Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 38

Regulation of Eukaryotic Gene Expression by Small RNAs (RNA Interference, RNAi)

Expression *vs* Repression

Lecture 29	Cloning and Expression vectors
Lecture 30	Eukaryotic Protein Expression Systems –I
Lecture 31	Eukaryotic Protein Expression Systems –II
Lecture 32	Eukaryotic Protein Expression Systems –III
Lecture 33	Human Gene Therapy
Lecture 34	DNA Vaccines
Lecture 35	Transgenic Animals
Lecture 36	Transgenic Plants
Lecture 37	Knockout Mice
Lecture 38	Regulation of eukaryotic gene expression by small RNAs

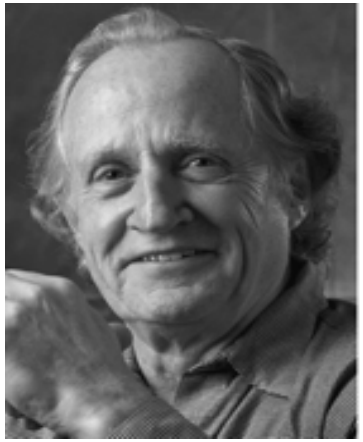
RNA interference

RNA interference (RNAi) is an evolutionally highly conserved process of post-transcriptional gene silencing (PTGS) by which double stranded RNA (dsRNA) causes sequence-specific degradation of mRNA sequences.

Lecture 37 Knockout Mice

Researchers who developed the technology for the creation of knockout mice won Nobel Prize in the year 2007

The Nobel Prize in Physiology or Medicine 2007 was awarded jointly to Mario R. Capecchi, Sir Martin J. Evans and Oliver Smithies *"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"*.



Mario R. Capecchi

Sir Martin J. Evans

Oliver Smithies

The ability to delete or mutate any gene of interest in mice has transformed the landscape of mammalian biology research.

2006

Nobel Prize in Physiology or Medicine for 2006 was awarded to **Andrew Fire**, of Stanford University School of Medicine, and **Craig C. Mello** of the University of Massachusetts Medical School, for their discoveries related to RNA interference.

A. Fire

C. Mello

History of RNAi

1990

mRNA
++++++

Introduction of extra copies of a gene encoding an enzyme involved in production of pigment

mRNA
++

How does introduction of additional copies of gene involved in pigment synthesis result in decreased pigmentation?

How does introduction of additional copies of gene lead to A decrease in mRNA levels?

Postranscriptional gene silencing (PTGS)

Similar phenomenon observed in fungus *N. crassa*, called quelling

The answer actually came in the year 1998 from
researchers working on the nematode, *Caenorhabditis elegans*

It was observed that when double stranded RNA was
Injected into the worm, it lead to a decrease in the expression of homologous gene

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

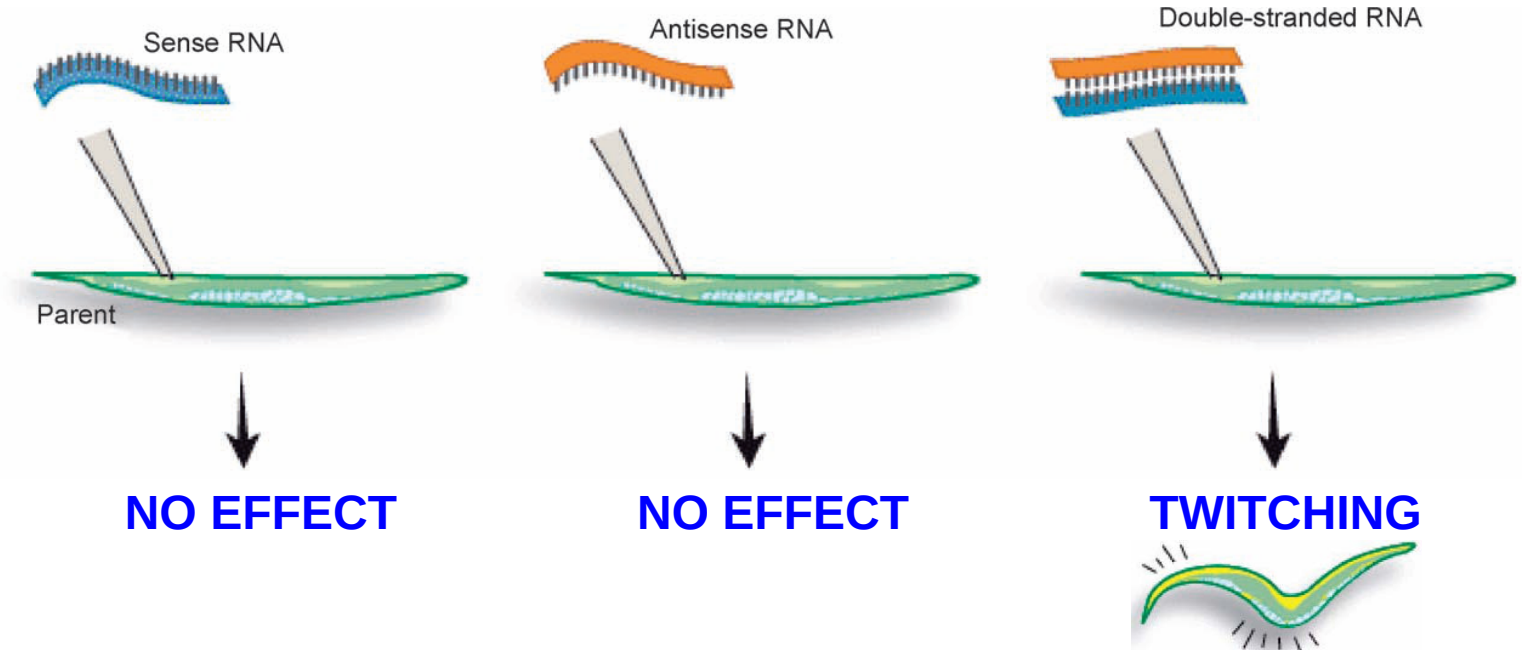
Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC.
Nature. 1998 Feb 19;391:806-11.

