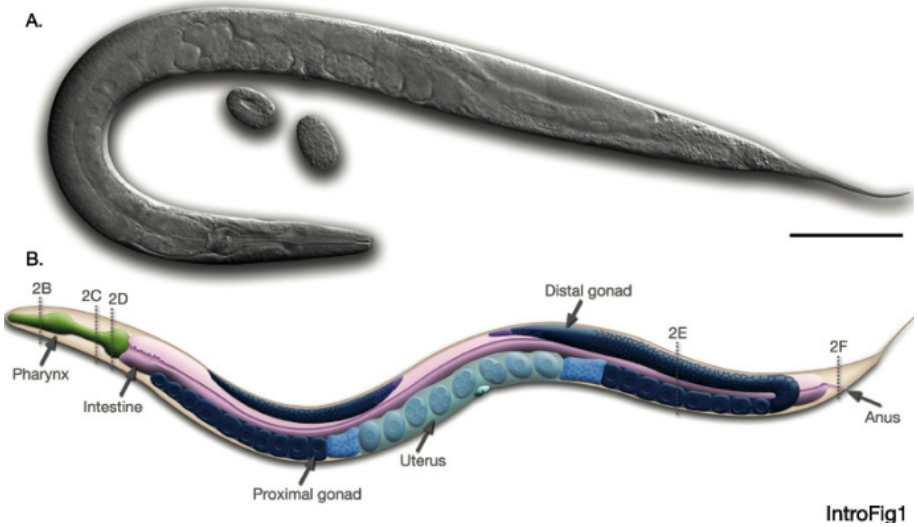
siRNA ve tedavide kullanımı

Ar.Gör.Dr.Pelin Özyavuz Çubuk
Gazi Üniv. Tıp Fakültesi
Tıbbi Genetik AD
2016



Caenorhahditis eiegans

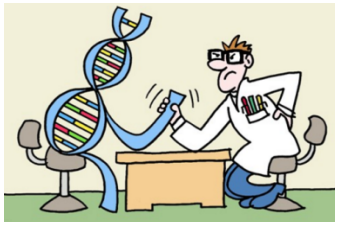
RNA → mRNA

RNA → %98 → ncRNA

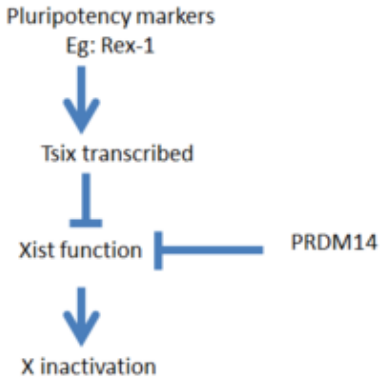
20.000

• ncRNA'lar hedef mRNA'ya bağlanır ve gen susturulması ile düzenleme yaparlar.

non-coding RNA lar

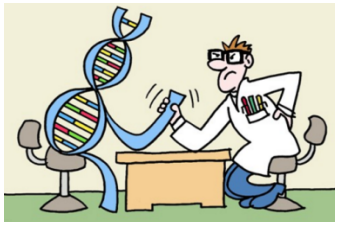


- Büyüklüklerine göre
  - Uzun ncRNA >200 nükleotid
  - Kısa ncRNA <200 nükleotid



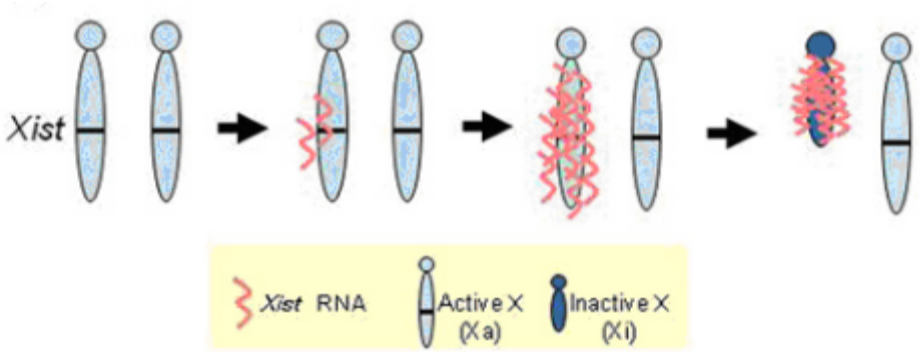
non-coding RNA lar

Büyüklüklerine göre



- Uzun
  - Xist (16bin)
  - Tsix (40bin)
  - Linc (1600)
- Histon deasetilasyonu ve metilasyonu
- Embriyonik kök hücreden gamet farklılaşması
- Evrimsel ol. korunmuş

