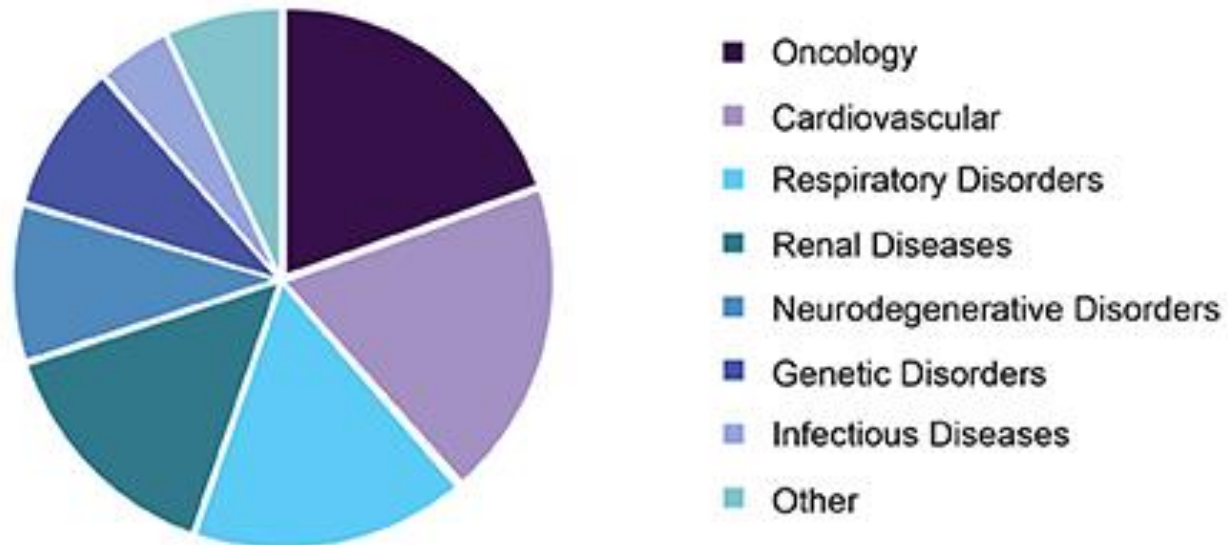


Studying Gene Expression

RNA interference (RNAi) technology

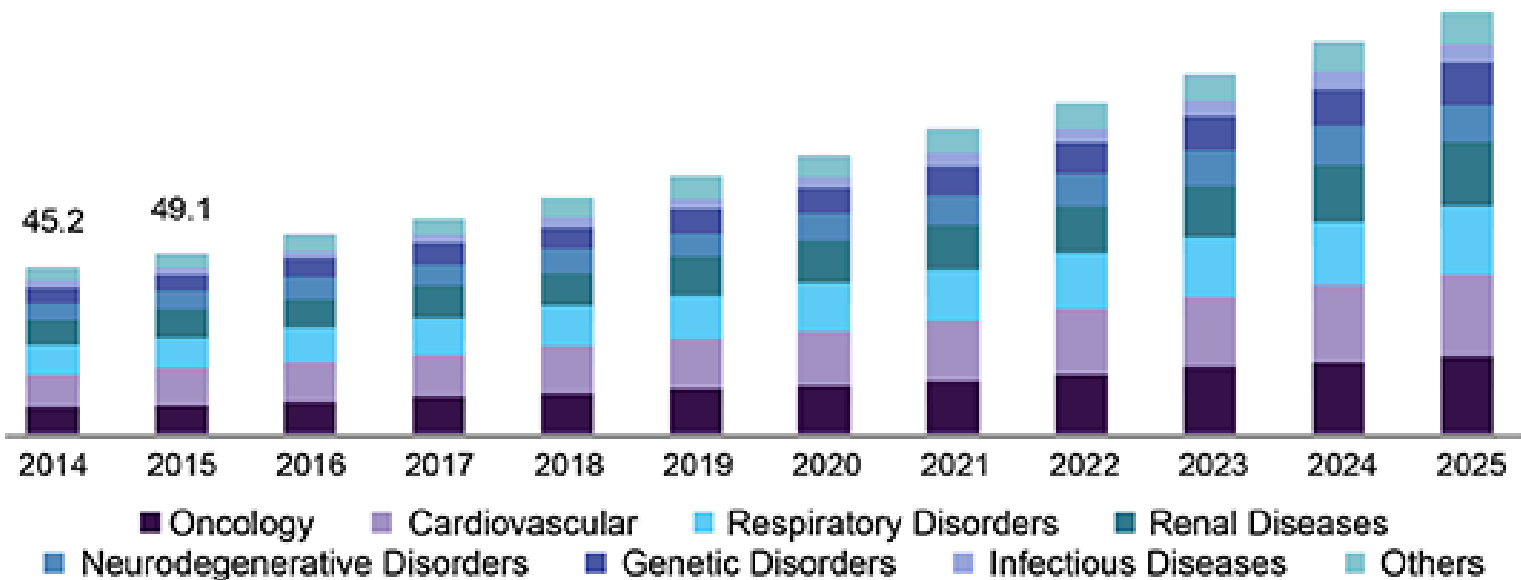
- Many terms used: post translational gene silencing (in plants), RNA interference (animal), quelling (in fungi)
- RNA studies represent a means of partial or complete loss-of-function phenotypes, possibly leading to identification of gene functions.