Nature. 1998 Feb 19;391:744-5.
Double-stranded RNA poses puzzle.
Wagner RW, Sun L.

Fire & Mello

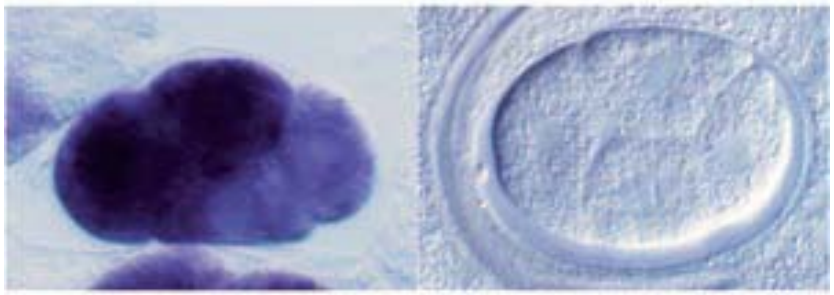
They injected *Caenorhabditis elegans,* a nematode, with a double stranded RNA and observed a drastic decrease in the expression of the gene bearing homologous sequence. Injection of single stranded RNA (Sense or antisense) had no or only marginal effect. These studies indicated that double-stranded RNA, which people didn't think normally existed in eukaryotic cells , had a biological function. shook up the research community.

Reducing expression of unc-22 results in a movement defect (twitching behavior)



Adapted from: Angew. Chem. Int. Ed. 2007, 46, 6966 – 6984

mex-3-mRNA



CONTROL dsRNA

This phenomenon of

double stranded RNA-induced gene silencing was termed

RNA INTERFERENCE

OR

RNAi

dsRNA-induced gene silencing (RNA interference or RNAi) was soon reported in a wide range of eukaryotes ranging from worms, insects, mammals and plants.

"Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway": J. Kennerdell, R. Carthew, Cell 1998, 95, 1017 – 1026.

"Targeted disruption of gene function in Drosophila by RNA interference [RNA-i]: a role for nautilus in embryonic somatic muscle formation": L. Misquitta, B. Paterson, Proc. Natl. Acad.Sci. USA 1999, 96, 1451 – 1456.

"Double-stranded RNA induces mRNA degradation in Trypanosoma brucei": H. Ngo, C. Tschudi, K. Gull, E. Ullu, Proc. Natl. Acad. Sci. USA 1998, 95, 14687 – 14 692.

"Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA": P. Waterhouse, M. Graham, M.Wang, Proc. Natl. Acad. Sci. USA 1998, 95, 13959 – 13964.

"Gene silencing in Neurospora crassa requires a protein homologous to RNA-dependent RNA polymerase": C. Cogoni, G. Macino, Nature 1999, 399, 166 – 169.

"EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in C. elegans": A. Smardon, J. Spoerke, S. Stacey, M. Klein, N. Mackin, E. Maine, Curr. Biol. 2000, 10, 169 – 178.

"Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance": P. Mourrain, C. Beclin, T. Elmayan, F. Feuerbach, C. et al., Cell 2000, 101, 533 – 542.

"An RNA-dependent RNA polymerase gene in Arabidopsis is required for posttranscriptional gene silencing mediated by a transgene but not by a virus": T. Dalmay, A. Hamilton, S. Rudd, S. Angell, D. Baulcombe, Cell 2000, 101, 543 – 553.

"On the role of RNA amplification in dsRNA-triggered gene silencing": T. Sijen, J. Fleenor, F. Simmer, K. Thijssen, S. Parrish, L. Timmons, R. Plasterk, A. Fire, Cell 2001, 107, 465 – 476.

It was shown that plants contain an RNA-dependent RNA Polymerase that synthesizes double stranded RNA in presence of very high levels of mRNA.

This double stranded RNA in turn is responsible for decrease in the expression of the homologous gene.