- Kısa (sRNAs veya dsRNA)
  - miRNA
  - siRNA
  - piRNA (Piwi-protein-interacting RNAs)



Pluripotency markers
Eg: Rex-1

↓

Tsix transcribed

⊣

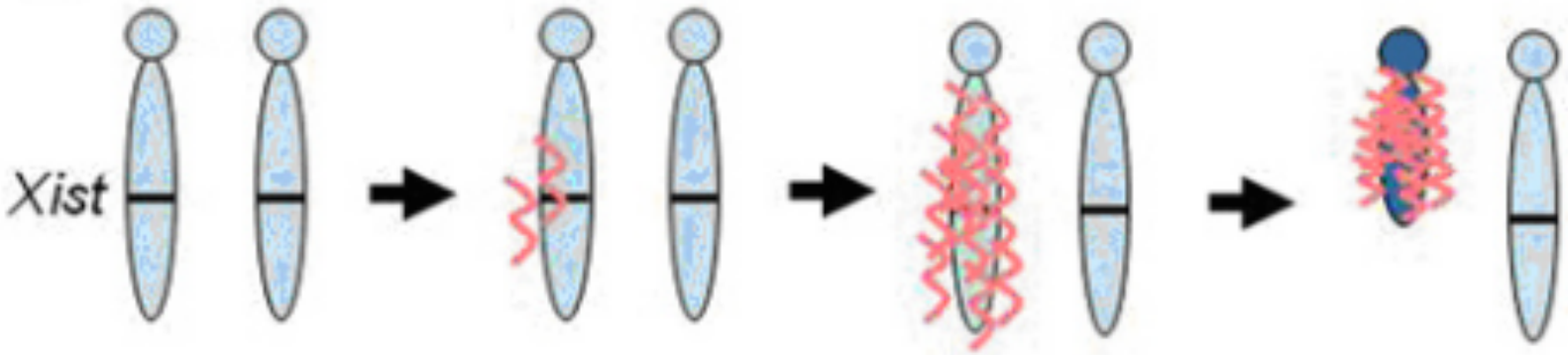
Xist function ⊣ PRDM14

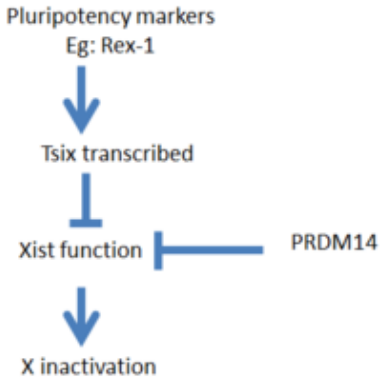
↓

X inactivation

TABLE 9.12 EXAMPLES OF LONG REGULATORY HUMAN RNAs

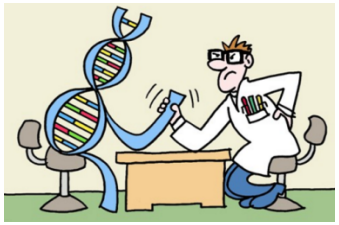
RNA	Size	Gene location	Gene organization	Function
XIST	19.3 kb	Xq13	6 exons spanning 32 kb	regulator of X-chromosome inactivation
TSIX	37.0 kb	Xq13	1 exon	antisense regulator of XIST
H19	2.3 kb	11p15	5 exons spanning 2.67 kb	involved in imprinting at the 11p15 imprinted cluster associated with Beckwith–Wiedemann syndrome
KCNQTOT1 (= LIT1)	59.5 kb	11p15	1 exon	antisense regulator at the imprinted cluster at 11p15
PEG3	1.8 kb[a]	19q13	variable number of exons but up to 9 exons spanning 25 kb	maternally imprinted and known to function in tumor suppression by activating p53
HOTAIR	2.2 kb	12q13	6 exons spanning 6.3 kb	*trans*-acting gene regulator; although part of a regulatory region in the *HOX-C* cluster on 12q13, HOTAIR RNA represses transcription of a 40 kb region on the *HOX-D* cluster on chromosome 2q31





