Lecture 8

Inhibition of gene expression by means of nucleic acids

5 December 2011

Inhibitory nucleic acids

1. Antisense oligonucleotides: short, 12-20 nts.

2. Triple helix-forming oligonucleotides (TFO)

   pyrmidine oligodeoxynucleotides that specifically bind to a major groove of polypurine region of dsDNA via the formation of triple helices according to recognition rules established by Hoogsten

   Modification: binding of camptothecin to TFO transforms topoisomerase to nuclease - topoisomerase is not able to re-ligate

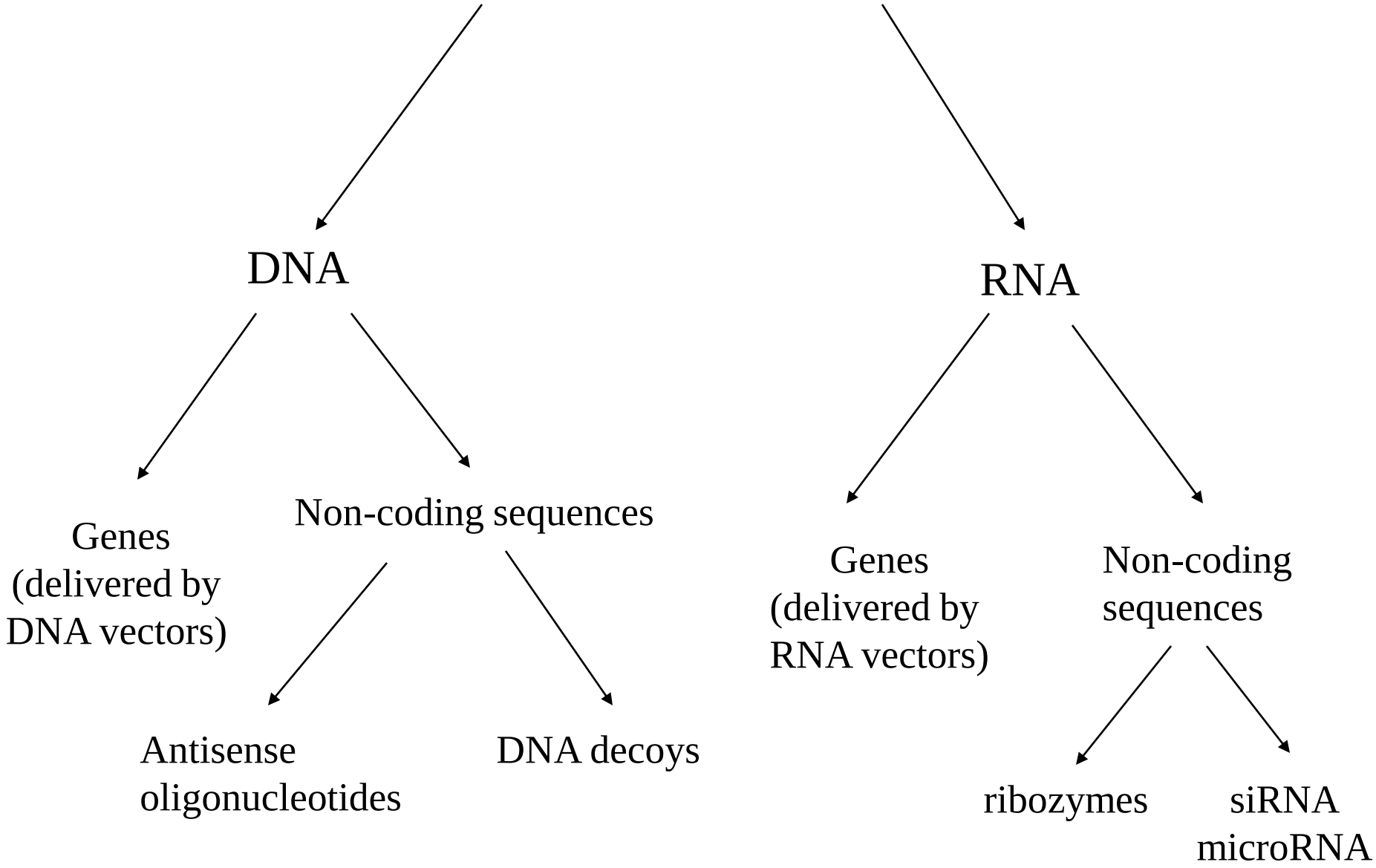
3. Ribozymes – short catalytically active RNSs

4. Deoxyribozymes (DNAzymes) – short catalytic DNA that cleave sequence-specifically ratget RNA.

   More stable than RNA, it is easier to synthesize and to modify them.

5. **siRNA**
6. **microRNA**

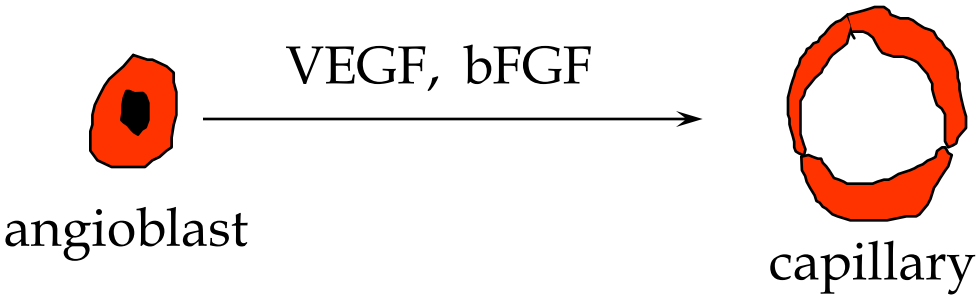
Therapeutic nucleic acids



MACUGEN (PEGAPTANIB)

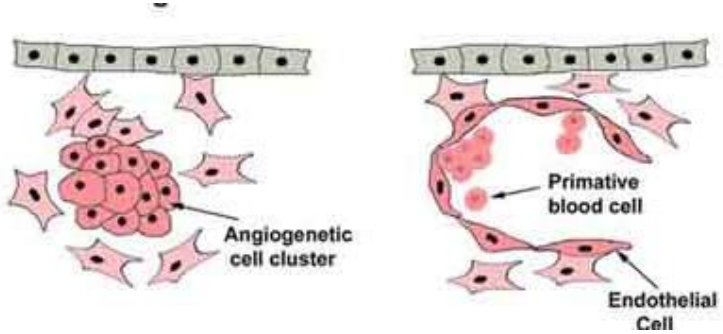
Pegaptinib sodium is a pegylated oligonucleotide aptamer that binds to and inactivates $VEGF_{165}$

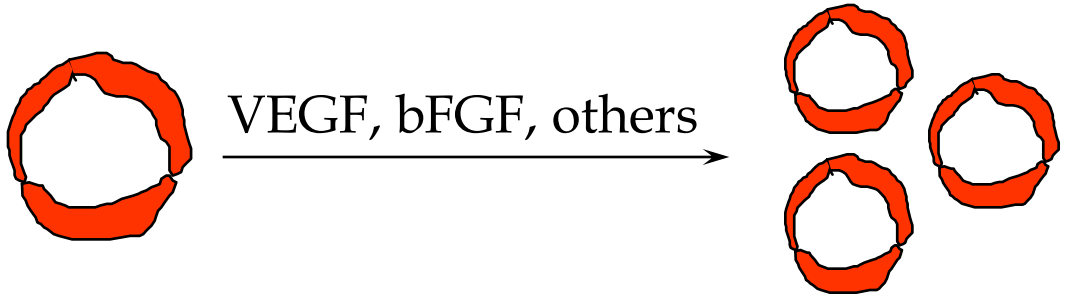
Mechanisms of blood vessels formation



Vasculogenesis

capillaries are formed from vascular progenitor cells

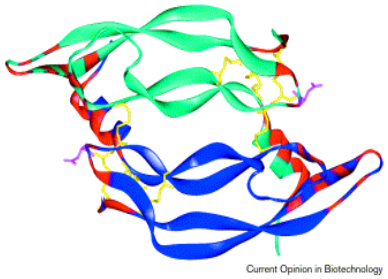




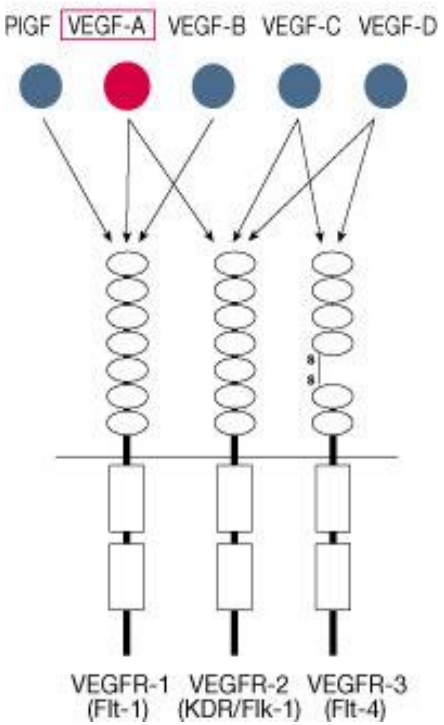
Angiogenesis

formation of new blood vessels from pre-existing capillaries

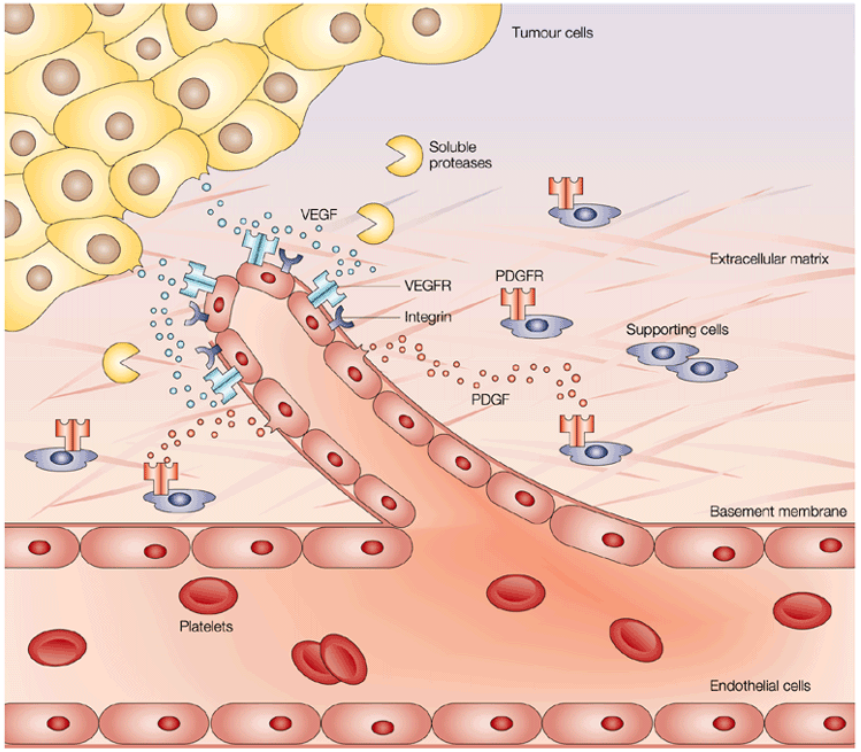
VEGF-A – a major mediator of blood vessels formation



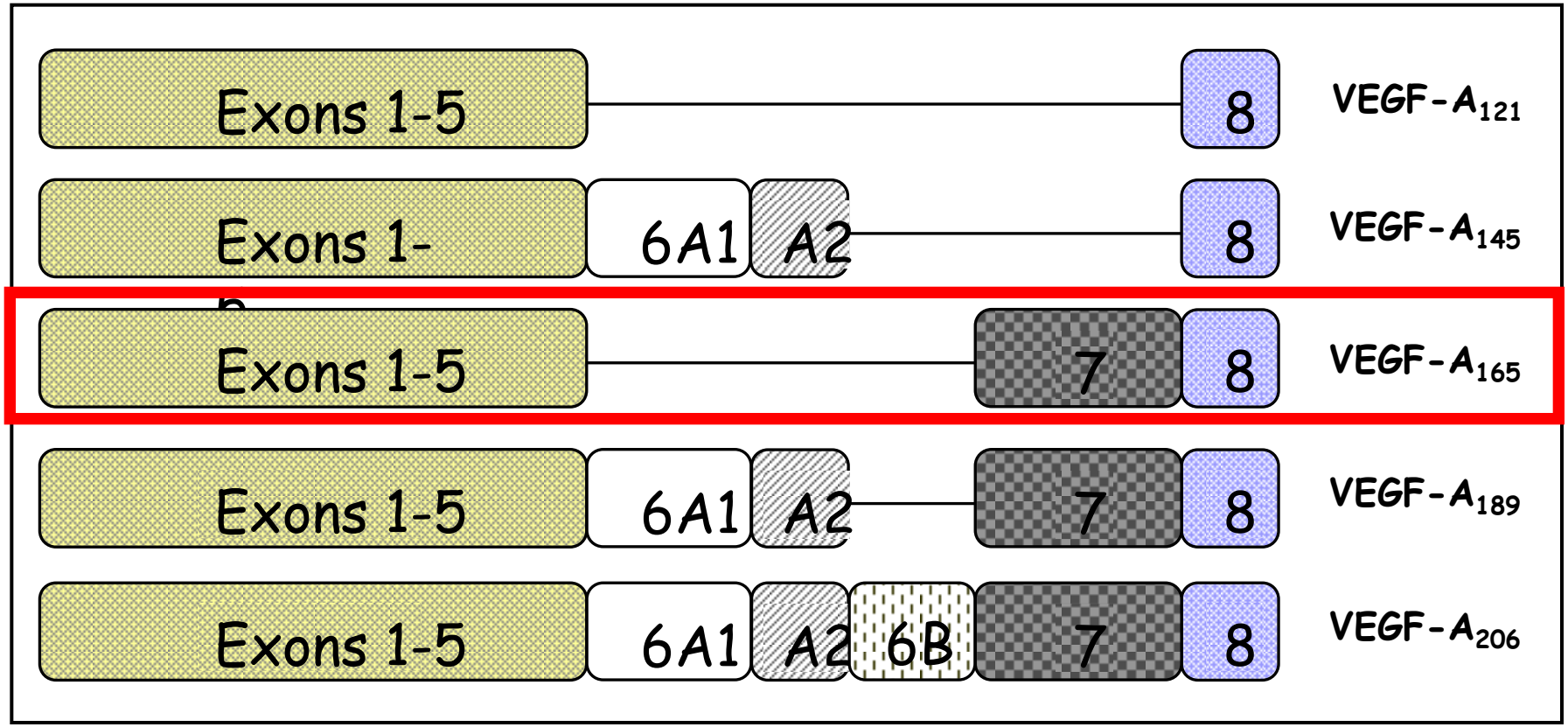
VEGF-A is a major angiogenic growth factor. It acts on endothelial cells, being produced by numerous cell types, including vascular smooth muscle cells (VSMC), fibroblasts or tumor cells.



