DNA Replication: Difference in Prokaryotes/ Eukaryotes

Feature / Component	Prokaryotes (E. coli)	Eukaryotes (Human)
Origin of Replication	Single origin (OriC)	Multiple origins on each chromosome
Helicase	DnaB	MCM complex (Mini-Chromosome Maintenance)
Primase	DnaG	DNA Polymerase α-primase complex
Leading Strand Polymerase	DNA Polymerase III	DNA Polymerase ε
Lagging Strand Polymerase	DNA Polymerase III	DNA Polymerase δ
DNA Polymerase I / RNase H	Removes RNA primers and fills gaps	RNase H + DNA Pol δ fills gaps
Sliding Clamp	β-clamp	PCNA (Proliferating Cell Nuclear Antigen)
Clamp Loader	γ-complex	RFC (Replication Factor C)
Topoisomerase	DNA Gyrase (Type II)	Topoisomerase I & II
Single-Strand Binding Protein (SSB)	SSB	RPA (Replication Protein A)
Telomere replication	Not required (circular DNA)	Telomerase needed for linear chromosome ends
Replication Speed	~1000 nucleotides/sec	~50 nucleotides/sec

Genome Size	Small, circular (~4.6 Mb in E. coli)	Large, linear (~3 billion bp in humans)

Eukaryotes have many polymerases. Some important ones:

1. DNA Pol α (alpha) – starts replication by adding RNA-DNA primer
2. DNA Pol δ (delta) – synthesizes lagging strand
3. DNA Pol ε (epsilon) – synthesizes leading strand
4. DNA Pol γ (gamma) – replicates mitochondrial DNA
5. DNA Pol β (beta) – base excision repair
6. DNA Pol η, ι, κ (eta, iota, kappa) – translesion synthesis (damage tolerance)

E. coli has 5 main DNA polymerases:

1. DNA Polymerase I
    - Role: Removes RNA primers (5′ → 3′ exonuclease activity)
      Fills in the gaps with DNA
      Direction: 5′ → 3′ synthesis
      Proofreading: 3′ → 5′ exonuclease activity
2. DNA Polymerase II
   Role: DNA repair
   Can help restart replication after damage
3. DNA Polymerase III
   Role: Main enzyme for DNA replication
   Synthesizes leading and lagging strands
   High processivity (works fast and long without falling off)
   Has 3′ → 5′ proofreading activity
4. DNA Polymerase IV
   Role: Error-prone DNA repair (translesion synthesis)
5. DNA Polymerase V
   Role: DNA repair, especially during SOS response to severe DNA damage

1. During DNA replication, which enzyme is responsible for unwinding the double helix?
A) DNA ligase
B) DNA helicase
C) DNA polymerase
D) RNA primase

2. Which strand is synthesized continuously during DNA replication?
A) Lagging strand
B) Leading strand

C) Okazaki strand
D) None of the above

3. What is the function of DNA polymerase III in prokaryotic replication?
A) Adding RNA primers
B) Synthesizing new DNA strands
C) Joining Okazaki fragments
D) Unwinding the DNA helix

4. Okazaki fragments are found on which strand of DNA?
A) Leading strand
B) Lagging strand
C) Both strands
D) Neither strand

https://www.youtube.com/watch?v=vP8-5Bhd2ag

🧬 DNA Repair — Damage & Types of Repair

🔹 What is DNA Repair?

DNA repair is the process by which cells detect and fix damage in their DNA.
It is essential for maintaining genetic stability, preventing diseases, ageing, and cancer.

⚠️ DNA Damage

DNA damage means any change or break in the normal structure of DNA.
It can lead to mutations, cell malfunction, or cancer if not repaired.

