BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biomedical science

Course Title : Cancer Biology

Course Code : 18BMS59C16

Unit-V

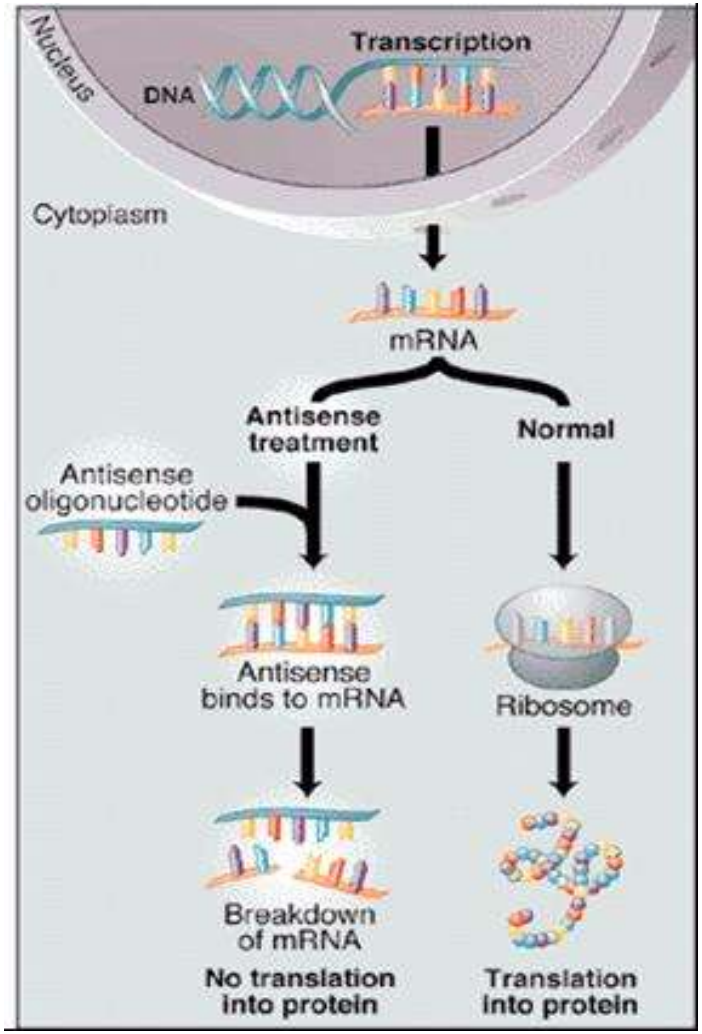
TOPIC: RNAi Strategy

Dr. G.MATHAN
Professor
Department of Biomedical Science

What is the antisense oligonucleotides?

The term antisense oligonucleotides refers to molecules made of synthetic genetic material, which interact with natural genetic material (DNA or RNA) harboring the information for production of proteins.

What is the antisense oligonucleotides?



The term antisense oligonucleotides refers to molecules made of synthetic genetic material, which interact with natural genetic material (DNA or RNA) harboring the information for production of proteins.

Molecular Biology's Central Dogma

DNA

Plasmid

Virus

AAAA

RNA

messenger RNA
ribosomal RNA
transfer RNA
other noncoding RNAs

protein

History of RNA-mediated suppression

Curious findings in plants led the way…

Adding a pigment gene to petunias made them less pigmented! Biology works in mysterious ways…

Jorgensen 1990
van der Krol 1990

Gene injection (pigmentation Enzyme-petunias)
Expectation: more red color
Co-suppression of transgene and endogenous gene.

Bill Douherty and Lindbo 1993

Gene injection with a complete tobacco etch virus particle.
Expectation: virus expression
Co-suppression of transgene and virus particles via RNA.

Fire and Mello 1998

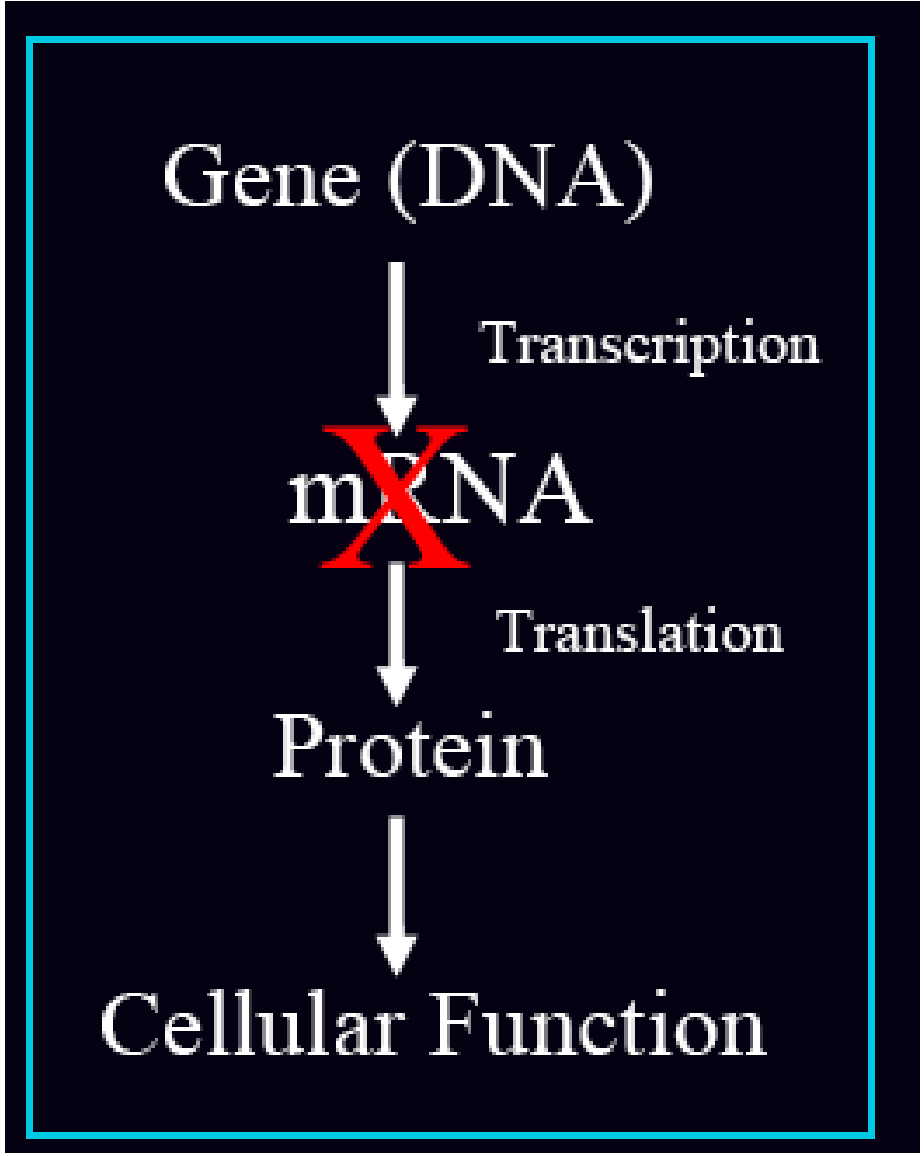
Injection of dsRNA into C. elegans
RNA interference (RNAi) or silencing

Hamilton and Baulcombe 1998

Identification of short antisense RNA sequences
dsRNA?
How?

Ambros 1993 (2000)

Identification of small RNA in C. elegans (micro RNA)



RNA interference:

--A type of gene regulation

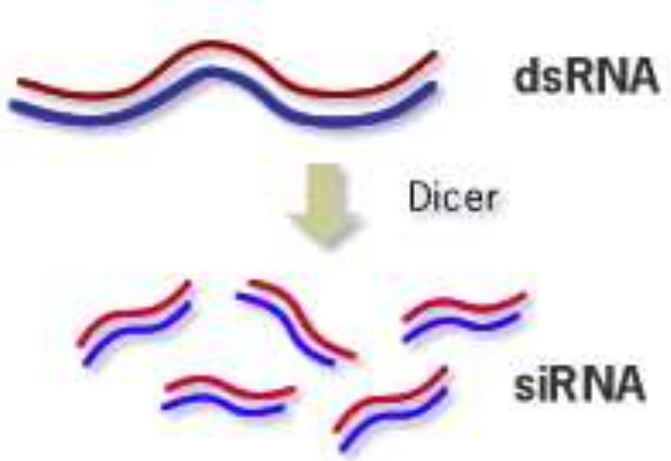
--Involve small RNA molecules

--Induce a double stranded RNA

Shooting mRNA means **RNA interference**

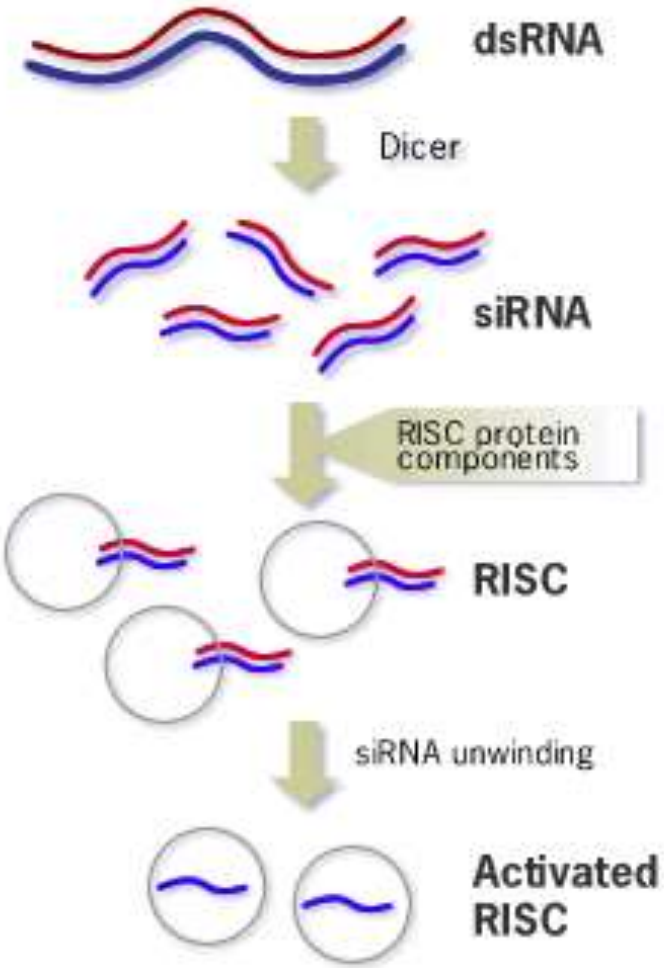
Step 1

- dsRNA is processed into sense and antisense RNAs
  - 21-25 nucleotides in length
  - have 2-3 nt 3’ overhanging ends
  - Done by *Dicer* (an RNase III-type enzyme)



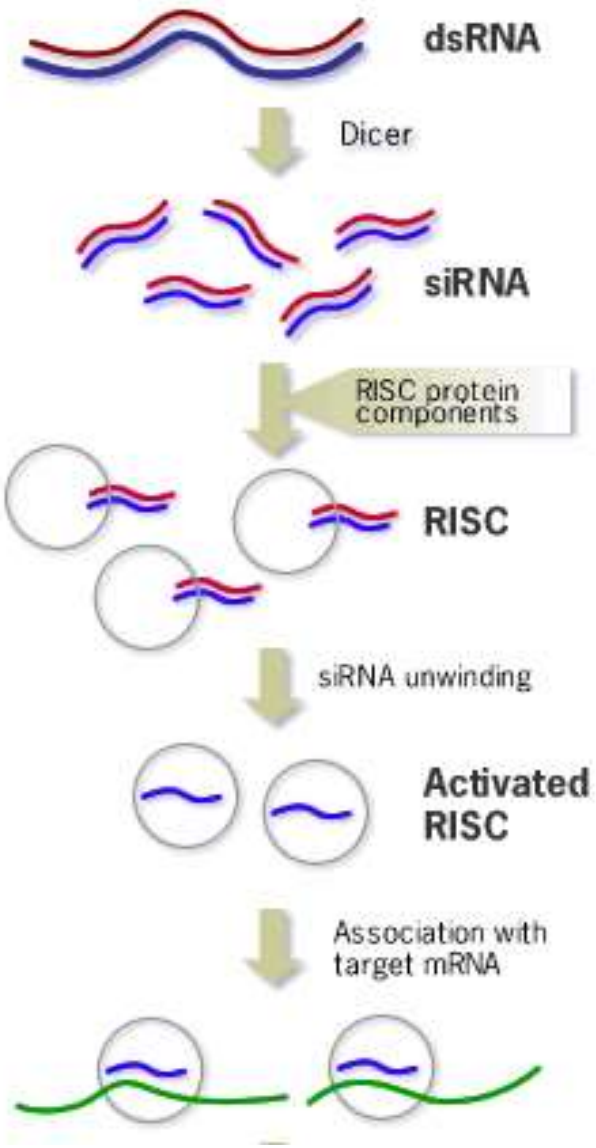
Step 2

- The siRNAs associate with *RISC* (RNA-induced silencing complex) and unwind



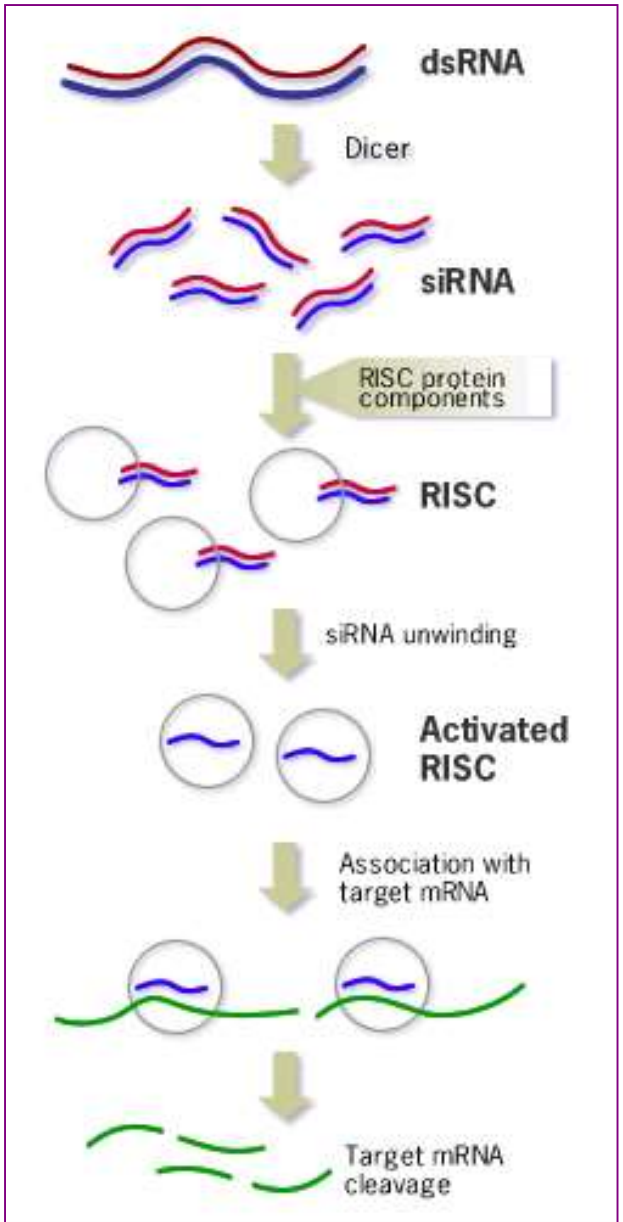
Step 3

- the antisense siRNAs act as guides for *RISC* to associate with complimentary single-stranded mRNAs.