Receptors on endothelial cells



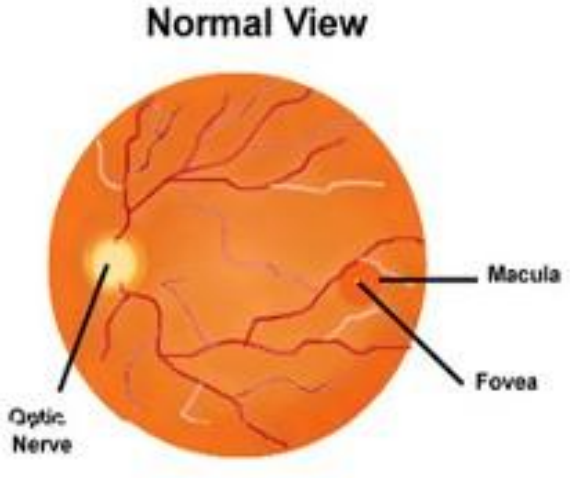
Various isoforms of VEGF

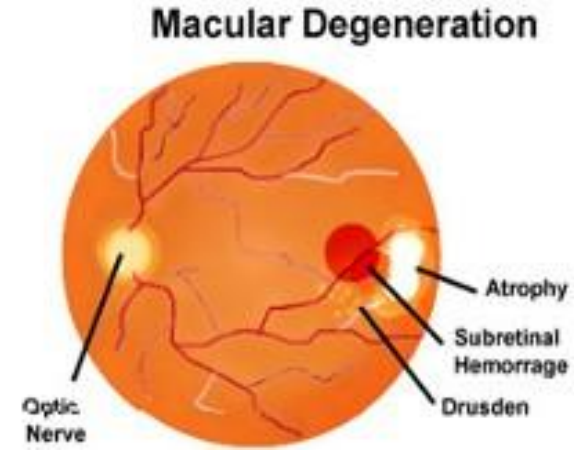


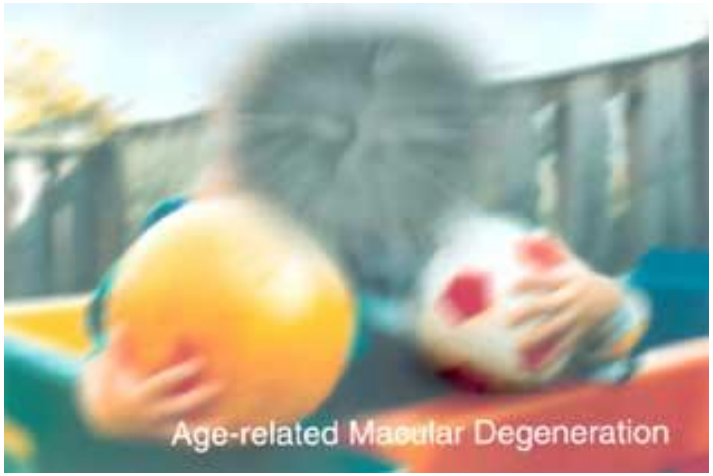
VEGF-A isoforms

Isoform	Size (amino acid)	Coding exons	Features
VEGF-A121	121	1-5, 8	Secreted
VEGF-A145	145	1-6, 8	Binds NRP2 but not NRP1; secreted
VEGF-A165	165	1-5, 7, 8	The most abundant and biologically active isoform; secreted; binds NRP1 and NRP2
VEGF-A165b	165	1-5, 7, alternative exon 8	Secreted, endogenous inhibitory form of VEGF-A165
VEGF-A183	183	1-5, short exon 6, 7, 8	Sequestered in ECM but released by cleavage
VEGF-A189	189	1-8	Sequestered in ECM but released by cleavage
VEGF-A206	206	1-8 plus additional exon	Sequestered in ECM but released by cleavage

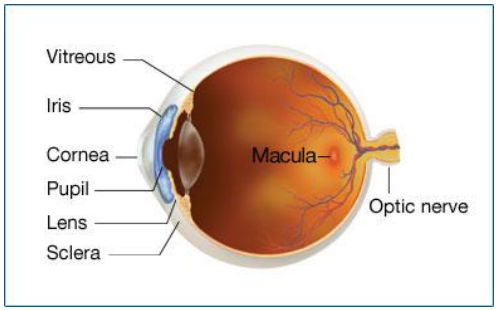
Macular degeneration







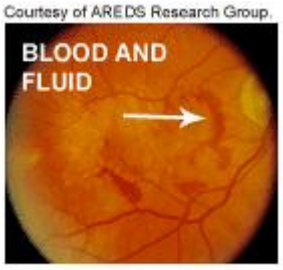
Types of AMD



AMD is the #1 cause of severe vision loss in people over age 60. Today, at least 15 million people in the United States have this health problem.

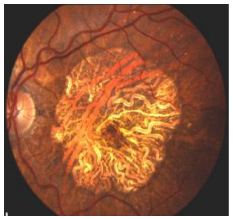
Dry AMD

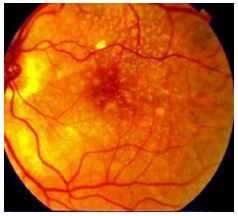
Dry AMD is the most common form of AMD, representing approximately 90% of all AMD cases. However, dry AMD accounts for only 10% of the severe vision loss associated with macular degeneration. Dry AMD is characterized by development of yellow-white deposits underneath your retina, known as drusen, and can also be determined by deterioration of your retina. There is no generally accepted treatment for dry AMD, although vitamins, antioxidants and zinc supplements may slow its progression. Over time, dry AMD cases often develop into wet macular degeneration



Wet AMD

Wet AMD occurs when abnormal blood vessels start to grow under the center of your retina. These new blood vessels may be very fragile and often leak blood and fluid. The blood and fluid can damage your macula or create a scar on your retina, causing vision problems. Damage to the macula can occur rapidly, causing a noticeable blurring or even loss of central vision. The vision loss may be permanent, because abnormal blood vessels and scar tissue are actually destroying normal retina tissue. Once lost, these light-sensitive cells in your retina cannot be replaced.





It is estimated that about 1.6 million people in the United States currently have wet AMD, with 200,000 new cases per year.

Treatment for AMD

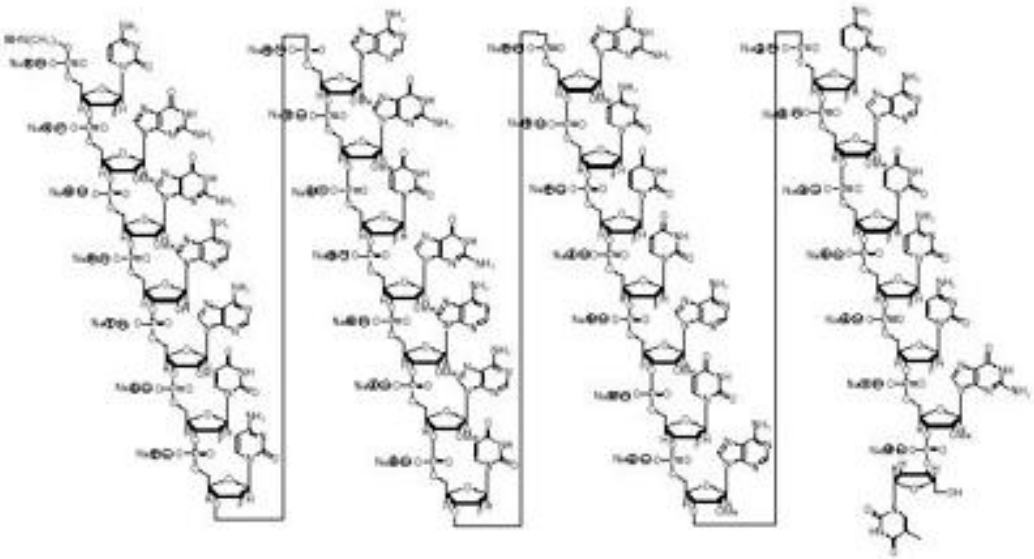
1. Photodynamic therapy

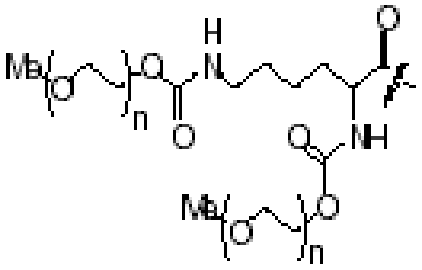
1. Antibodies – anti-VEGF
    2.1. Bevacizumab (Avastin)
    2.2. Rivacizumab (Lucentis)

3. Aptamers – pegaptanib sodium (Macugen)

Pegaptanib sodium

Pegaptanib sodium is a covalent conjugate of an oligonucleotide of twenty-eight nucleotides in length that terminates in a pentylamino linker, to which two 20-kilodalton monomethoxy polyethylene glycol (PEG) units are covalently attached via the two amino groups on a lysine residue.





Pegaptanib sodium

The chemical name for pegaptanib sodium is as follows: RNA, ((2'-deoxy-2'-fluoro)C-Gm-Gm-A-A-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2' -fluoro)C-Am-Gm-(2'-deoxy-2-fluoro)U-Gm-Am-Am-(2'-deoxy-2' -fluoro)U-Gm-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)U-Am-(2'-deoxy-2' -fluoro)U-Am-(2'-deoxy-2'-fluoro)C-Am-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2' -fluoro)C-Gm-(3'® 3')-dT), 5'-ester with a ,a '-[4,12-dioxo-6-[[[5-(phosphoonoxy)pentyl]amino]carbonyl]-3,13-dioxa-5,11-diaza-1,15-pentadecanediyl]bis[w -methoxypoly(oxy-1,2-ethanediyl)], sodium salt.

The molecular formula for pegaptanib sodium is
$C_{294}H_{342}F_{13}N_{107}Na_{28}O_{188}P_{28}$ $[C_2H_4O]n$

(where n is approximately 900)
and the molecular weight is approximately 50 kilodaltons.

Macugen

- Specifically binds VEGF-A_{165}

-registered by FDA in December 2004

MACUGEN treatment given every 6 weeks for up to 2 years has been shown to significantly reduce the risk of moderate vision loss in patients with all types of wet AMD.

administered by intravitreal injection every six weeks for at least two years

Ribozymes

Ribozymes

Small RNA molecules which allow sequence-specific endoribonucleolytic cleavage in a catalytic manner

Processing of rRNA in Tetrahymena

Self-cleaving RNAs from:

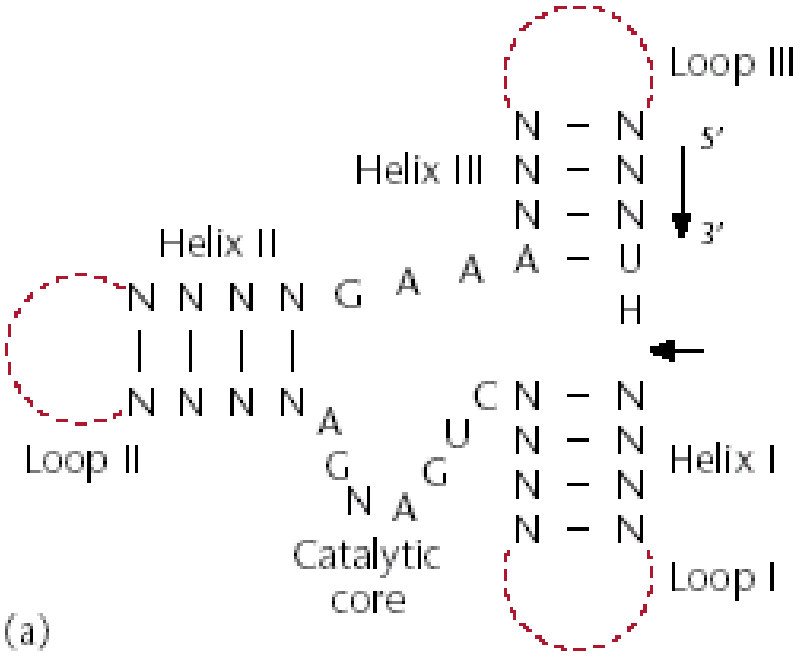
- bacteriophages
- plant satellite virusoids
- satellite tobacco ringspot virus
- human delta hepatitis virus

RNA components of a polysaccharide branching enzyme and RNAse P

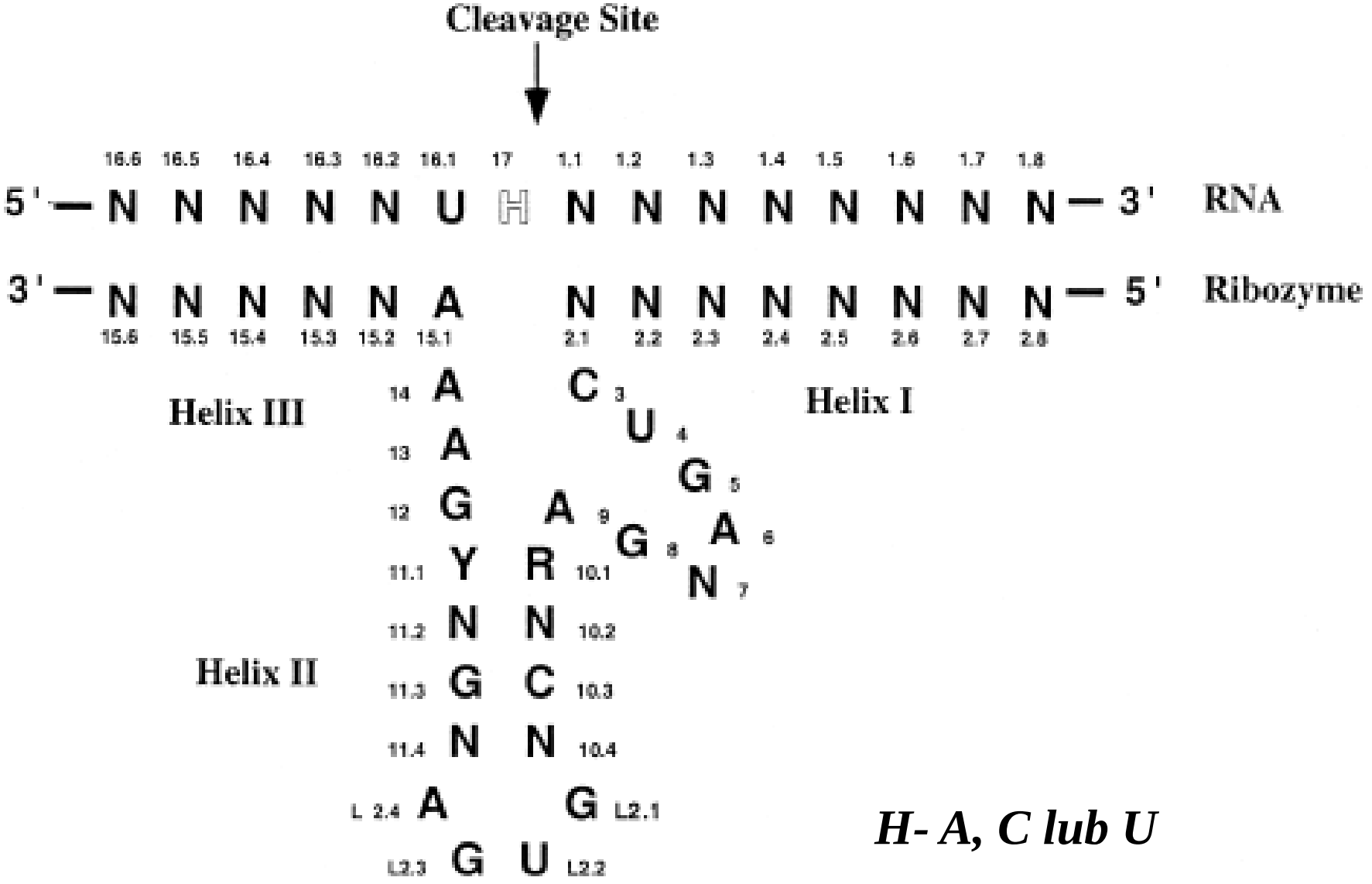
Discovered in the begining of 80's by Thomas Cech and Sydney Altman

„Hammerhead" ribozymes

-They are present in genomes of viroids and virusoids, small single-stranded RNA pathogens of plants
- they are built from three helices connected by loops
- modified ribozymes have only one loop