🌍 Sources of DNA Damage

Source	Example	Effect
☀️ UV Radiation	Sunlight	Forms thymine dimers (distorts DNA)
💨 Chemicals	Tobacco smoke, pollutants	Change DNA structure
☢️ Ionizing Radiation	X-rays, gamma rays	Breaks DNA strands

⚙️ Replication Errors	DNA copying mistakes	Wrong base pairing
🍔 Environmental / Lifestyle	Stress, poor diet	Promotes DNA instability

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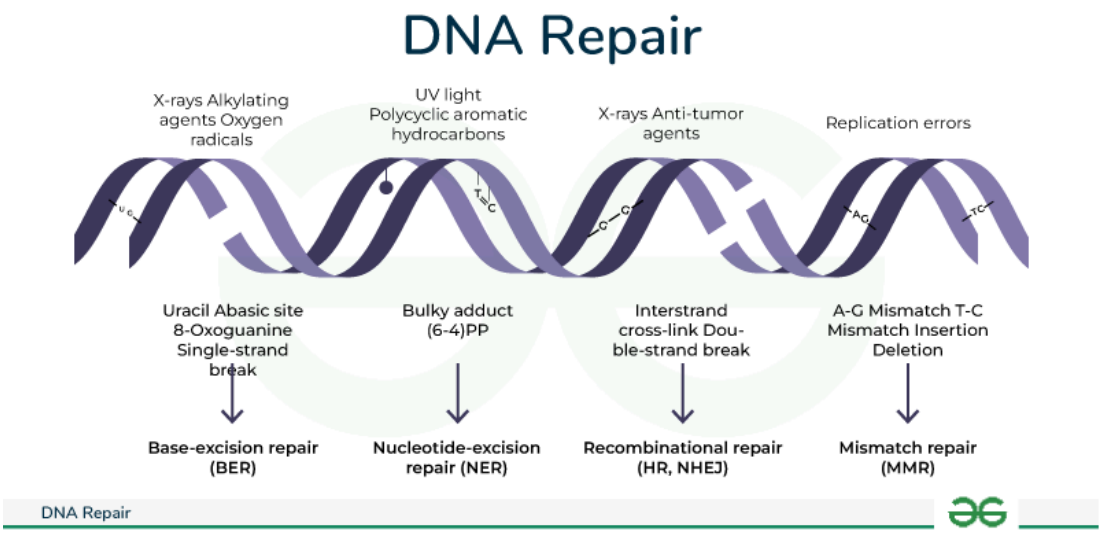
🧩 Types of DNA Repair Mechanisms

Type	What It Fixes	How It Works
🧪 Base Excision Repair (BER)	Small, single-base damage	Removes wrong base → fills with correct one
💡 Nucleotide Excision Repair (NER)	Bulky lesions (e.g., thymine dimers)	Cuts damaged strand → replaces short section
🔍 Mismatch Repair (MMR)	Replication errors (mismatched bases)	Detects mismatches → removes and corrects them
🔗 Homologous Recombination (HR)	Double-strand breaks (error-free)	Uses sister DNA as template for repair
⚙️ Non-Homologous End Joining (NHEJ)	Double-strand breaks (error-prone)	Joins DNA ends directly, may cause small errors



🧠 Bonus:
In some organisms, an enzyme called photolyase can directly fix UV-induced thymine dimers — but humans lack this enzyme.

💪 Significance of DNA Repair

Role	Description
🧬 Preserves Genetic Information	Fixes replication errors → maintains genome stability
🚫 Prevents Diseases	Avoids mutations and cancer development
⏳ Slows Ageing	Reduces DNA damage buildup linked to ageing
🌞 Protects from UV Damage	Prevents UV-related skin cancers
🧠 Supports Immune Function	Helps in antibody gene rearrangements
🌱 Drives Evolution	Some mutations that escape repair create variation for evolution