Global antisense & RNAi therapeutics market, by application, 2017 (%)



Source: www.grandviewresearch.com

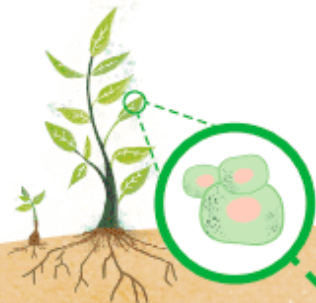
Japan antisense & RNAi therapeutics market share, by application, 2014 - 2025 (USD Million)



Source: www.grandviewresearch.com

UNDERSTANDING RNAi

RNAi, or "RNA Interference," is a natural process that occurs in the cells of plants, animals, and people.



All living things - like this plant - are made up of **cells**, the basic units of life.

Inside the nucleus of each cell is a detailed genetic blueprint, encoded in **DNA**...

... which is transcribed (copied) into **messenger RNA (mRNA)** ...

...which gets translated by the cell's machinery to make a specific **protein**.

Proteins are the building blocks of tissues and they carry out many essential biological functions. In some cases, decreasing the production of specific proteins can be beneficial. RNAi is a natural process that works like a "dimmer switch" to dial down the level of a protein. It likely evolved to protect cells from viruses.

Cytoplasm

Nucleus

DNA

mRNA

Protein

Protein

HOW DOES RNAi WORK?

- 1 It begins when a form of RNA made of two strands (**double-stranded RNA, or dsRNA**) is introduced into the cell, for example by a virus, or produced in the cell.
- 2 When a cell "sees" dsRNA, it activates structures that work like scissors to **cut it up**.
- 3 Next, **other structures attach** to these small pieces of RNA and turn them back into single-stranded RNA.
- 4 These structures then bind to **mRNA with a matching code**.
- 5 As a result, **production of the protein** encoded by that mRNA is prevented.

When we know the gene that encodes a certain protein, we can use RNAi to target that protein and dial it down in a highly specific way. In agriculture, for example, this can potentially impact the production of proteins responsible for the development of a disease or essential for a pest's survival, thus protecting plants from such disease or pest infestations.

