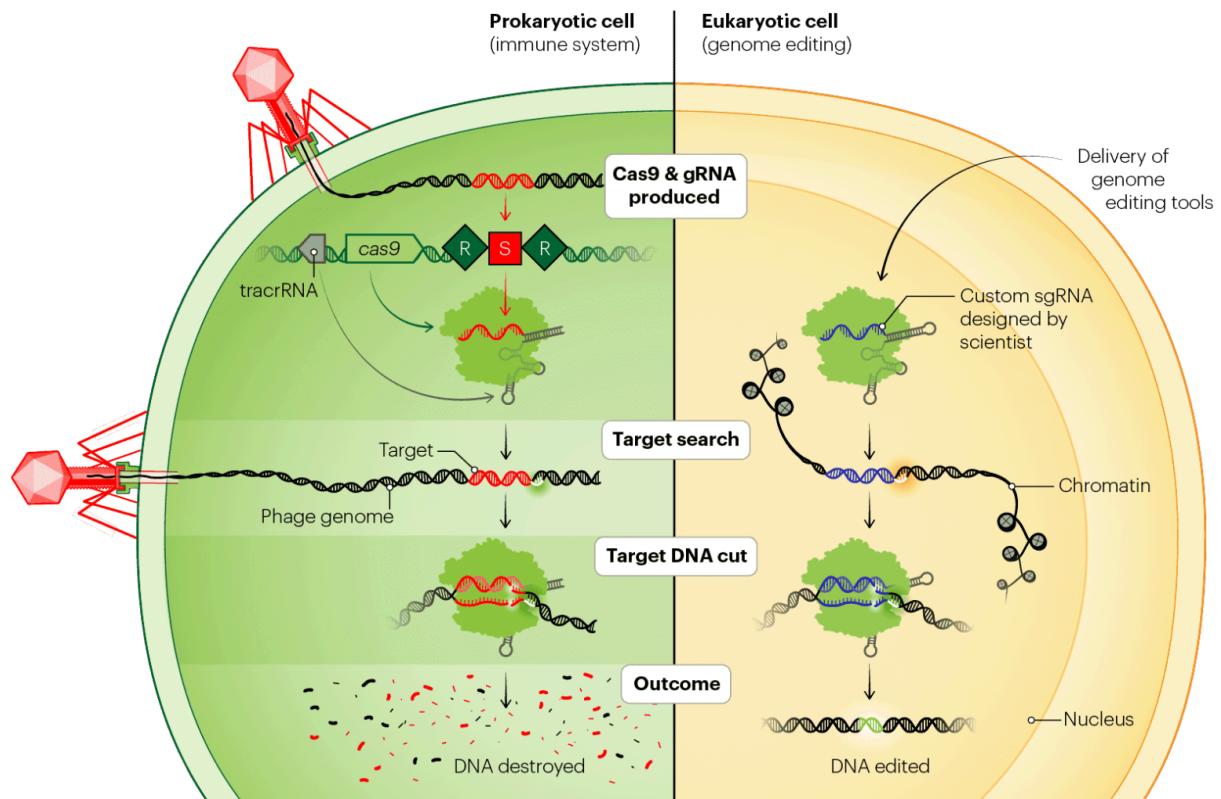
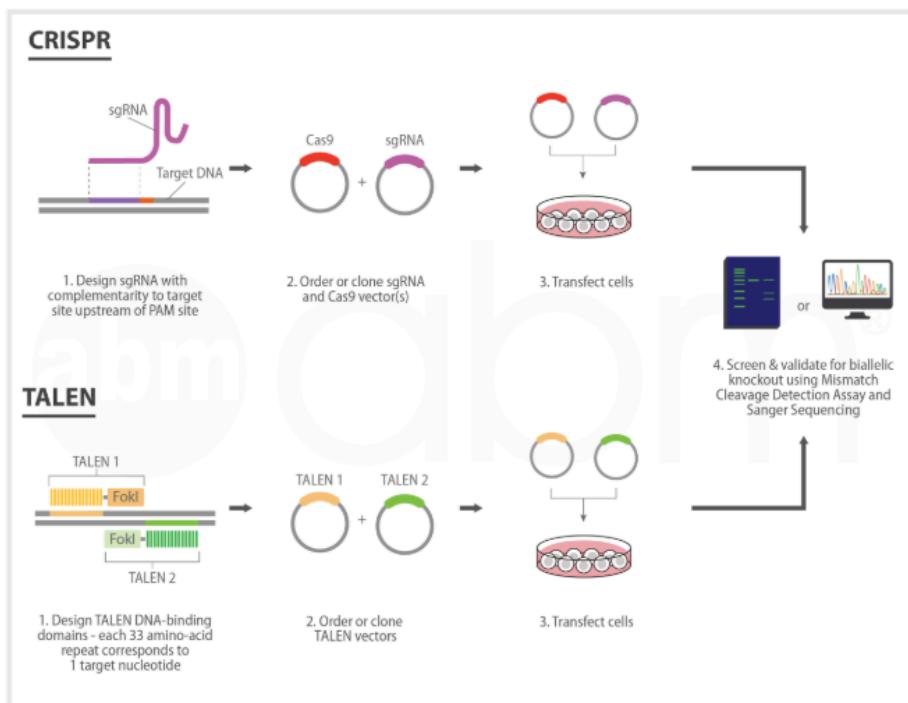
CRISPR is easier and faster to use than ZFNs and TALENs because it only requires changing the guide RNA, not engineering new proteins.