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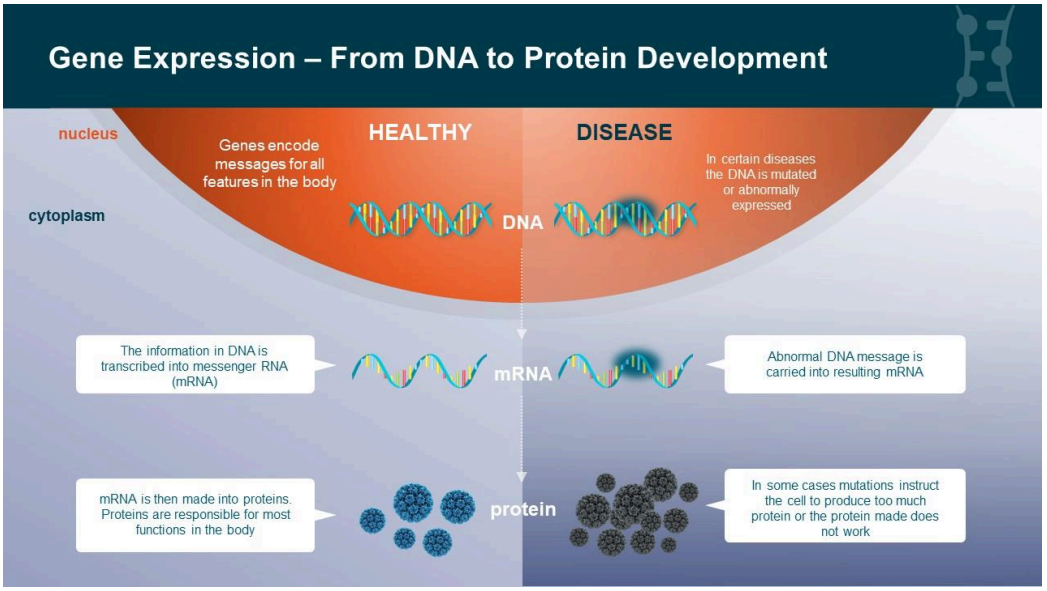
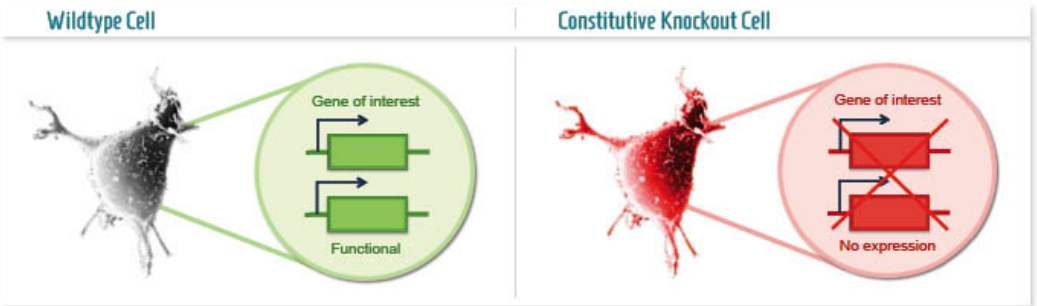
What is Gene Silencing?

- Gene silencing is a treatment that blocks specific genes from producing harmful proteins.
- It temporarily stops a gene's message instead of permanently changing DNA.
- Different from gene therapy: Gene therapy adds new DNA; gene silencing reversibly blocks gene activity.

Why is it needed?

- Sometimes cells make faulty proteins or too much protein due to genetic errors.
- This can cause diseases like cystic fibrosis, Huntington's disease, thalassemia, and some cancers.
- Gene silencing targets the root cause, not just symptoms.