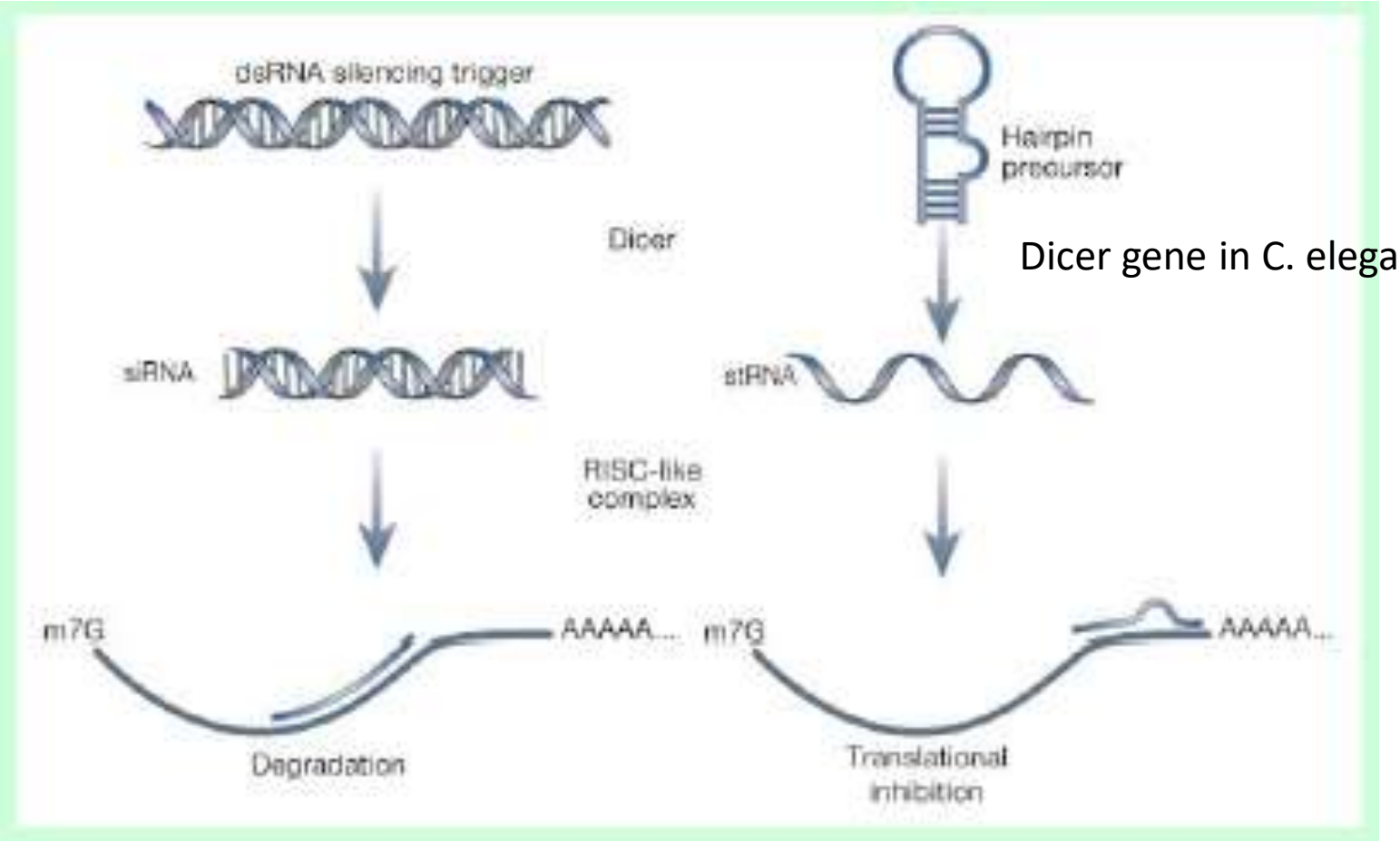


Step 4

- *RISC* cuts the mRNA approximately in the middle of the region paired with the siRNA
- The mRNA is degraded further



Gene regulation by small RNAs



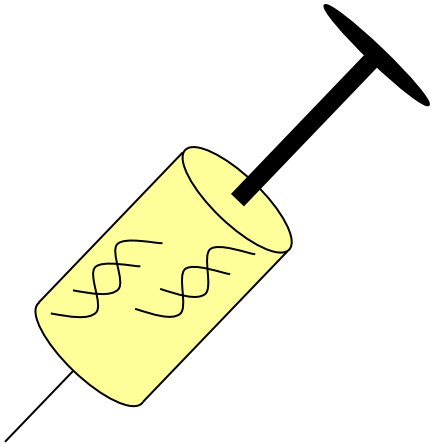
Dicer gene in C. elegans

siRNAs degrade mRNA to stop gene expression quickly

Small temporal(St) RNAs prevent translation to stop gene expression quickly

The discovery of RNA-mediated interference

- Antisense technology was used in worms...
- Difficult to explain: sense and antisense RNA preparations are each sufficient to cause interference.



inject worms with dsRNA corresponding to a gene involved in wiggling (*unc-22*)

QuickTime™ and a GIF decompressor are needed to see this picture.

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

Fire et al., Nature **391**. (1998)

The discovery of RNA-mediated interference

Working hypothesis: the silencing effect by antisense or sense RNA might be due to low-level contaminations of **double-stranded RNA**.

Gene segment	Size (kilobases)	Injected RNA	F_1 phenotype
unc-22			*unc-22*-null mutants: strong twitchers[78]
*unc22A** Exon 21-22	742	Sense	Wild type
		Antisense	Wild type
		Sense + antisense	Strong twitchers (100%)
unc22B Exon 27	1,033	Sense	Wild type
		Antisense	Wild type
		Sense + antisense	Strong twitchers (100%)
unc22C Exon 21-22†	785	Sense + antisense	Strong twitchers (100%)

Results:

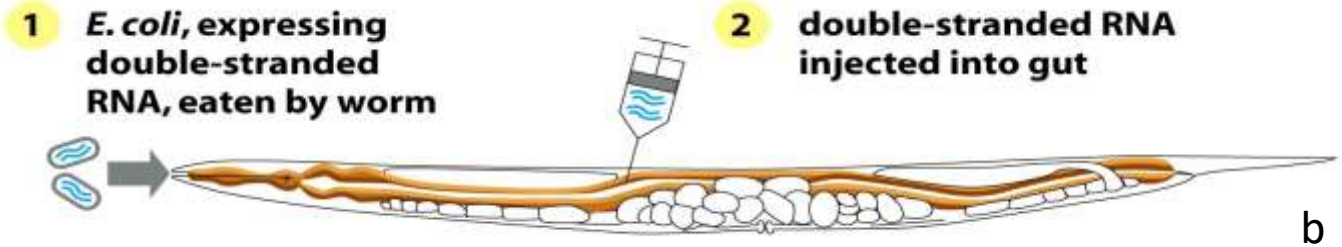
- double-stranded RNA is far more effective than single-stranded RNA.
- The sense or antisense RNAs lose their silencing effect if they are purified from the contaminating Double-stranded RNA (dsRNA).
- only a few molecules of dsRNA are required per cell → non-stochiometric effect that implies an amplification component.

RNAi –Rapid way to test Gene Function

RNAi used to inactivate genes in Drosophilla and mammalian cell culture lines

To study the consequences and importance of the particular gene function through this study

Dominant negative mutation created by RNAi



A. Double stranded RNA introduced in to *c. elegans*
1. Either by feeding the E.coli expressing dsRNA or
2. By injecting directly in to gut

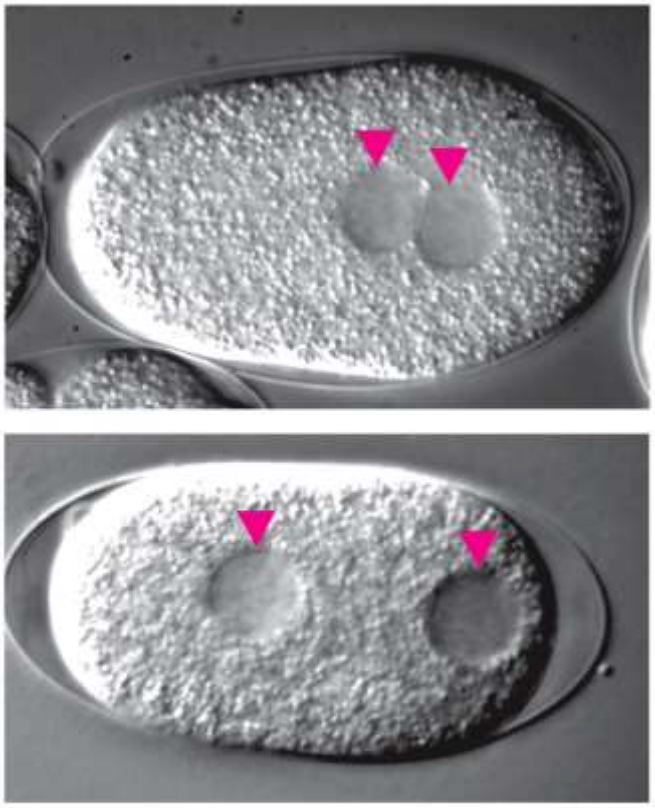
It inhibit the expression of target gene in different tissue TYPE

• The RNAi effect is capable of **spreading** whithin the whole nematode.
→ this observation led to new discoveries that allowed the identification of genes and Therefore to the mechanisms that are involved in RNAi

B. Wild type worm-embryo after the egg has been fertilized
Egg and sperm pronuclei (red arrow) migrated and come Together in the posterior half of the embryo

c. Worm embryo at the same stage in which a gene involved In cell division has been inactivated by RNAi
2 pronuclei have failed to migrate

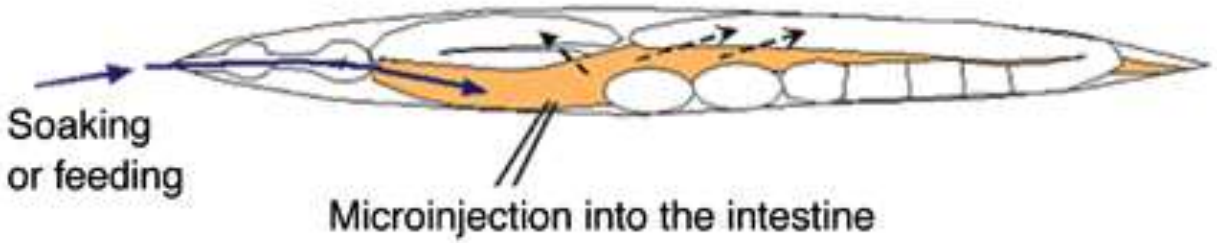
b

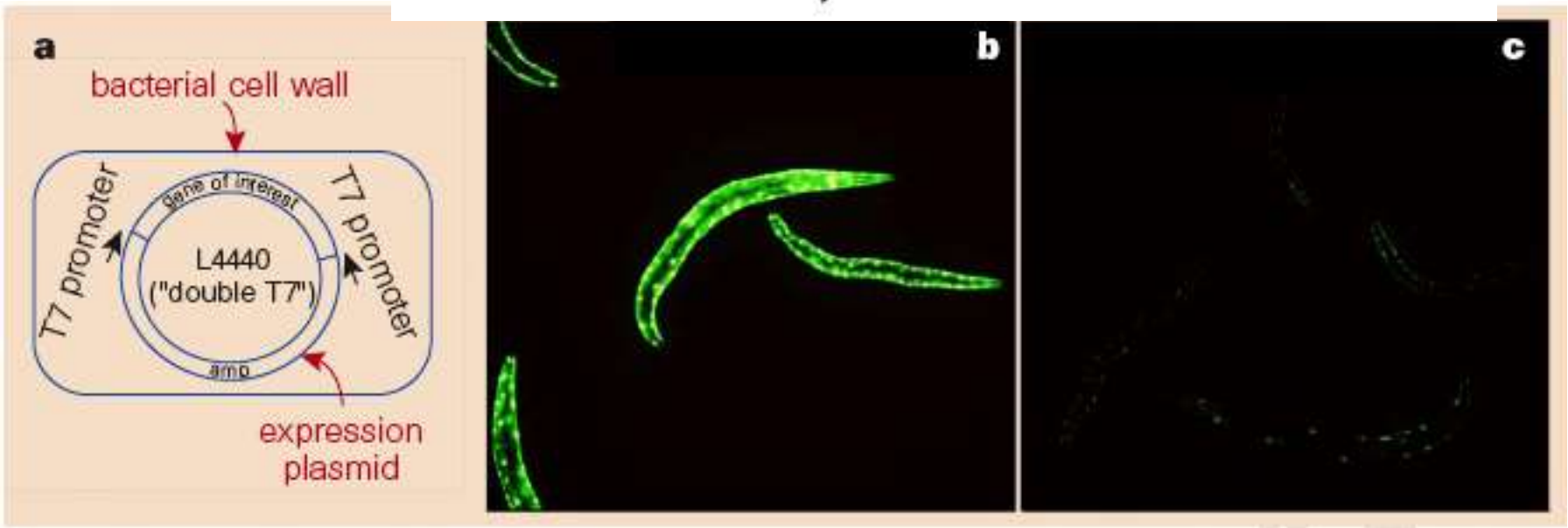


B.C from P.GONCZY et.al., Nature 408:331-336,2000

RNAi does not require microinjection

Transgenes and endogenous genes can be inactivated through soaking worms in dsRNA or by feeding them with bacteria that produce dsRNA.



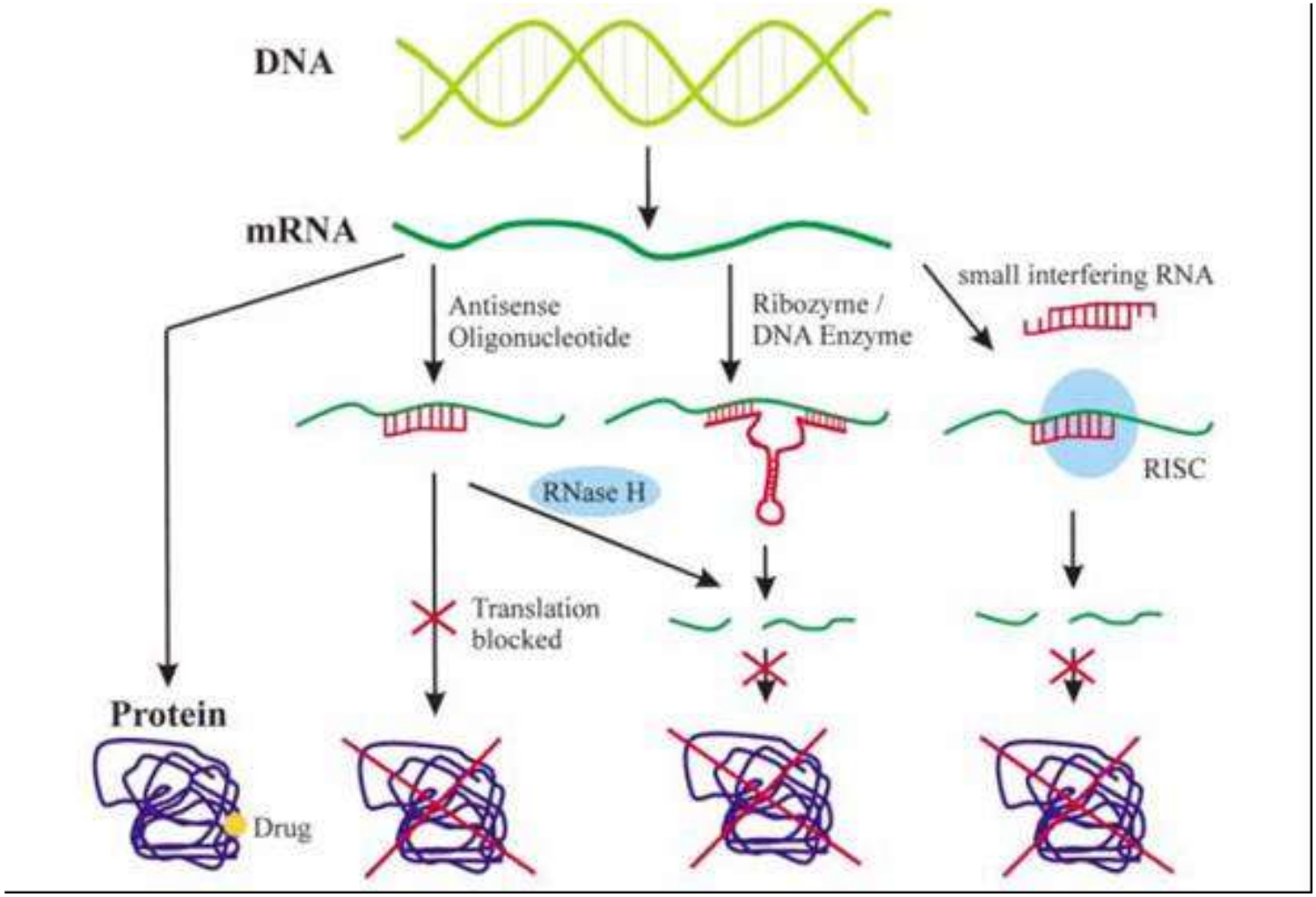


dsRNA production in bacteria

GFP-expressing strain fed on naive bacteria (b) and on bacteria expressing dsRNA corresponding to the gfp coding region