The players in interference

RNA

- siRNA (small interfering RNA): double stranded; 21-22 nt
- miRNA (micro RNA): single stranded; 19-25 nt; encoded by non-coding protein genome

RISC

RNA induced silencing complex that cleaves mRNA

Enzymes

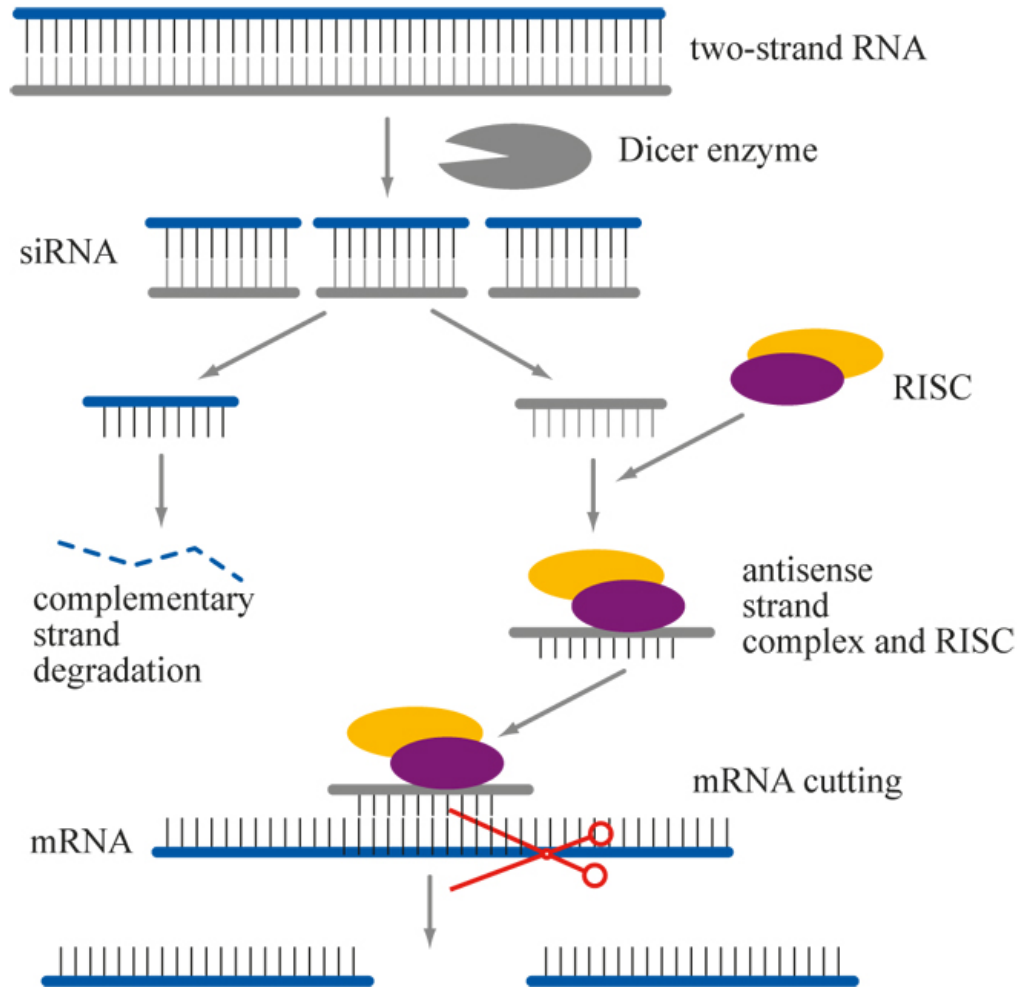
Dicer: produce 21-22 nt clavaiges that initiate RNAi

Summary and Comparison of siRNA and miRNA

Different:

	siRNA	miRNA
structure	Double-stranded	Single-stranded with hair pin
precursor	Exogenous long dsRNA	Endogenous transcribed RNA
Targeting	Perfect base pairing Specific one mRNA Coding region	Imperfect or perfect basepairing Multiple mRNAs Coding or 3'-UTR region
effect	mRNA cleavage	Translational repression or cleavage
Significance	Viral defense and genome stability	Endogenous gene expression regulation