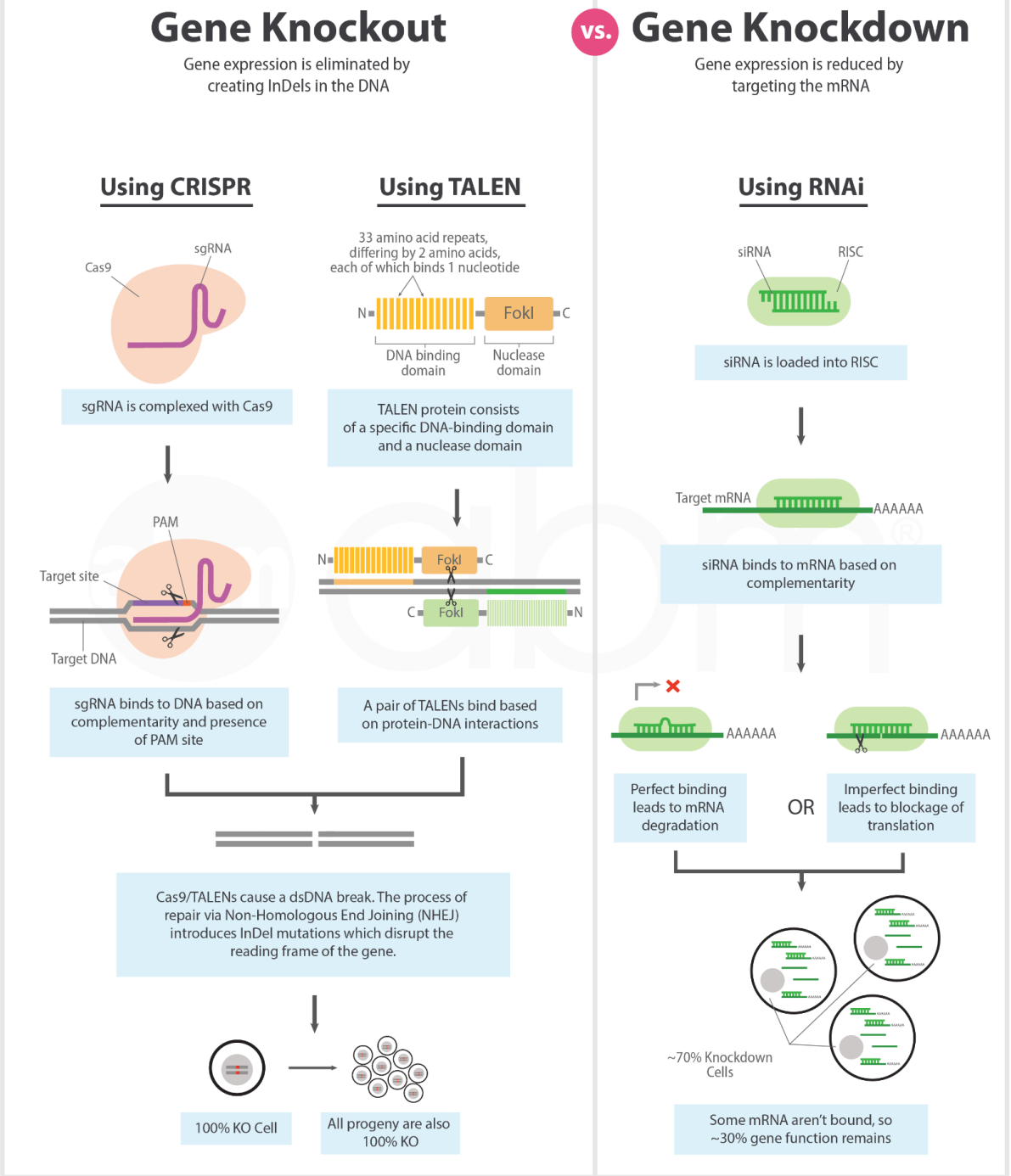




Gene Silencing Methods – CRISPR, TALEN & RNAi

1. Knockout vs Knockdown

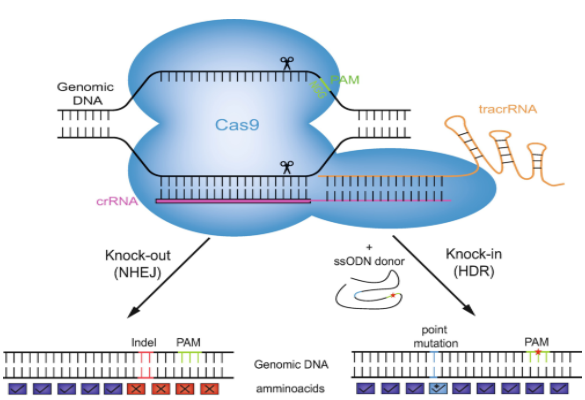
- Knockout: Completely stops a gene from making its protein. Done by CRISPR or TALEN.
- Knockdown: Reduces gene expression but doesn't fully stop it. Done by RNAi.