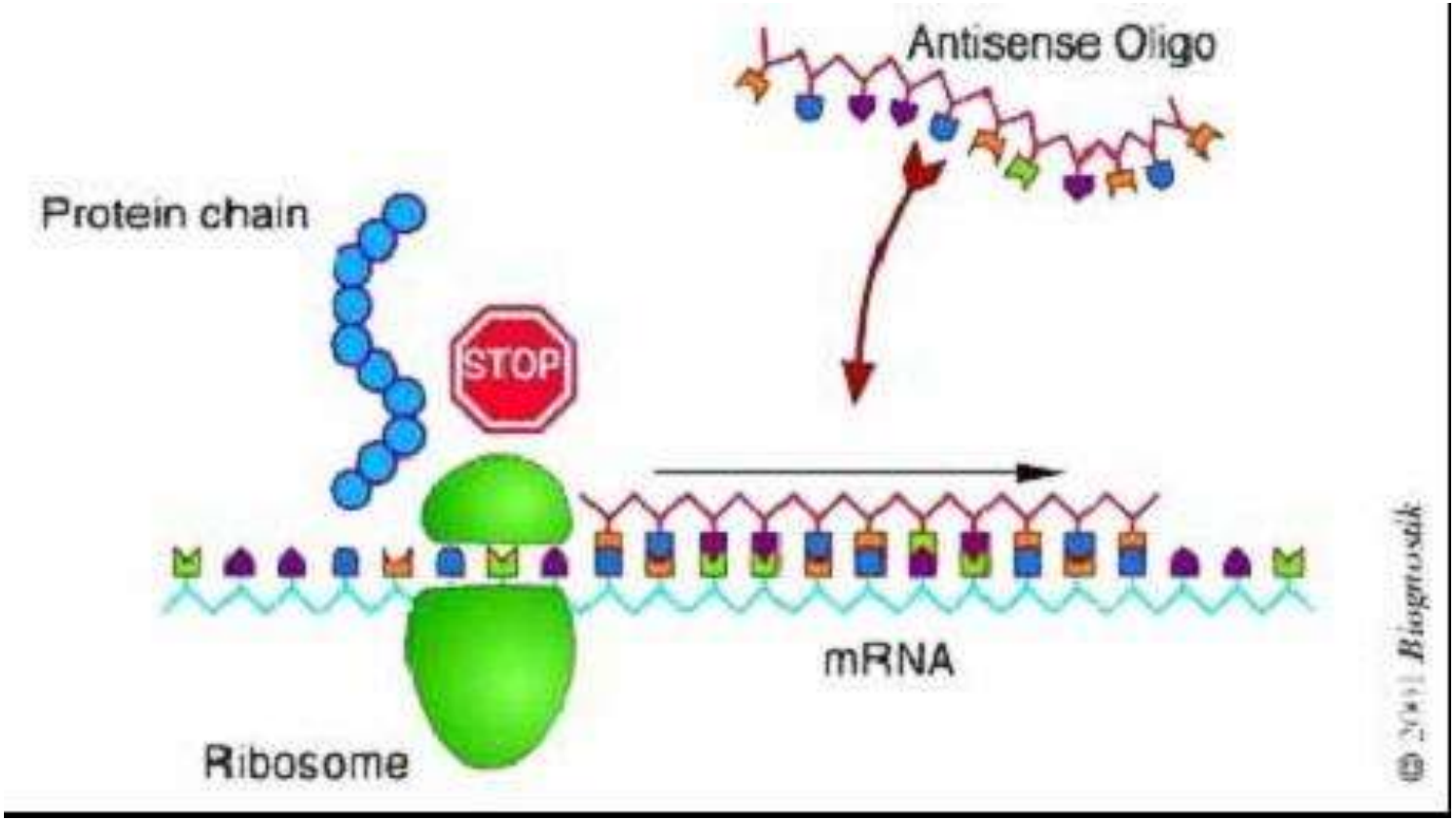
Timmons and Fire, Nature **395**. (1998)
Tabara et al, Science **282** (1998)

Anti-mRNA Strategies

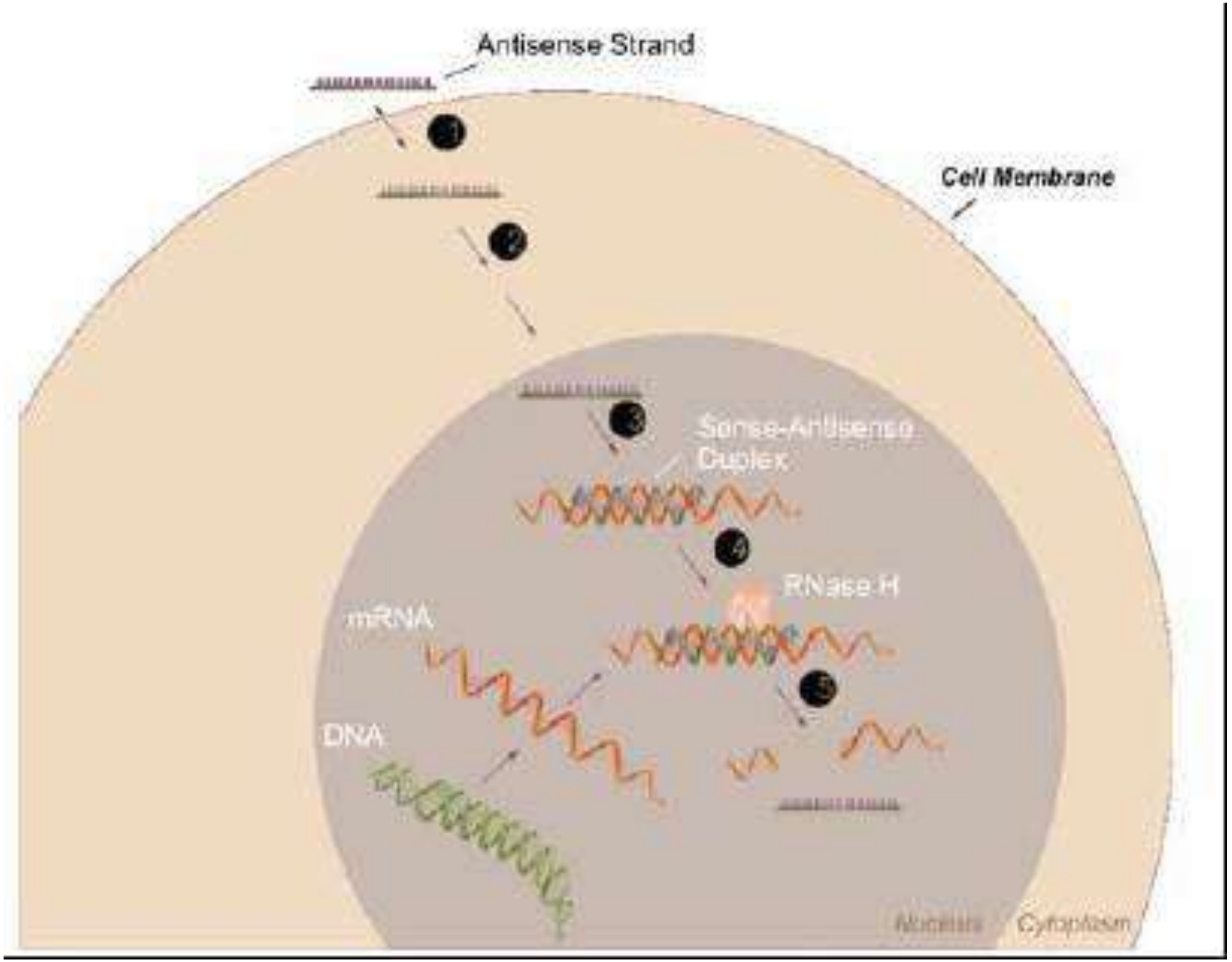


Mechanism of Action of Antisense Oligonucleotides.

1. Translational Arrest by Blocking the Ribosome.



2. Activation of RNase H

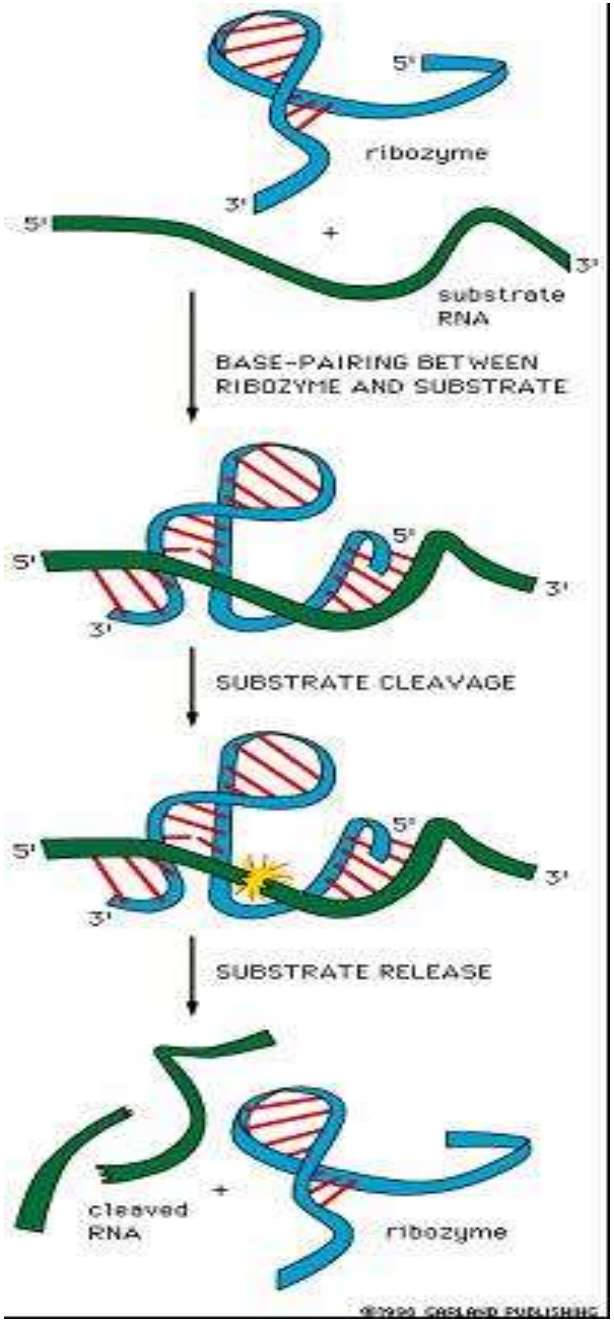


Ribozymes

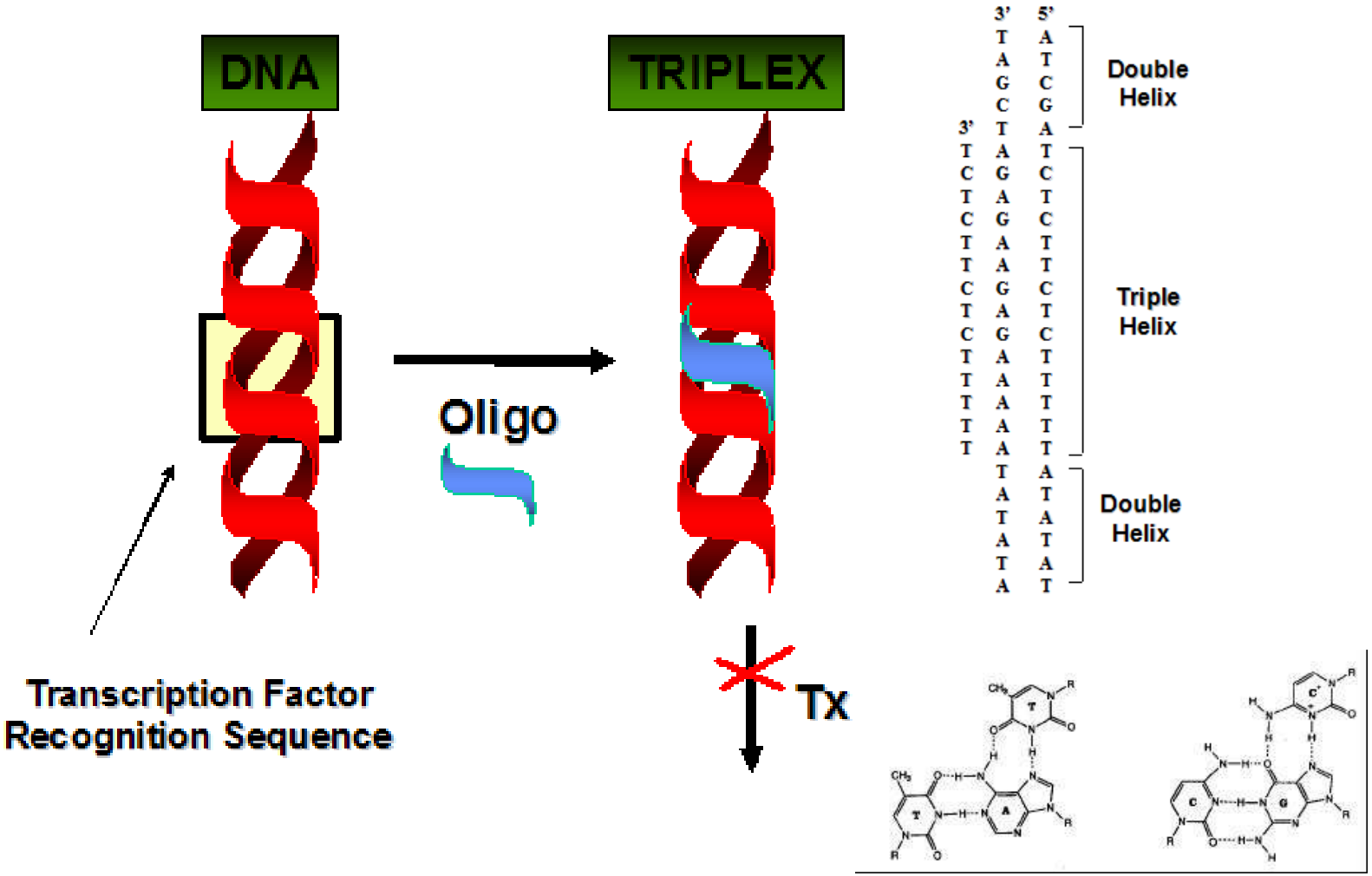
- Ribozymes are RNA molecules that catalyze biochemical reactions.

1982 T. Cech shows that the intron of one Tetrahymena pre-rRNA is self splicing. He proposes the term "ribozyme" to refer to catalytic RNA

- Ribozymes cleave single-stranded regions in RNA through transesterification or hydrolysis reactions that result in cleavage of phosphordiester bonds



Triplex Antisense Technology (ANTIGENE)



Limitations of Practical Applications of Antisense Oligonucleotides

Despite the simplicity of the idea behind the Antisense, several problems have to be overcome for successful application:

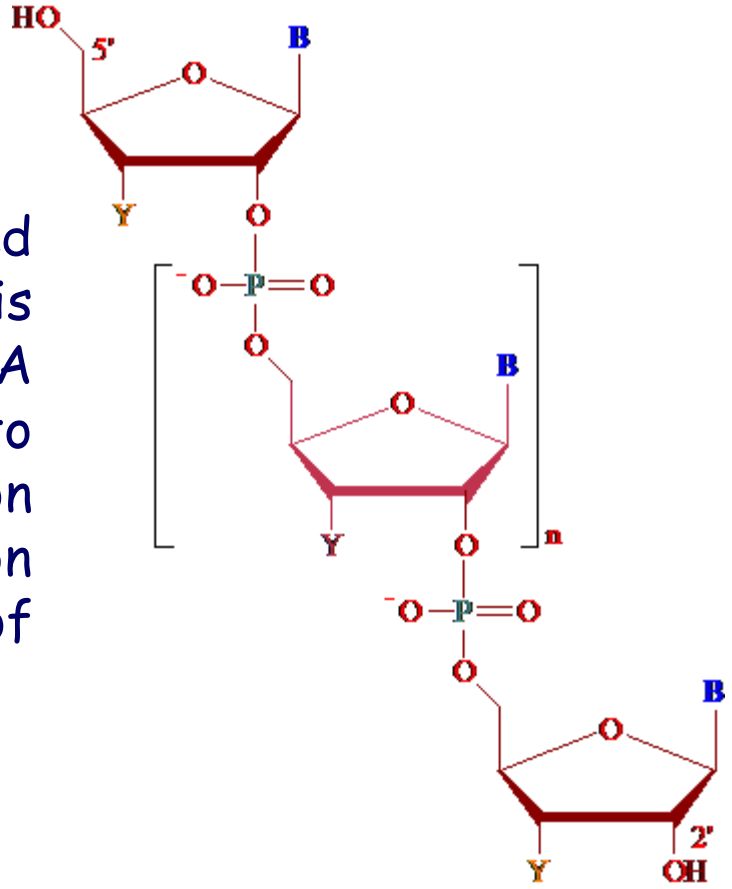
1. Accessible sites of the target RNA for oligonucleotide binding have to be identified.

2. Antisense agents have to be protected against nucleolytic attack.

3. Cellular uptake and correct intracellular localization.

Modification of Backbone Linkage Sites (2',5'-Oligonucleotides)

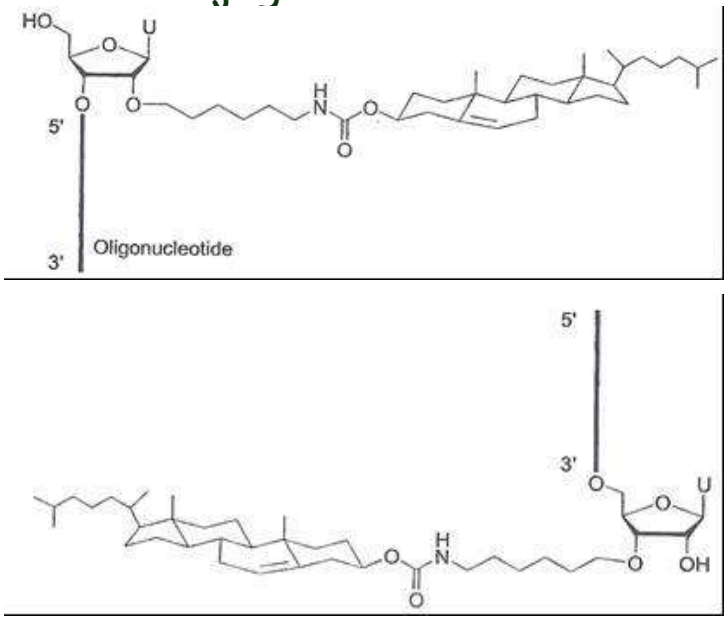
The 2′,5′-backbone modified oligonucleotide system is naturally occurring RNA isomers that are thought to be involved in the regulation of cell growth/differentiation and the antiviral effect of interferon.