KNOCKDOWN

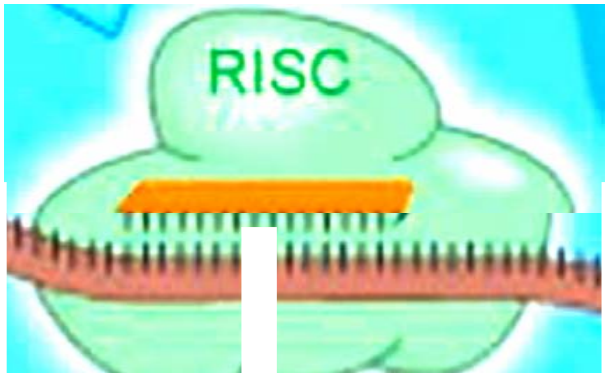
VS

KNOCKOUT

THE MECHANISM OF RNAi

First the double-stranded RNA teams up with a protein complex named Dicer, which cuts the long RNA into short pieces.

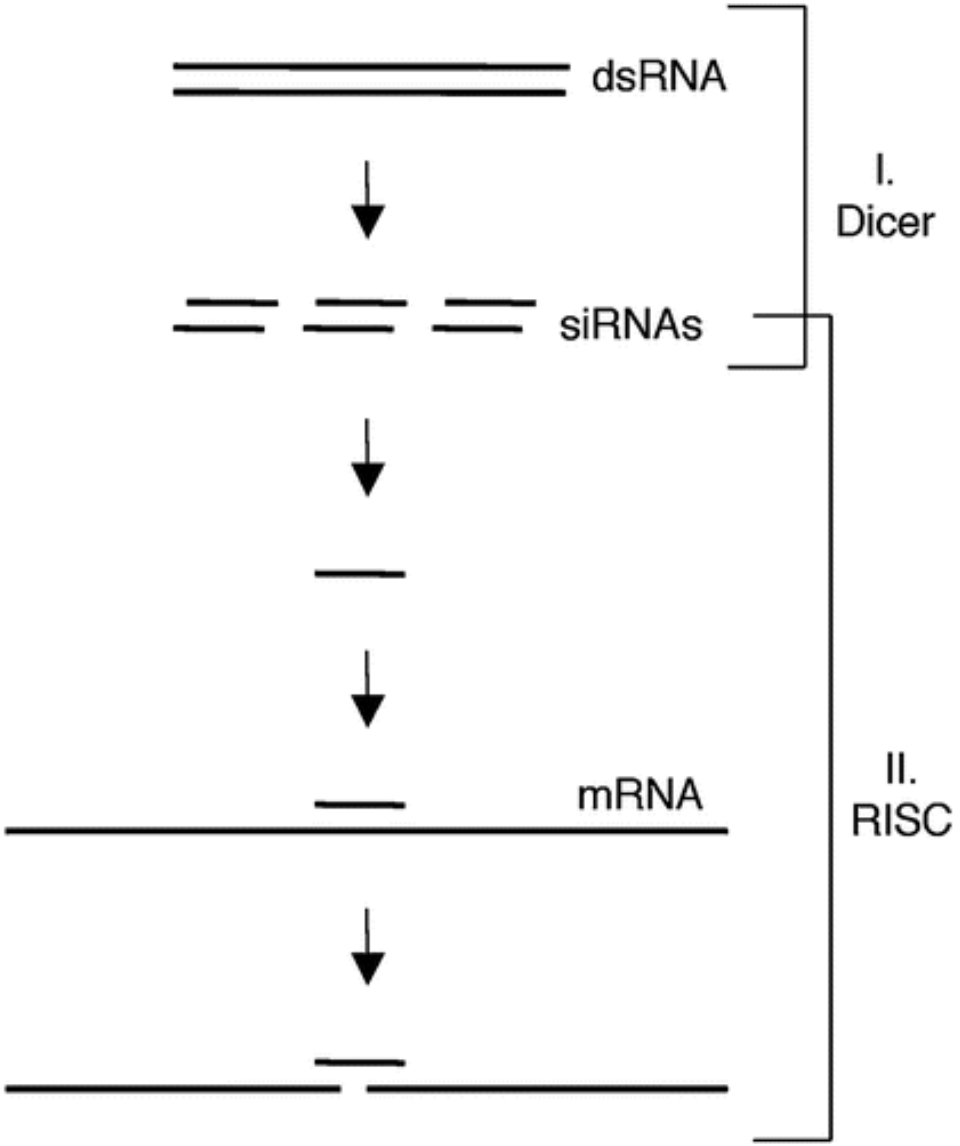
Then another protein complex called RISC (RNA-induced silencing complex) discards one of the two RNA strands.



The RISC-docked, single-stranded RNA then pairs with the homologous mRNA and destroys it.

Gene silencing

THE MECHANISM OF RNAi



Argonaute: Central Component of the
RNA-Induced Silencing Complex (RISC)

One strand of the dsRNA produced by Dicer is retained in the RISC complex in association with Argonaute

Induction of RNAi using dsRNA quickly became a powerful tool for the study the function of genes in many lower eukaryotes especially the *C. elegans and D. melanogaster.*

However, knockdown of gene expression using dsRNA was not possible in mammalian cells, since dsRNA induced an immune response leading to apoptosis.

This mechanism is used by mammalian cells during viral infection by destroying virus-infected cells and preventing the spread of the virus to neighboring cells.

Thus, for quite some time, RNAi using dsRNA was largely confined to lower eukaryotes.

A new strategy to trigger RNAi in mammalian cells was developed using relatively small dsRNAs which are long enough to induce RNAi, but small enough to avoid inducing an immune response.

