RNA Interference (RNAi)

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Road Map

I. Definition of RNAi
II. History
III. Mechanism
IV. Applications
Definition
What is RNAi?

According to the National Center for Biotechnology Information (NCBI),

“RNA interference (RNAi) or Post-Transcriptional Gene Silencing (PTGS) is a conserved biological response to double-stranded RNA that mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes” (1).
What is RNAi?

A biological process observed in a number of eukaryotic organisms including protozoa, flies, nematodes, insects, parasites, and mouse and human cell lines \(^{(2)}\).

Also known as,

- co suppression or posttranscriptional gene silencing (PTGS) in plants
- quelling in fungi
- RNAi in the animal kingdom
What is RNAi?

• Can be used for gene knockdown.

• This mechanism promises to “revolutionize” experimental biology and may have important practical applications in functional genomics, therapeutic intervention, agriculture and other areas (1)
History
History

• Early observations of RNAi in various organisms:
  1. Petunias
  2. *Neurospora crassa*

• 1998 *Nature* paper by Craig Mello and Andrew Fire on gene silencing in *C. elegans*.

• For their work, Mello and Fire were awarded the Nobel Prize for Physiology or Medicine in 2006.
Mechanism
Exogenous Mechanism

1. Exogenous dsRNA initiates the process by activating the ribonuclease protein DICER. dsRNA is imported into the cytoplasm and cleaved by DICER into short fragments called siRNAs [7].
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2. siRNAs are integrated into RISC via the RISC-loading complex. [7] Around this stage, siRNAs are degraded into single stranded RNA molecules.
Exogenous Mechanism

1. Exogenous dsRNA initiate the process by activating the ribonuclease protein DICER. dsRNA is imported into the cytoplasm and cleaved by DICER into short fragments called siRNAs (7).

2. siRNAs are separated into single strands and integrated into RISC via the RISC-loading complex (7).

3. After being integrated into the RISC, siRNAs base-pair to corresponding target mRNA and cleave it (7).

4. The cleaved mRNA cannot be used as a translation template, therefore regulating gene expression (7).
Endogenous Mechanism

Endogenous pre-micro RNAs are processed into stem loops in the nucleus and then exported to the cytoplasm (8).

Different than the exogenous process, the miRNA-loaded RISC scans cytoplasmic mRNAs for complementarity (8).

Instead of cleaving corresponding mRNA sequences, miRNAs target the 3’ untranslated regions of mRNA and binds with imperfect complementarity, which blocks access of ribosomes for translation (8).
Applications
Applications of RNAi

- Gene Knockdown
- Medicine
- Biotechnology
- Functional genomics
- Agriculture
Gene Knockdown

• Gene knockdown is when gene expression is reduced but not completely eliminated \(^{(10)}\).

• Knockdown is done by degrading/blocking translation of mRNA \(^{(10)}\).

• Results in low levels of gene expression.

• siRNA are designed to be complementary to an RNA sequence of interest \(^{(10)}\).

• siRNA is loaded into RISC. RISC guides system to bind to mRNA and cleaves it. Results in gene knockdown! If mRNA is not cleaved, like in the endogenous process, translation is simply inhibited \(^{(10)}\).
RNAi

- qRT-PCR to measure mRNA levels
- Western Blot to measure protein levels
Applications of RNAi: Agriculture

RNAi has resulted in the invention of novel crops:

• nicotine-free tobacco
• decaffeinated coffee
• nutrient fortified vegetation
• hypoallergenic crops
Thank you!!
References


10. CRISPR vs. TALENs vs. RNAi: Which system is best for your gene silencing project? Available at: https://www.abmgood.com/marketing/knowledge_base/Gene-Silencing-CRISPR-TALEN-RNAI. (Accessed: 16th November 2019)