Pendant (Conjugated) Oligonucleotides

Various molecules (pendants) have been attached (conjugated) to oligonucleotides to modify their pharmacokinetic properties.

The cholesterol conjugates have received the most attention.



The attachment at the 3'-O of the 3'-terminal nucleotide has been shown to provide greater nuclease resistance than the 2'-O of the 5'-terminal nucleotide.

Cell, Vol. 101, 25–33, March 31, 2000, Copyright ©2000 by Cell Press

RNAi: Double-Stranded RNA Directs the ATP-Dependent Cleavage of mRNA at 21 to 23 Nucleotide Intervals

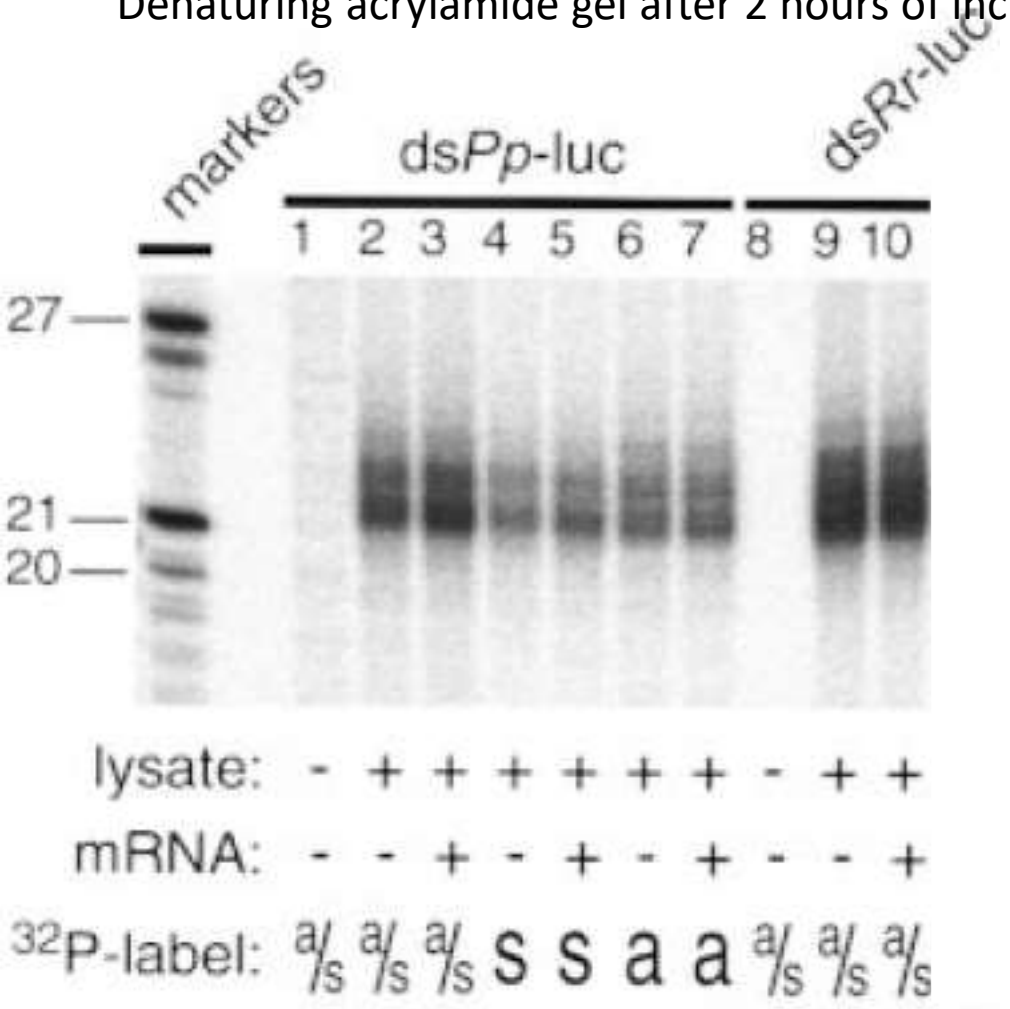
Phillip D. Zamore,*# Thomas Tuschl,†#
Phillip A. Sharp,‡§ and David P. Bartel§||

During the RNAi reaction, both strands of the dsRNA are processed to RNA segments 21-23 nucleotides in length.
Processing of the dsRNA to the small RNA fragments does not require the targeted mRNA.

The mRNA is cleaved only within the region of identity with the dsRNA. Cleavage occurs at sites 21-23 nucleotides apart, the same interval observed for the dsRNA itself, suggesting that the 21-23 nucleotide fragments from the dsRNA are guiding mRNA cleavage

21-22 nt RNA fragments are produced

Experiment: *Drosophila* cell lysate + labelled dsRNA (one or both strands).
Denaturing acrylamide gel after 2 hours of incubation.

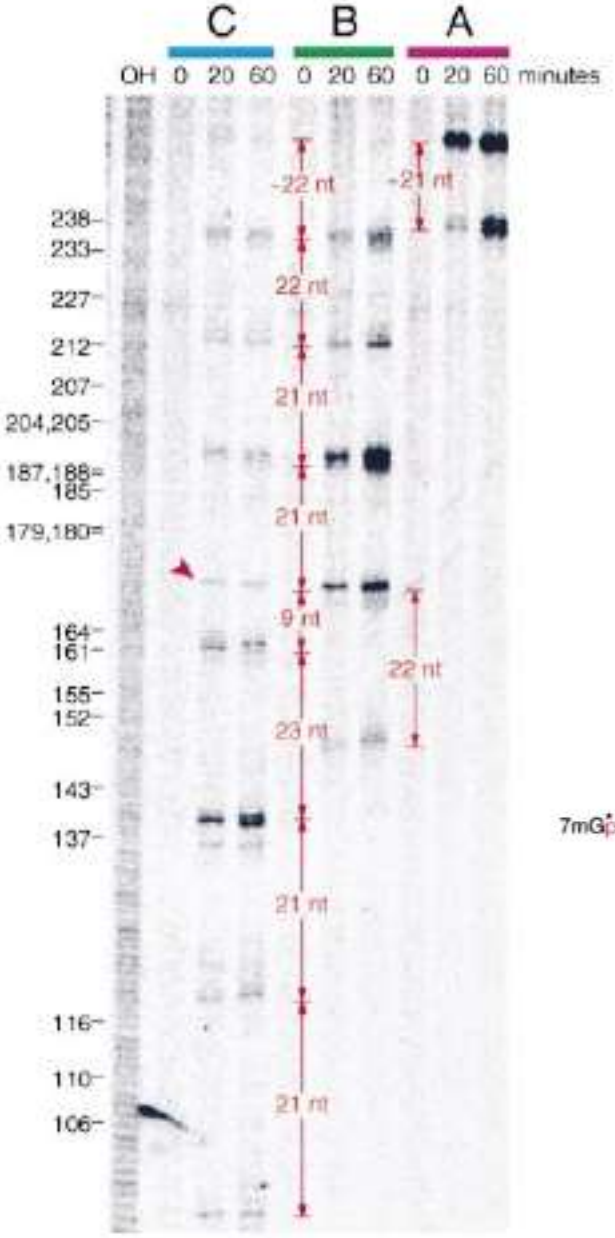


Results:

- 21-mer formation does not require the presence of the corresponding mRNA.
- both strands are equally processed.

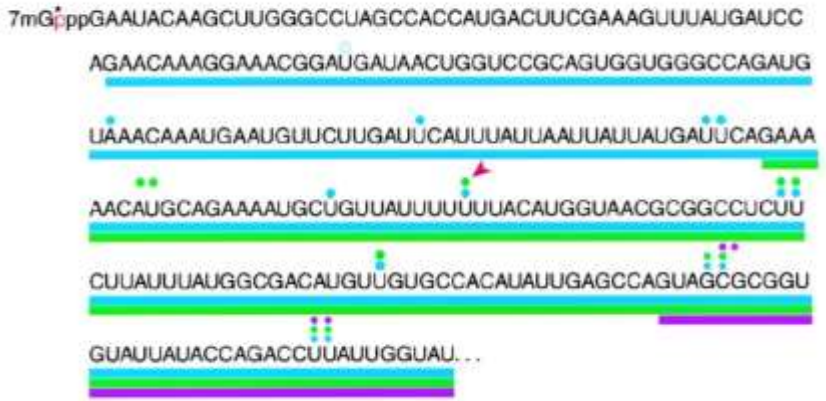
Zamore et al. Cell **101** (2000)

The traget mRNA is cleaved in 21-23 nt intervals



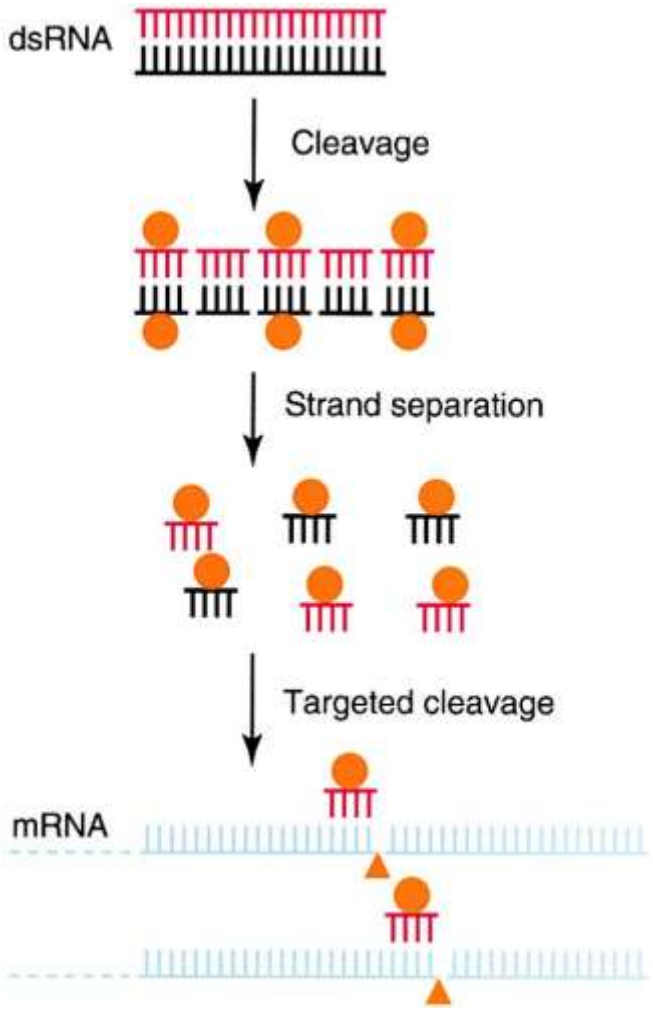
3 different target RNAs were tested (A, B, C).

Intermediate digestion products show differences of 21-23 nt.



Zamore et al. Cell 101 (2000)

A first model for the mechanism RNAi



Zamore et al, Cell, v101 pp25-33 (2000)

miRNA

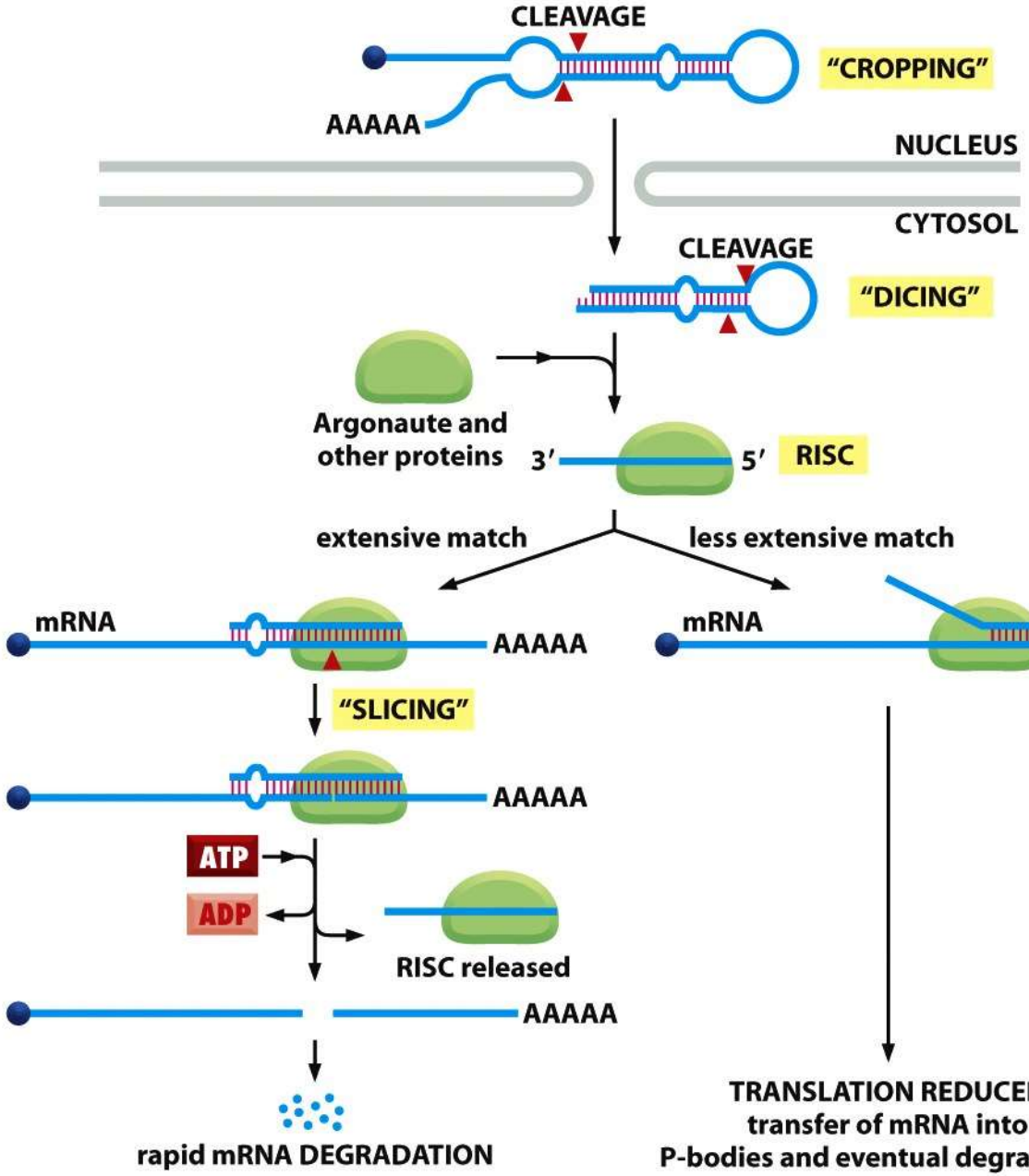
MicroRNAs (miRNAs) are genomically encoded non-coding RNAs that help regulate gene expression, particularly during development.

Mature miRNAs are structurally similar to siRNAs produced from exogenous dsRNA, but before reaching maturity, miRNAs must first undergo extensive post-transcriptional modification.

An miRNA is expressed from a much longer RNA-coding gene as a primary transcript known as a *pri-miRNA* which is processed, in the cell nucleus, to a 70-nucleotide stem-loop structure called a *pre-miRNA* by the microprocessor complex.