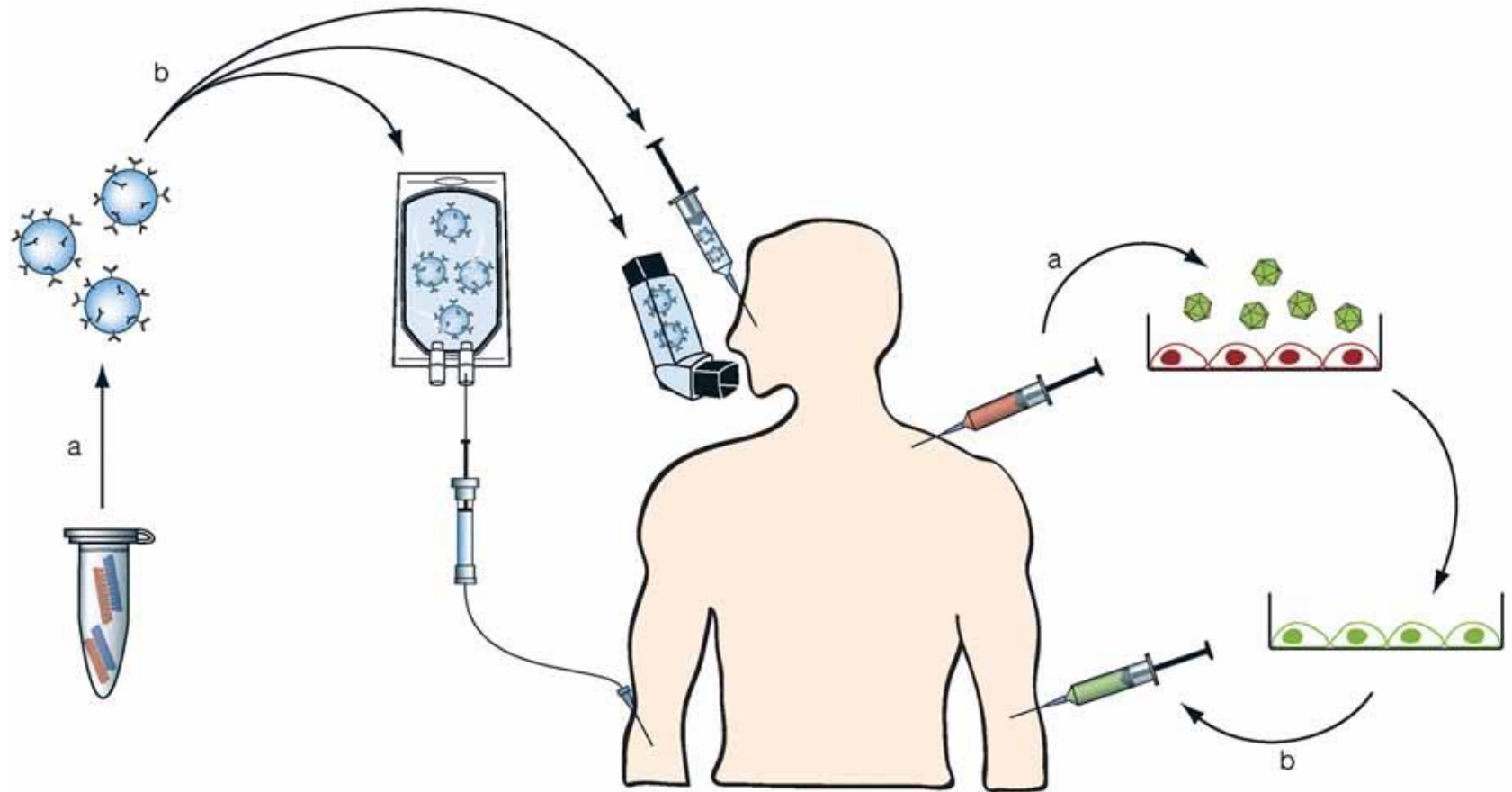
Dicer and RISC



RNAi-based therapeutics delivery

A SYSTEMIC DELIVERY

B *ex-vivo* DELIVERY



Genome editing

- Genetic engineering in which DNA is inserted, replaced, deleted in a genome of living organism by using engineered nuclease (“molecular scissors”)

CRISPR

- Clustered Regularly Interspaced Short Palindromic Sequence
- In 2011, selected as Method of The Year by Nature Methods. In 2013, first demonstration in mammalia