CRISPR mechanism to induce loss-of-function gene mutation.

gene mutation. The Cas9-mediated induction of double-strand break (DSB) activates endogenous mechanisms of DNA repair, aiming to fix the genomic damage produced at the locus of interest. These error-prone mechanisms can induce different outcomes in the target locus.

Gene Knockout Workflow



Innovative Genomics Institute | CRISPRpedia

<https://www.synthego.com/blog/rnai-vs-crispr-guide>

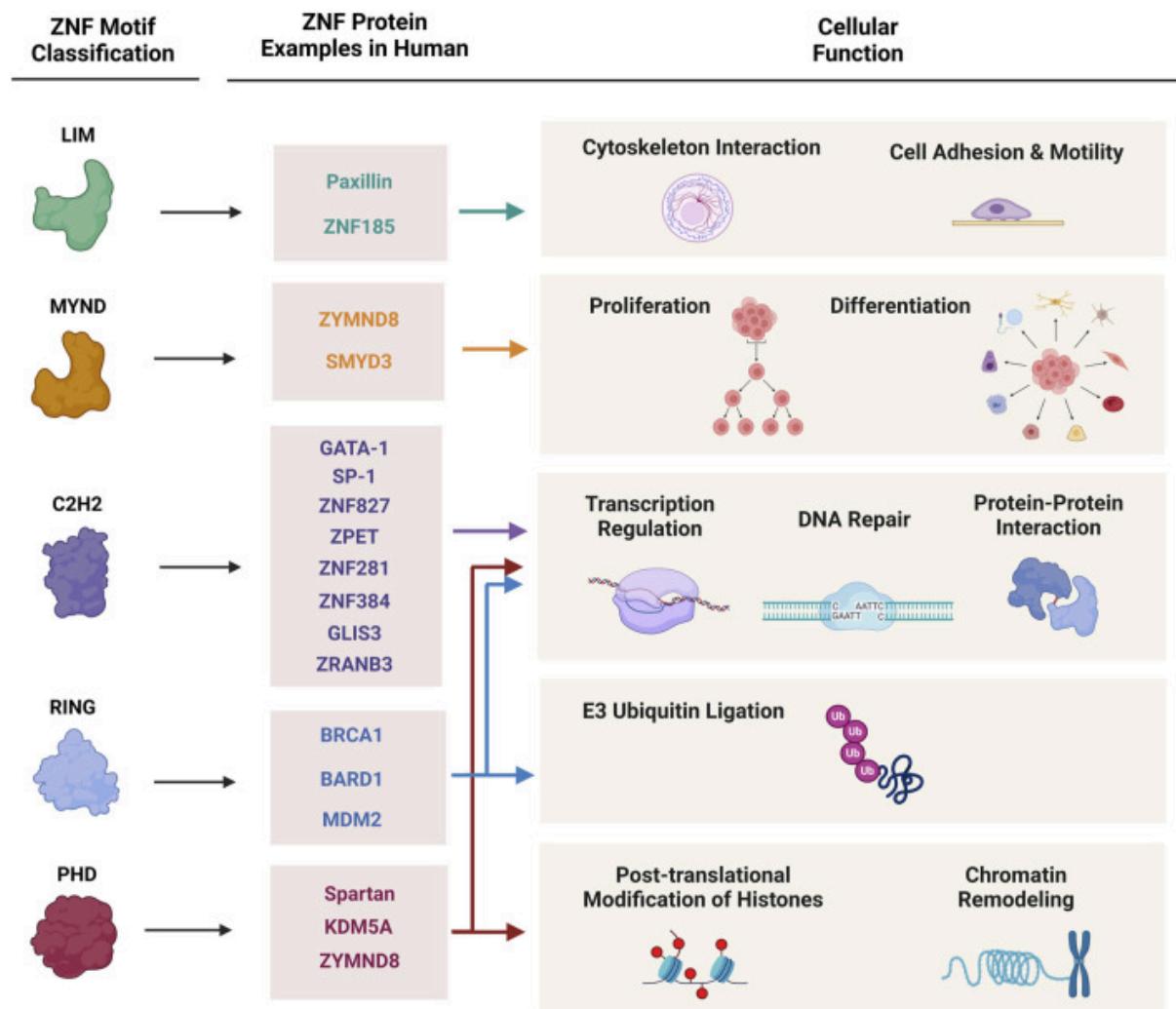
Zinc finger proteins: guardians of genome stability