MicroRNAs (miRNAs) are endogenous approximately 22 nt RNAs that can play important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression.

miRNA processing and mechanism

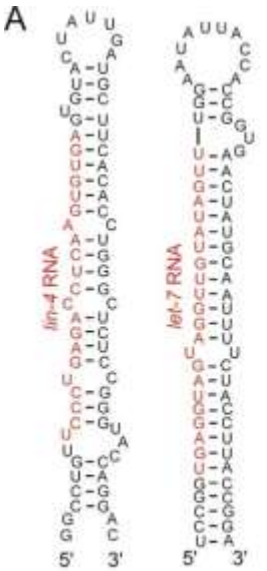


Precursor miRNA –complementarity
Between one part of sequence & another
Form double strand structure
in cytocol it cleaved by dicer enzyme
And form miRNA.
CONJUCATE WITH RNA INDUCED
SILENCING COMPLEX
In plants RNA:RNA match is extensive-
Cleaves the target m RNA and rapid
degradation

Less extensive does not slice the m RNA
TRANSLATION OF m RNA is repressed
Effect is associated with shortening of
Poly A tail
The destabilized m RNA moved to cytosol
Structure called processing bodies, which
Contains RNA and m RNA degrading enzymes

Figure 7-112 *Molecular Biology of the Cell* (© Garland Science 2008)

First miRNA
in *C.elegans*

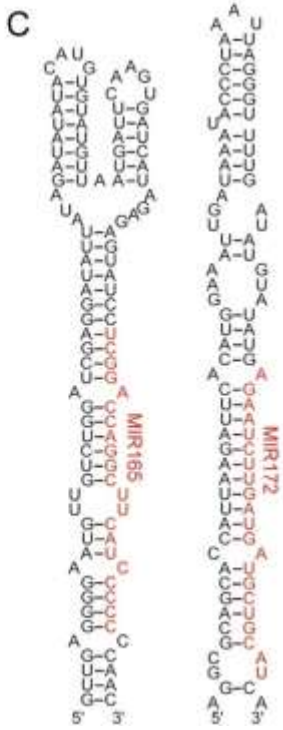


Human: 200-255 miRNA

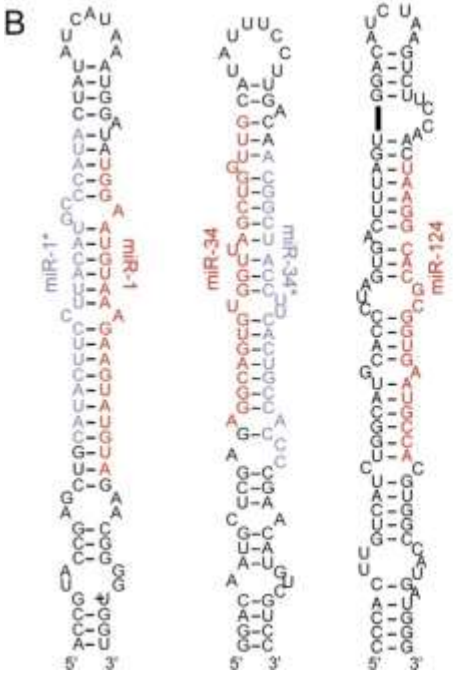
C.elegans: 103-120 miRNA

Drosophila: 96-124 miRNA

miRNA
in Plants



miRNA
in *C.elegans*
with homologs
In flies and human



Functions ??

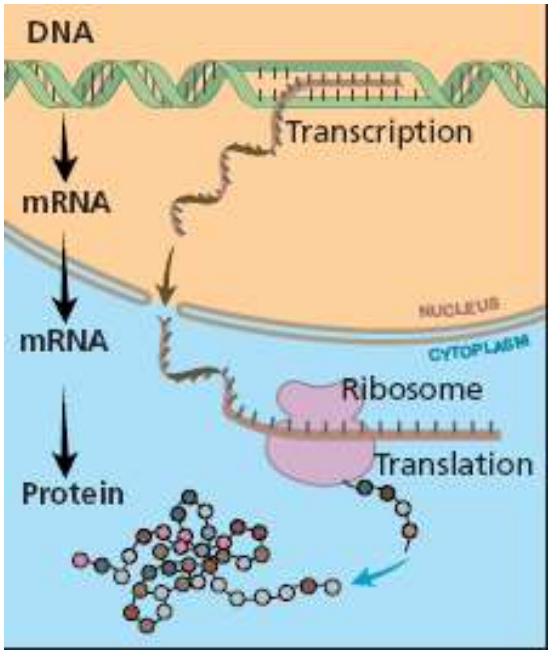
Table 1. MicroRNAs and Their Functions: Examples for which Phenotypic Consequences of Disrupted or Ectopic miRNA Regulation Are Known

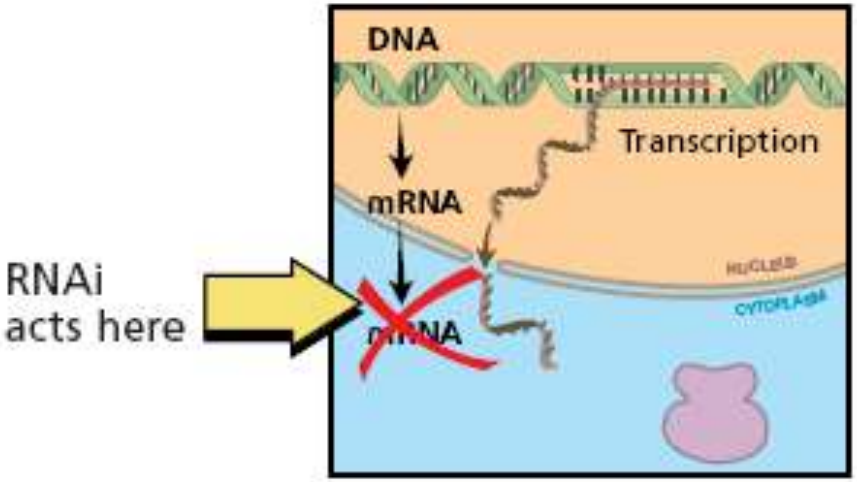
miRNA	Target Gene(s)	Biological Role of miRNA/Target Gene	Refs
Nematodes			
lin-4 RNA	*Ce lin-14* probable transcription factor	Timing of early larval developmental transitions	1,2
	Ce lin-28 cold shock domain protein	Timing of early larval developmental transitions	3
let-7 RNA	*Ce lin-41* probable RNA-binding protein	Timing of late larval developmental transitions	4,5
	Ce hbl-1 transcription factor	Timing of late larval developmental transitions	6,7
lsy-6 RNA	*Ce cog-1* transcription factor	Left/right asymmetry of chemoreceptor expression	8
Insects			
bantam miRNA	*Dm hid* pro-apoptotic protein	Apoptosis and growth control during development	9
miR-14	unknown	Apoptosis and fat metabolism	10
Mammals			
miR-181	unknown	Hematopoietic differentiation	11
Plants			
miR165/166	*At REV* and relelated transcription factors	Axial meristem initition and leaf development	12-14
miR172	*At AP2* and related transcription factors	Flower development; timing transition to flowering	15-18
miR-JAW	*At TCP4* and releated transcription factors	Leaf development, embryonic patterning	19
miR159	*At MYB33* and related transcription factors	Leaf development	12,15,19

Species abbreviations: *Caenorhabditis elegans*, *Ce*; *Drosophila melanogaster*, *Dm*; *Arabidopsis thaliana*, *At*.
1 (Lee et al., 1993); 2 (Wightman et al., 1993); 3 (Moss et al., 1997); 4 (Reinhart et al., 2000); 5 (Slack et al., 2000); 6 (Abrahante et al., 2003); 7 (Lin et al., 2003); 8 (Johnston and Hobert, 2003); 9 (Brennecke et al., 2003); 10 (Xu et al., 2003); 11 (Chen et al., 2004); 12 (Rhoades et al., 2002); 13 (Tang et al., 2003); 14 (Emery et al., 2003); 15 (Park et al., 2002); 16 (Kasschau et al., 2003); 17 (Chen, 2003); 18 (Aukerman and Sakai, 2003); 19 (Palatnik et al., 2003)

What is RNA interference (RNAi)?

- *"The Process by which dsRNA silences gene expression..."*
- Degradation of mRNA or translation inhibition



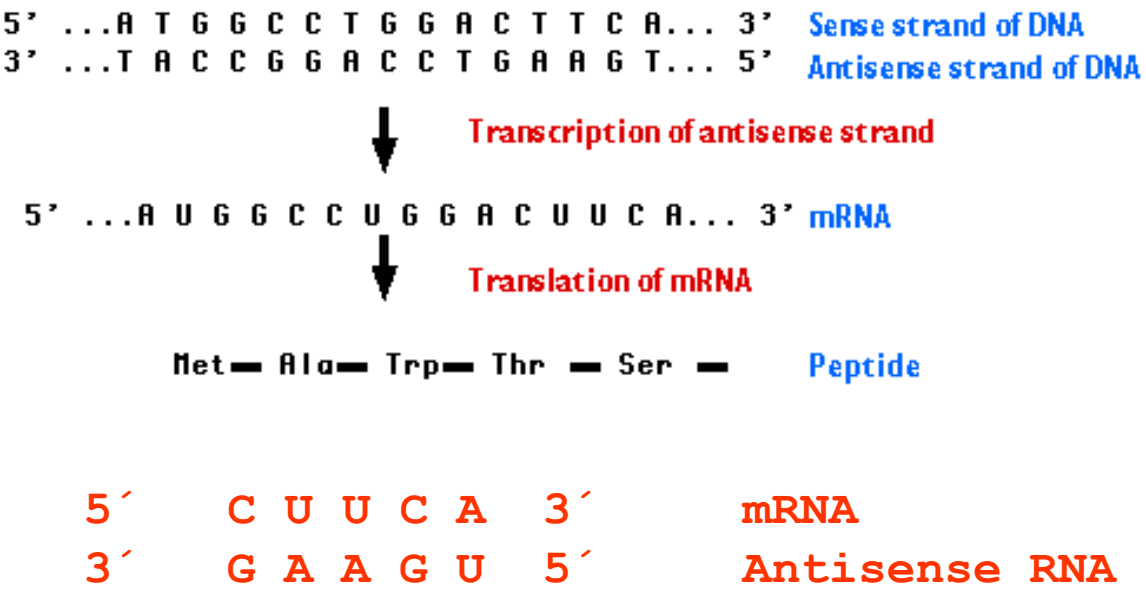


In RNA interference, RNA in double-stranded form breaks down the mRNA for a specific gene, thus stopping production of protein.

www.nobelprize.org

What are sense and antisense RNA?

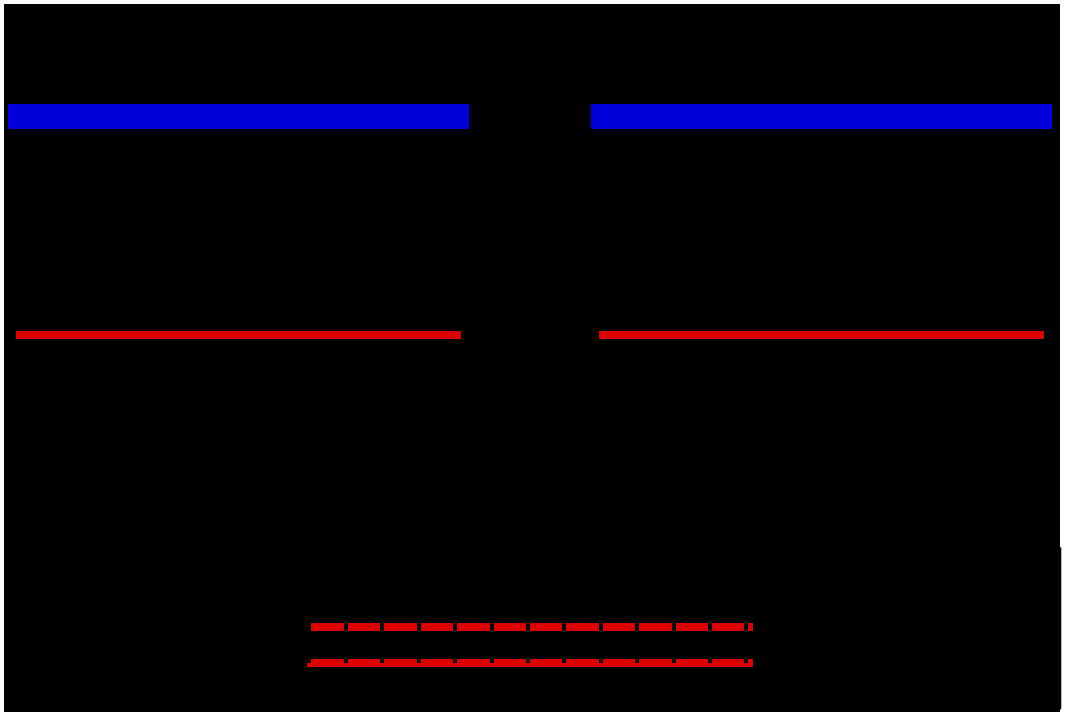
- Messenger RNA (mRNA) is single-stranded, called "sense" because it results in a gene product (protein).



What are sense and antisense RNA?

- Antisense molecules interact with complementary strands of nucleic acids, modifying expression of genes.

```
5´   C U U C A  3´     mRNA
3´   G A A G U  5´     Antisense RNA
```



RNAi terms

- dsRNA: double stranded RNA, longer than 30 nt
- miRNA: microRNA, 21-25 nt.
  - Encoded by endogenous genes
- siRNA: small-interfering RNA, 21-25 nt.
  - Mostly exogenous origin

RNAi like phenomena

- Plants
  - Petunias
- Fungi
  - *Neurospora*
- Animals
  - *Caenorhabditis elegans*

Alternate terms to RNAi

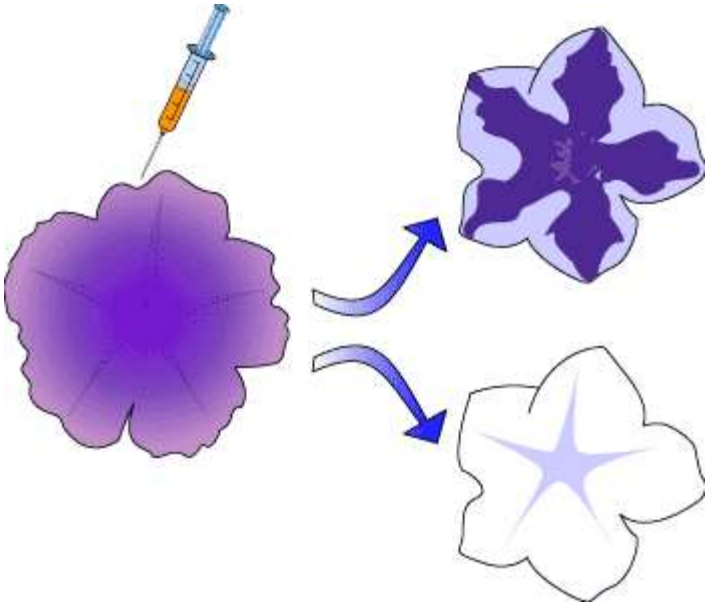
- PTGS (Posttranscriptional Gene Silencing)
- Cosuppression
- Quelling
- Virus-induced gene silencing

1990-Petunias

- Napoli et al. defined an RNAi-like phenomenon and called it "*cosupression*
- chalcone synthase (CHS), a key enzyme in flavonoid biosynthesis, the rate-limiting enzyme in anthocyanin biosynthesis, responsible for the purple coloration.

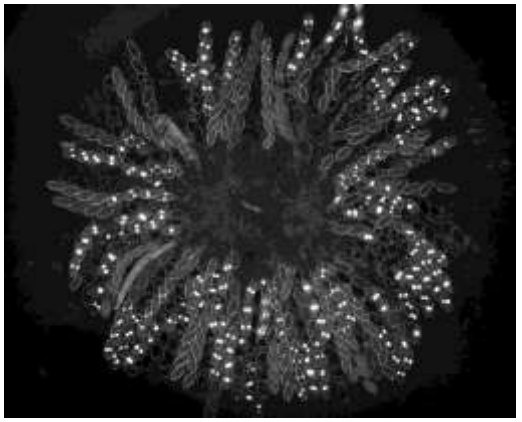
Overexpression of chalcone synthase in petunias unexpectedly resulted in white petunias

- The levels of endogenous as well as introduced CHS were 50-fold lower than in wild-type petunias, which led the authors to hypothesize that the introduced transgene was "**<u>cosuppressing</u>**" the endogenous CHS gene.



http://www.scq.ubc.ca/?p=265

1992-The mold

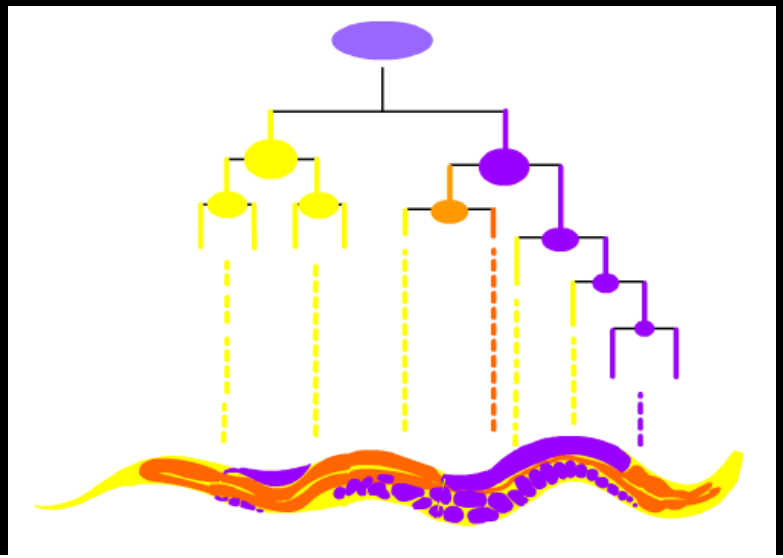


A rosette of the asci

- Carlo Cogoni and Guiseppe Macino of the Università di Roma La Sapienza in Italy introduced a gene needed for carotenoid synthesis in the mold *Neurospora crassa:*
  - The introduced gene led to inactivation of the mold's own gene in about 30% of the transformed cells. They called this gene inactivation "**quelling**."