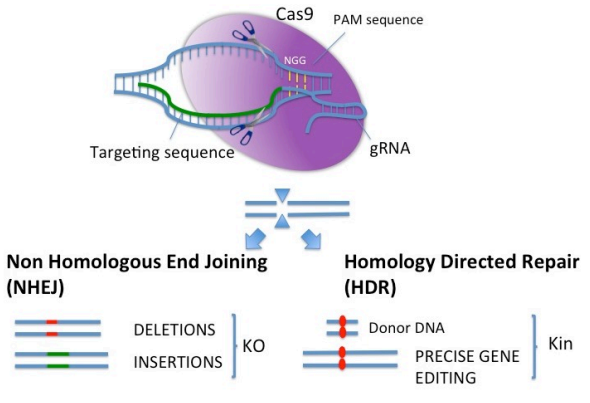


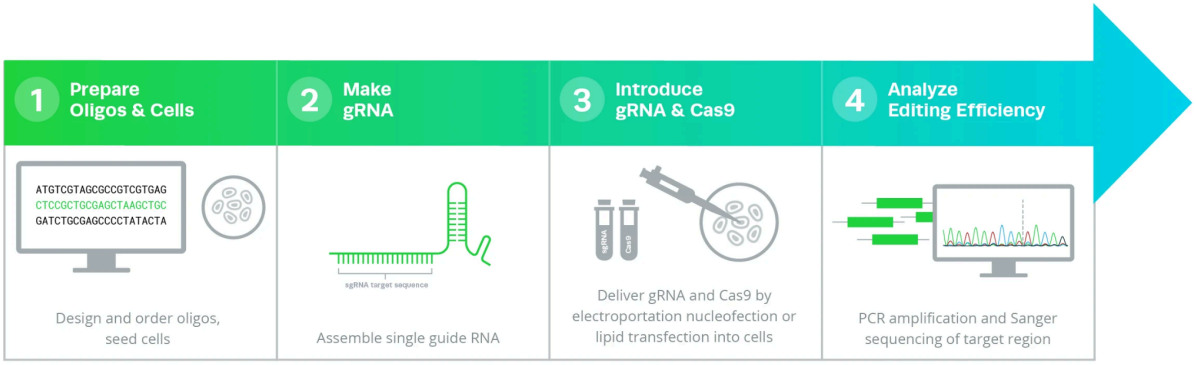
2. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

- Uses sgRNA (guides) + Cas9 (cuts DNA).
- Cas9 cuts DNA at a specific PAM sequence.
- DNA repair via NHEJ can cause frameshift mutations, leading to gene knockout.

- Flexible: can also do gene activation, repression, or imaging with modified Cas9 (dCas9).







3. TALEN (Transcription Activator-Like Effector Nucleases)

- Artificial restriction enzymes: DNA-binding domain + FokI nuclease.
- Two TALENs bind opposite DNA strands and cut it → repaired via NHEJ → knockout.
- Design is harder: TALENs must be used in pairs and are sensitive to methylation.

4. RNAi (RNA Interference)

- Uses siRNA or shRNA to target mRNA, not DNA.
- Blocks translation or degrades mRNA → gene knockdown.
- Works fast (24 hours), simpler experimental setup.
- Can have off-target effects and may trigger immune responses.