These smaller dsRNAs, known as "small interfering RNAs" (siRNAs), bind to messenger RNAs (mRNAs) and silence the homologous gene.

This opened the door for knockdown of genes in mammalian cells leading to the exploitation of RNAi as a novel therapeutic strategy.

Synthetic siRNAs

Synthetic siRNAs are typically 21 bp double-stranded RNA molecules with dinucleotide 3' overhangs.

Synthetic siRNAs are loaded directly into RISC upon introduction into the cytoplasm by transfection or electroporation.

The RNAi effect achieved is transient, lasting typically for 3–7 days.

Three Ways to Trigger the RNAi Pathway in Eukaryotic Cells

In non-mammalian systems, the RNAi pathway is turned on when double-stranded RNA (dsRNA; >30 bp) is introduced into cells.

In mammalian systems, RNAi can be triggered by introducing DNA based expression vectors designed to express short hairpin RNA (shRNA) molecules or by direct transfection of synthetic short interfering RNA (siRNA) molecules.

In each case, gene silencing results from destruction of mRNA that is complementary to the input siRNA or the siRNA molecules created by Dicer cleavage of longer dsRNA or shRNA molecules.

‘Off-target' effects of RNAi.

It was observed that RNAi could also change expression of RNAs that closely resembled a target RNA — not just the target RNA itself
(A. L. Jackson *et al*. *Nature Biotechnol*. **21,** 635–637; 2003).

Thus, RNAi can change the levels of proteins that are not related to the target RNA (P. C. Scacheri *et al*. PNAS **101,** 1892–1897; 2004).

‘Off-target' effects of RNAi.
A. L. Jackson and P. S. Linsley
B. Trends Genet. **20,** 521–524; 2004).

Careful design of the siRNA is required to maximize silencing of the target gene while minimizing off-target effects.

With improved understanding of the mechanism of RNAi, algorithms for designing effective siRNAs have improved, decreasing the number of sequences that need to be tested to elicit gene-specific silencing.

It is necessary to independently test multiple distinct siRNA sequences targeting the same transcript to confirm that observed gene silencing is due to specific rather than off-target effects.

Double-stranded RNAs are naturally produced in eukaryotic cells during development, and they have a key role in regulating gene expression by acting as negative regulators.

These double stranded RNAs are processed by the Dicer machinery to generate small single stranded RNAs which are known as

microRNAs (miRNAs)

– The first naturally occurring microRNA was discovered in C. elegans:

lin-4 (22 bases)

– Identified in screen for defects in timing of larval development
– *lin-4* partially complementary to conserved sites in *lin-14* 3'UTR
[Lee *et al.,* 1993]

- *lin-4* binds these sites and negatively regulates *lin-14* translation

– No other miRNAs found for 7 years!

– Second miRNA (21 bases) named ***let-7*** *was discovered by* Reinhart *et al.,* in the year 2000, which regulates *lin-14* in same way as lin-4

MicroRNA genes are transcribed by RNA polymerase II as a long double stranded precursor called the primary microRNA, or pri-microRNA.

The microRNA portion of the pri-microRNA transcript forms a hairpin with signals for double-stranded RNA specific nuclease cleavage. A double-stranded RNA specific ribonuclease known as Drosha processes the pri-microRNA to release the hairpin into a precursor microRNA, or pre-microRNA.

The pre-microRNA is exported into the cytoplasm by a nuclear export protein termed Exportin 5.