non-coding RNA lar

Büyüklüklerine göre



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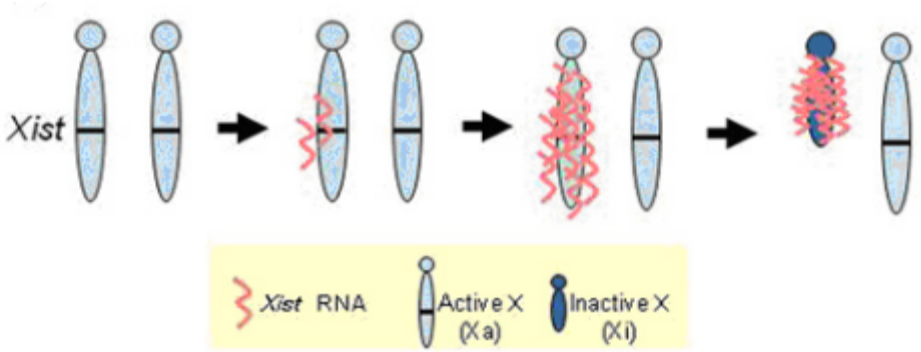


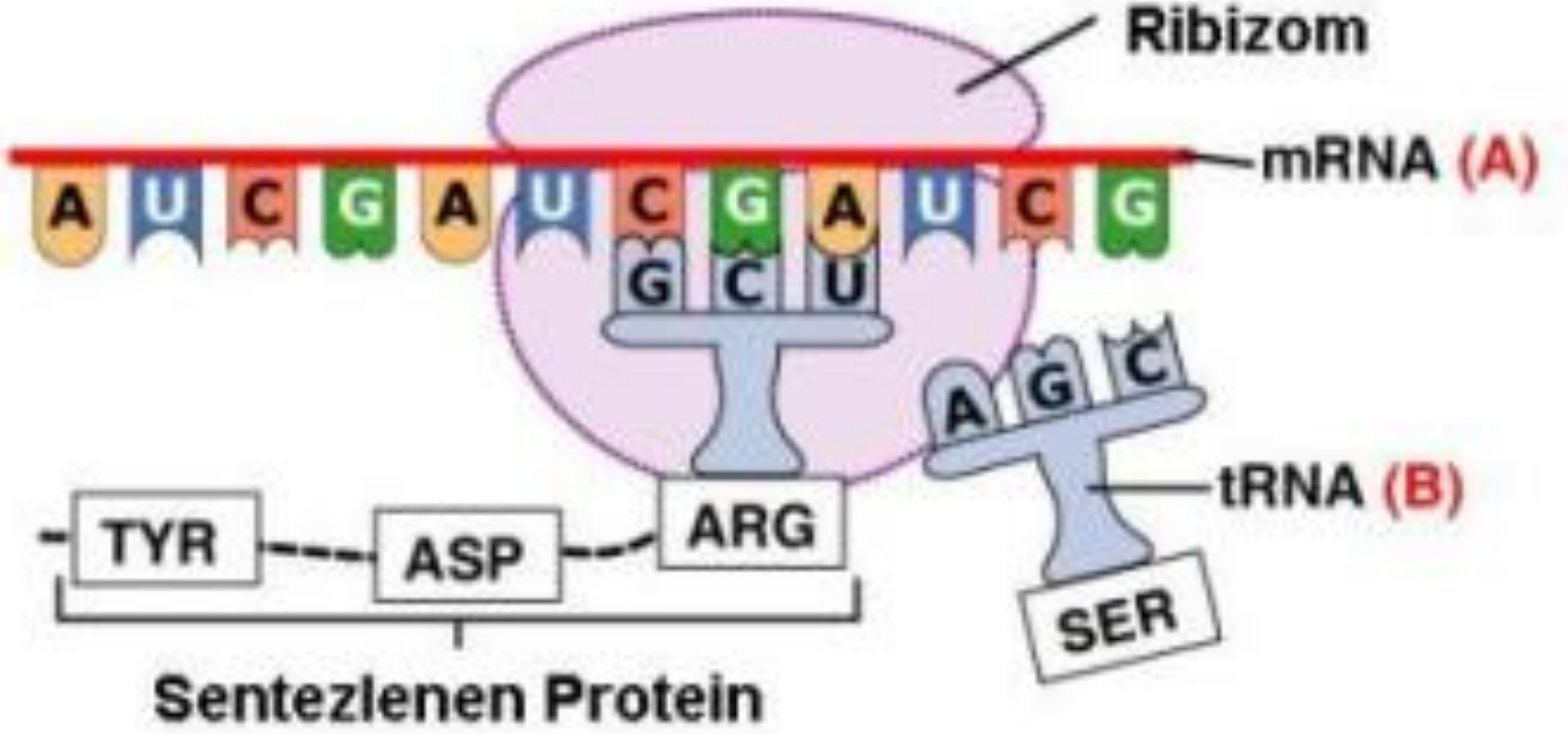
TABLE 9.9 (*cont.*) MAJOR CLASSES OF HUMAN NONCODING RNA