Zinc Finger Proteins (ZNFs)

Zinc finger proteins (ZNFs) are a large and diverse group of proteins that make up about 5% of the human genome. These proteins contain small structures, called zinc fingers, that include zinc ions bound to histidine and cysteine amino acids. These structures help ZNFs bind to various molecules like DNA, RNA, proteins, and lipids. Due to their flexibility, ZNFs play important roles in essential cellular processes such as gene regulation, cell adhesion, protein breakdown, DNA repair, and chromatin remodeling. There are several types of ZNFs, each involved in different biological functions, and they can have multiple roles within a cell.

Types of Zinc Finger Domains:

1. **C2H2 Domain:**
This type of zinc finger has two cysteine and two histidine residues and is mainly found in transcription factors. These proteins can bind to specific DNA sequences and are useful in genome editing. Some C2H2 proteins, like GATA-1, can also switch between different functions depending on their protein partners.
2. **LIM Domain:**
LIM domains contain two zinc-binding sites and are involved in regulating gene expression, cell movement, and interactions with the cytoskeleton. For example, Paxillin helps regulate actin, and ZNF185 has been proposed as a tumor suppressor due to its effects on cell proliferation.
3. **MYND Domain:**
The MYND domain is similar to the LIM domain and is involved in protein interactions, particularly with proline-rich proteins. ZMYND8, a protein with a MYND domain, plays a critical role in DNA repair, helping fix DNA damage by interacting with other repair proteins.
4. **RING Domain:**
The RING domain is found in proteins that regulate other proteins by adding ubiquitin molecules, a process important for DNA repair and cancer progression. BRCA1, a well-known protein in this category, is involved in repairing DNA and regulating gene expression.
5. **PHD Domain:**
PHD domains help regulate DNA repair and chromatin structure. They play a role in histone modification, which affects gene expression and DNA repair processes. ZYMD8, a protein with both MYND and PHD domains, contributes to chromatin remodeling and DNA damage repair.



Zinc Finger Proteins in DNA Repair

DNA is constantly exposed to damage from various sources. To protect our genome, cells use DNA damage repair (DDR) mechanisms. These processes include repairing mismatched DNA, correcting damaged bases, and fixing broken DNA strands. Zinc finger proteins, like PARP1, BRCA1, and ATM, play essential roles in these repair pathways. PARP1, for instance, helps recruit repair proteins to DNA damage sites and is involved in several repair mechanisms.

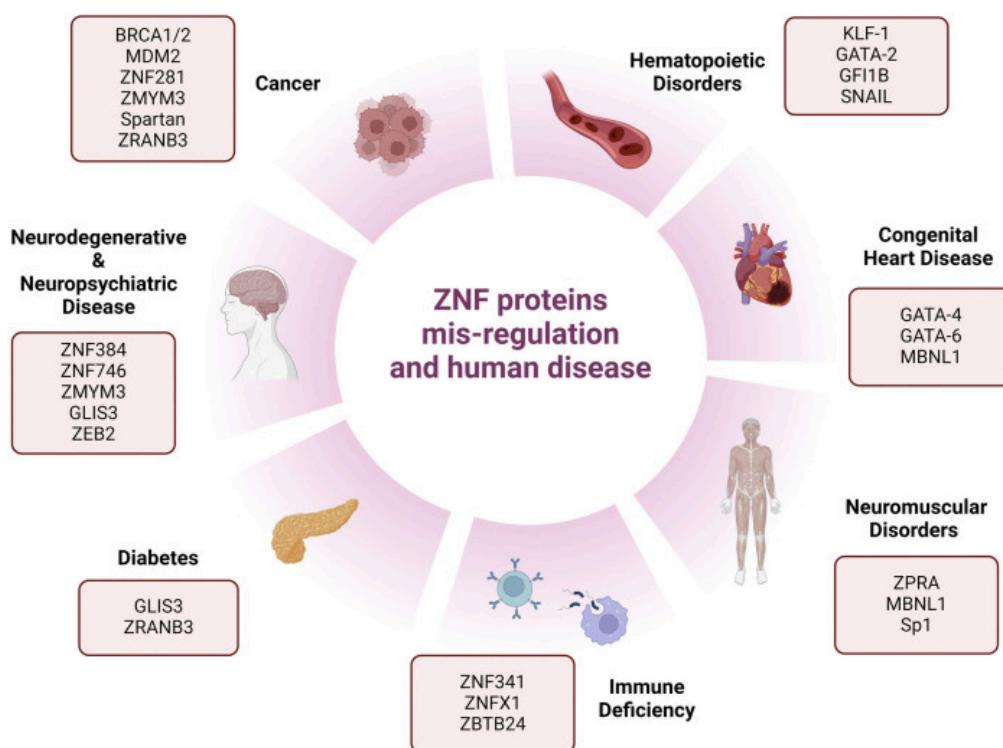
PARP Inhibitors (PARPi)