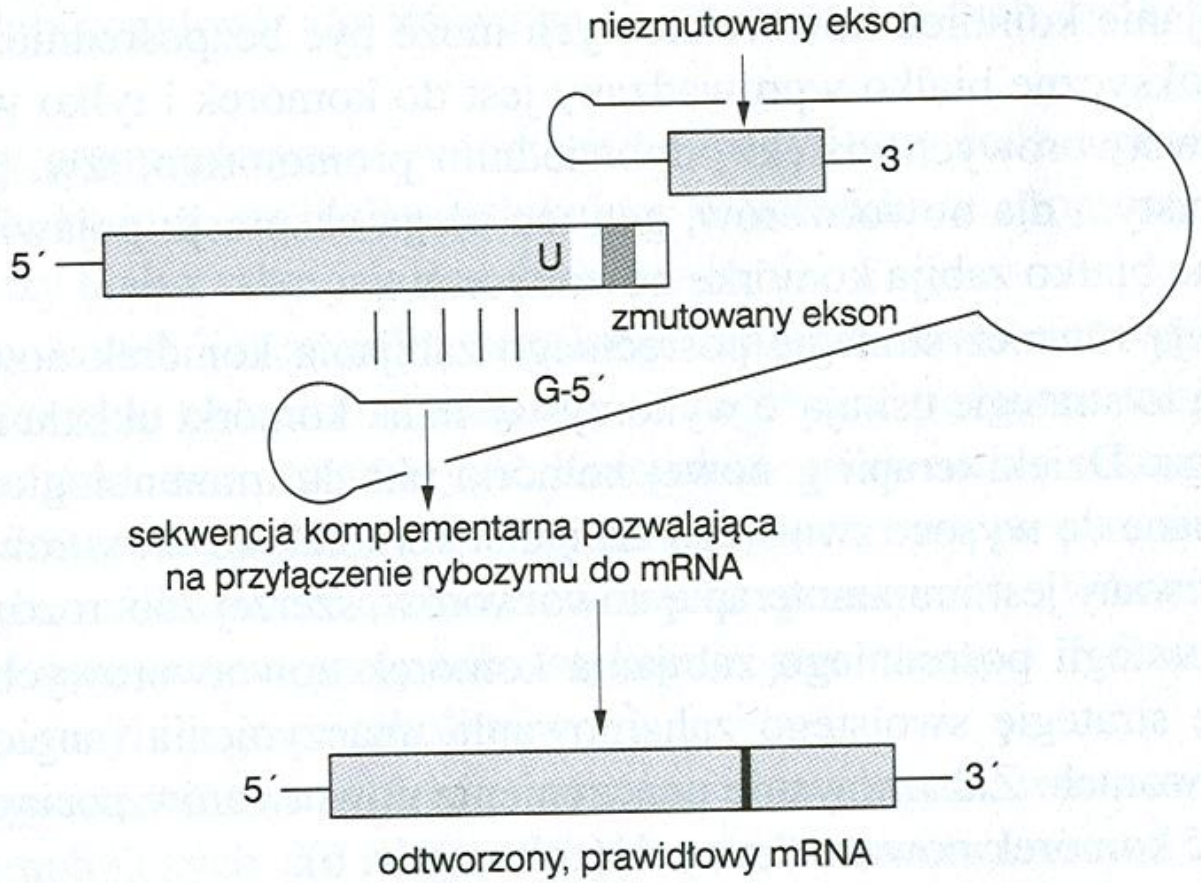


„Hammerhead” ribozymes



H- A, C lub U

Correction of mutation by means of ribozymes



Ryc. 2.6. Naprawa eksonu zawierającego mutację przez rybozymy należące do tzw. grupy I intronów. Rybozym przyłącza się do ściśle określonej sekwencji znajdującej się w zmutowanym mRNA. W wyniku reakcji następuje odłączenie fragmentu (eksonu) mRNA zawierającego mutację i przyłączenie w to miejsce eksonu prawidłowego (wg Lewina i Hanswirtha, 2001)

Delivery of ribozymes to target cells

- exogenous - ribozymes must be chemically modified
- endogenous - coding sequences for ribozymes are delivered with viral/plasmid vectors

Degradation of ribozymes in the serum

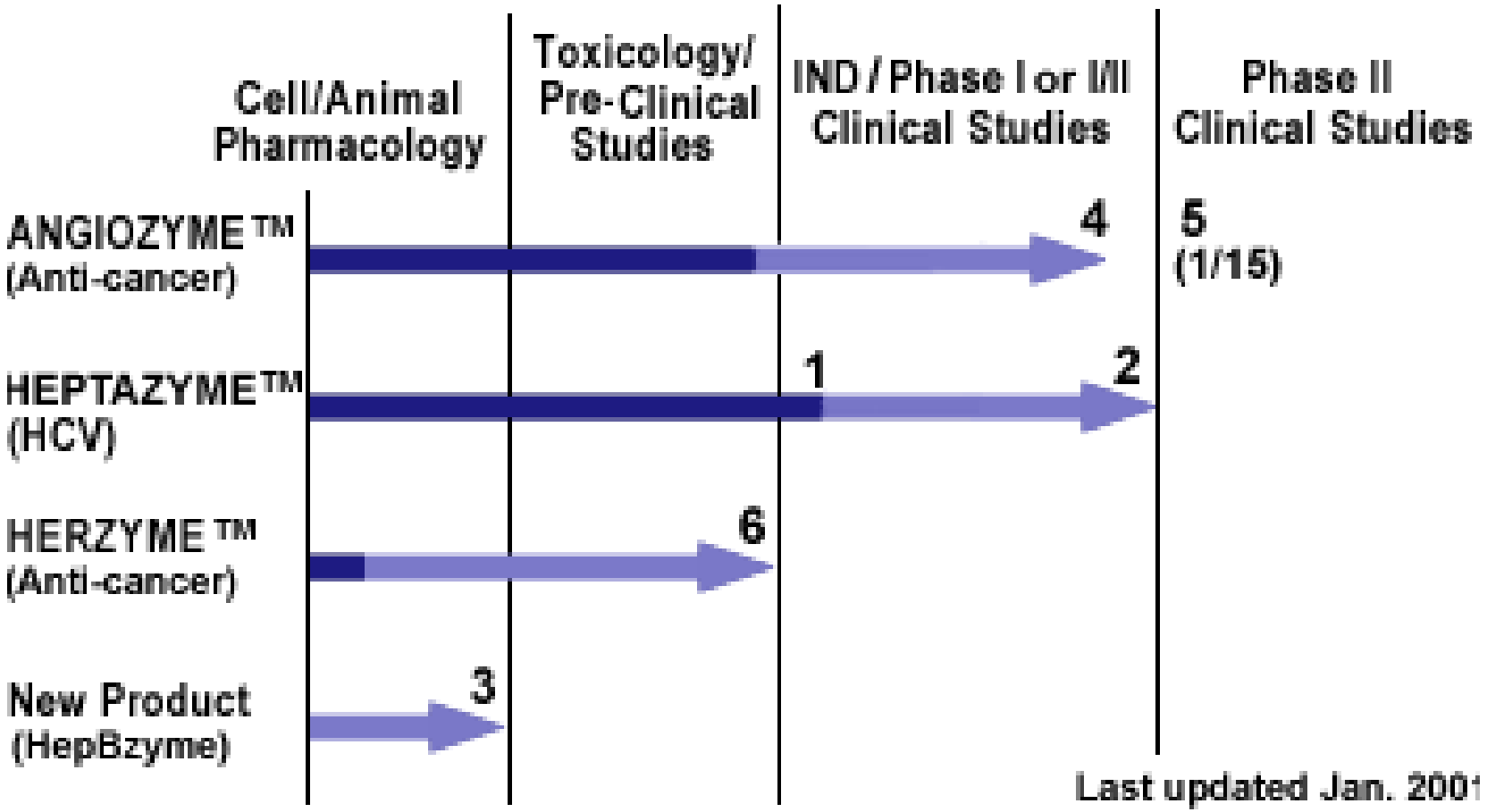
Ribozymes - application

1. Inhibition of gene expression

HIV
HBV
HCV
CML - chronic myelogenous leukemia
tumors - ANGIOZYME

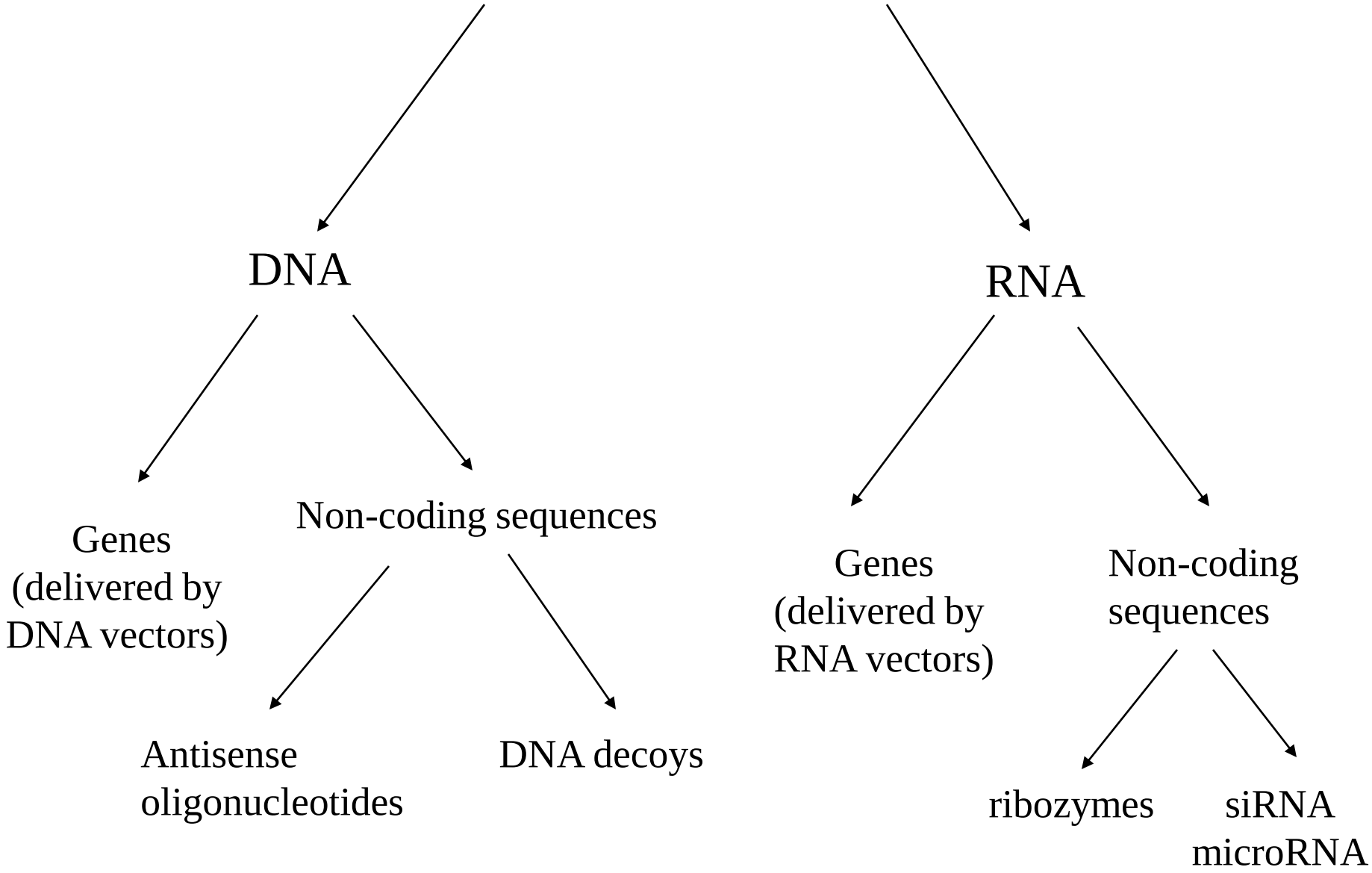
2. Correction of mutations

Ribozyme Pharmaceuticals Inc.



Sirna Therapeutics, Inc. (NASDAQ: RNAI), formerly Ribozyme Pharmaceuticals, Inc.

Therapeutic nucleic acids



Anti-HIV ribozymes

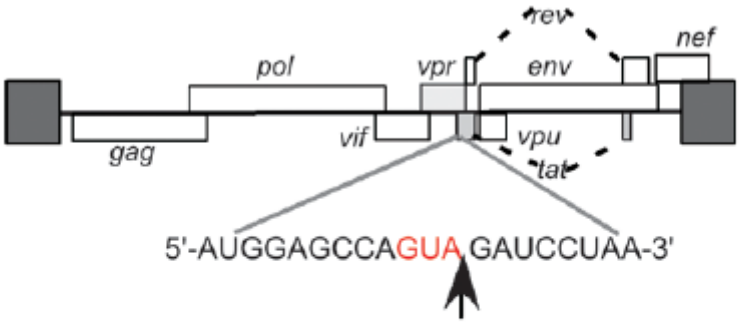
OZ1 comprises a Moloney Murine Leukemia Virus-based, replication-incompetent gamma retroviral vector (LNL6) containing a gene that encodes a ribozyme that targets the overlapping *vpr and tat reading frames of HIV-132,35. OZ1 inhibits the replication of laboratory and clinical* isolates of HIV-1 *in vitro*

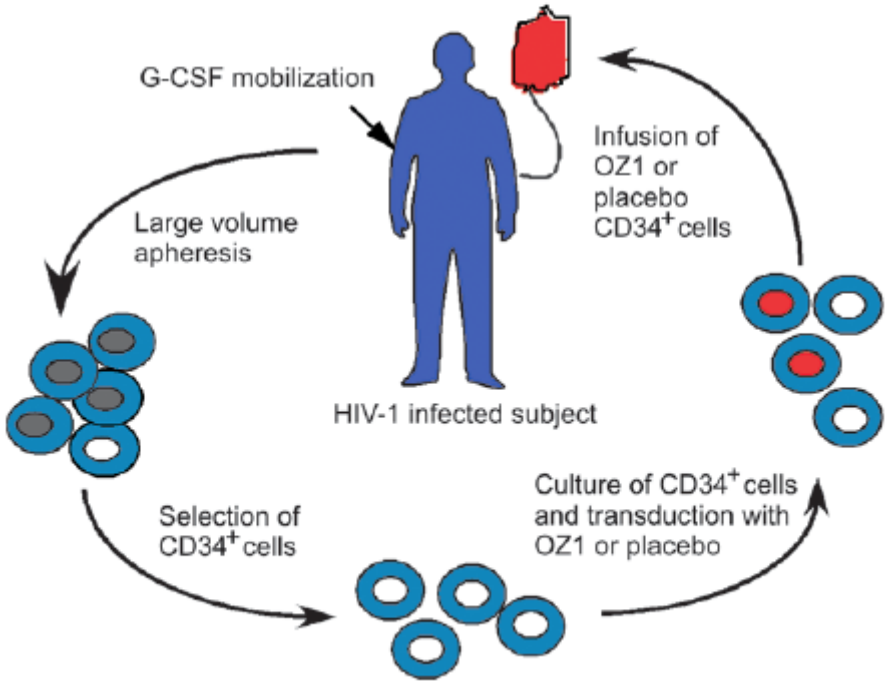
Nat Med. 2009 March ; 15(3): 285–292. doi:10.1038/nm.1932.

Safety and Efficacy of Autologous CD34+ Hematopoietic Progenitor Cells Transduced with an Anti-*Tat* Ribozyme in a Multi-Center, Randomized, Placebo-Controlled, Phase II Gene Therapy Trial for the Human Immunodeficiency Virus

Ronald T Mitsuyasu[1], Thomas C Merigan[2], Andrew Carr[3], Jerome A Zack[4], Mark A Winters[2], Cassy Workman[5], Mark Bloch[6], Jacob Lalezari[7], Stephen Becker[8], Lorna Thornton[8], Bisher Akil[9], Homayoon Khanlou[10], Robert Finlayson[11], Robert McFarlane[12], Don E Smith[13], Roger Garsia[14], David Ma[3], Matthew Law[15], John M. Murray[15,16], Christof von Kalle[17,18], Julie A Ely[19], Sharon M Patino[19], Alison E Knop[19], Philip Wong[19], Alison V Todd[19], Margaret Haughton[19], Caroline Fuery[19], Janet L Macpherson[19], Geoff P Symonds[19], Louise A Evans[19], Susan M Pond[19], and **David A Cooper[3,15]**

Anti-HIV ribozymes





Gene transfer has potential as a once-only treatment that reduces viral load, preserves the immune system, and avoids lifetime highly active antiretroviral therapy. This study, the first randomized, double-blind, placebo-controlled, phase II cell-delivered gene transfer clinical trial, was conducted in 74 HIV-1 infected adults who received a *tat/vpr* specific anti-HIV ribozyme (OZ1) or placebo delivered in autologous CD34+ hematopoietic progenitor cells. There were no OZ1-related adverse events. There was no statistical difference in viral load between the OZ1 and placebo group at the primary end-point (average at weeks 47 and 48) but time weighted areas under the curve from weeks 40-48 and 40-100 were significantly lower in the OZ1 group. Throughout the 100 weeks, CD4+ lymphocyte counts were higher in the OZ1 group. This study provides the first indication that cell-delivered gene transfer is safe and biologically active in HIV patients and can be developed as a conventional therapeutic product.

Mitsuyasu et al., Nature Med. 2009

RNA interference

PSTG – post-transciptional gene silencing

Specific inhibition of gene expression by double-stranded RNA, which stimulates the degradation of a target mRNA

Phenomena first observed in petunia

Attempted to overexpress chalcone synthase (anthrocyanin pigment gene) in petunia. (trying to darken flower color)

Caused the loss of pigment.