RNA class	Subclass or evolutionary/ functional subfamily	No. of different types	Function	Gene organization, biogenesis, etc.
MicroRNA (miRNA), ~22 nucleotides	> 70 families of related miRNAs	~1000	multiple important roles in gene regulation, notably in development, and implicated in some cancers	see Figure 9.17 for examples of genome organization, and Figure 9.16 for how they are synthesized
Piwi-binding RNA (piRNA), ~24–31 nucleotides	89 individual clusters	> 15,000	often derived from repeats; expressed only in germ-line cells, where they limit excess transposon activity	89 large clusters distributed across the genome; individual clusters span from 10 kb to 75 kb with an average of 170 piRNAs per cluster
Endogenous short interfering RNA (endo-siRNA), ~21–22 nucleotides	many	probably more than 10,000[a]	often derived from pseudogenes, inverted repeats, etc.; involved in gene regulation in somatic cells and may also be involved in regulating some types of transposon	clusters at many locations in the genome

non-coding RNA lar

İşlevine göre

- Housekeeping ncRNAs
  - rRNA, tRNA
    - translasyon
  - snRNA
    - Small nuclear RNA
    - İntron uzaklaştırılması
  - snoRNA
    - Small nucleolar RNA
    - rRNA modifikasyonu
  - gRNA
    - RNAnın hedefe yönlendirilmesi
- Düzenleyici ncRNAs
  - tmRNA
    - tRNA ve mRNA karışımı
    - Ribozomların kırık mRNAdan arınması
  - shRNA
    - Short hairpin RNA
    - Genetik imprinting
  - SNP RNA
    - Enzimatik aktivite göstermesi için ligand oluşturma


Ribizom
mRNA (A)
A U C G A U C G A U C G
G C U
A G C
tRNA (B)
TYR
ASP
ARG
SER
Sentezlenen Protein