Key component

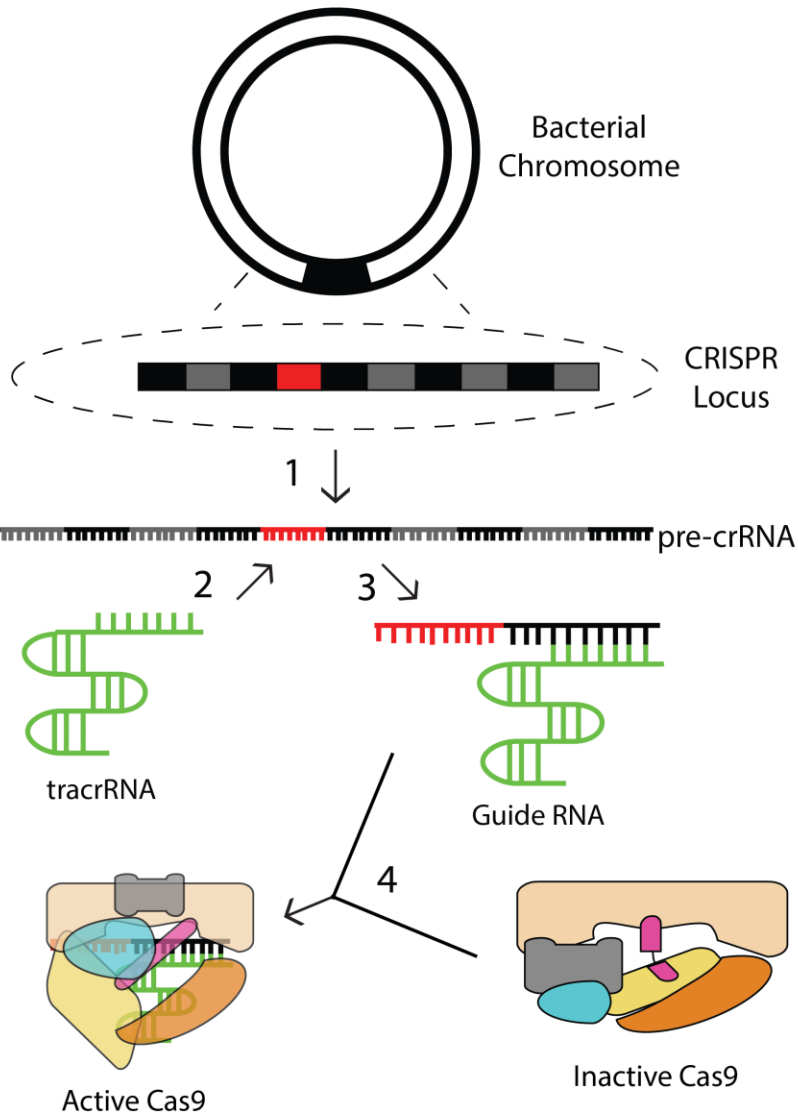
sgRNA

- Single guide RNA. A combination of crRNA and tracrRNA

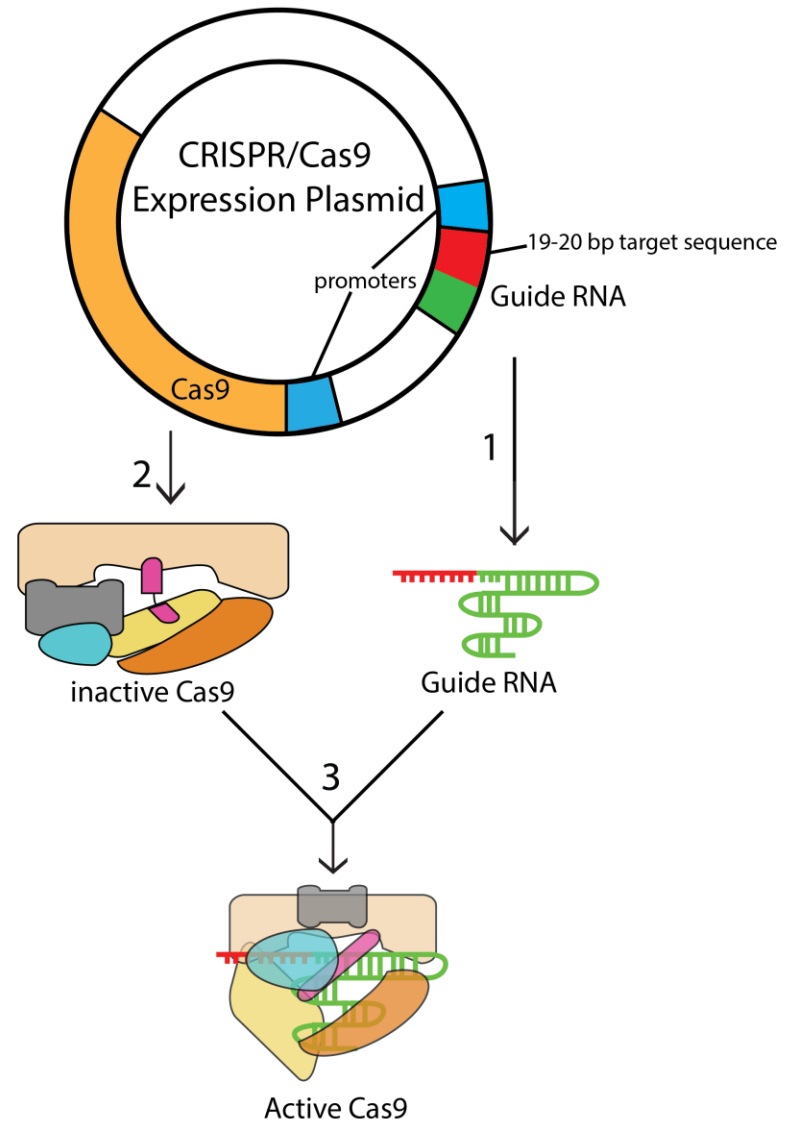
Cas9

Protein that can modify DNA in its active form

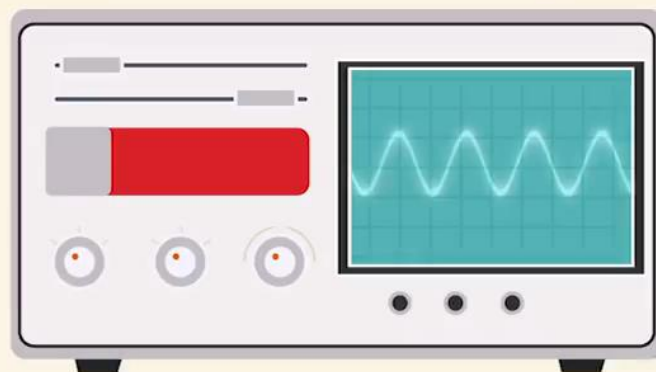
Natural CRISPR Pathway



Engineered CRISPR/Cas9 System



CRISPR for diagnostic





The shorter lawn grasses on the left (perennial ryegrass) need to be mowed less frequently than their conventional counterpart, shown on the right. The shorter grasses were produced using a traditional plant breeding technique. Yi Li is currently using the CRISPR technique to create grasses of other species that require less maintenance. Yi Li, [CC BY-SA](#)

Naturally decaffeinated coffee



	siRNA	CRISPR
Targeting sequence	siRNA Oligonucleotide shRNA plasmid	(<i>Synthetic sgRNA</i>) sgRNA plasmid
Effector	- (Endogenous)	Cas9 Endonuclease
Effect	Knockdown (variable)	Knockout (Silencing, Activation)
Time to effect	2–3 days	Days; may require selection
Libraries	Lentiviral shRNA Pools Lentiviral shRNA arrays Arrayed siRNA Sub-panels	Lentiviral sgRNA Pools
Off-target effects	High	No consensus reached – Depending on policy of lab off-targets may be absent or very high

For an overview of key differences between siRNA and CRISPR technologies, please see Taylor and Woodcock (2015).