PARP inhibitors, like Olaparib, are used in cancer treatment, particularly for cancers with defects in DNA repair pathways, such as those with BRCA mutations. These drugs prevent the repair of DNA damage, leading to cell death in cancer cells. However, some tumors develop resistance to PARPi treatments, often due to mutations or other changes in the DNA repair proteins. Understanding how resistance develops is crucial for improving these therapies.

Newly Identified Zinc Finger Proteins in DNA Repair and Disease

Recent research has revealed new zinc finger proteins involved in DNA repair. For example, ZNF384 plays a role in repairing DNA double-strand breaks, and ZNF281 interacts with repair proteins to help fix DNA damage. Some of these proteins are associated with cancer and may influence how patients respond to treatment. The growing understanding of these proteins could lead to new ways of improving cancer therapies and overcoming resistance to DNA-damaging treatments.

Given the challenges of PARPi resistance, there is a need to identify new DNA repair proteins that can be targeted in cancer treatment, either alone or in combination with other drugs, to overcome resistance in tumor cells. Recent studies have highlighted the broader roles of zinc finger proteins (ZNFs) in human health, beyond their involvement in cancer. ZNFs have been linked to various diseases such as Parkinson's disease (ZNF746), Spinal Muscular Atrophy (ZPR1), diabetes (GLIS3), congenital heart disease (GATA4), and more.



<https://pmc.ncbi.nlm.nih.gov/articles/PMC4358406>

KRAB-containing zinc finger proteins (KRAB-ZFPs) silence transposable elements (TEs) by binding specific DNA sequences and recruiting the KAP1 corepressor, triggering histone and DNA methylation to form repressive chromatin. Here, we demonstrate that **ZFP809**, a KRAB-ZFP, initiates silencing of endogenous retroviruses (ERVs) in a **sequence-specific** way by recruiting **heterochromatin-inducing complexes**. These proteins play key roles in genome stability, development, and cancer.

Knockins and Knockouts in Mice

Genetically modifying mice has become crucial in research, medicine, and biotechnology. Transgenic mice are used to study genetic disorders, development, and test therapies. They help scientists understand gene functions, genetic pathways, and protein properties.

Gene Targeting in Mice

Gene targeting involves modifying the mouse genome at specific locations, often using homologous recombination in embryonic stem (ES) cells. A piece of DNA with a desired change is introduced to the ES cells, which then replace the target gene with this altered DNA. This method allows researchers to create mice with specific gene deletions, mutations, or other changes.

Knockin Mice

Knockin mice are created by inserting a transgene (a gene from another organism or modified mouse gene) into a specific location in the genome. This results in more consistent gene expression across generations, as the transgene is present as a single copy in one location. Knockin mice are helpful for studying protein overexpression or adding human genes to the mouse genome.

Knockout Mice

Knockout mice are created by removing or inactivating a gene, which can help study the effects of gene loss. This can be done using homologous recombination to delete specific parts of the gene. Knockout mice may show complex traits or even embryonic lethality, depending on which gene is knocked out.