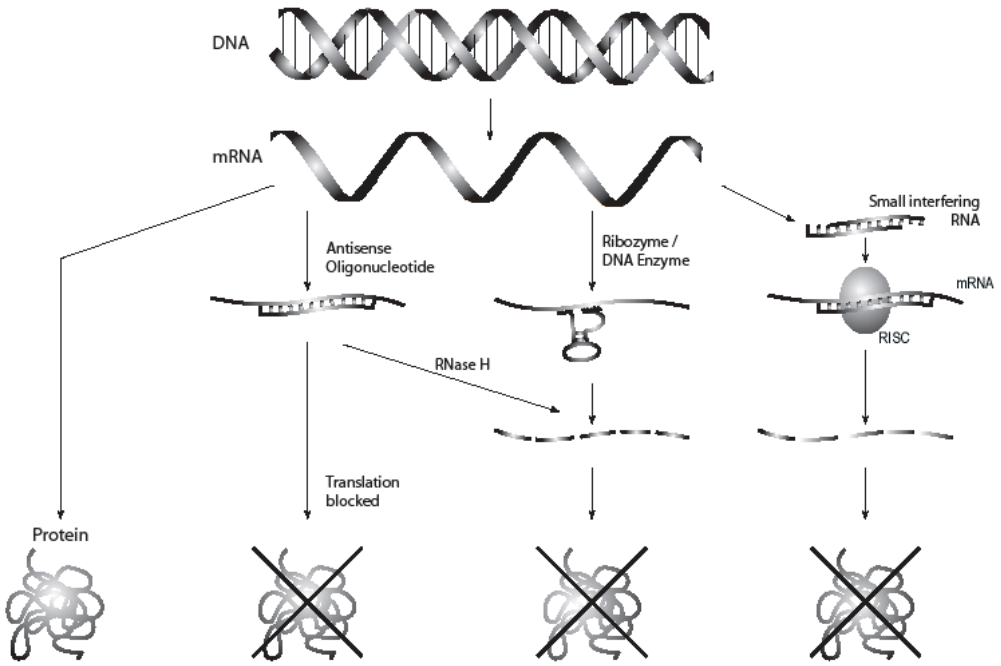
Comparison of Methods

Feature	RNAi	CRISPR	TALEN
Target	mRNA	DNA	DNA
Effect	Knockdown	Knockout	Knockout
Ease of design	Easiest	Moderate	Hardest
Experimental setup	Simple	Moderate	Complex
Efficiency	High for knockdown	High for knockout	Moderate
Off-target effects	High	Moderate (can reduce with nickase)	Low
Flexibility	Limited to silencing	Very high (activation, repression, editing)	Moderate (editing only)

Type	Role	Size (nt)
Scan RNAs (scnRNA)	Genome rearrangement, chromosome segregation, meiotic prophase	~29
Small nuclear RNA (snRNA)	Splicing (removal of introns from genes)	120–300
Small nucleolar RNA (snoRNA)	rRNA processing	Variable 87–275
Repeat-associated siRNA (rasiRNA)	Silencing of genetic repeat	~24–26
Transacting short interference RNA (tasiRNA)	Post -transcriptional gene regulation	~21
Natural antisense transcript-associated siRNA (natsiRNA)	Derived from antisense transcript region	20–25
Piwi -intracting RNAs(piwiRNA)	Fertility of male mammals, male fish or fly of either sex	26–31
X- inactive specific transcript RNAs (xistRNAs)	Inactivation of X chromosome, more severe in females	16500
Pregnancy- induced non-coding RNA (pincRNA)	Effective during pregnancy	22–25

Table 17.1 Types of RNA in a Eukaryotic Cell

Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes.
Transfer RNA (tRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes.
Ribosomal RNA (rRNA)	Plays structural and catalytic (ribozyme) roles in ribosomes.
Primary transcript	Serves as a precursor to mRNA, rRNA, or tRNA and may be processed by splicing or cleavage . In eukaryotes, pre-mRNA commonly contains introns, noncoding segments that are spliced out as the primary transcript is processed. Some intron RNA acts as a ribozyme, catalyzing its own splicing.
Small nuclear RNA (snRNA)	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA in the eukaryotic nucleus.
SRP RNA	Is a component of the signal-recognition particle (SRP), the protein-RNA complex that recognizes the signal peptides of polypeptides targeted to the ER.