1995-The worm

- Guo and Kemphues studied par-1 gene during embryogenesis
- The worm, *C. elegans*
  - has a fixed lineage: hypodermis, intestine, gonads
  - asymmetric divisions



The Laureates

Andrew Fire, born in 1959, is a US citizen. Since 2003 he has been professor of Pathology and Genetics at Stanford University School of Medicine, Stanford, California, USA.

In 1983 he took his PhD in Biology at the Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. He began his research on the nematode *C. elegans* during his time as visiting scientist in Cambridge, England, at the laboratory of Sydney Brenner (Nobel Laureate 2002). When Fire and Mello made their key discoveries about RNA interference, Fire was working at the Carnegie Institution of Washington.

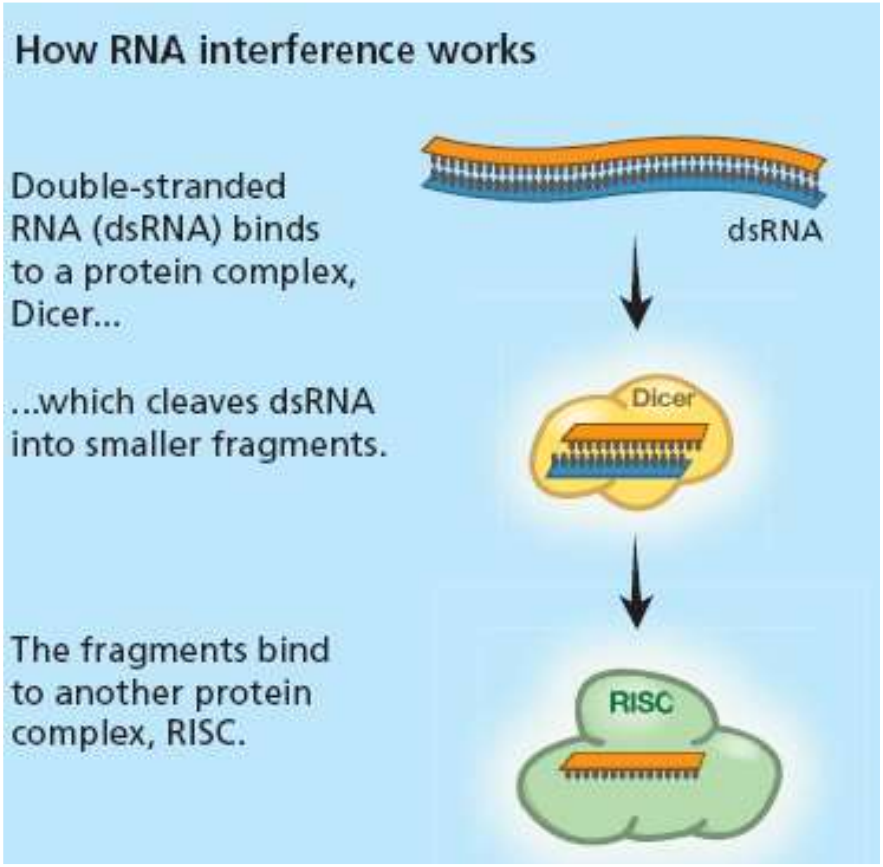
L. CICERO/STANFORD

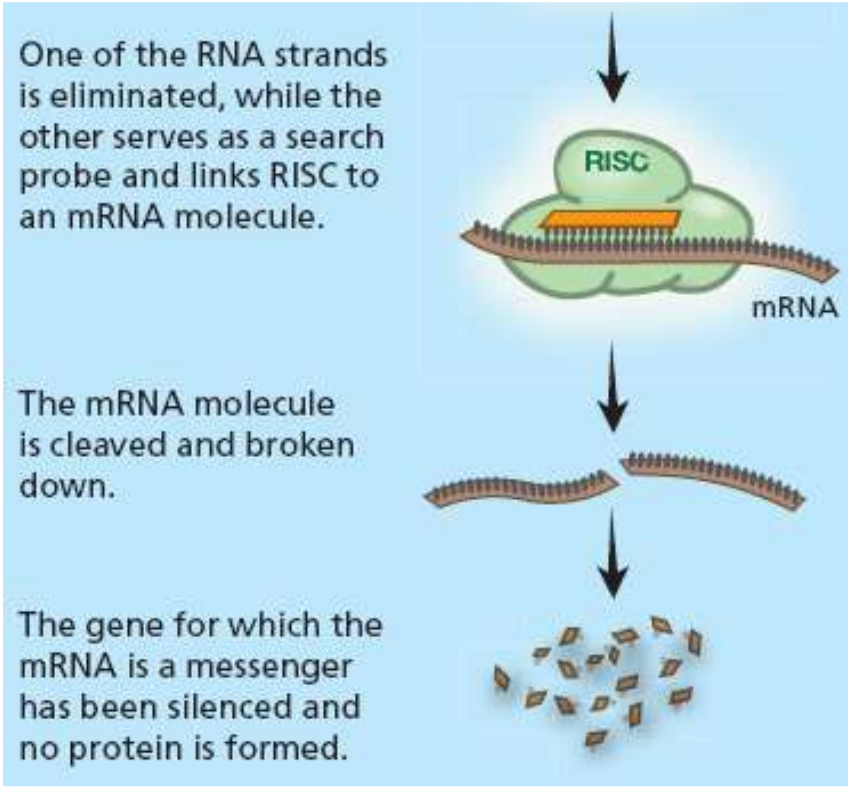
R.CARLIN/UMMAS

Craig Mello, born in 1960, is a US citizen and a professor of Molecular Medicine. Since 1994 he has worked within the Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA. He is also a Howard Hughes Medical Institute Investigator.

In 1990 he took his PhD in Cellular and Developmental Biology at Harvard University, Boston, Massachusetts. Before he moved to the University of Massachusetts Medical School in Worcester, he worked at the Fred Hutchinson Cancer Research Center.

How does RNAi work?

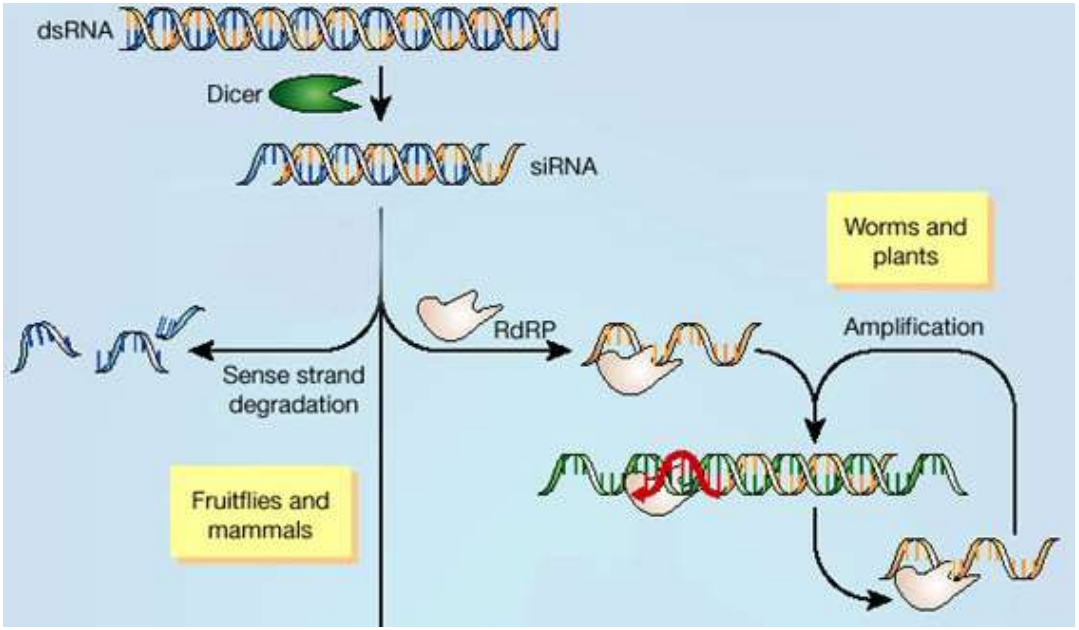




www.nobelprize.org

siRNA biogenesis

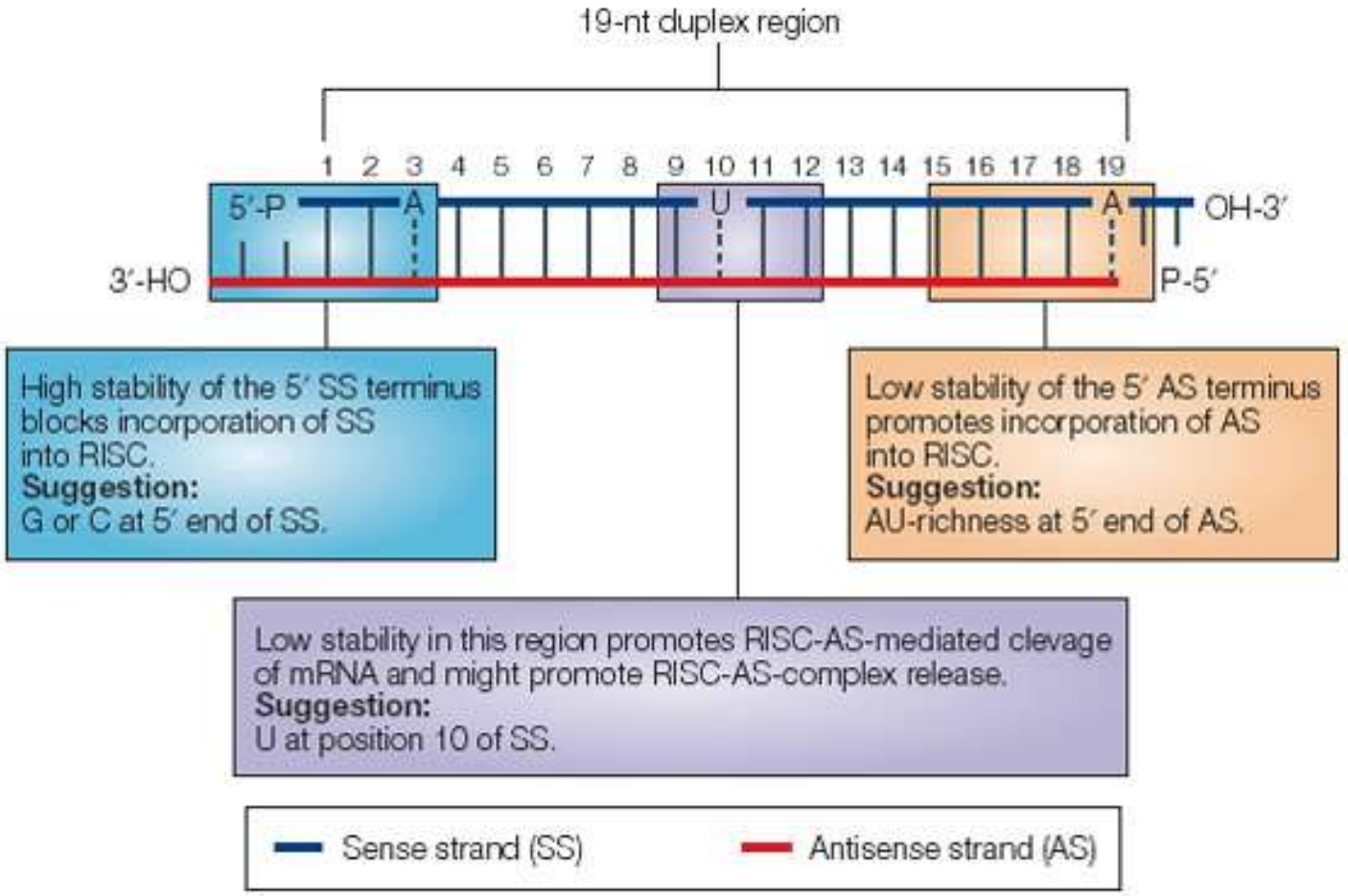
- Dicer (type III RNAse III) cleaves long dsRNA into siRNA 21-25nt dsRNA from exogenous sources
  - Symmetric 2nt 3’ overhangs, 5’ phosphate groups
  - Evidence for amplification in C. elegans and plants



RNA Induced Silencing Complex (RISC)

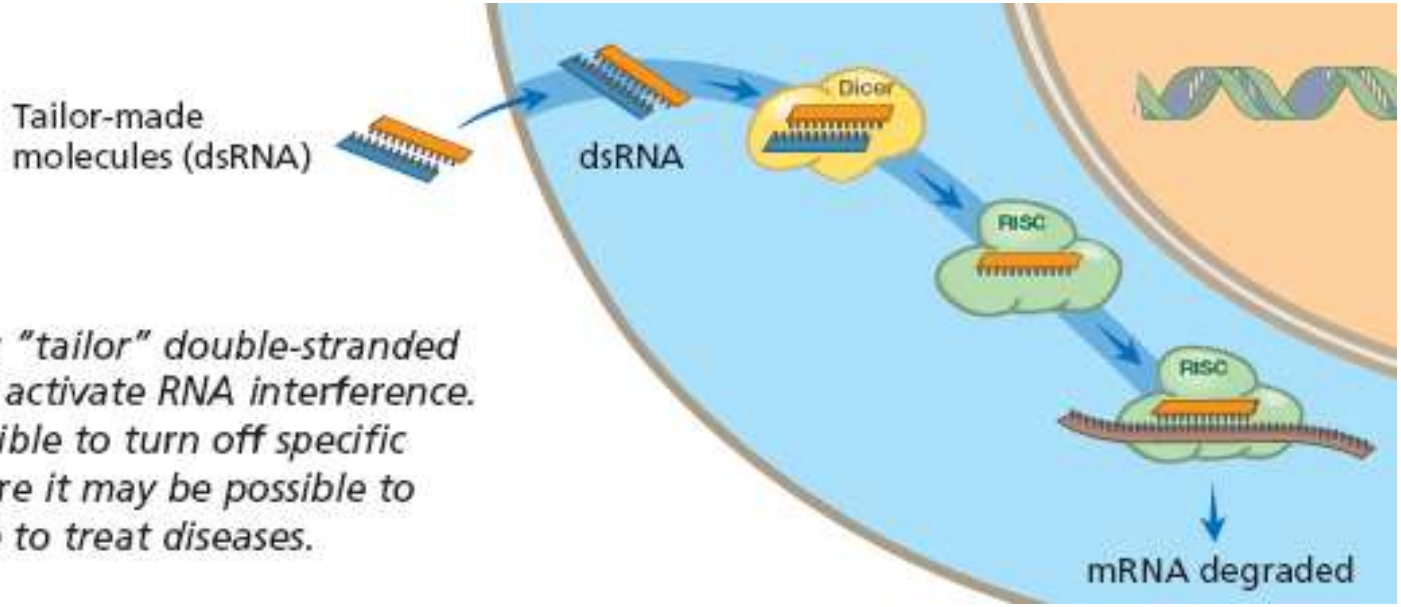
- RNAi effector complex
- Preferentially incorporates one strand of unwound RNA [Khvorova et al., 2003]
  - Antisense
- How does it know which is which?
  - The strand with less 5’ stability usually incorporated into RISC [Schwarz et al., 2003]

siRNA design



Mittal, 2004

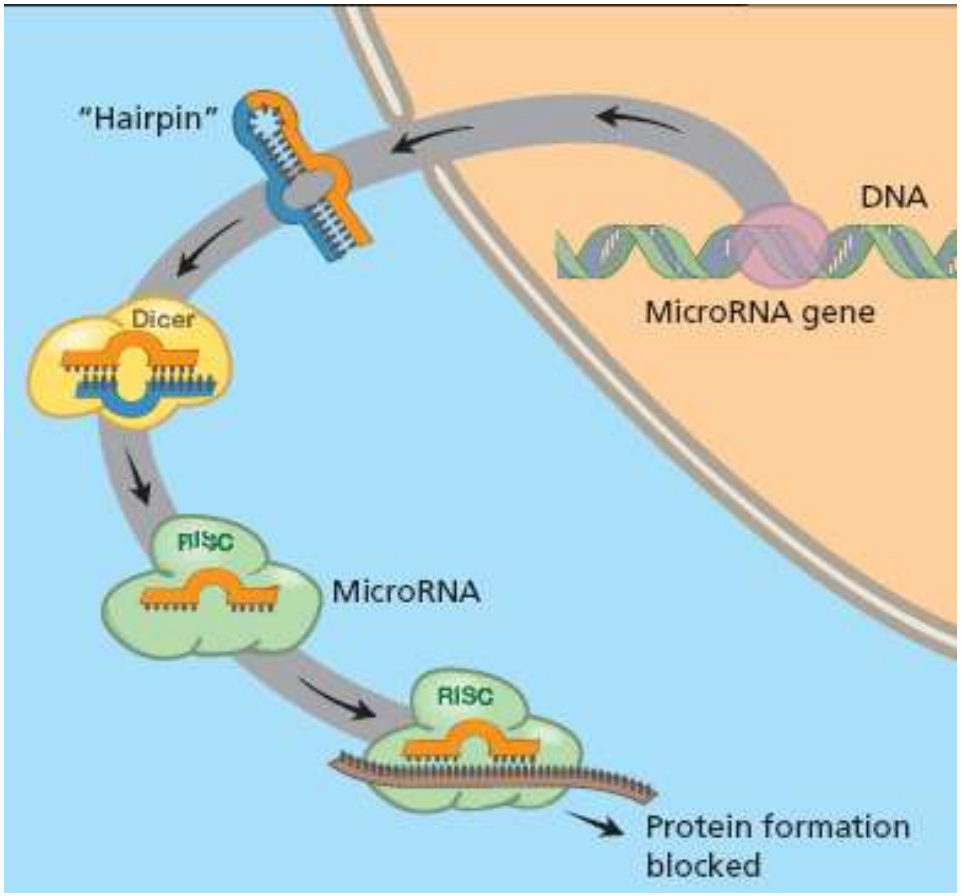
Custom-made siRNAs



Scientists can now "tailor" double-stranded RNA molecules to activate RNA interference. This makes it possible to turn off specific genes. In the future it may be possible to use this technique to treat diseases.