Jorgensen i wsp, 1990

THE PLANT CELL, Vol 2, Issue 4 279-289, Copyright © 1990 by American Society of Plant Biologists

Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes in trans

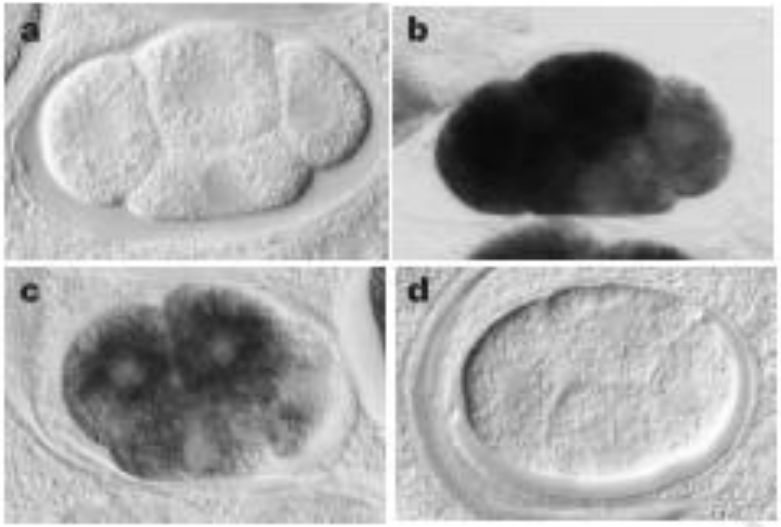
C. Napoli, C. Lemieux and R. Jorgensen DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, California 94608

We attempted to overexpress chalcone synthase (CHS) in pigmented petunia petals by introducing a chimeric petunia CHS gene. Unexpectedly, the introduced gene created a block in anthocyanin biosynthesis. Forty-two percent of plants with the introduced CHS gene produced totally white flowers and/or patterned flowers with white or pale nonclonal sectors on a wild-type pigmented background; none of hundreds of transgenic control plants exhibited such phenotypes. Progeny testing of one plant demonstrated that the novel color phenotype co-segregated with the introduced CHS gene; progeny without this gene were phenotypically wild type. The somatic and germinal stability of the novel color patterns was variable. RNase protection analysis of petal RNAs isolated from white flowers showed that, although the developmental timing of mRNA expression of the endogenous CHS gene was not altered, the level of the mRNA produced by this gene was reduced 50-fold from wild-type levels. Somatic reversion of plants with white flowers to phenotypically parental violet flowers was associated with a coordinate rise in the steady-state levels of the mRNAs produced by both the endogenous and the introduced CHS genes. Thus, in the altered white flowers, the expression of both genes was coordinately suppressed, indicating that expression of the introduced CHS gene was not alone sufficient for suppression of endogenous CHS transcript levels. The mechanism responsible for the reversible co-suppression of homologous genes in trans is unclear, but the erratic and reversible nature of this phenomenon suggests the possible involvement of methylation.

dsRNA inhibits gene expression more effectively than ssRNA

Control Embryo

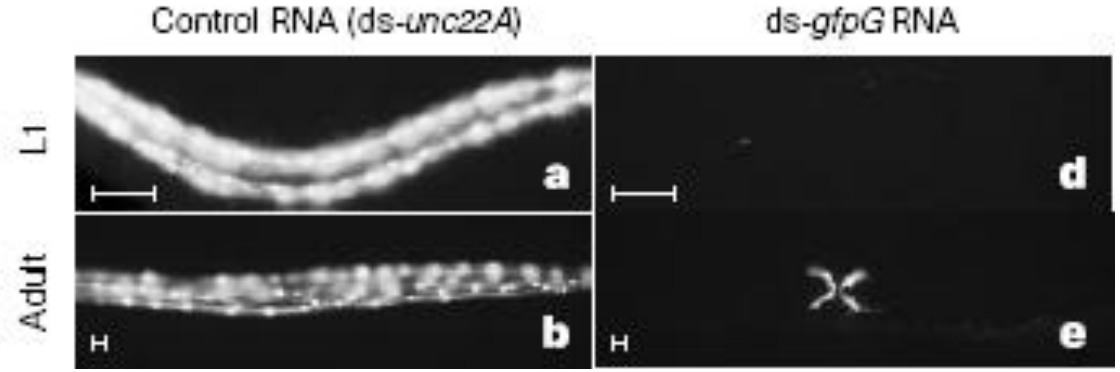
Embryo with Visualised mRNA of mex-3



Embryo injected with single-stranded antisense RNA

Embryo injected with dsRNA

Nematode after injection of unrelated dsRNA



Nematode injected with specific dsRNA

dsRNA several hundred nucleotides long

Fire at al., 1998

DsRNA is more effective than single stranded

2. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998;391:806-11.

- RNA interference is an inherited mechanism

- Only few dsRNA were required for inhibition of gene expression, indicating for an amplification process

Nobel prize 2007

Andrew Fire

Craig Mello

Patent for RNA interference

Genetic inhibition by double-stranded RNA

Abstract

A process is provided of introducing an RNA into a living cell to inhibit gene expression of a target gene in that cell. The process may be practiced ex vivo or in vivo. The RNA has a region with double-stranded structure. Inhibition is sequence-specific in that the nucleotide sequences of the duplex region of the RNA and of a portion of the target gene are identical. The present invention is distinguished from prior art interference in gene expression by antisense or triple-strand methods

Inventors: ***Fire*****; Andrew (Baltimore, MD), Kostas; Stephen (Chicago, IL), Montgomery; Mary (St. Paul, MN), Timmons; Lisa (Lawrence, KS), Xu; SiQun (Ballwin, MO), Tabara; Hiroaki (Shizuoka, JP), Driver; Samuel E. (Providence, RI), Mello; Craig C. (Shrewsbury, MA)**

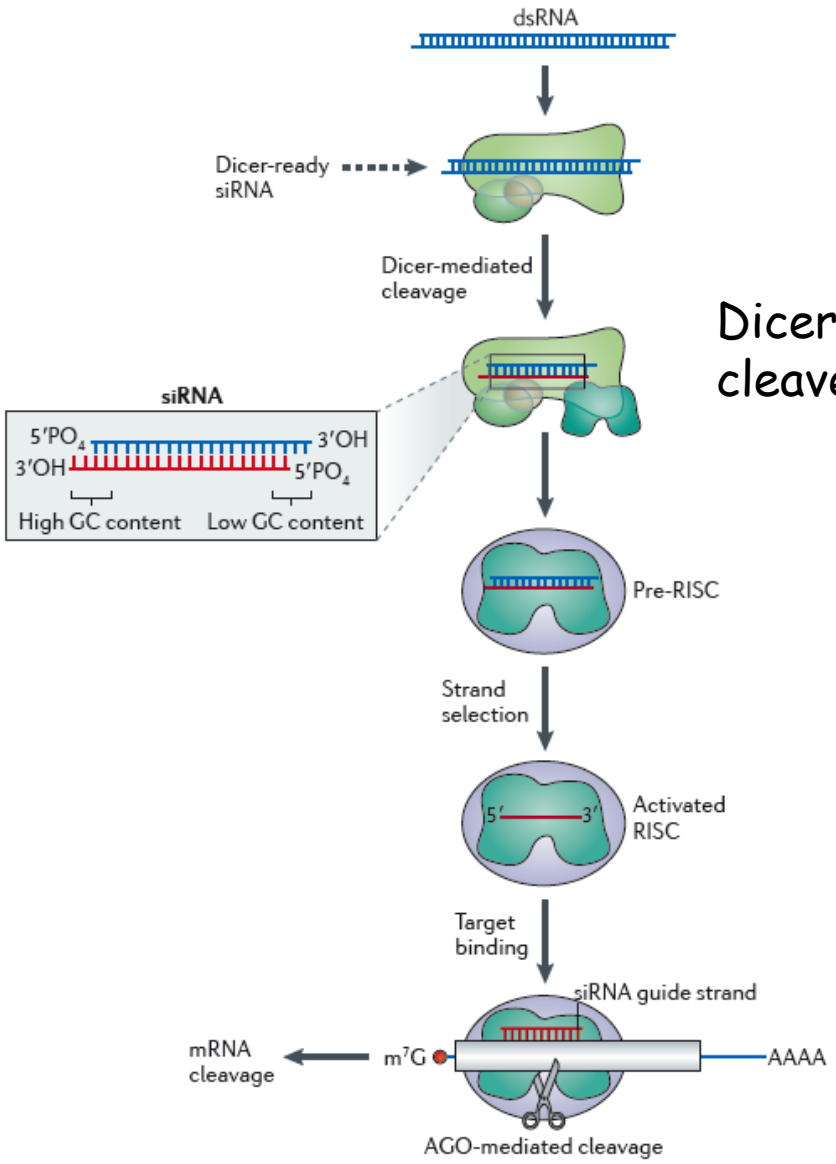
Assignee: Carnegie Institute of Washington (Washington, DC)

Appl. No. 09/215,257

Filed: December 18, 1998

This application claims the benefit of U.S. Provisional Appln. No. 60/068,562, filed Dec. 23, 1997.

Mechanisms of RNA interference



Dicer – an enzyme that cleaves long dsRNA

BL Davidson & PB McCray Jr, Nature Rev Genet, 2011

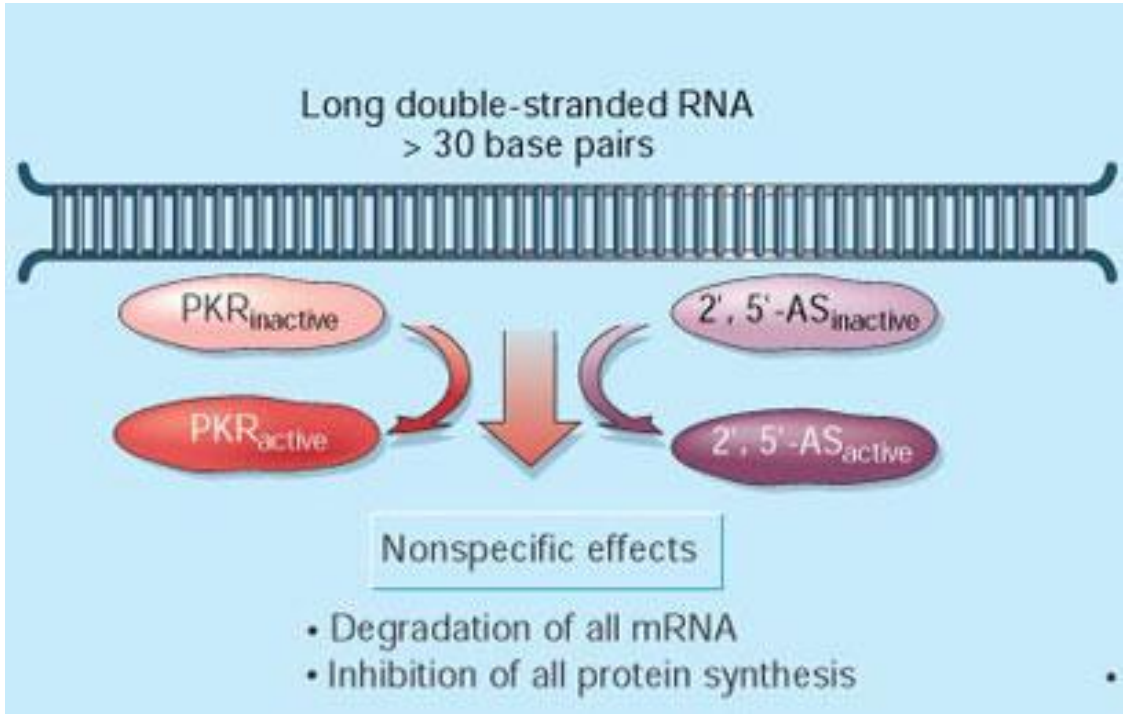
3.1.6. Biological functions of RNAi pathway – summary

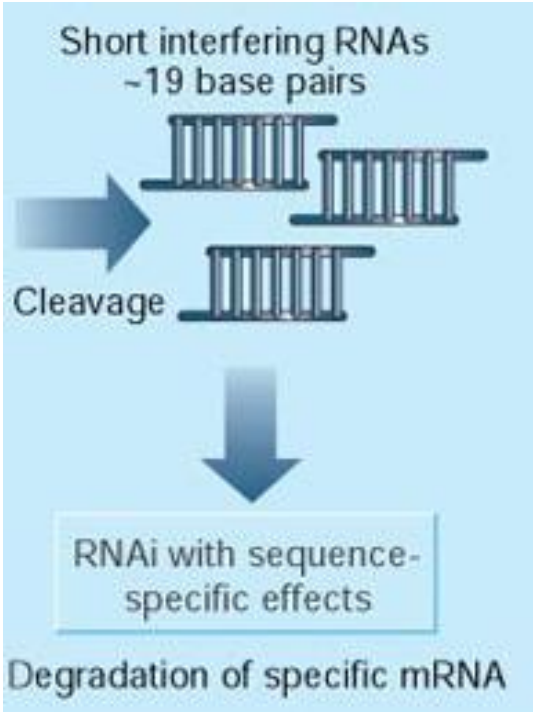
In a wide range of species RNAi-based mechanisms appear to be involved in the multiple physiological processes such as:

- The control of mobile genetic elements, such as transposons, in *C. elegans* and *D. melanogaster*;
- The defence against viral infection (plants, insects);
- The regulation of heterochromatin formation, mitosis and meiosis (investigated mainly in yeast);
- The regulation of gene expression during development (invertebrates and vertebrates).