RNA Interference (RNAi) & Gene Silencing

RNAi:

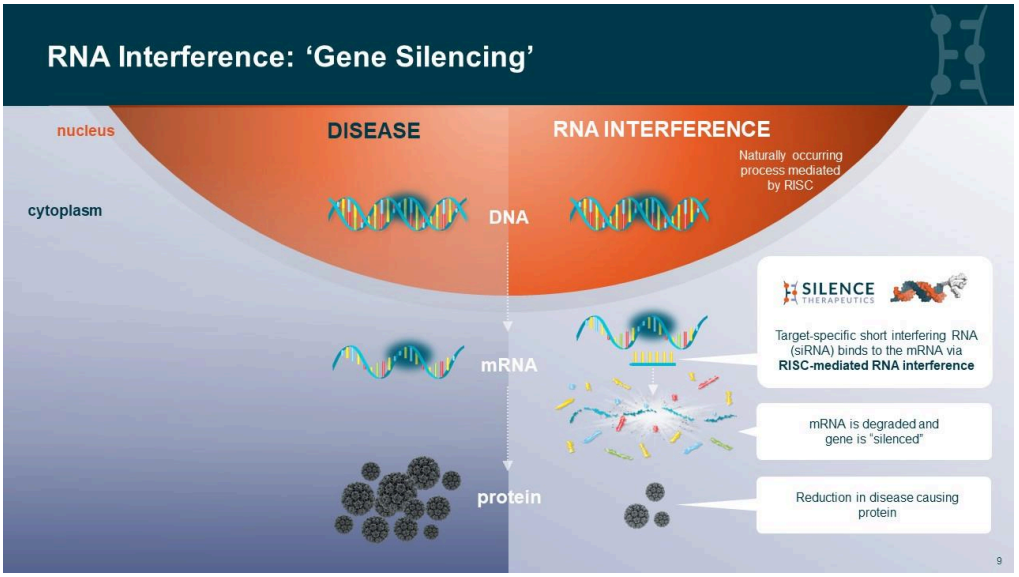
- Natural process discovered in the 1990s (Nobel Prize 2006).
- Helps cells control gene activity and defend against viruses.
- Works by blocking or reducing mRNA so protein production is silenced.

Process:

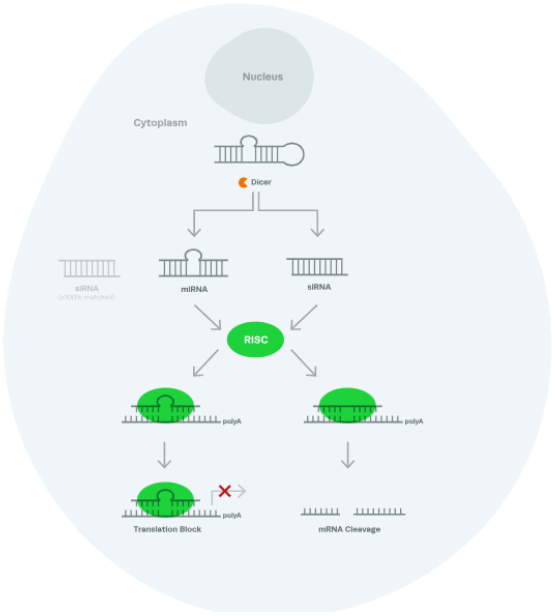
1. RNA molecules are cut into small interfering RNAs (siRNA).
2. siRNA binds to the target mRNA.
3. This prevents protein production and "silences" the gene.