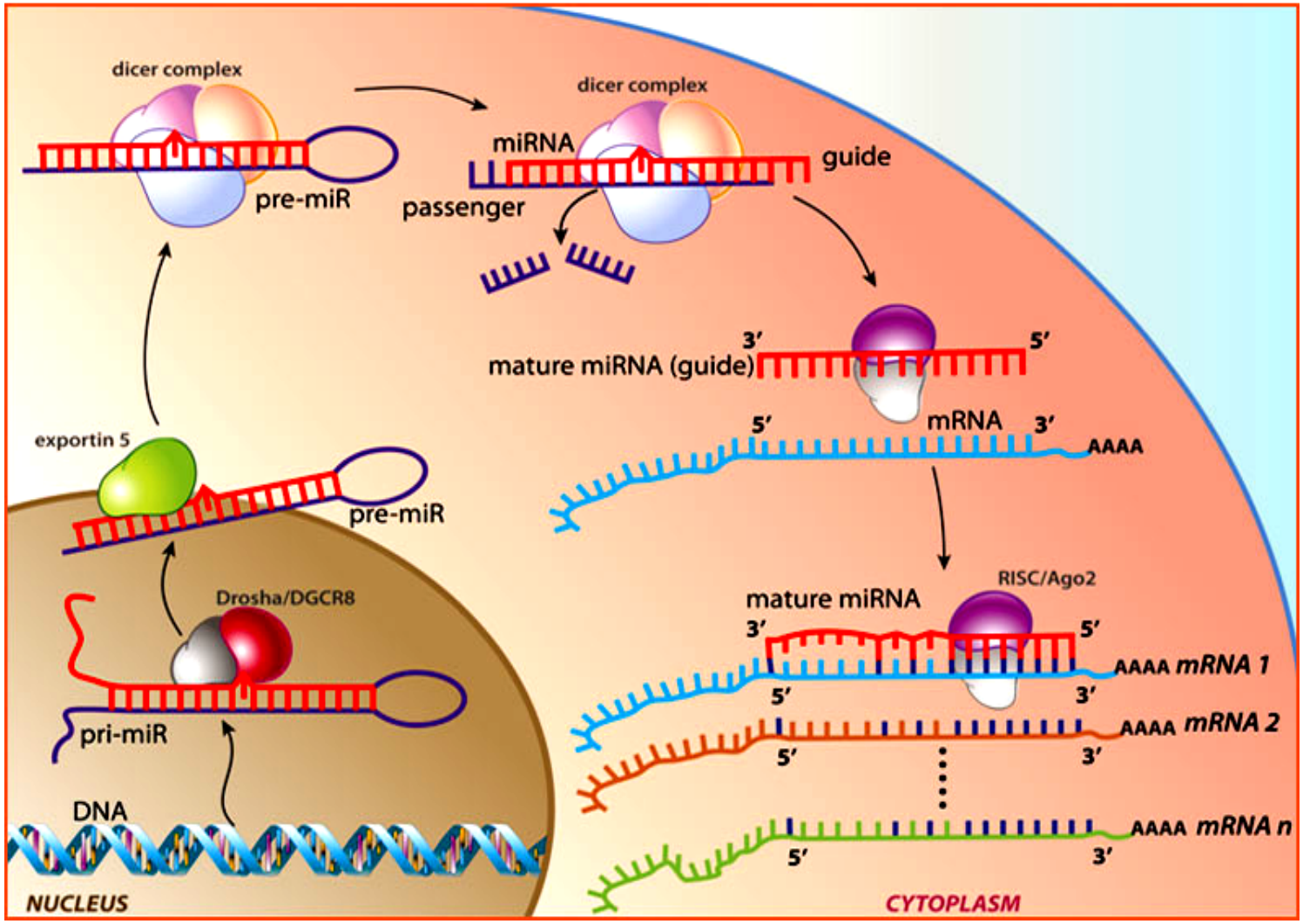
In the cytoplasm the pre-microRNA is cleaved by the enzyme Dicer into a 20 to 25 nucleotide long double-stranded RNA.

The double-stranded RNA produced by Dicer are then separated. The single-stranded mature microRNA assembles into a protein-RNA complex called the RNA-induced silencing complex (RISC).

Through the RISC complex the microRNA targets messenger RNAs by direct base pairing.

The 5' region of a microRNA, (nucleotides 1 through 8), is the most critical for targeting and function. The microRNA target sites are often imperfect matches and located in the 3' UTR of target messenger RNAs. Since microRNAs do not require perfect complementarity for target recognition, a single microRNA is able to regulate multiple messenger RNAs and often produces measurable phenotype.

microRNAs Are Small Endogenous Non-coding RNAs that Regulate Gene Expression



http://www.regulusrx.com/

MicroRNAs have been shown to play an integral role in numerous biological processes including the immune response, cell-cycle control, metabolism, viral replication, stem cell differentiation and human development.

Many microRNAs are conserved across multiple species indicating the evolutionary importance of these molecules as modulators of critical biological pathways.

Indeed, microRNA expression or function is significantly altered in many disease states, including cancer, heart failure, and viral infections.

Key Points

1. Small RNAs, in the forms of siRNAs and miRNAs, play large roles in the regulation of gene expression in eukaryotes. They are important in normal cell metabolism, development, and defense against invaders.

2. siRNAs are produced from longer segments of dsRNA by Dicer, assembed into RISC, and targeted to mRNAs with perfect complementarity, giving silencing by cleavage and degradation of the RNA or by formation of heterochromatin.

3. The pathway for miRNA has many steps in common with that for siRNA. However, miRNAs are processed from hairpin structures by Drosha and then by Dicer, and they most often have imperfect complementarity with their targets.

Key Points

4. A miRNA is a ssRNA of ~22 nucleotides in length, which is generated by the RNase-III-type enzymes Drosha and Dicer from an endogenous transcript that contains a local hairpin structure. It originate as a primary RNA (pri-miRNA) of several hundred base pairs

5. miRNAs contributes significantly to regulation of cellular gene expression.
   - Silencing of endogenous genes regulates basic biological processes, including the transition from one developmental stage to the next.
     - the archetype miRNAs, let-7 and lin-4, regulate *C. elegans* larval development
     - miRNAs are expressed in a specific spatial and temporal pattern during development in *D. melanogaster* or differentiation of mouse embryonic stem cells

6. The function of most miRNAs remains unknown…

miRNA *vs* siRNA

- miRNA: microRNA.
  - Encoded by endogenous genes.
  - Hairpin precursors - pre-miRNAs
    - The pre-miRNAs are hairpins with imperfect complementarity in their stems and frequent bulges, mismatches and G:U wobble base pairings.
  - Recognize multiple targets.