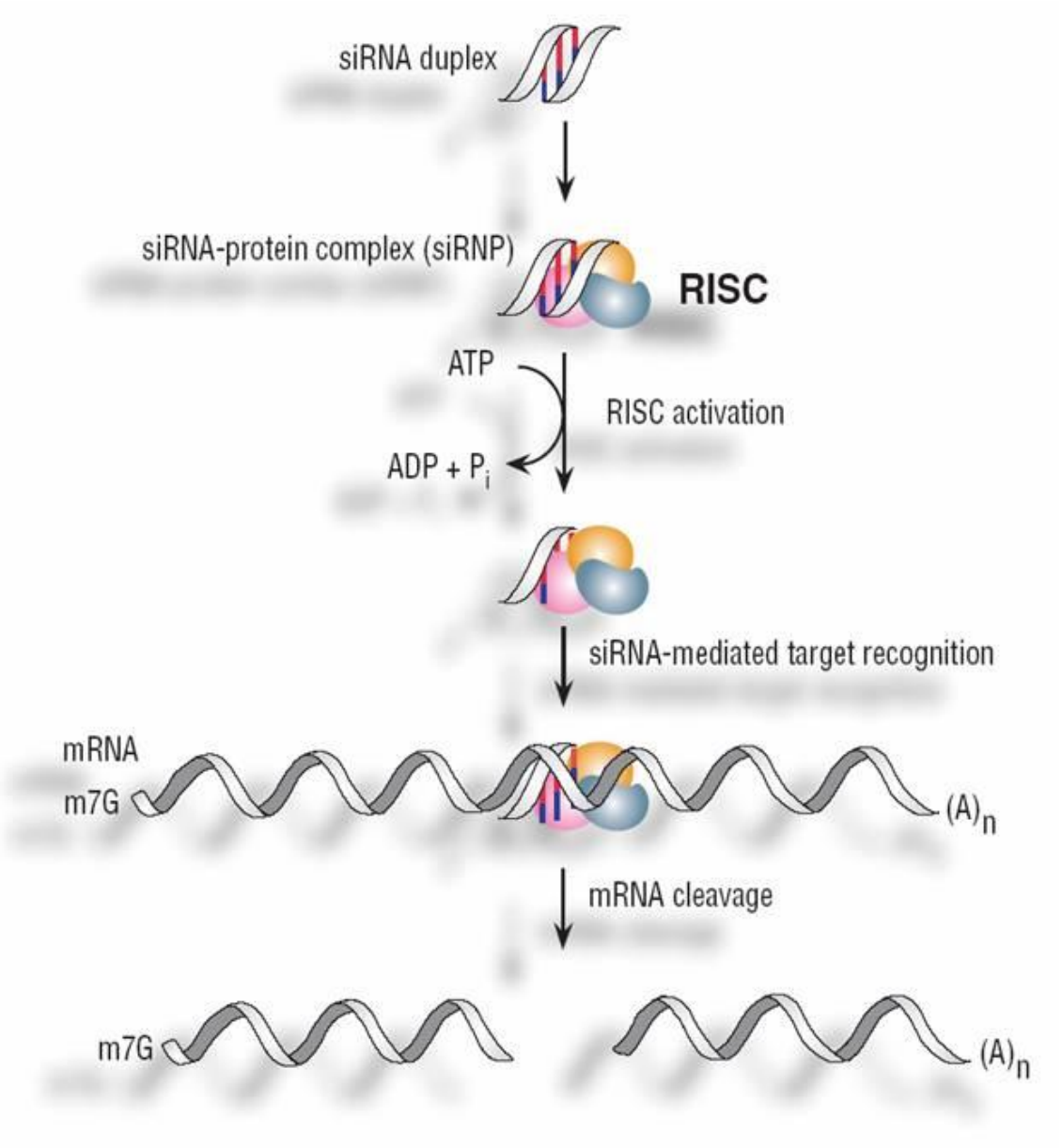
Application of siRNA in mammalian cells

Delivery of long dsRNA is not effective in mammalian cells

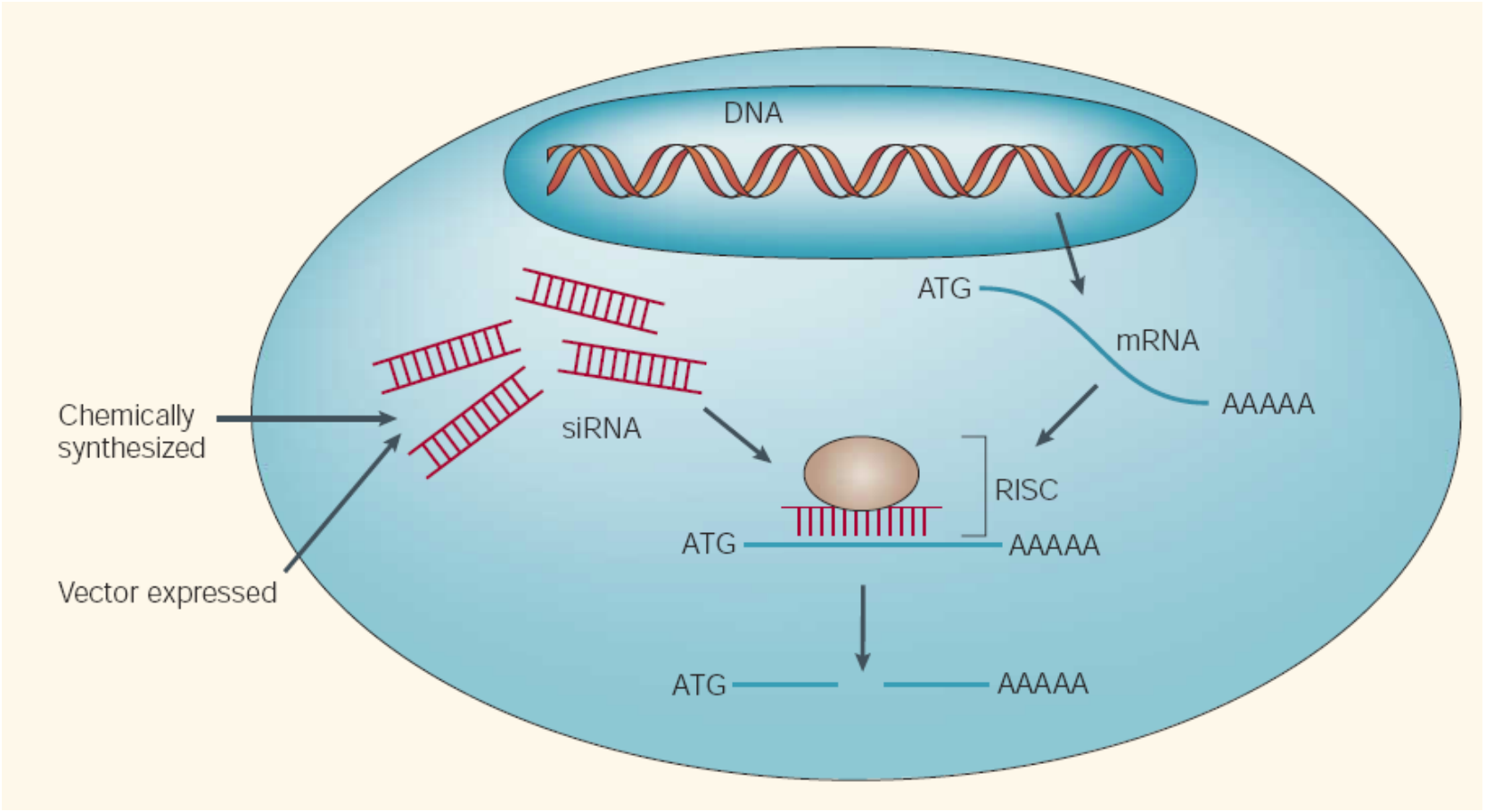




Mechanisms of action of siRNA in mammalian cells



Delivery of siRNA to mammalian cells



Chemically synthesised siRNA – short inhibition
siRNA-encoding vectors – long-term inhibition

Several ways to obtain siRNA

1. Chemical synthesis of RNA
2. Synthesis of DNA oligos, annealing, adding T7 polymerase promoter, transcription in vitro
3. Additing T7 polymerase promoter, transcription in vivo
4. Cloning of sequence coding antisense RNA into plasmid, transfection and expression in cells
5. Cloning into adenoviral/AAV/retroviral vectors

Generation of siRNA by overexpression in vectors

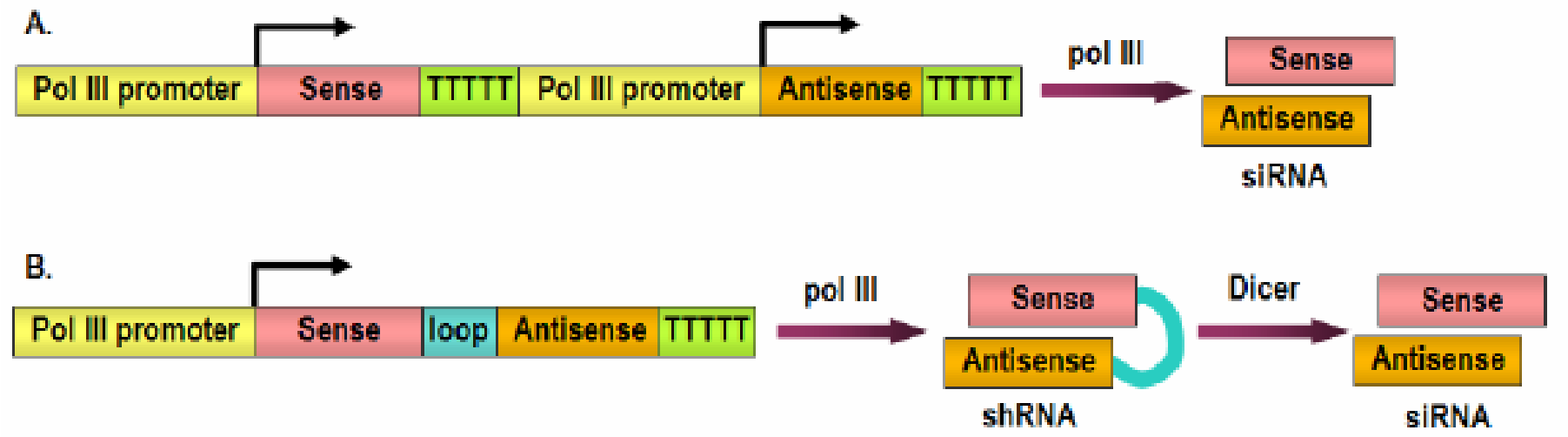
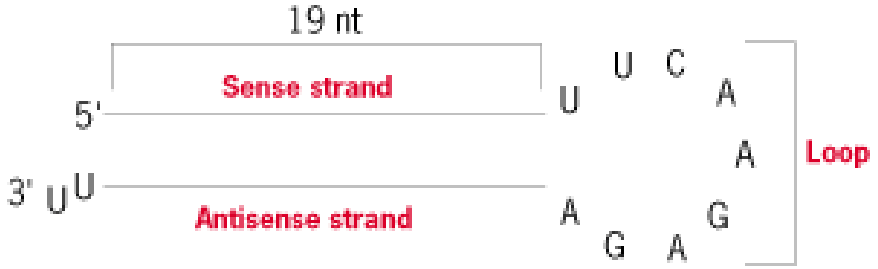


Figure 3.4. The strategies of siRNA expression from pol III vectors: tandem promoters (A) and single promoter followed by hairpin structure (B).

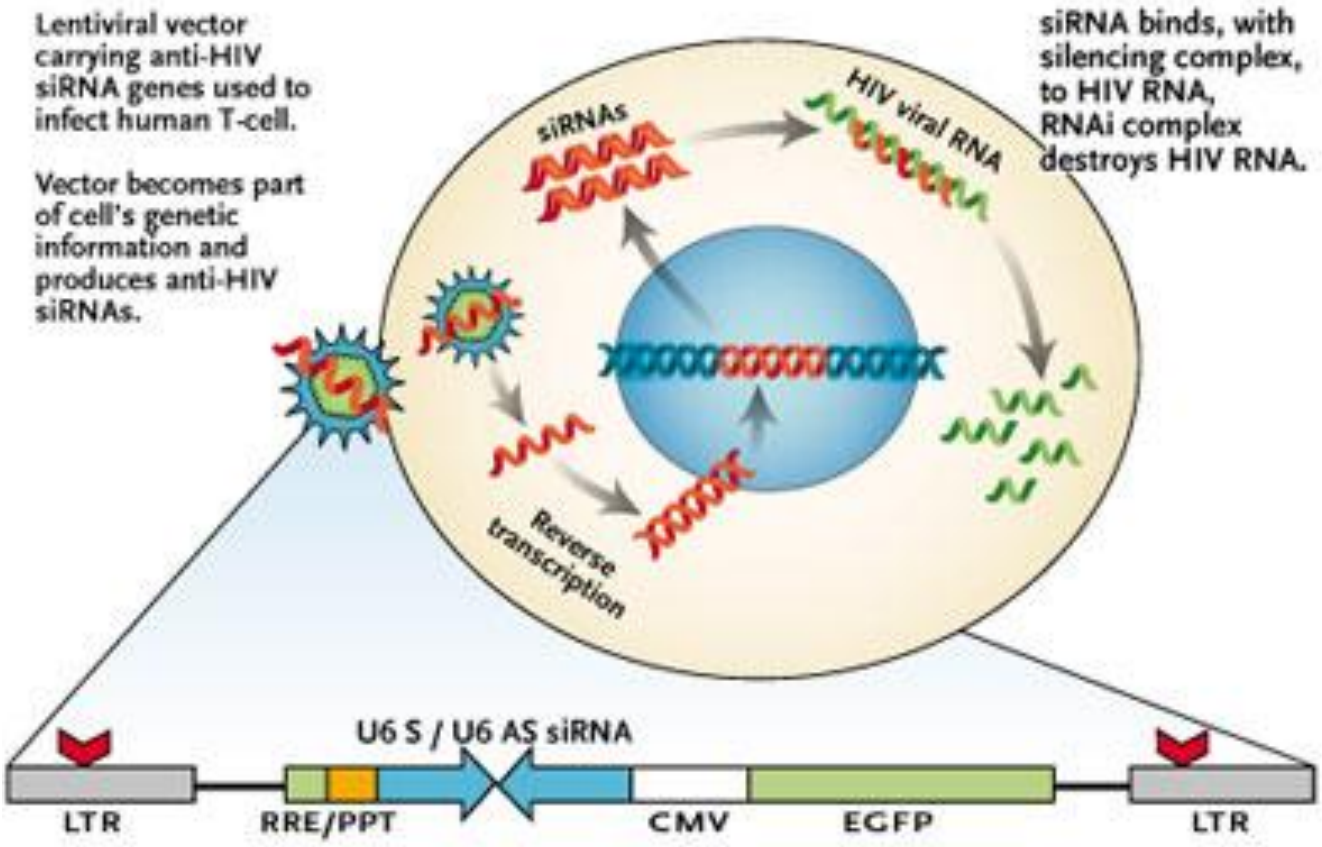
Plasmid vector for overexpression of siRNA



Viral vectors for overexpression of siRNA

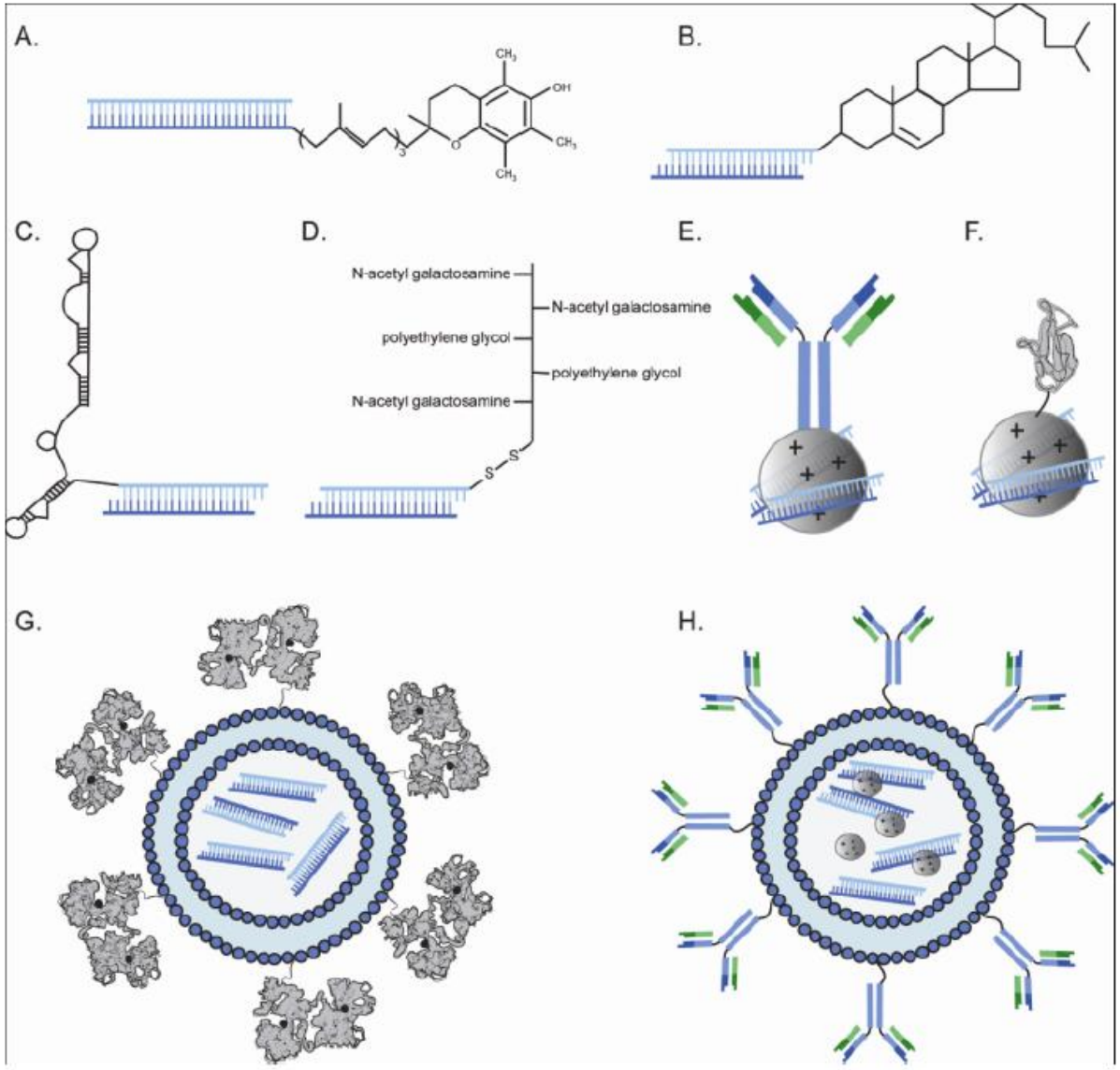
1. Adenoviral vectors
2. Retroviral/lentiviral vectors
3. AAV vectors

Lentiviral vector for overexpression of siRNA targeting HIV



SYSTEMIC RNAi: Citing concerns over efficacy, stability, and specificity, many researchers develop localized RNAi therapeutics strategies, such as for use in the eye. A novel variantion on this approach is being developed in which a patient's blood stem cells are transfected with a lentiviral vector expressing an anti-HIV siRNA. Those cells are then reintroduced to the patient, where the hope is that the cells will propagate and develop into mature blood cells capable of fending off HIV infection.