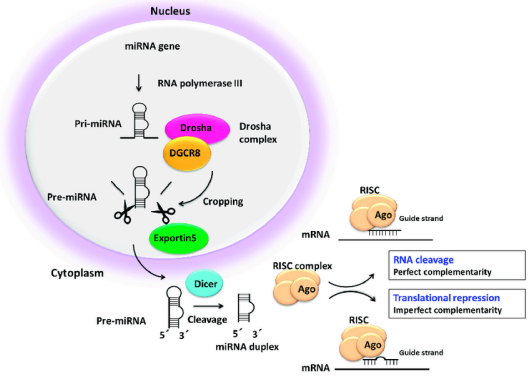
Gene Silencing Therapy:

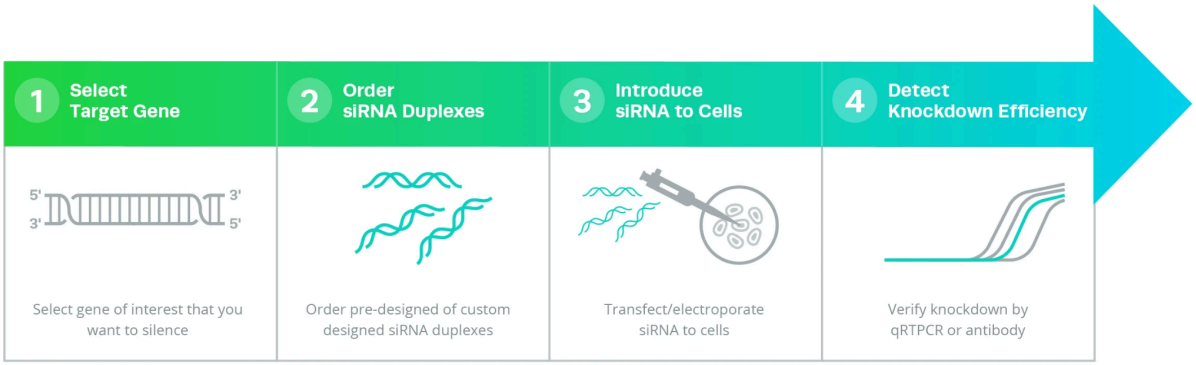
- Scientists mimic siRNA to block disease-causing genes.
- Stops harmful protein production and reverses disease effects.
- Effect is temporary, controllable, and specific.



	RNAi	CRISPR
Benefits	· 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)	· 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	· 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	· Cannot be used to study essential genes







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1. Which of the following enzymes directly seals nicks in the DNA backbone during repair?
   a) DNA polymerase
   b) DNA ligase
   c) Restriction endonuclease
   d) Helicase

2. Base excision repair (BER) mainly fixes:
   a) Bulky DNA adducts
   b) Single base modifications
   c) Cross-linked DNA
   d) Chromosomal translocations

3. Homologous recombination repair (HRR) requires:
   a) A sister chromatid
   b) RNA template
   c) Random ligation
   d) Single-strand DNA only

4. Non-homologous end joining (NHEJ) is:
   a) Error-free

b) Error-prone
c) Only in prokaryotes
d) Repairing single-strand breaks

5. Which of the following statements is correct?

   A) CRISPR-Cas9 works by degrading target mRNA to reduce protein levels.
   B) RNA interference (RNAi) uses small RNAs to block translation or degrade specific mRNAs.
   C) DNA repair mechanisms cannot fix double-strand breaks in cells.
   D) Small RNAs like tRNA and rRNA are directly involved in DNA repair.

6. RNA interference (RNAi) works by:
   a) Mutating DNA
   b) Degrading or blocking mRNA
   c) Inserting new genes
   d) Activating transcription

7. CRISPR-Cas9 differs from RNAi because it:
   a) Silences mRNA
   b) Knocks out DNA permanently
   c) Uses siRNA
   d) Works only in plants

8. TALENs require:
   a) Single binding site
   b) Pair of nucleases targeting opposite DNA strands
   c) PAM sequence
   d) miRNA machinery

9. Gene knockdown refers to:
   a) Complete elimination of gene expression
   b) Temporary reduction of gene expression
   c) DNA deletion
   d) Insertion of a reporter gene

10. Small interfering RNAs (siRNAs) function by:
    a) Binding DNA to block transcription
    b) Binding mRNA to prevent translation
    c) Editing protein structure
    d) Increasing gene expression

1(B), 2(B), 3(A), 4(B), 5(B), 6(B), 7(B), 8(B), 9(B), 10(B)