- **siRNA: short-interfering RNA.**
  - Mostly exogenous origin.
  - dsRNA precursors
  - May be target specific

- Discovered in different ways
- Similar biogenesis
- Share common pathway components and outcomes

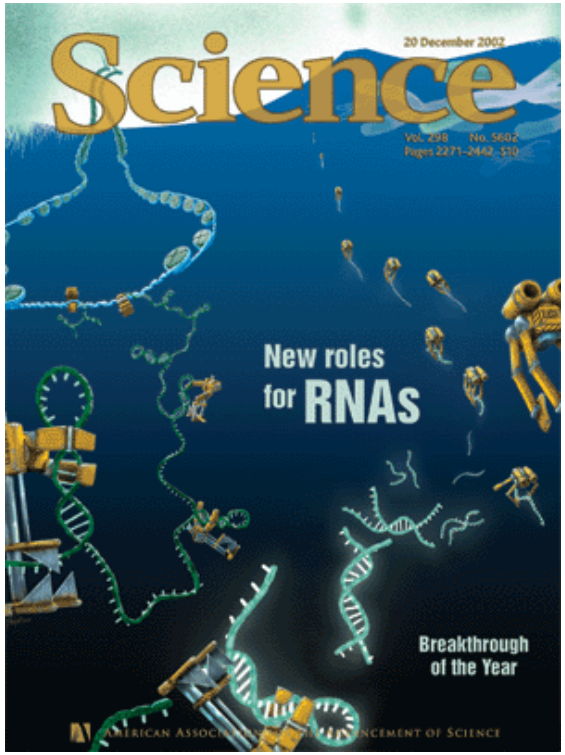
RNA interference (RNAi) is an important biological process for modulating eukaryotic gene expression.

From being named Science's 'Breakthrough of the Year' in 2002, advances in our understanding of the natural role of RNAi in a range of cellular processes, both protective and destructive, have revealed the potential for a novel class of drugs based on small, inhibitory RNAs and their derivatives.

The natural ability of eukaryotic cells to repress gene expression via RNAi was exploited for silencing target genes in vitro, in tissues, and in whole organisms.

http://www.sciencemag.org/cgi/content/full/298/5602/2296

2002



BREAKTHROUGH OF THE YEAR:
Small RNAs Make Big Splash
Jennifer Couzin

Benefits of RNAi

RNAi therapy

Gene therapy was restricted to diseases such as hemophilia or cystic fibrosis, where a normal copy of a gene could replace a mutated gene and cure the disease.

RNAi opened up possible gene therapy for diseases caused by an overproduction of normal protein.

RNAi could also be harnessed to block viral infections and to stop the overproduction of the protein that drives macular degeneration, the leading cause of blindness.

Haibin Xia et al. Nature Medicine 10, 816 - 820 (2004)

RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia (SCA1)

Spinocerebellar ataxia type 1 (SCA1) and Huntington disease, are progressive, untreatable, neurodegenerative disorders caused by the expansion of paolyglutamine repeats.

In inducible mouse models of SCA1 and Huntington disease, repression of mutant allele expression improves disease phenotypes. Thus, therapies designed to inhibit expression of the mutant gene would be beneficial.

Upon intracerebellar injection, recombinant adeno-associated virus (AAV) vectors expressing short hairpin RNAs profoundly improved motor coordination, restored cerebellar morphology and resolved characteristic ataxin-1 inclusions in Purkinje cells of SCA1 mice.

Our data demonstrate *in vivo* the potential use of RNAi as therapy for dominant neurodegenerative disease.

Raoul, C. *et al. Nature Medicine* **11**, 423 - 428 (2005)

Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS

Mutations in Cu/Zn superoxide dismutase (encoded by *SOD1*), one of the causes of familial amyotrophic lateral sclerosis (ALS), lead to progressive death of motoneurons through a gain-of-function mechanism.

We report that in *SOD1*^{G93A} transgenic mice, a model for familial ALS, intraspinal injection of a lentiviral vector that produces RNAi-mediated silencing of *SOD1* substantially retards both the onset and the progression rate of the disease.

Vira Bitko et al., *Nature Medicine* **11**, 50 - 55 (2004)

Inhibition of respiratory viruses by nasally administered siRNA

Respiratory syncytial virus (RSV) and parainfluenza virus (PIV) are two respiratory pathogens of paramount medical significance that exert high mortality.

Using an RNA interference (RNAi) approach, we show that individual as well as joint infection by RSV and PIV can be specifically prevented and inhibited by short interfering RNAs (siRNAs), instilled intranasally in the mouse, with or without transfection reagents.

When targeting both viruses in a joint infection, excess of one siRNA moderated the inhibitory effect of the other, suggesting competition for the RNAi machinery.

Our results suggest that, if properly designed, low dosages of inhaled siRNA might offer a fast, potent and easily administrable antiviral regimen against respiratory viral diseases in humans.

Alnylam Pharmaceuticals

RNAi can lower cholesterol levels in mice.

The study is particularly encouraging because the treatment was injected directly into the bloodstream — a delivery method whose simplicity appeals to clinicians.

http://www.alnylam.com/

Erika Check Nature 432, 136 (11 November 2004)
Hopes rise for RNA therapy as mouse study hits target

Acuity Pharmaceuticals

Acuity hopes to use a small interfering RNA to treat patients with wet age-related macular degeneration, a condition that is caused by extended growth of blood vessels in the retina.