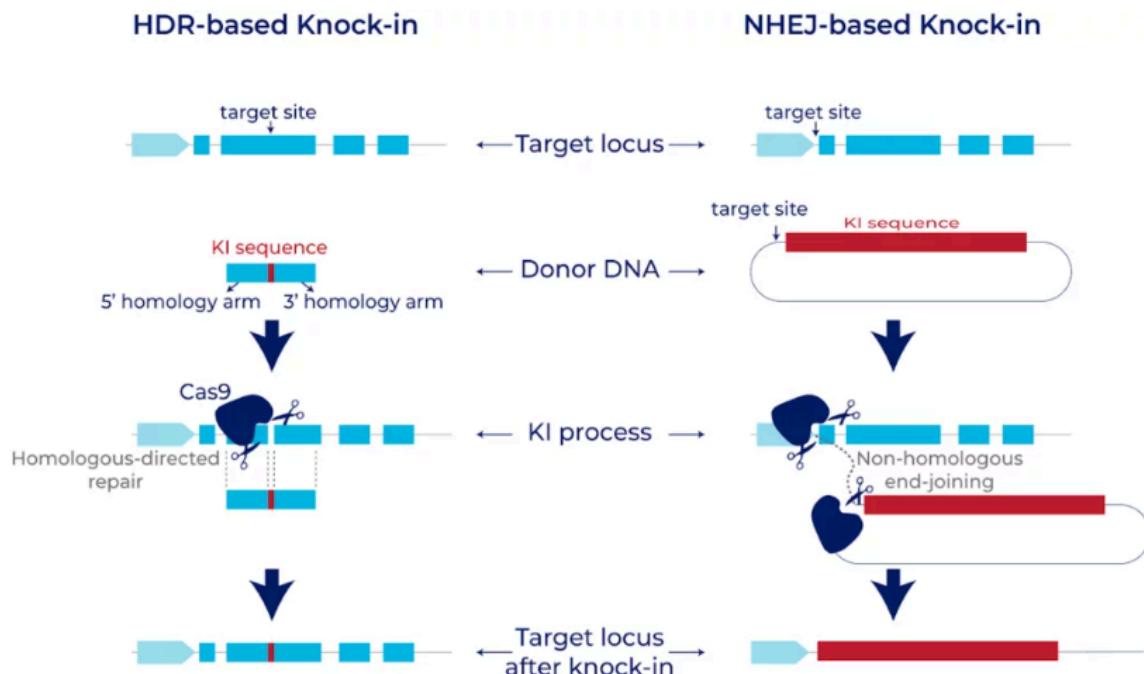
Conditional Gene Modification

Some genes are essential for development or survival, so knocking them out directly can be fatal. In these cases, scientists use conditional gene modification, such as the Cre-lox or Flp-frt system, to knock out genes in specific tissues or at specific times. This allows researchers to study gene function without affecting the mouse's survival.

- **Cre-lox System:** Cre recombinase catalyzes recombination between loxP sites, allowing targeted gene alterations.
- **Flp-frt System:** Flp recombinase is used similarly to Cre but provides another tool to manage gene alterations, especially to remove unwanted markers from genes.

These systems enable more precise studies of gene function in mice, making them powerful tools for research.

<https://go.genetargeting.com/conditional-ko-quick-guide>



Differences Between Knockouts and Knockins

Despite their similar names, knockouts (KO) and knockins (KI) are different genetic modifications:

- **Knockout (KO):** The goal is to **remove** a DNA sequence, disrupting a gene's function.
- **Knockin (KI):** The goal is to **insert** a DNA sequence into a specific location in the genome.

Both techniques often use **CRISPR/Cas9** technology to cut the DNA at a specific site, which then triggers the cell's natural repair mechanisms.

Knockout Techniques

- Knockout uses the **NHEJ (non-homologous end joining)** DNA repair pathway. This repair is error-prone and can result in small insertions or deletions (INDELS), causing a **frameshift** and often a **nonfunctional protein**.
- If the gene is at the start of the coding sequence, the knockout typically prevents any protein from being made. If the gene is further along, the protein may be truncated or modified, likely losing its function.

Applications of Knockouts:

- **Understanding gene function:** Observing the effects of missing proteins.
- **Disease modeling:** Mimicking human diseases that result from missing or dysfunctional proteins, and testing potential treatments. **Zebrafish** are a helpful model for visualizing and studying gene knockouts due to their transparency and small size.

Knockin Techniques

- Knockins use either **NHEJ** or **HDR (homology-directed repair)** to insert a new DNA sequence.
 - **NHEJ:** One DNA end is joined with the donor DNA, potentially leading to random insertion.
 - **HDR:** Uses the donor DNA as a template, requiring two matching sequences (homology arms) to insert the new sequence precisely.

Applications of Knockins:

- **Point mutations:** Introducing small changes in genes to study specific alterations, such as those in human diseases where a mutation causes a toxic gain of function.