Methods of delivery of siRNA



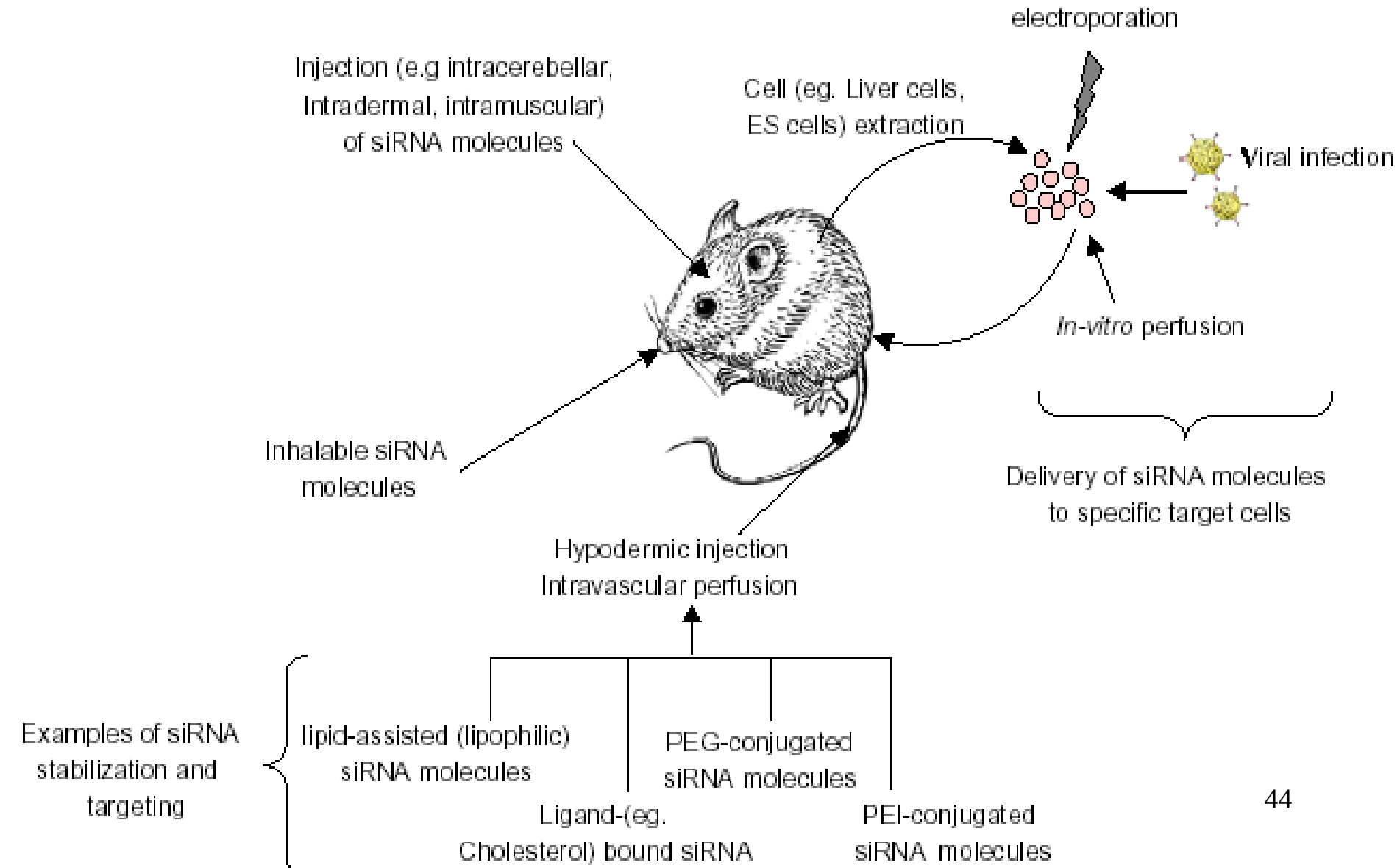
The delivery of siRNA is facilitated by directly conjugating the siRNA to the targeting reagent (A-D), non-covalent binding of siRNA with a highly positively charged motif fused to the targeting reagent (E and F), or encapsulation of the siRNA within a nanoparticle coated with the targeting reagent (G and H

α-tocopherol
cholesterol
N-acetyl-galactosamine
antibody
ligand
transferrin
antibody

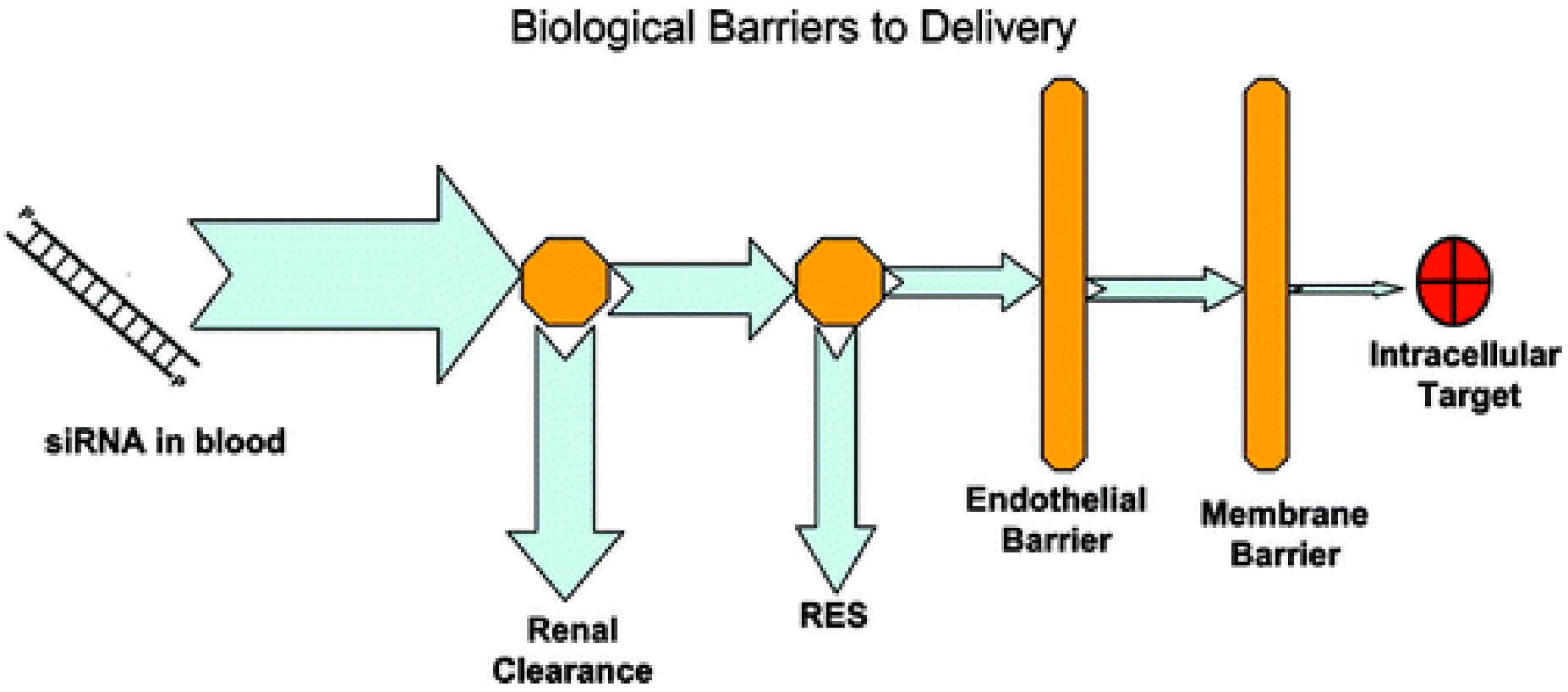
Manjutah & Dyskhoorn, Disc Med., 2011

Strategies for delivery of siRNA in vivo

R.K.M. Leung, P.A. Whittaker / Pharmacology & Therapeutics 107 (2005) 222–239



Barriers to overcome for siRNA delivery



Clinical trials for RNAi therapy (1)

Clinical setting	Drug	Indication(s)	Target(s)	Sponsor	Status
Ocular and retinal disorders	TD101	Pachyonychia congenita	Keratin 6A N171K mutant	Pachyonychia Congenita Project	Completed, Phase I
	QPI-1007	Non-arteritic anterior ischaemic optic neuropathy	Caspase 2	Quark Pharm., Inc.	Active, Phase I
	AGN211745	Age-related macular degeneration; choroidal neovascularization	VEGFR1	Sirna Therapeutics, Inc.	Completed, Phase I, II
	PF-655	Diabetic macular oedema (DME); age-related macular degeneration (AMD)	RTP801	Quark Pharm., Inc.	Active, Phase I
	SYL040012	Glaucoma	β2 adrenergic receptor	Sylentis	Active, Phase I, II
	Bevasiranib	Diabetic macular oedema	VEGF	Opko Health, Inc.	Completed, Phase II
	Bevasiranib	Macular degeneration	VEGF	Opko Health, Inc.	Completed, Phase II
Cancer	CEQ508	Familial adenomatous polyposis	β-catenin	MDRNA, Inc.	Active, Phase I
	ALN-PLK1	Liver tumours	PLK1	Alnyam Pharm.	Active, Phase I
	FANG	Solid tumours	Furin	Gradalis, Inc.	Active, Phase II
	CALAA-01	Solid tumours	RRM2	Calando Pharm.	Active, Phase I
	SPC2996	Chronic myeloid leukemia	BCL-2	Santaris Pharm.	Ongoing, Phase I, II
	ALN-VSP02	Solid tumours	VEGF, kinesin spindle protein	Alnylam Pharm.	Active, Phase I
	NCT00672542	Metastatic melanoma	LMP2, LMP7, and MECL1	Duke University	Active, Phase I
	Atu027	Advanced, recurrent or metastatic solid malignancies	PKN3	Silence Therapeutics	Active, Phase I

BL Davidson & PB McCray Jr, Nature Rev Genet, 2011

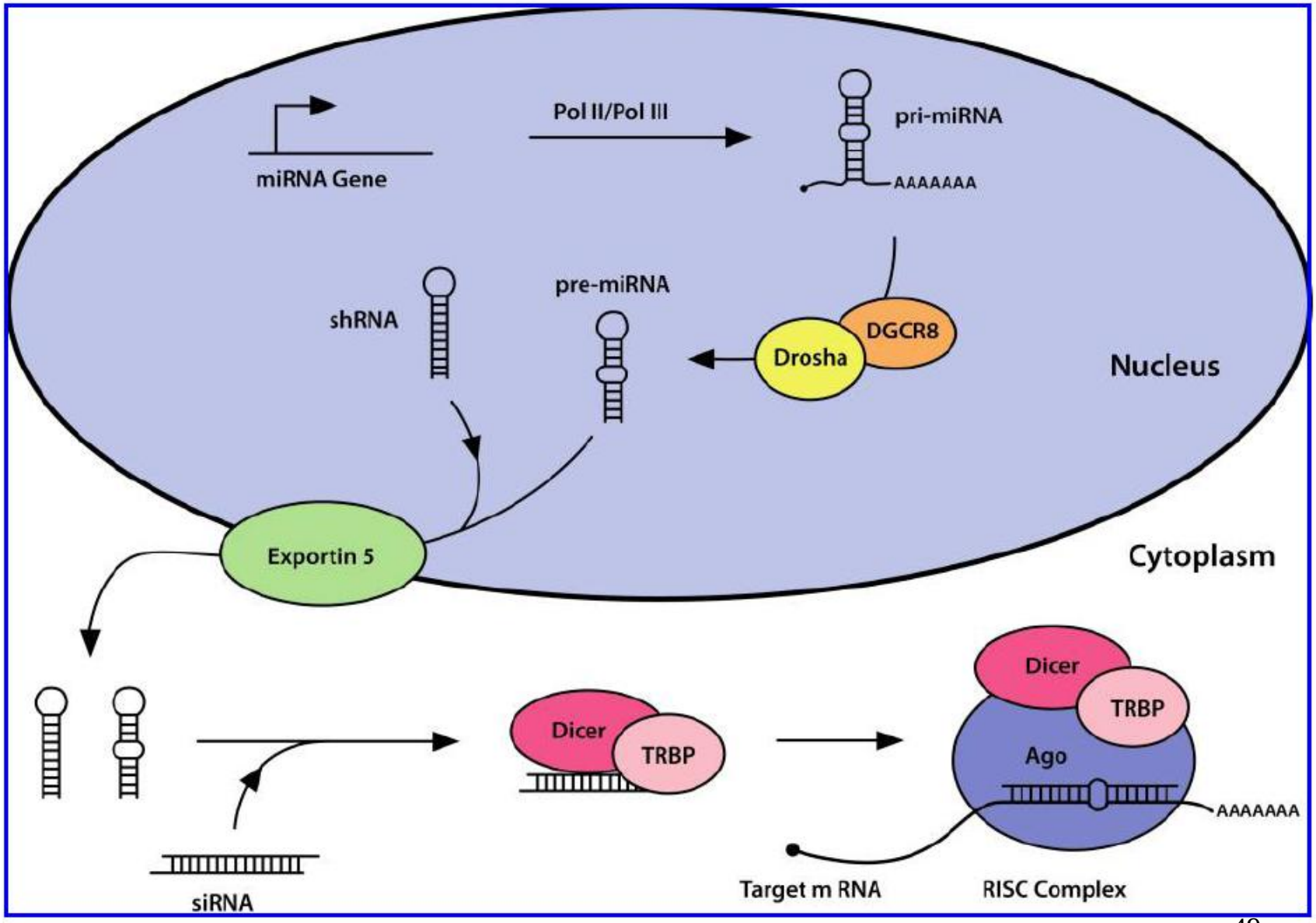
Clinical trials for RNAi therapy (2)

Clinical setting	Drug	Indication(s)	Target(s)	Sponsor	Status
Kidney disorders	QPI-1002/I5NP	Acute kidney injury	p53	Quark Pharm., Inc.	Terminated, Phase I
	QPI-1002/I5NP	Delayed graft function kidney transplant	p53	Quark Pharm., Inc.	Active, Phase I, II
	QPI-1002/I5NP	Kidney injury acute renal failure	p53	Quark Pharm., Inc.	Completed, Phase I
LDL lowering	TKM-ApoB	Hypercholesterolaemia	APOB	Tekmira Pharm. Corp.	Terminated, Phase I
	PRO-040,201	Hypercholesterolaemia	APOB	Tekmira Pharm. Corp.	Terminated, Phase I
Antiviral	SPC3649	Hepatitis C virus	miR-122	Santaris Pharm	Active, Phase II
	pHIV7-shI-TAR-CCR5RZ	HIV	HIV Tat protein, HIV TAR RNA, human CCR5	City of Hope Medical Center/Benitec	Active, Phase 0
	ALN-RSV01	RSV in volunteers	RSV nucleocapsid	Alnylam Pharm.	Completed, Phase II
	ALN-RSV01	RSV in lung transplant patients	RSV nucleocapsid	Alnylam Pharm.	Completed, Phase I
	ALN-RSV01	RSV in lung transplant patients	RSV nucleocapsid	Alnylam Pharm.	Active, Phase II