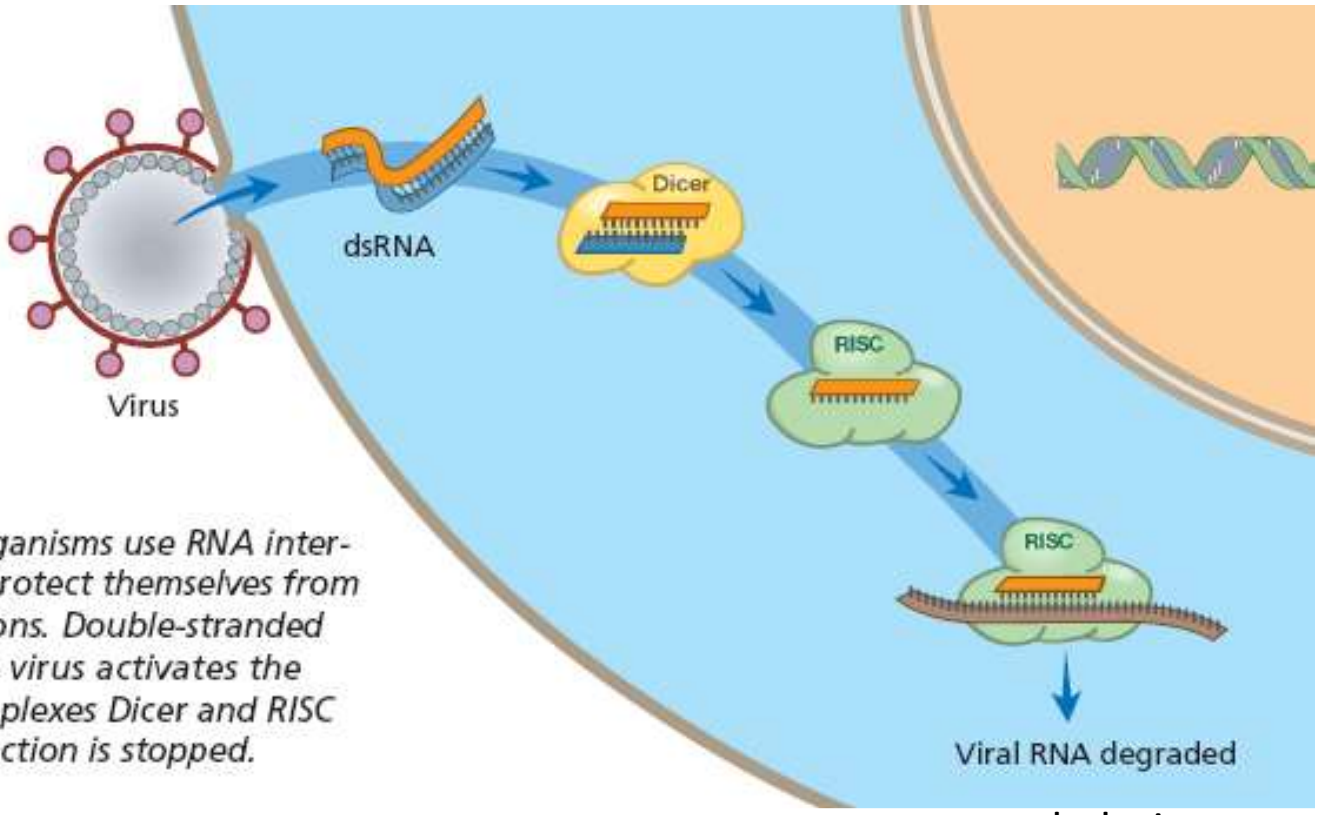
Endogenous RNAi-miRNA

- We have hundreds of different genes that encode small RNA (collectively, microRNA) whose precursors can form double-stranded RNA. These can activate the RNA interference process and thus switch off the activity of various genes with matching segments.
- First miRNA is lin-4



www.nobelprize.org

Defense Against Viruses



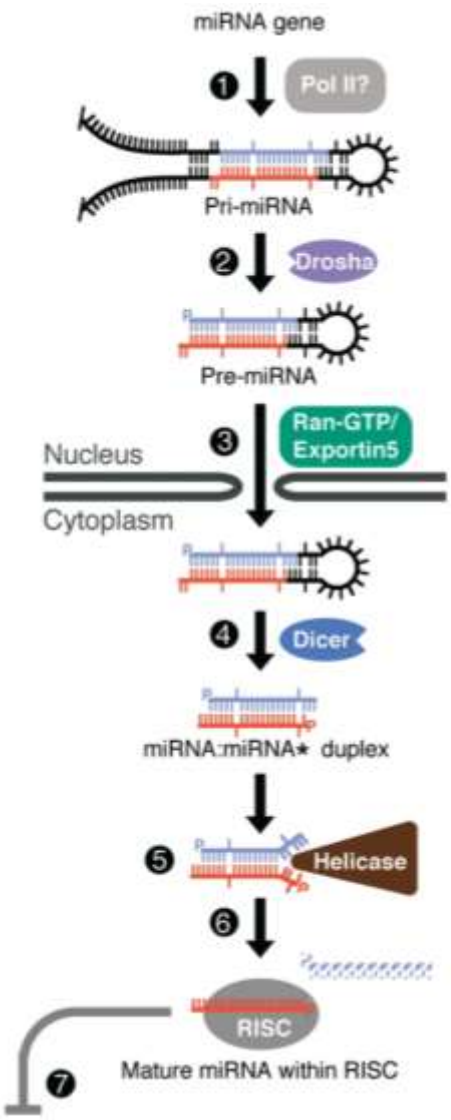
Primitive organisms use RNA interference to protect themselves from virus infections. Double-stranded RNA from a virus activates the protein complexes Dicer and RISC and the infection is stopped.

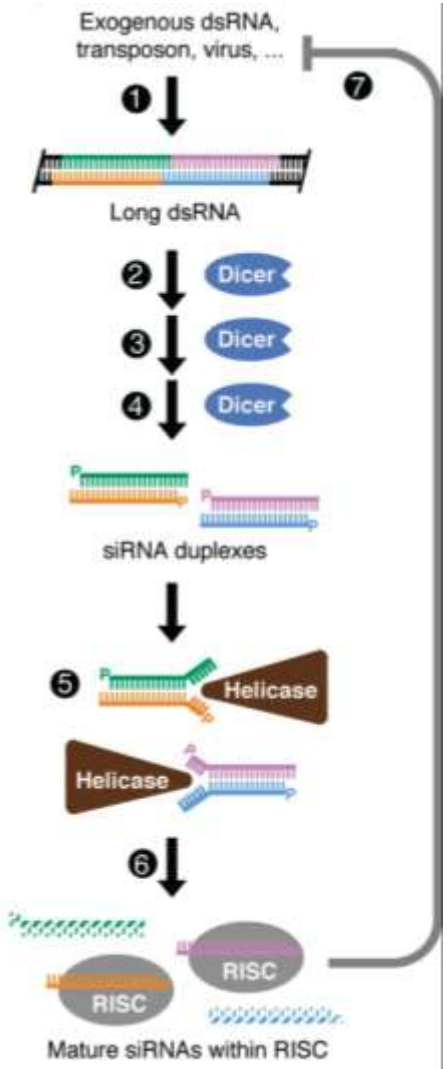
www.nobelprize.org

- **Indeed, Baulcombe, Vance, and others have shown that, in the continuing evolutionary war to survive and reproduce, plant viruses have evolved genes that enable them to suppress silencing.**

miRNA

siRNA





A. miRNAs are produced by the successive actions of two RNAseIII ribonucleases:

Drosha (RNAse) and Pasha (sdRNA binding protein) act as a heterodimeric complex in the nucleus.

The resulting pre-miRNA is then exported into the cytoplasm where it is cleaved by Dicer.

The miRNA duplex must be unwound and the miRNA strand be incorporated into RISC.

B. long dsRNA is a substrate for Dicer but not for Drosha. Dicer cut two times to yield the siRNA duplex.

The guide strand is incorporated into RISC.

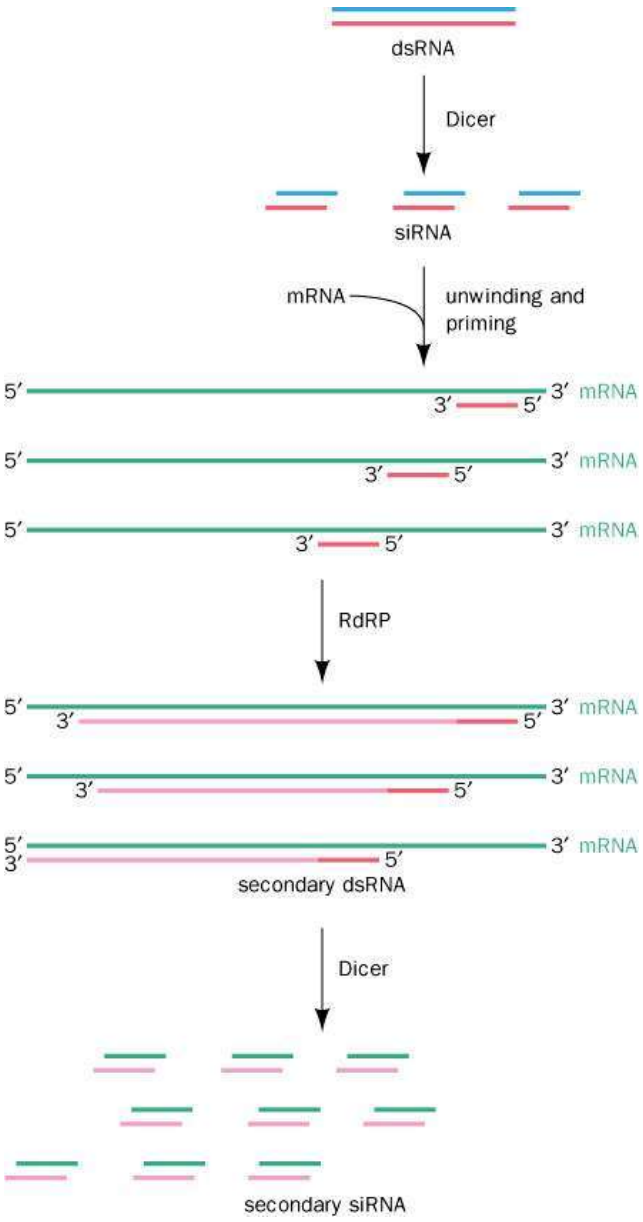
Nobel Prize Physiology- 2024

For the discovery of microRNA and its role in post-transcriptional gene regulation

1.Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843-854. doi:10.1016/0092-8674(93)90529-y

2.Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin14 by lin-4 mediates temporal pattern formation in C. elegans. Cell. 1993;75(5):855-862. doi:10.1016/0092-8674(93)90530-4

A model for transitive RNAi



1) siRNA resulting from the action of Dicer is unwound and binds to its target mRNA.

2) There it acts as a primer for RNA-dependent RNA polymerase (RdRP).

3) 5'-3' extension.

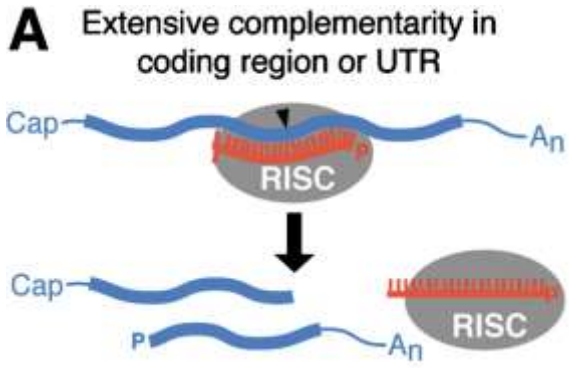
4) The resulting secondary dsRNA is then cleaved by Dicer to form secondary siRNAs.

→ amplification of the siRNA
→ genes that are located 5' to the initial target gene may also be silenced.

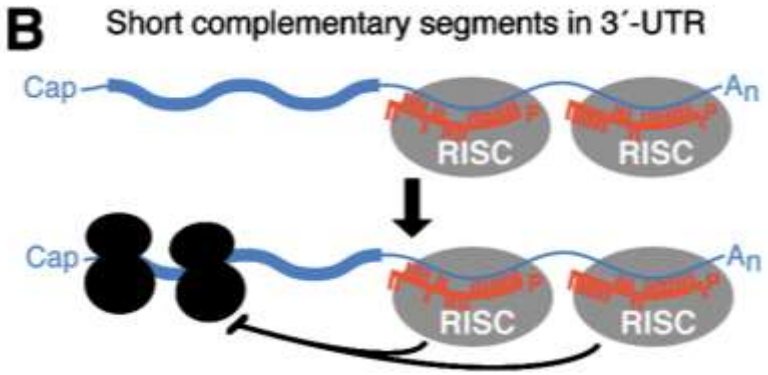
The effect is limited to 300-500 nt 5' to the initial target site.

Voet 31-69

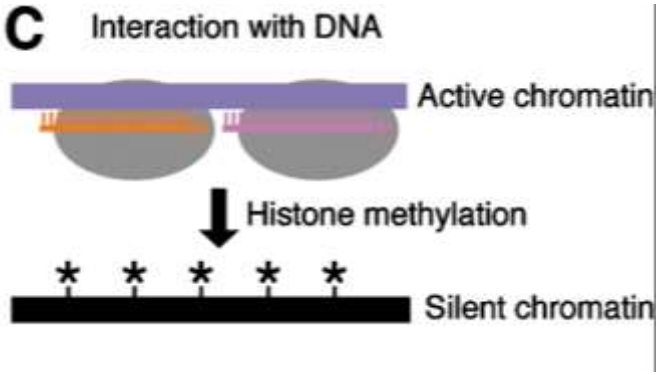
Post-transcriptional
Cleavage of mRNA



Translational repression
of the mRNA



Transcriptional
silencing



Designing siRNA

- The target sequence should be 50-100 bp downstream of start codon or in the 3’ UTR
- Search for a 23nt long sequence
- Ensure that your target sequence is not homologous to any other genes
- Avoid more than three guanosines or three cytosines in a row
- avoid stretches of > 4 T's or A's
- secondary structure of the target mRNA does not appear to have a strong effect on silencing
- Designing several siRNA’s helps to find a highly efficient one

Example for siRNA's

CEP

targeted region (cDNA): 5' AACTGGACTTCCAGAAGAACATC

sense siRNA: 5' CUGGACUUCCAGAAGAACAdt

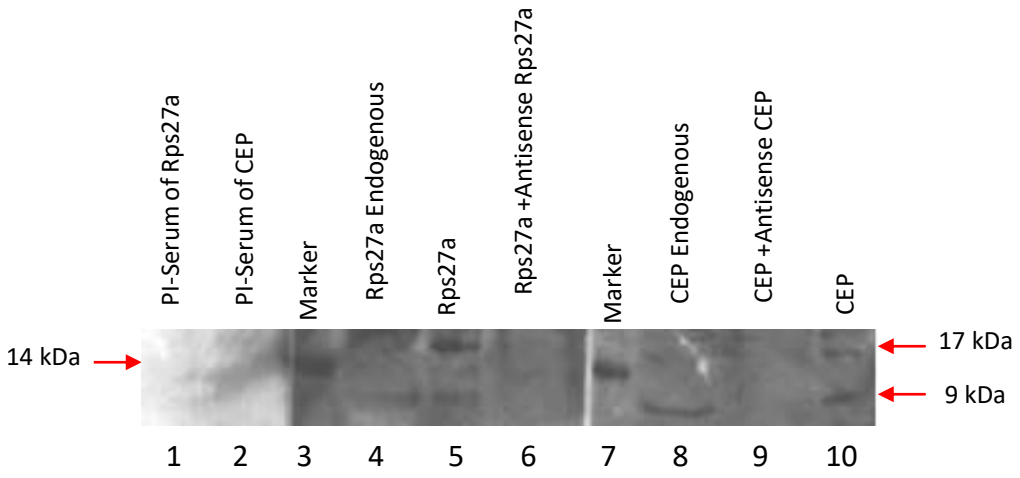
antisense siRNA: 5' UGUUCUUCUGGAAGUCCAGdt