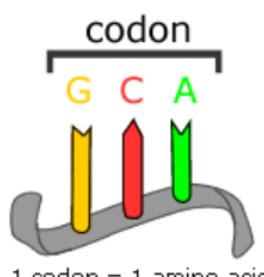
- **Conditional knockout:** A specific gene is removed only in certain tissues or at certain times, helping to study its role in specific contexts.
- **Reporter lines:** Inserting sequences that allow proteins or tissues to be labeled with a tag or fluorescent marker for easier visualization.

Unlike knockouts, which are relatively straightforward, **knockins are more challenging** and often require optimization of the technique.

Understanding Mutations - DNA: The Basis of Mutations

Mutations are changes in DNA, so to understand them, we need to know how DNA works. DNA contains instructions for building and operating the body, written in a genetic code.

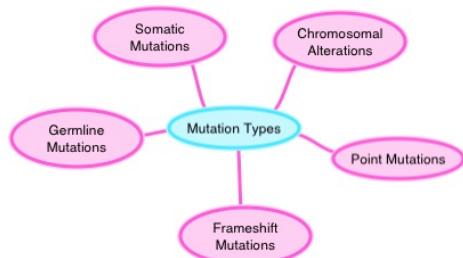
- **DNA Structure:** DNA is made up of smaller units called **bases**. There are four types of bases: **A (adenine), T (thymine), G (guanine), and C (cytosine)**. These bases form a long sequence that encodes the instructions for creating proteins.
- **Genes and Function:** Some parts of DNA control when genes are turned on or off, some parts don't have a known function, and other parts are genes that carry the instructions for making **proteins**. Proteins are chains of **amino acids** that help build and maintain the body.
- **Codons:** The coding parts of DNA are divided into **codons**, groups of three bases that specify an amino acid or signal the end of the protein. For example, the codon **GCA** codes for the amino acid **alanine**. There are 20 different amino acids in humans.



- **1 codon = 1 amino acid**

Protein Synthesis: The cellular machinery reads these codons and uses them to build a protein by linking the corresponding amino acids together. A **stop codon** signals the end of the protein.

Types of Mutations



What Causes Albinism?

Albinism is caused by a mutation in the gene responsible for producing melanin, a protein that gives color to the skin and eyes. This mutation can result in little or no melanin production.

Mutations

A mutation is a change in the DNA or RNA sequence. While mutations are often linked to science fiction, they happen naturally in everyone. Most people have many mutations, and they are essential for evolution as they create new genetic variations. Although most mutations have no impact, some can be beneficial, and even harmful mutations rarely cause drastic changes.

Types of Mutations

- **Germline Mutations:** These occur in reproductive cells (sperm or eggs). They are significant because they can be passed on to offspring, affecting every cell in the next generation.
- **Somatic Mutations:** These happen in other body cells and are not passed to offspring. They usually have little effect, as they only affect the individual where they occur.

Chromosomal Alterations

These mutations change the structure of chromosomes, often when a part of a chromosome breaks off and reattaches incorrectly. They can be serious and may result in the death of an organism or lead to abnormalities, like Down Syndrome, which is caused by a chromosomal duplication.

Point Mutations

A point mutation changes a single nucleotide in the DNA sequence. These mutations are typically less serious than chromosomal alterations. There are three types of point mutations:

- **Silent Mutation:** No effect; the change doesn't alter the protein.
- **Missense Mutation:** Changes one amino acid, which may or may not affect the protein's function.
- **Nonsense Mutation:** A change that creates a stop codon, usually leading to a nonfunctional protein.

| Type | Description | Example | Effect |
|----------|--|-----------------------------------|-----------------|
| Silent | mutated codon codes for the same amino acid | CAA (glutamine) → CAG (glutamine) | none |
| Missense | mutated codon codes for a different amino acid | CAA (glutamine) → CCA (proline) | variable |
| Nonsense | mutated codon is a premature stop codon | CAA (glutamine) → UAA (stop) | usually serious |

Frameshift Mutations

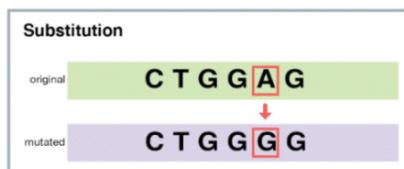
A frameshift mutation occurs when nucleotides are added or removed from the DNA sequence, shifting the reading frame. This alters how the codons are read and can drastically change the protein produced.