BL Davidson & PB McCray Jr, Nature Rev Genet, 2011

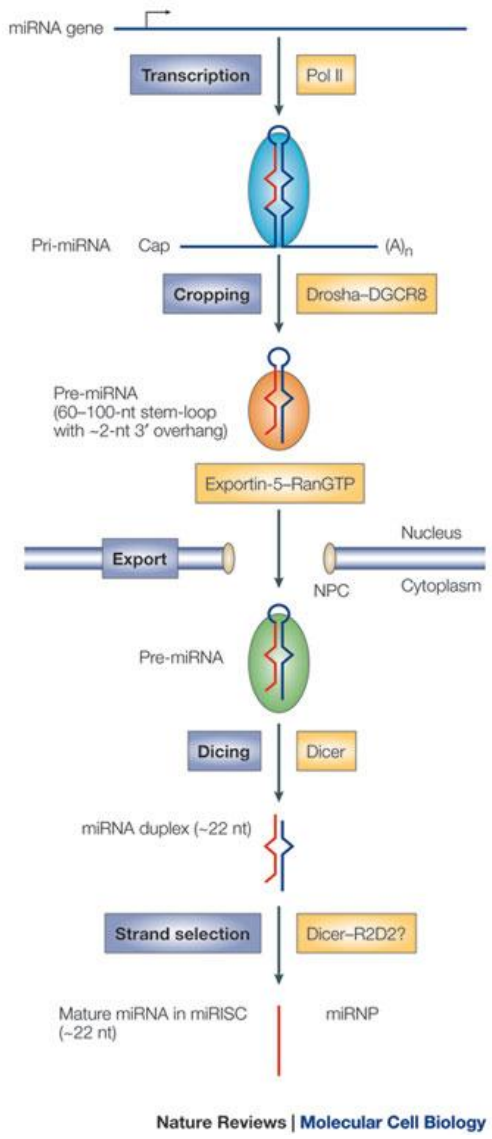
microRNA

The microRNA/RNA interference pathway



Marquez & McCaffrey, Hum Gene Therapy 2008

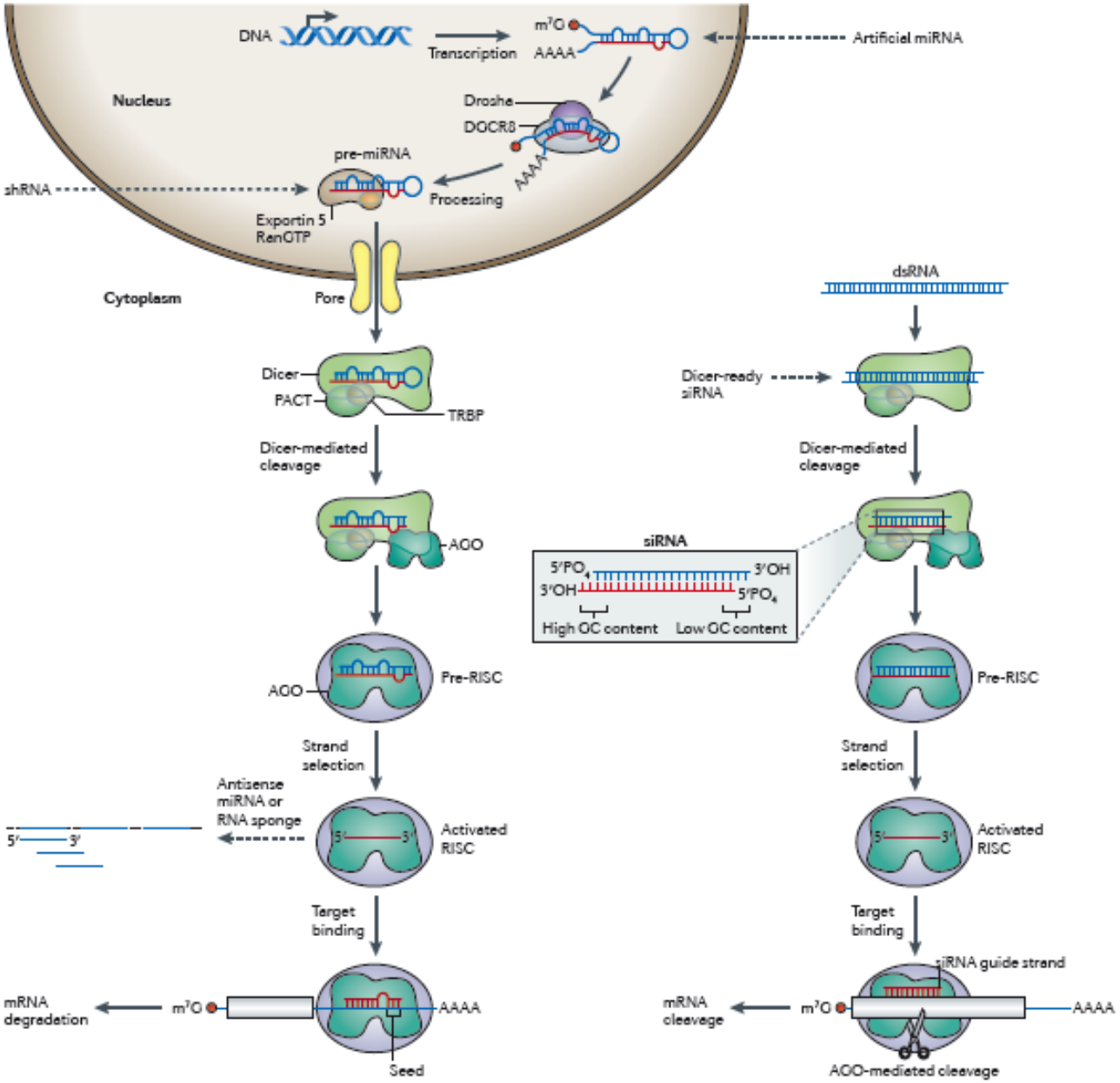
microRNA biogenesis



MicroRNA (miRNA) genes are transcribed by RNA polymerase II (pol II) to generate the primary transcripts (pri-miRNAs). The initiation step ('cropping') is mediated by the Drosha–DGCR8 complex (also known as the Microprocessor complex). Drosha and DGCR8 are both located mainly in the nucleus. The product of this nuclear processing step is a 70-nucleotide (nt) pre-miRNA, which possesses a short stem plus a 2-nucleotide 3' overhang. This structure might serve as a signature motif that is recognized by the nuclear export factor exportin-5. Pre-miRNA constitutes a transport complex together with exportin-5 and its cofactor Ran (the GTP-bound form). Following export, the cytoplasmic RNase III Dicer participates in the second processing step ('dicing') to produce miRNA duplexes. The duplex is separated and usually one strand is selected as the mature miRNA, whereas the other strand is degraded. In ***Drosophila melanogaster***, R2D2 forms a heterodimeric complex with Dicer and binds to one end of the small interfering RNA duplex. It thereby selects one strand of the duplex. It is not known if miRNAs use the same machinery for strand selection (see question mark). It is also unclear as to whether an R2D2 homologue functions in other animals apart from ***D. melanogaster***. miRISC, miRNA-containing RNA-induced silencing complex; NPC, nuclear pore complex

VN Kim, Nature Rev Mol Cell Biol 2010

The siRNA and miRNA pathway of RNAi in mammals



A little bit of history…

- 1981 – lin4 identified in a genetic screen in C. elegans
- 1993 – lin4 characterized molecularly, downregulation of lin14
- 1998 – RNAi silencing of genes
- 2000 – miRNA let7 with homologs in other species including humans
- Plants – 120 miRNA encoding genes
- Invertebrates – 150
- Humans - 500

Physiological roles of miRNA

C. elegans – lin-4 & let-7 – encode miRNA

↓

Mutation in those genes – disturbances in development of *C. elegans*

Involvement in:

- programmed cell death (apoptosis)
- neuron specification
- controlling of fat accumulation
- counteraction of genetic parasites

microRNA

1. Regulate the expression of about 50% (or more…) mRNA genes

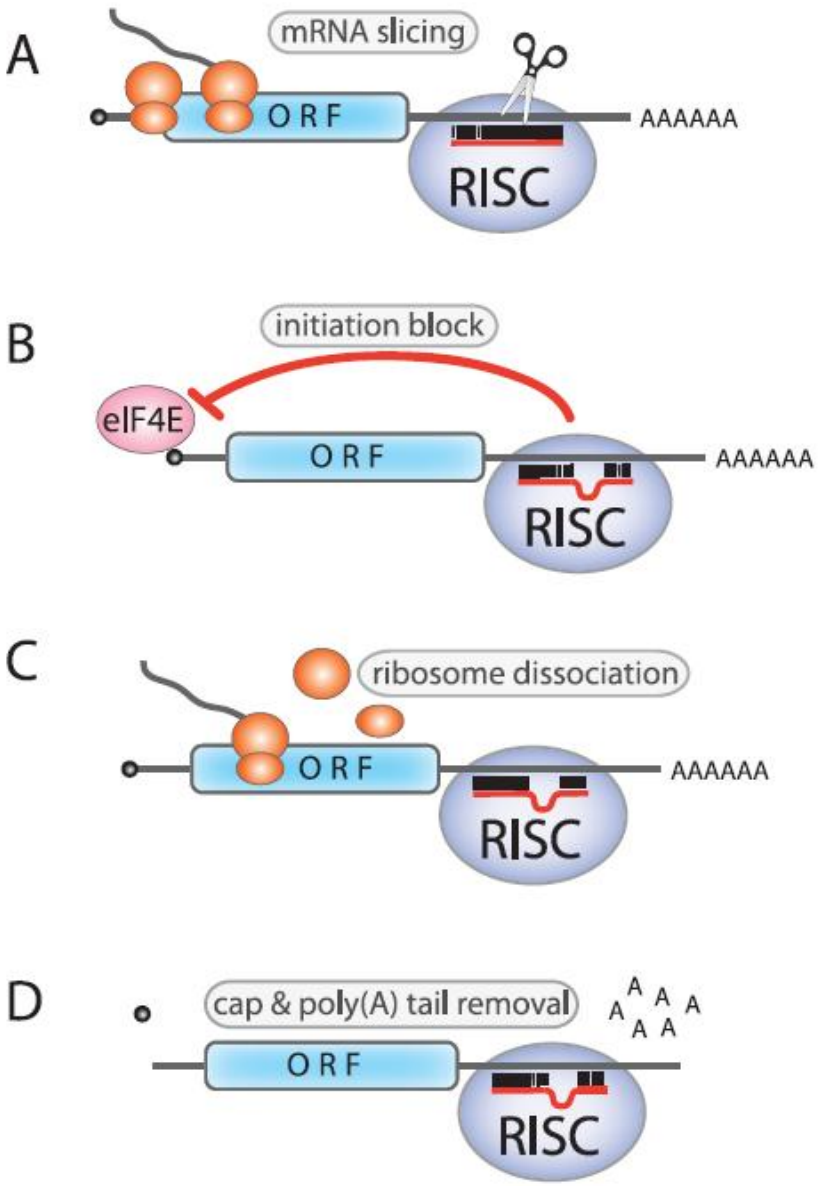
2. Regulation is strong and dependent on endogenous RNA interference system

3. microRNAs target specific sequences in mRNA

4. microRNAs are often expressed in a cell-specific manner

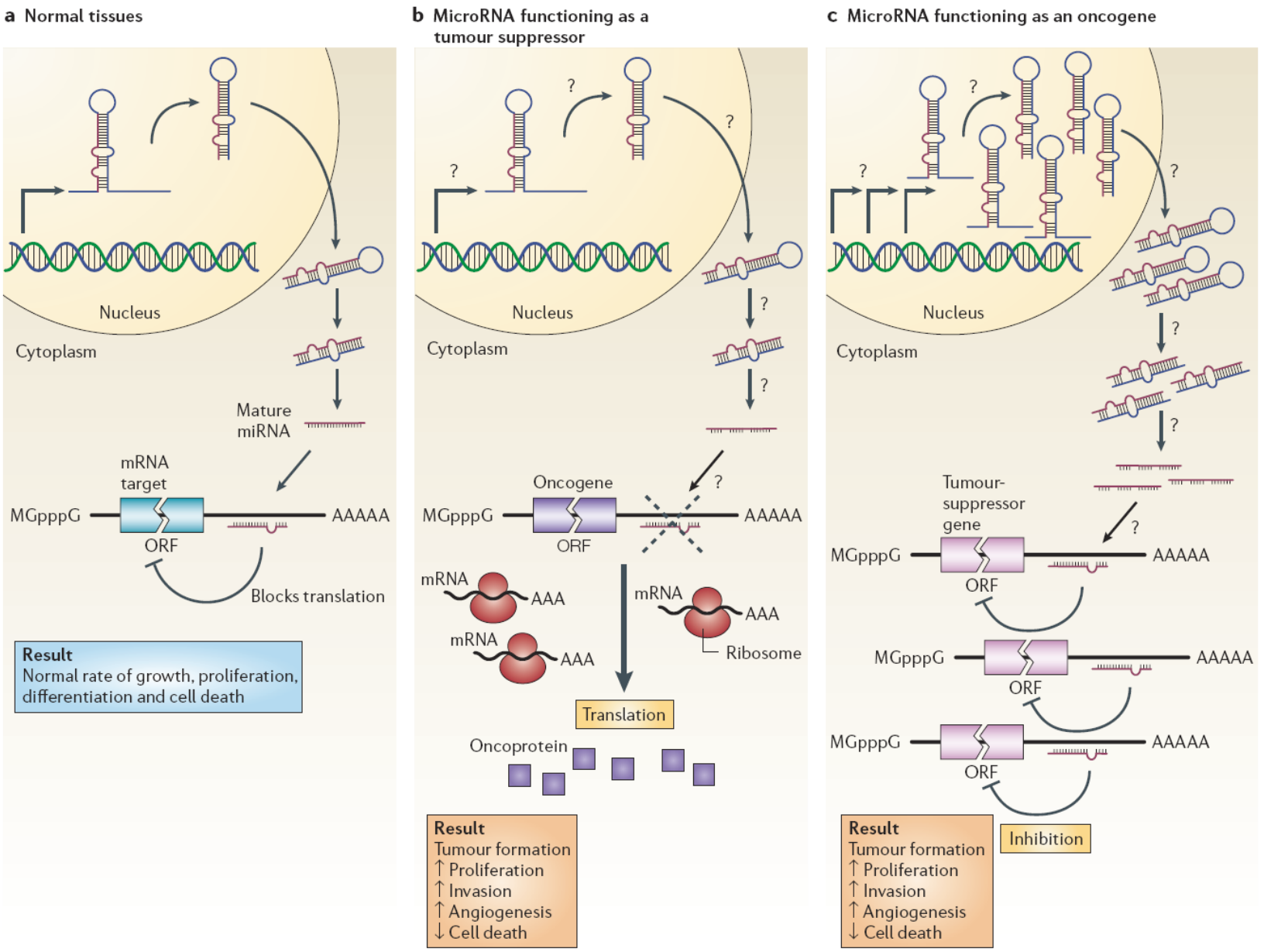
5. microRNA targeted vectors may allow cell-specific regulation of gene expression

Multiple mechanisms of post-transcriptional gene silencing by microRNA



Ku and McManus, Hum Gene Therapy, 2008

Role of miRNA in tumor development



Esquela-Kerscher & Slack, Nature Rev Cancer 2006

Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications

Cell-specific regulation of gene expression

1. **Cell specific promoters**

-eg, Flk-1, Tie -2: endothelial-specific

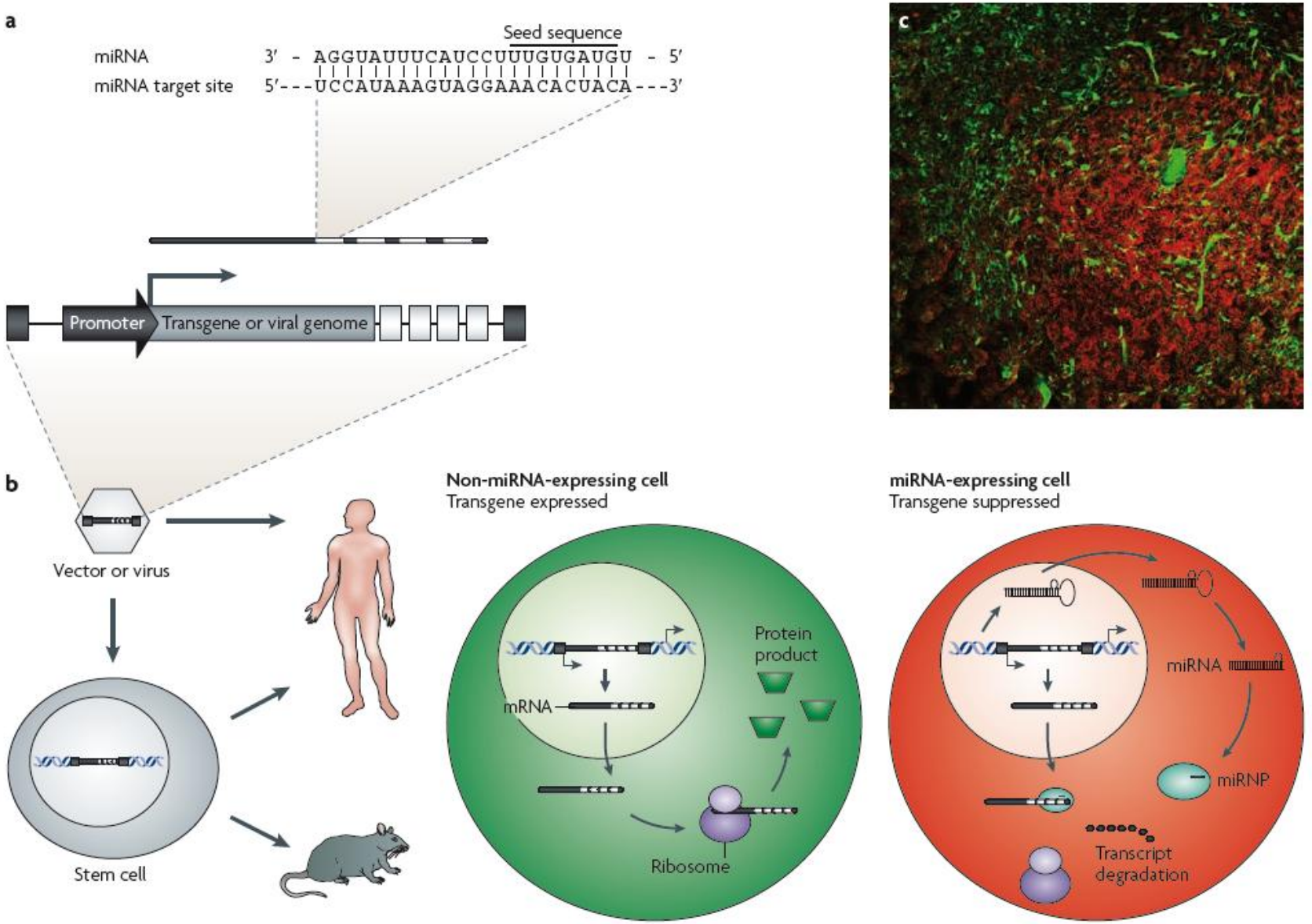
-Keratin 14 – keratinocyte specific

2. Vectors inhibited by microRNA

In 3' region of the transgene the sequence recognized by microRNA expressed in the cells, in which we do not want to express the transgene is incorporated