RPS27a

targeted region (cDNA): 5' AACGTACGCGGAATACTTCGATT

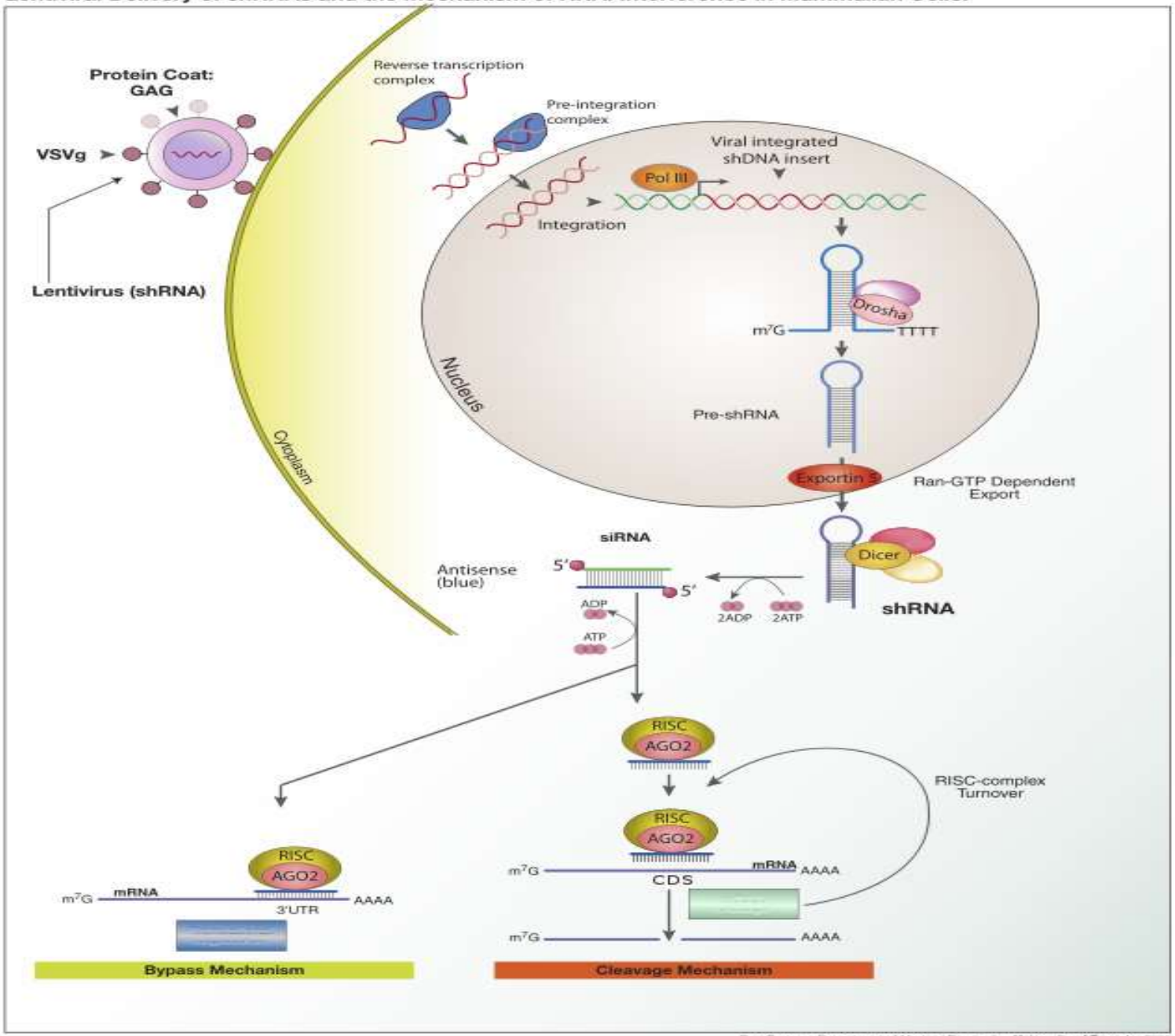
sense siRNA: 5' CGUACGCGGAAUACUUCGAdTdT

antisense siRNA: 5' UCGAAGUAUUCCGCGUACGdTdT



Detection of Rps27a and CEP expression in Huh7 cell lines. Total cells were lysed after 48hr posttransfection with Rps27a (lanes 5), anti-Rps27a (lanes 6), CEP (lanes10) and anti-CEP (lanes 9).

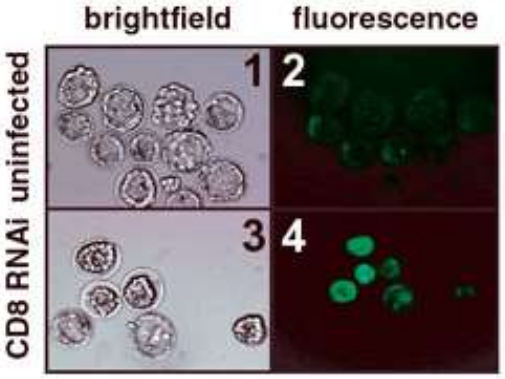
Lentiviral Delivery of shRNAs and the Mechanism of RNAi Interference in Mammalian Cells.



Dan Cojocari, Department of Medical Biophysics, **University of Toronto** 2010

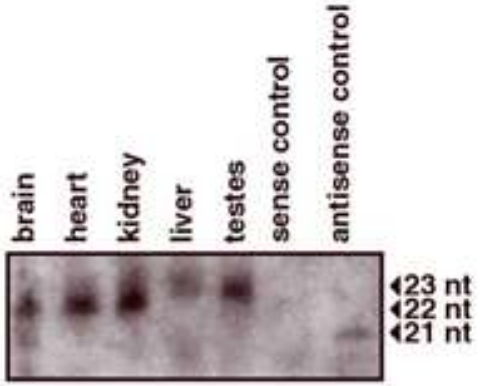
Functional silencing of genes in mice by Lentivirus-infection

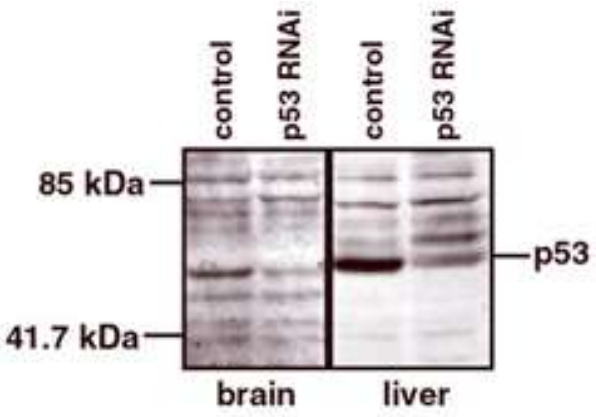
Generation of lentivirus infected zygotes



Silencing of p53:

Tissue was harvested from 8-wk-old mices





Application of Antisense Oligonucleotides

Functional Genomics and Target Validation:

Antisense oligonucleotides can be used to selectively manipulate the expression of chosen gene or genes. The process results in :

- A pharmacophore with a well-understood mechanism of action.

- Well characterized distribution and a safe side effect profile which could be used as a human therapeutic.

Application of Antisense Oligonucleotides

Potential Therapeutic Applications of Antisense Oligonucleotides

- A wide variety of potential therapeutic applications of antisense oligonucleotides has been reported in the last few years.
- Major areas of these therapeutic applications include:

2.1. Antiviral

2.2. Antibacterial

2.3. CNS Therapeutics: Antisense Oligonucleotides will address unmet medical needs for CNS diseases.

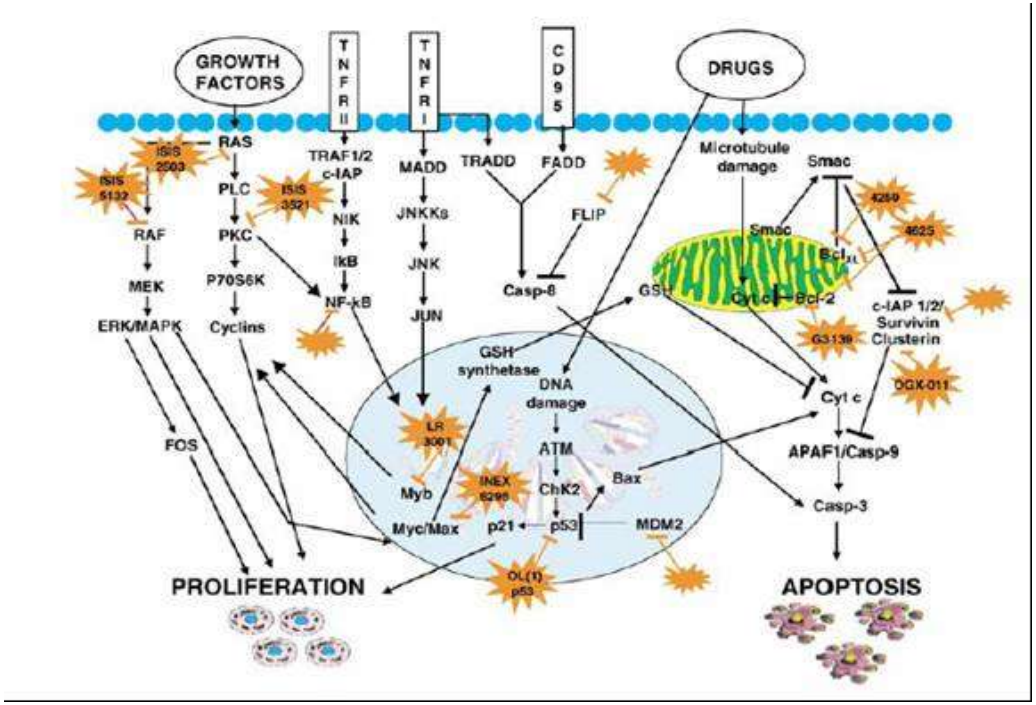
2.4. Inflammation Therapeutics: e.g. Colitis, Lupus, Lung inflammation, Skin inflammation, Transplantation rejection, Reperfusion injury, Rheumatoid Arthritis and Ocular disease.

2.5. Cardiovascular Therapeutics: e.g. prevention of restenosis, myocardial infarction, rejection in heart transplantation, hypertension and atherosclerosis.

2.6. Regulation of Apoptosis: which will address treatment of cancer, psoriasis,fibrosis, atherosclerosis, restenosis and others

2. Potential Therapeutic Applications of Antisense Oligonucleotides

2.7. Anticancer:



- The most recent antisense application as therapeutic tool is aimed to treat the SARS and bird Flu

RNA interference in biotechnology

Engineering food plants.

For example, cotton seeds are rich in dietary protein but naturally contain the toxic terpenoid product gossypol, making them unsuitable for human consumption.

RNAi has been used to produce cotton stocks whose seeds contain reduced levels of delta-cadinene synthase, a key enzyme in gossypol production, without affecting the enzyme's production in other parts of the plant, where gossypol is important in preventing damage from plant pests.

Similar efforts have been directed toward the reduction of the cyanogenic natural product linamarin in cassava plants.

Clinical Trials of Antisense Oligonucleotides

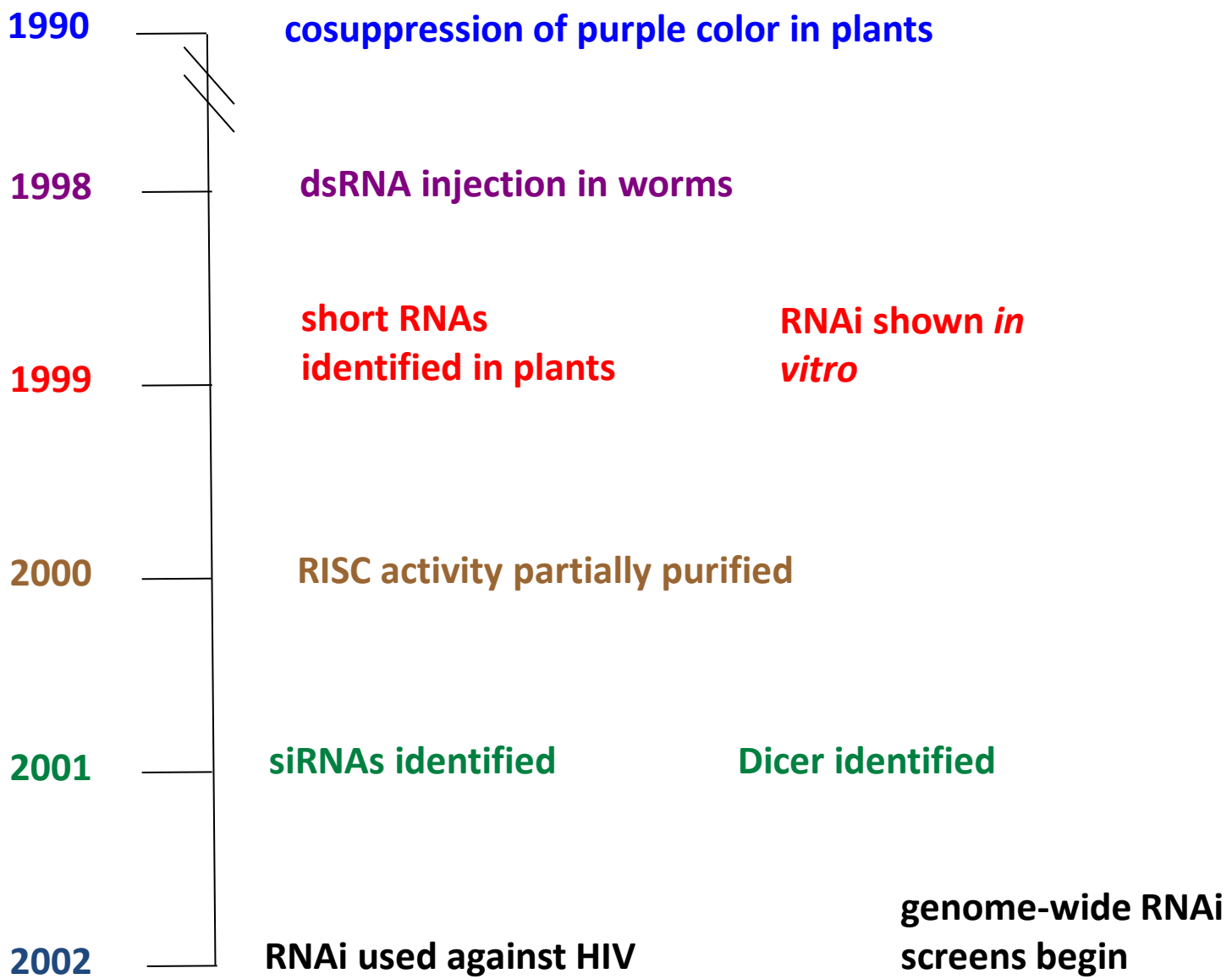
Table 4: Antisense Compounds Approved or in Clinical Trials (completion based on references 7,12,33,205)

Product	Company	Sequence	Chemistry	Target	Disease	Rout of Administration	Status (Phase)
Vitravene™	Isis/Novartis	GCGTTTGCTCTTCTTCTTGCG	PS	IE2	CMV Retinitis	Intravitreal	On Market
Affintak™	Isis/Lilly	GTTCTCGCTGGTGAGTTTCA	PS	PKC-α	Cancer–NSCLC, others	Parenteral	III
Alicaforsen™	Isis	GCCCAAGCTGGCATCCGTCA	PS	ICAM-1	Crohn's Disease	Parenteral	III
ISIS 2302	Isis	GCCCAAGCTGGCATCCGTCA	PS	ICAM-1	Topical Psoriasis	Topical	II
ISIS 2302	Isis	GCCCAAGCTGGCATCCGTCA	PS	ICAM-1	Ulcerative Colitus	Enema	II
ISIS 2503	Isis	TCCGTCATCGCTCCTCAGGG	PS	H-ras	Cancer-pancreatic, others	Parenteral	II
ISIS 14803	Isis/Elan	GTGCTCATGGTGCACGGTCT	PS-DNA	Antiviral	Hepatitis C	Parenteral	II
ISIS 104838	Isis/Elan	GCTGATTAGAGAGAGGTCCC	2nd Gen. Chimeric PS	TNF-α	Rheumatoid Arthritis	Parenteral/Oral	II
ISIS 104838	Isis	GCTGATTAGAGAGAGGTCCC	2nd Gen. Chimeric PS	TNF-α	Psoriasis	Topical	II
OGX-011	Isis	N/A	N/A	Clusterin	Cancer	Parenteral	I
Genasense™	Genta	TCTCCCAGCGTGCGCCAT	PS	Bcl-2	Cancer	Intravenous	II/III
E2F Decoy	Corgentech	N/A	N/A	E2F	Atherosclerosis	*Ex-vivo*	II/III

antisense oligonucleotide (fomivirsen) to treat Cytomegalovirus (CMV) retinitis

.

Timeline


1990
cosuppression of purple color in plants
1998
dsRNA injection in worms
short RNAs identified in plants
RNAi shown *in vitro*
1999
2000
RISC activity partially purified
2001
siRNAs identified
Dicer identified
2002
RNAi used against HIV
genome-wide RNAi screens begin

Thanks for your Attention

Acknowledgement

- The presentation is being used for educational and non-commercial purposes.
- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.