Erika Check Nature 430, 819 (19 August 2004)
Firm sets sights on gene silencing to protect vision

RNAi in basic and applied research

Testing Hypotheses of Gene Function

RNA interference allows scientists to knock down expression of a targeted gene and observe the effects of partial to full loss of function.

Engagement of the interleukin-1 (IL-1) receptor and the Toll-like receptor (TLR) induces mitogen-activated protein kinase (MAPK) pathways and activation of transcription factors such as NFkB and AP-1.

Three members of the novel Pellino protein family have been shown to be mediators in these pathways, but show pathway specificity in their function.

Using pre-designed siRNA targeting human Pellino3, RNA interference was used to show a physiological role for Pellino3 in mediating IL-1 activation of p38 MAPK in HEK293 cells:

Pellino3-specific siRNA caused a considerable reduction in IL-1 induced phosphorylation of p38 MAPK

Butler MP, Hanly JA, and Moynagh PN (2005)
J Biol Chem **280**:27759–27768.

Functional Screening and Target Identification

Cellular pathways that have been characterized biochemically or genetically can be further dissected using RNA interference. Libraries of siRNAs targeting large collections of genes enable screening experiments that tie genes to cellular function

A collection of siRNAs targeting 650 known and putative kinases was screened in HeLa cells using a histone-DNA fragmentation ELISA to provide a quantitative cell-based assay for apoptosis.

These experiments identified 73 kinases (11% of the kinase library) whose knockdown resulted in increased apoptosis; the collection was termed “survival kinases.”

Mackeigan, JP, Murphy, LO, Blenis, J
(2005)
Nature Cell Biology **7(6):**591–600.

Target Validation

Drug development follows the path of target identification -> target validation -> assay development -> drug lead identification -> lead optimization -> pre-clinical testing -> clinical trials.

Once a potential therapeutic target has been identified through a variety of biochemical or genetic methods, including screening using RNAi, siRNAs can be used to reduce its expression.

If the desired phenotype results, this outcome provides confidence that an inhibitor of the same target should have therapeutic value. In some cases, the siRNA itself may become the therapeutic lead.

Target Validation

Wet age-related macular degeneration (AMD) is characterized by neovascularization (overgrowth of new blood vessels under the retina) causing fluid leakage and scar tissue, leading to central vision impairment.

The vascular endothelial growth factor (VEGF) pathway is thought to play a central role in retinal and choroidal neovascularization.

Both VEGF and VEGF receptors (VEGFRs) are potential therapeutic targets.

A panel of chemically stabilized siRNAs targeting *vegfr1* (but not *vegfr2*) mRNA was screened in cultured endothelial cells for the ability to specifically knockdown expression of this gene, leading to identification of Sirna-027.

Sirna-027 was shown to reduce levels of *vegfr1* mRNA after intravitreous or periocular injection, compared to injection of an inverted control sequence.

Shen J, et al., (2005) Suppression of ocular neovascularization with siRNA targeting VEGF receptor 1. *Gene Ther* **Sep 29**:1–10.

siRNAs as Therapeutics

siRNAs have the potential to act as therapeutic products.

RNAi-based drugs currently in pre-clinical development include those targeting respiratory syncytial virus, hepatitis C, HIV, Huntington's disease and several other neurodegenerative disorders

siRNAs as Therapeutics

Reducing serum cholesterol and LDL levels is a primary clinical strategy for preventing and managing coronary artery disease.

Apolipoprotein B (apoB) binds to the LDL receptor and is essential for formation of low-density lipoproteins (LDL) in cholesterol metabolism.

84 siRNAs targeting mouse and human apoB in HepG2 liver cells were screened for their ability to reduce apoB mRNA and protein levels.

Two of the most potent siRNAs were chemically modified and conjugated to cholesterol and these siRNAs were shown to reduce apoB mRNA in liver and jejunum (the primary sites of apoB expression), decrease plasma levels of apoB protein, and reduce total cholesterol after intravenous injection in mice.

Cleavage of apoB mRNA was shown to occur specifically at the site predicted by current models of RNAi, 10 nt downstream of the 5' end of the siRNA antisense strand.

Soutschek J, etal., (2004) Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* **432(7014)**:173–178.

siRNAs as Therapeutics

Mutations in the Cu/Zn superoxide dismutase, encoded by SOD1, have been identified in approximately 20% of familial cases of amyotrophic lateral sclerosis (ALS).

A lentiviral vector encoding SOD1 shRNA (LV-shSOD1) targeting all forms of human SOD1 was shown in mouse embryonic fibroblasts derived from transgenic mice expressing human SOD1G93A to reduce mRNA and protein levels of SOD1G93A.

In vitro, LV-shSOD1 protected SOD1G93A motoneurons from nitric oxide-induced cell death.

SOD1G93A mice injected in the lumbar spinal cord with LV-shSOD1 showed significantly delayed disease onset, delayed onset of the decline of neuromuscular function, and delayed progression rate of motor deficit and ameliorated motor strength.

LV-shSOD1G93A mice showed increased motoneuron survival near injection sites and significantly increased the number of large motor fibers in mice.