eg. if we want the expression in hepatocyte but not in Kuppfer cell, we have to add to the 3' mRNA of the transgene the sequence recognized specifically by microRNA expressed in Kuppfer cells

microRNA regulated vectors to drive expression in a specific cell



Naldini i wsp. 2009

Inhibiting of endogenous microRNA functions

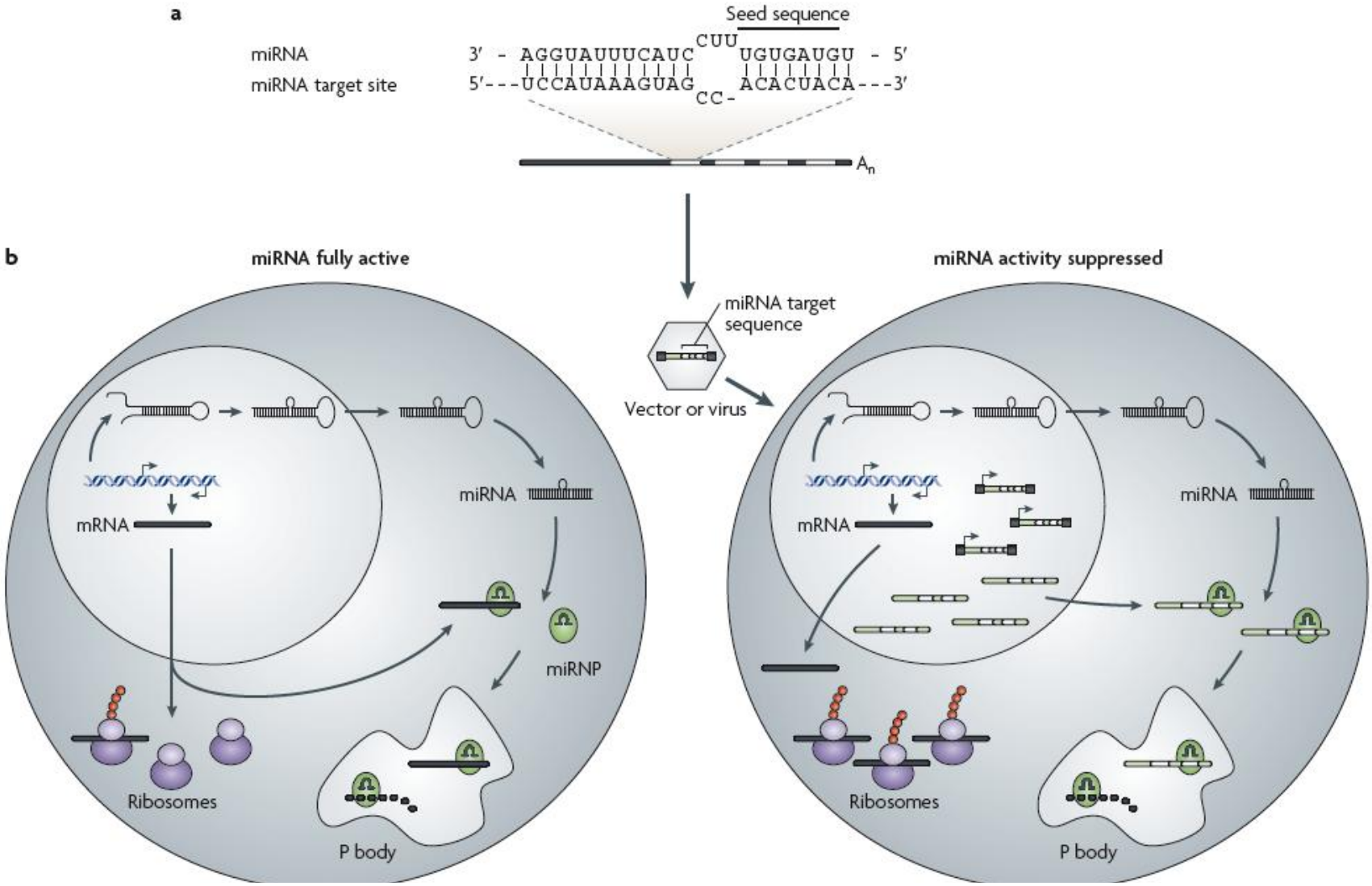


Figure 2 | **Inhibiting endogenous microRNA function. a** | Schematic of a vector or virus used to inhibit a microRNA (miRNA). The vector encodes multiple copies of perfectly complementary or bulged target sites (as shown) for a miRNA or miRNA family. **b** | The target sites are expressed at high levels by a strong RNA polymerase II or III promoter or by introducing a large number of vectors into a cell. The target-containing transcripts bind to the cognate miRNA, and because of excess target concentration saturate the miRNA. This limits the availability of the miRNA, and inhibits the regulation of its natural target mRNAs. The processing body (P body) is a region rich in enzymes involved in mRNA turnover. miRNP, miRNA–ribonucleoprotein complex.

Oligonucleotide manipulation of microRNA function

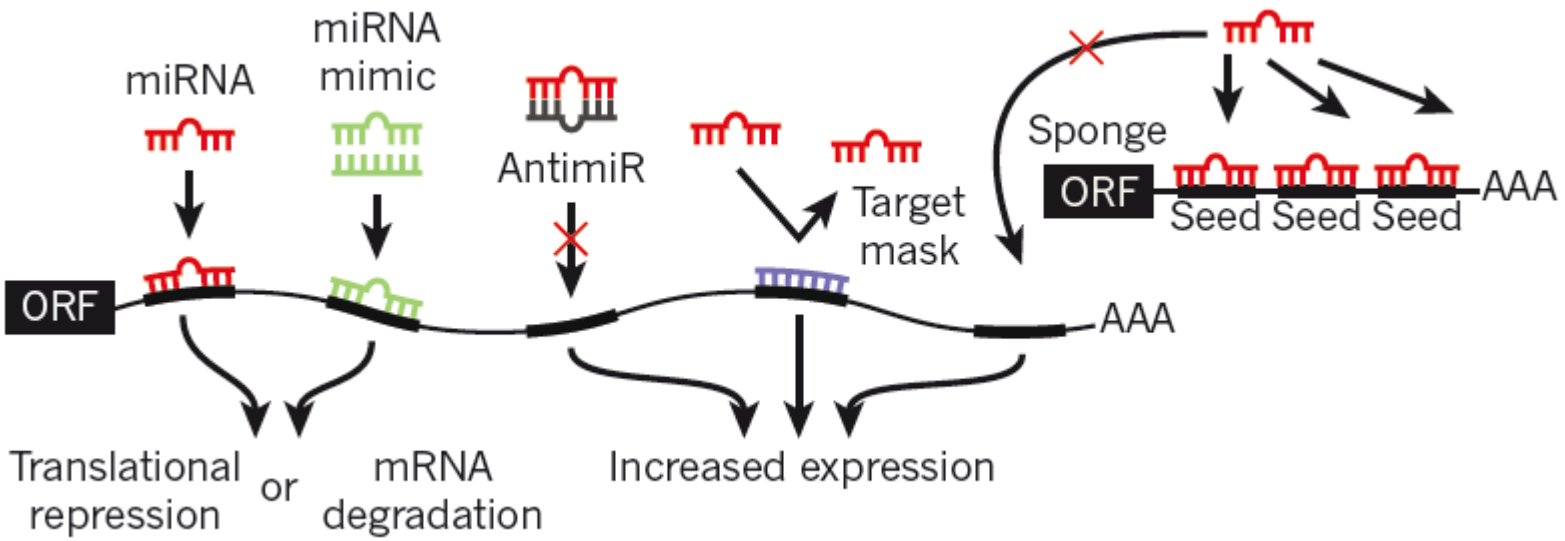


Figure 2 | Oligonucleotide manipulation of miRNA function. The various methods of artificially modulating miRNA expression or activity are shown. Endogenous miRNA (red) binds to complementary sequences in the 3′ UTR of a target gene, resulting in translational repression or mRNA degradation. An miRNA mimic (green) consists of an oligonucleotide duplex of the miRNA and a passenger strand. The miRNA mimic comprises the same nucleotide sequence as an endogenous miRNA, and is designed to target the same mRNAs as that miRNA. An antimiR (grey) is an oligonucleotide that is complementary to an endogenous miRNA, thereby designed to bind and inhibit its function. A target mask (blue) is an oligonucleotide designed to bind to a portion of an endogenous miRNA target without initiating mRNA degradation or translational inhibition. This strategy rescues one particular mRNA from miRNA-mediated repression. miRNA sponges consist of an open reading frame (ORF) linked to a 3′ UTR that contains several binding sites for a particular miRNA, acting as competitive inhibitors for miRNA binding.

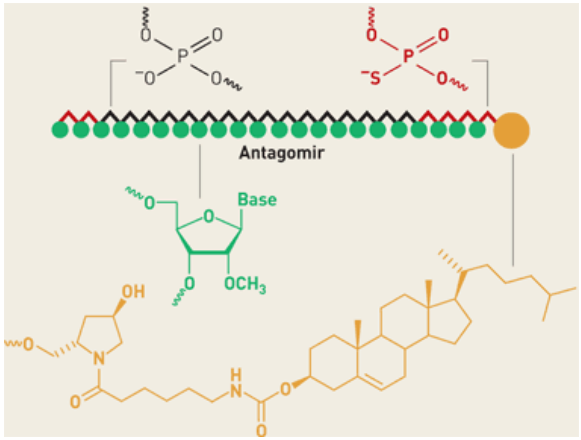
Small & Olson, Nature 2011

Antagomirs (antmirs)

Antagomirs are RNA-like oligonucleotides that harbor various modifications for RNAse protection and pharmacologic properties such as enhanced tissue and cellular uptake. They differ from normal RNA by complete 2′-*O*-methylation of sugar, phosphorothioate backbone and a cholesterol-moiety at 3′-end. Antagomirs efficiently silence miRNAs in most tissues after three injections at 80 mg/kg bodyweight (bw) on consecutive days.

. Antagomirs of mir-122 and mir-16*

Sequence	Description
5′-UGGAGUGUGACAAUGGUGUUUGU-3′	Mir-122
5′-$a_s c_s$aaacaccauugucacacu$_s c_s c_s a_s$-Chol-3′	Antagomir-122 (23 nt, 6 × P=S)
5′-$c_s a_s$caaacaccauugucacacuc$_s c_s a_s c_s$-Chol-3′	Antagomir-122 (25 nt, 6 × P=S)
5′-$c_s a_s$aacaccauugucacac$_s u_s c_s c_s$-Chol-3′	Antagomir-122 (21 nt, 6 × P=S)
5′-$a_s a_s$acaccauugucaca$_s c_s u_s c_s$-Chol-3′	Antagomir-122 (19 nt, 6 × P=S)
5′-$a_s a_s$caccauugucac$_s a_s c_s u_s$-Chol-3′	Antagomir-122 (17 nt, 6 × P=S)

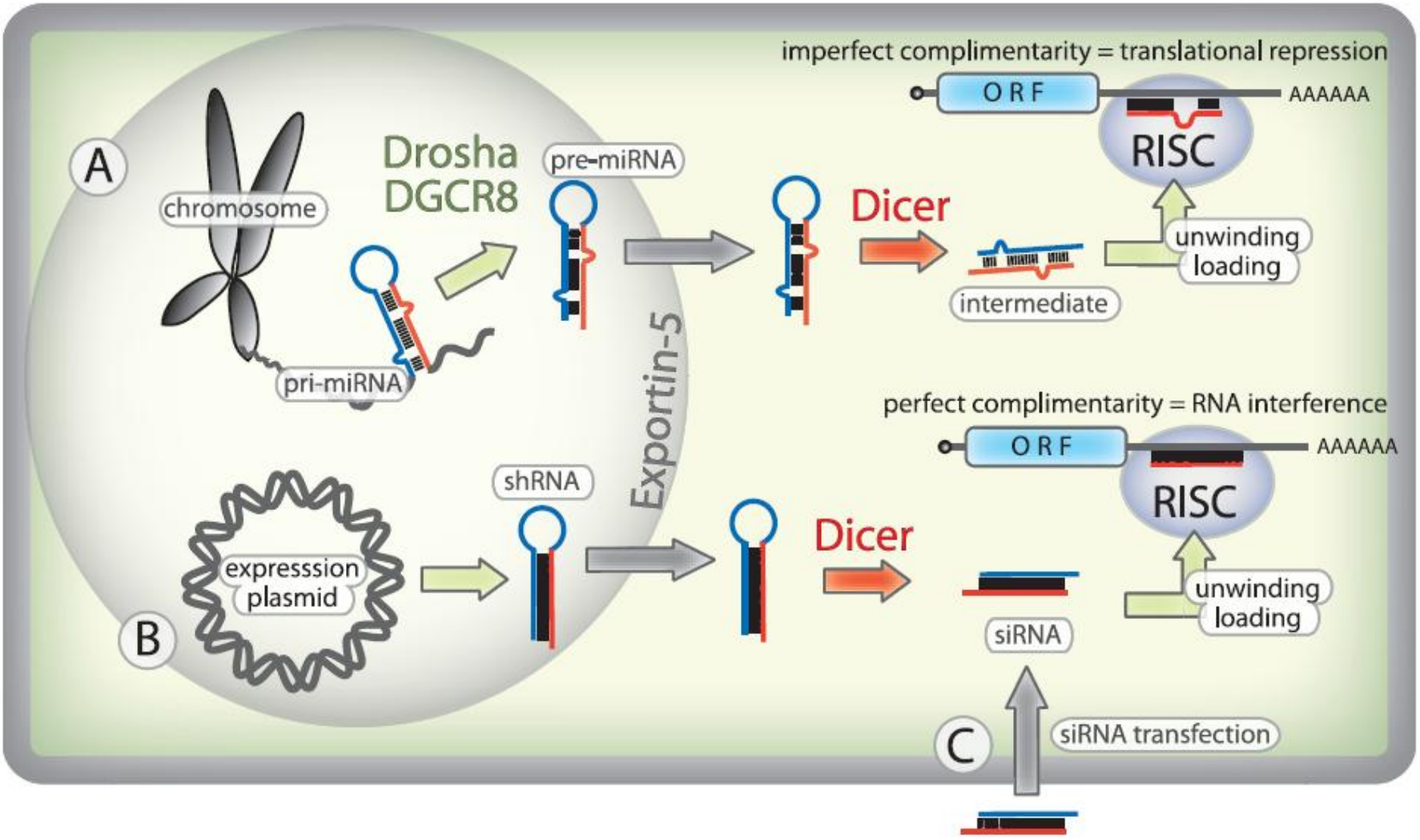


Methods for delivery of RNAi triggers

Species/ formulation	Packaging capacity	Applications and considerations
Viral vector		
Adenovirus	Up to ~35 kb, usually <10 kb	dsDNA vector with large packaging capacity, transient expression, highly immunogenic
Adeno-associated virus (AAV)	~4.5 kb	ssDNA vector, small packaging capacity, mildly immunogenic, lasting expression in nondividing cells, capsid pseudotyping/engineering facilitates specific cell-targeting
Lentivirus	Up to 13.5 kb (larger inserts will decrease titre)	RNA vector, integration competent and incompetent forms available, less immunogenic than adenovirus or AAV, envelope pseudotyping facilitates cell targeting, clinical production more difficult than for adenovirus or AAV
Herpes simplex virus	150 kb	DNA vector, episomal, lasting expression, immunogenic
Bacterial vector species[‡]		
Escherichia coli, *S.* Typhymurium[§]		Delivery of short hairpin RNA or small interfering RNA to gut tissue
Non-viral formulations[‖]		
Nanoparticle		Self-assembling, may target specific receptors, requires technical expertise to prepare
Stable nucleic acid lipid particle (SNALP)		Stable for systemic delivery, broad cell-type delivery
Aptamer		Targeting of specific receptors, requires sophisticated screening to develop
Cholesterol		Stable for systemic delivery, broad cell-type delivery

BL Davidson & PB McCray Jr, Nature Rev Genet, 2011

Mechanisms of microRNA, shRNA and siRNA are similar



Ku and McManus, Hum Gene Therapy, 2008