non-coding RNA lar

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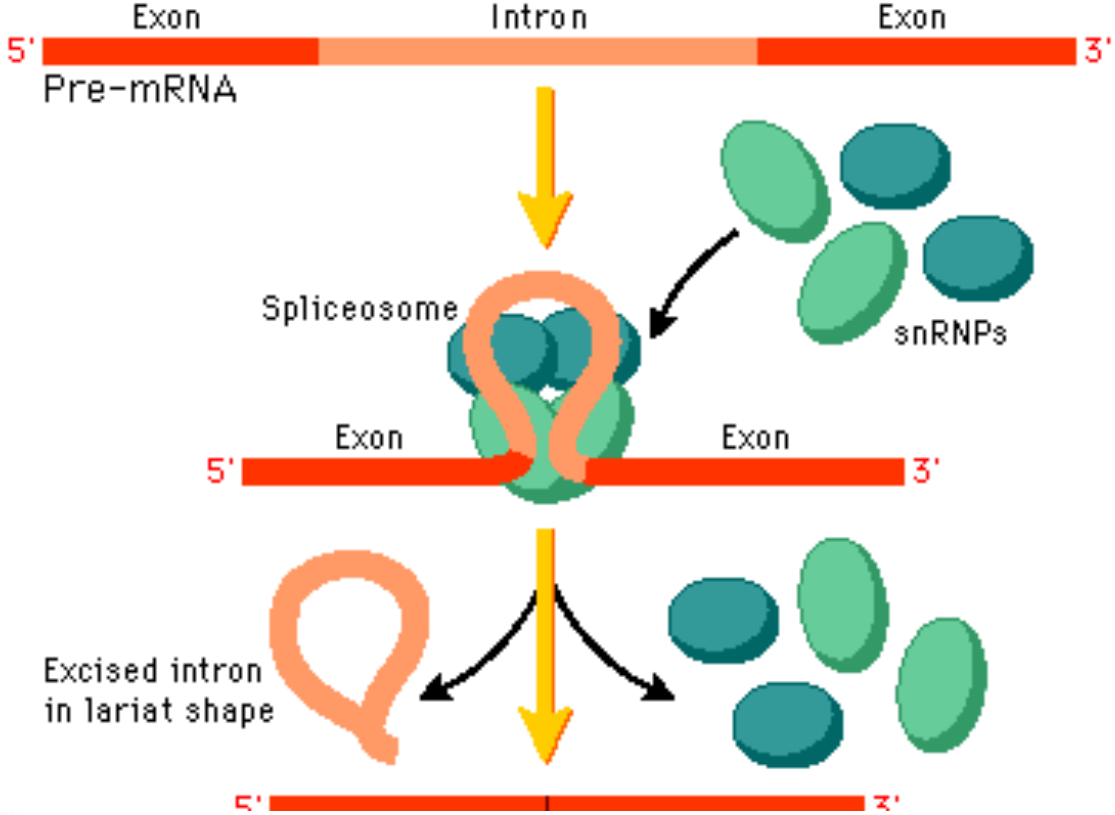

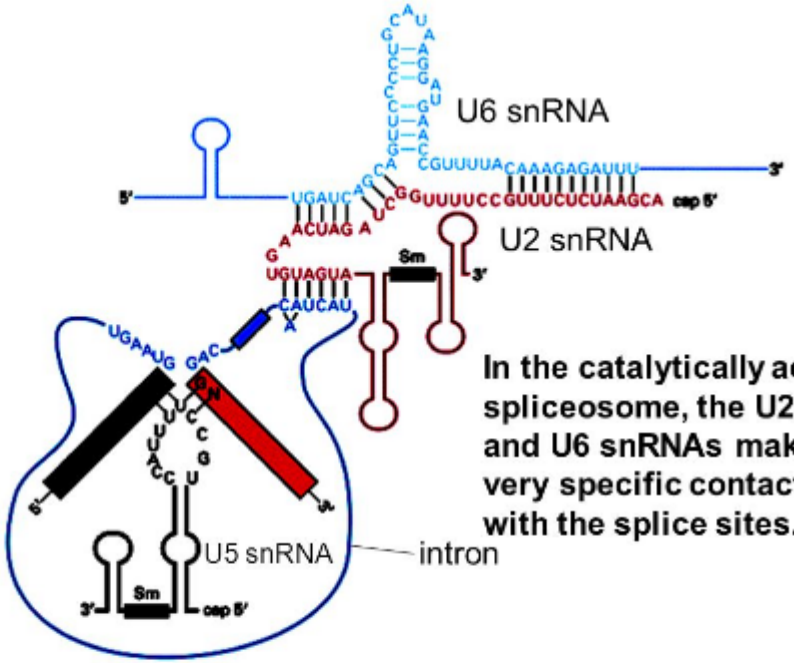

In the catalytically active spliceosome, the U2, U5 and U6 snRNAs make very specific contacts with the splice sites.