Raoul C, et al., (2005) Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nat Med* **4(11)**:423–428.

siRNAs as Therapeutics

Infection by HCV, an RNA virus that infects 1 in 40 people worldwide, is the most common reason for liver transplantation in the United States and Europe.

McCaffrey et al., fused the NS5B region (non-structural protein 5B, viral-polymerase-encoding region) of this virus with luciferase RNA and monitored RNAi by co-transfection *in vivo*.

An siRNA targeting the NS5B region reduced luciferase expression from the chimaeric HCV NS5B protein–luciferase fusion by 75%

This result suggests that it may be feasible to use RNAi as a therapy against other important human pathogens.

Gene expression: RNA interference in adult mice
Anton P. McCaffrey et al., Nature 418, 38-39 (4 July 2002)

New small RNAs
and
Novel functions for small RNAs

siRNA and heterochromatin formation

- The repeat-associated siRNA (rasiRNA) pathway
  - Transcription from opposing promoters found in repetitive DNA elements, such as centromeric repeats and satellite DNA, leads to the formation of long dsRNAs.
  - These long dsRNAs are cleaved by **Dicer**, into siRNAs.
  - These are unwound and taken up by the RNA-induced transcriptional silencing complex (**RITS**)
  - **RITS** directs the establishment of silenced chromatin over the region of DNA homologous to the siRNAs.
  - This silenced chromatin is characterized by sequence-specific DNA methylation and histone methylation and by recruiting heterochromatin-associated proteins.

Small RNAs may play a major role in regulation of eukaryotic gene expression

- New classes of small RNAs:
  Tiny non-coding RNA [Ambros *et al.*, 2003]
    - tncRNA – 20-22nt
    - Discovered in *C. elegans*
    - Not likely generated from hairpin loops
    - Not conserved among species
    - Many complementary to mRNAs
    - Function is not well understood.

  – **RNA as a Molecular Switch:**
  Small Modulatory RNA – smRNA [Kuwabara *et al.*, 2004]
    - Discovered in mice
    - Conserved in vertebrates
    - Interacts with regulatory protein
    - Turns transcriptional repressor into activator

Fire AZ. **Gene silencing by double-stranded RNA (Nobel Lecture)**
Angew Chem Int Ed Engl. 2007;46(37):6966-84.

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

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC.
Nature. 1998 Feb 19;391:806-11.

Nature. 1998 Feb 19;391:744-5.
Double-stranded RNA poses puzzle.
Wagner RW, Sun L.

RNAi - Key developments

- **1990**
  - **co-suppression of purple color in plants.**
- **1995** Guo S, and Kemphues KJ.
  - First noticed that sense RNA was as effective as antisense RNA for suppressing gene expression in worm C. elegans
- **1998** Fire et al.
  - First described RNAi phenomenon in C. elegans by injecting dsRNA into C. elegans which led to an efficient sequence-specific silencing and coined the term "RNA Interference".
- **2000** Zamone et al.
  - Reported processing of long dsRNA by Rnase III (Dicer) into shorter fragments of 21-23-nt intervals in Drosophila extracts
- **2001** Bernstein et al.
  - Cloned Dicer, the RNase III enzyme that is evolutionarily conserved and contains helicase and PAZ domains, as well as two dsRNA-binding domains.
- **2002** Tuschl T and colleagues
  - First described RNAi in mammalian cells
- **2003** Paddison et al. Sui et al. Paul et al.
  - Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells.
- **2003** Song et al.
  - First reported that siRNAs can be used therapeutically in whole animals
- **2004** Kawasaki and Taira Morris et al.
  - First observed that siRNA silences gene at transcriptional level possibly through directing *de novo* DNA methylation.

NATURE COLLECTIONS
microRNAs

3 A skin microRNA promotes differentiation by repressing 'stemness'. Yi, R., Poy, M. N., Stoffel, M. & Fuchs, E. *Nature* **452,** 225–229 (2008)

8 SMAD proteins control DROSHA-mediated microRNA maturation. Davis, B. N., Hilyard, A. C., Lagna, G. & Hata, A. *Nature* **454,** 56–61 (2008)

14 Widespread changes in protein synthesis induced by microRNAs. Selbach, M. *et al. Nature* **455,** 58–63 (2008)

20 MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Umbach, J. L. *et al. Nature* **454,** 780–783 (2008)

24 MicroRNAs to *Nanog*, *Oct4* and *Sox2* coding regions modulate embryonic stem cell differentiation. Tay, Y., Zhang, J. Q., Thomson, A. M., Lim, B. & Rigoutsos, I. *Nature* **455,** 1124–1128 (2008)

29 Endogenous human microRNAs that suppress breast cancer metastasis. Tavazoie, S. F. *et al. Nature* **451,** 147–152 (2008)

35 MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Yoo, A. S., Staahl, B. T., Chen, L. & Crabtree, G. R. *Nature* **460,** 642–646 (2009)

40 LNA-mediated microRNA silencing in non-human primates. Elmén, J. *et al. Nature* **452,** 896–899 (2008)

44 MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Thum, T. *et al. Nature* **456,** 980–984 (2008)

http://www.nature.com/nature/supplements/collections/micrornas/editorial.pdf

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RNAi movie

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