- Germline mutations affect reproductive cells and can be passed to offspring.
- Somatic mutations affect body cells and cannot be inherited.
- Chromosomal alterations change chromosome structure and can cause severe effects.
- Point mutations alter a single nucleotide, which can have varying effects.
- Frameshift mutations change the reading frame of the sequence, leading to major changes in the protein.

DNA can change in several ways, leading to different types of mutations. Here's a quick overview:

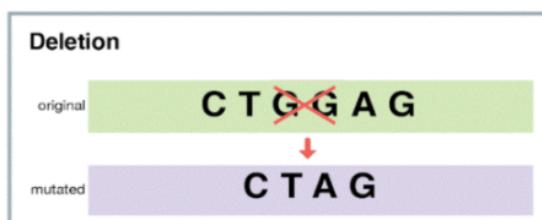
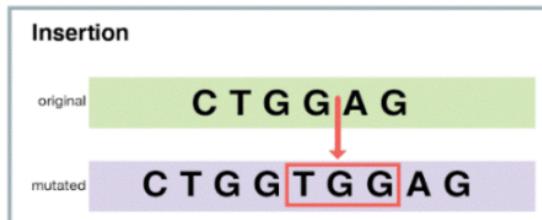
1. **Substitution:** One base is replaced with another (e.g., changing A to G). This can:

- Change a codon to code for a different amino acid, altering the protein (e.g., sickle cell anemia).
- Change a codon to one that still codes for the same amino acid, causing no change (silent mutation).
- Change a codon to a “stop” codon, leading to an incomplete protein that may not work.

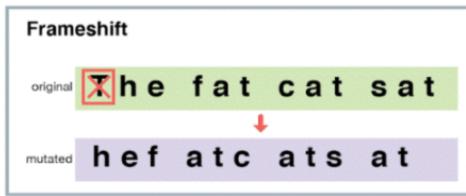


2. **Insertion:** Extra base pairs are added into the DNA sequence.

Deletion: A section of DNA is lost or removed.



3. **Frameshift:** Insertions or deletions can shift the reading of the gene, causing the codons to be read incorrectly. This often results in a non-functional or incomplete protein.

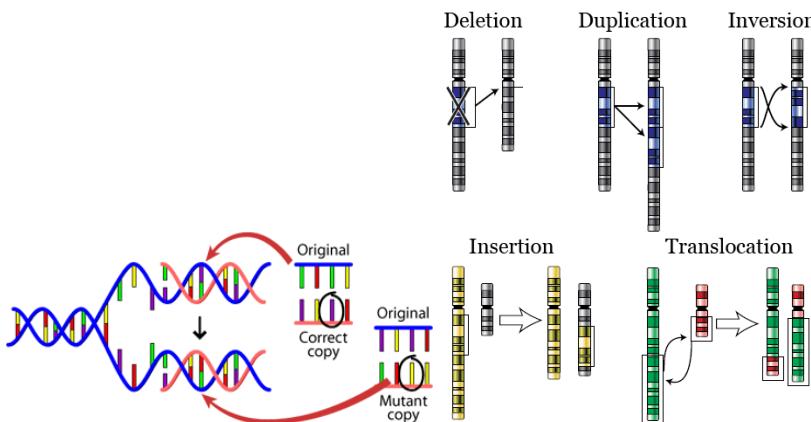


AUG-AAU-ACG-GCU = start-asparagine-threonine-alanine

Now, assume an insertion occurs in this sequence. Let's say an **A** nucleotide is inserted after the start codon **AUG**:

AUG-AAA-UAC-GGC-U = start-lysine-tyrosine-glycine

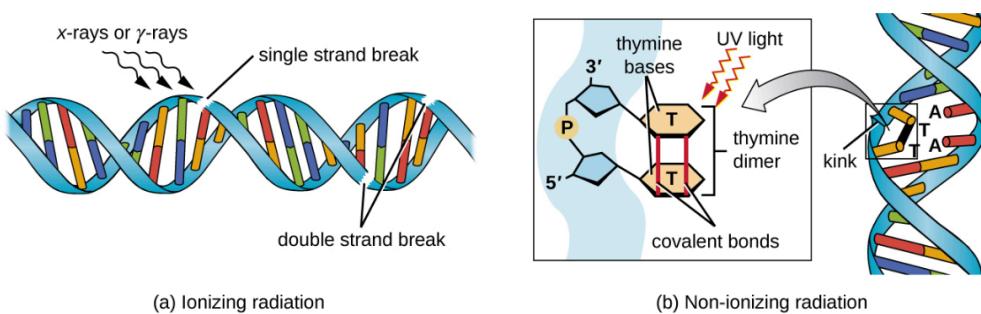
While there are other types of mutations, these are the main ones to understand.



Causes of Mutations

Mutations can happen for several reasons:

1. **DNA copying errors:** When a cell divides, it copies its DNA, but sometimes the copy is imperfect, leading to a mutation.
2. **External influences:** Exposure to chemicals or radiation can damage DNA. The cell tries to fix this, but the repair might not be perfect, causing a mutation.



Effects of Mutations

- **Somatic mutations:** These occur in non-reproductive cells and do not get passed to offspring. For example, a mutation causing a tulip's petal to have two colors won't affect the plant's seeds. Somatic mutations can also cause diseases like cancer.

Germ line mutations: These happen in reproductive cells (eggs and sperm) and can be passed to offspring, affecting evolution.

Effects of Germ Line Mutations



Neutral or no effect: Some mutations don't affect the organism. For example, a mutation might slightly change a cat's ear shape but have no impact on its health.

Detrimental effect: Some mutations harm the organism. For example, Marfan syndrome, caused by a mutation in connective tissue, leads to heart problems.

Beneficial effect: Some mutations help an organism. For example, insects might develop resistance to pesticides like DDT due to a mutation.

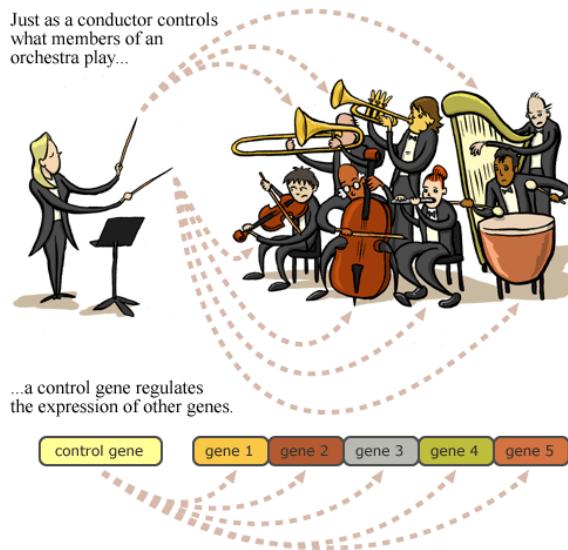
A Summary of Mutagenic Agents

| Mutagenic Agents | Mode of Action | Effect on DNA | Resulting Type of Mutation |
|---|--|---|---|
| Nucleoside analogs | | | |
| 2-aminopurine | Is inserted in place of A but base pairs with C | Converts AT to GC base pair | Point |
| 5-bromouracil | Is inserted in place of T but base pairs with G | Converts AT to GC base pair | Point |
| Nucleotide-modifying agent | | | |
| Nitrous oxide | Deaminates C to U | Converts GC to AT base pair | Point |
| Intercalating agents | | | |
| Acridine orange, ethidium bromide, polycyclic aromatic hydrocarbons | Distorts double helix, creates unusual spacing between nucleotides | Introduces small deletions and insertions | Frameshift |
| Ionizing radiation | | | |
| X-rays, γ -rays | Forms hydroxyl radicals | Causes single- and double-strand DNA breaks | Repair mechanisms may introduce mutations |
| X-rays, γ -rays | Modifies bases (e.g., deaminating C to U) | Converts GC to AT base pair | Point |
| Nonionizing radiation | | | |
| Ultraviolet | Forms pyrimidine (usually thymine) dimers | Causes DNA replication errors | Frameshift or point |

Mutations in Control Genes

Some mutations happen in genes that control when and where other genes are active. These mutations can have big effects because they can change how the entire body is built. For example, Hox genes control where body parts like heads and limbs develop. A mutation in these genes can cause major changes in an organism's body structure over generations.

- <https://evolution.berkeley.edu/dna-and-mutations/a-case-study-of-the-effects-of-mutation-sickle-cell-anemia/>



A beneficial mutation called CCR5-delta 32 provides resistance to HIV infection. The mutation affects a protein called CCR5 on T cells, which HIV needs to enter the cell. People with this mutation are less likely to get infected with HIV because the virus can't bind to the altered receptor. This mutation is most common in northern Europeans and may have been selected in the past due to protection against diseases like the plague and smallpox. It might have spread during Viking invasions.

Although this discovery is promising for HIV research, such as developing drugs that block CCR5, testing for the mutation is not widely recommended. Some individuals with the mutation have still contracted HIV, so relying on the mutation alone is not a foolproof method of protection. However, targeting CCR5 remains a potential strategy for new HIV treatments.