What are the proteins doing in catalysis?

non-coding RNA lar

İşlevine göre

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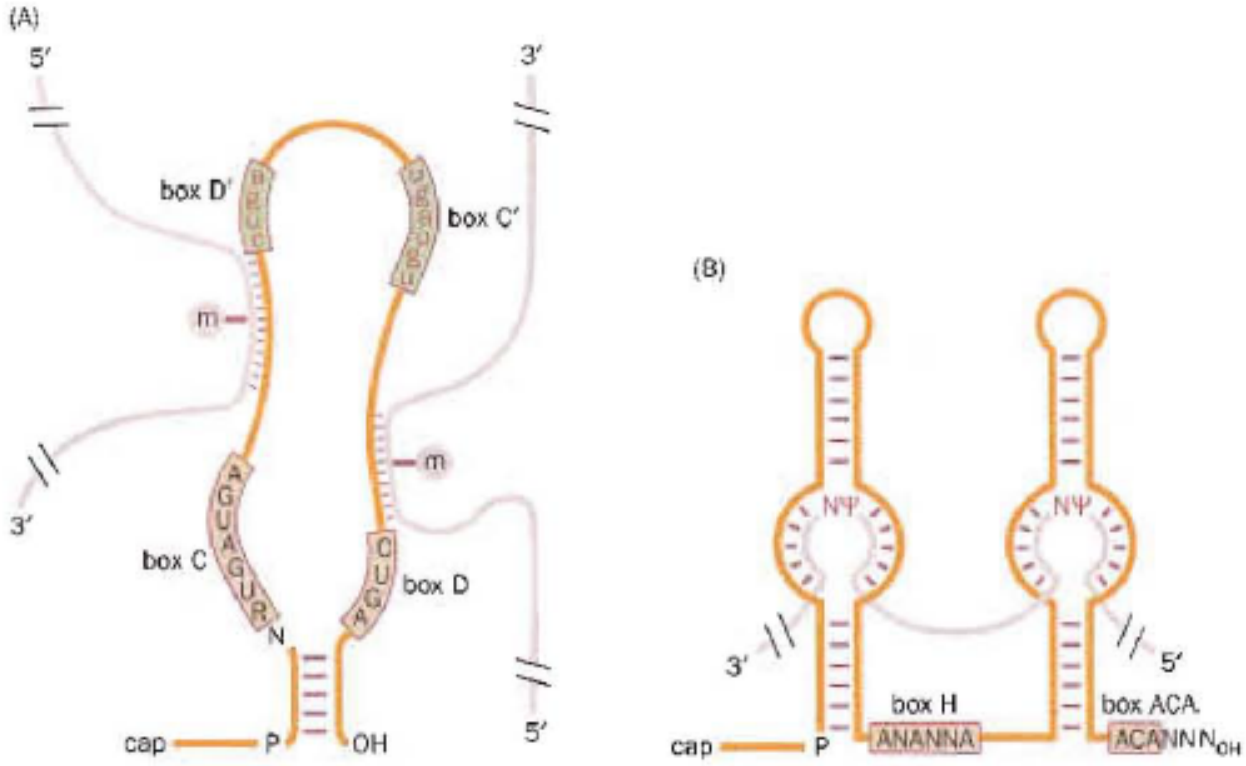

Figure 9.15 Structure and function of snoRNAs. (A) C/D box snoRNAs guide 2′-*O*-methylation modifications. The box C and D motifs and a short 5′, 3′-terminal stem formed by intrastrand base pairing (shown as a series of short horizontal red lines) constitute a kink-turn structural motif that is specifically recognized by the 15.5 kD snoRNP protein. The C′ and D′ boxes represent internal, frequently imperfect copies of the C and D boxes. C/D box snoRNAs and their substrate RNAs form a 10–21 bp double helix in which the target residue to be methylated (shown here by the letter m in a circle) is positioned exactly five nucleotides upstream of the D or D′ box. R represents purine. (B) H/ACA box snoRNAs guide the conversion of uridines to pseudouridine. These RNAs fold into a hairpin–hinge–hairpin–tail structure. One or both of the hairpins contains an internal loop, called the pseudouridylation pocket, that forms two short (3–10 bp) duplexes with nucleotides flanking the unpaired substrate uridine (ψ) located about 15 nucleotides from the H or ACA box of the snoRNA. Although each box C/D and H/ACA snoRNA could potentially direct two modification reactions, apart from a few exceptions, most snoRNAs possess only one functional 2′-*O*-methylation or pseudouridylation domain.

non-coding RNA lar

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  - snoRNA
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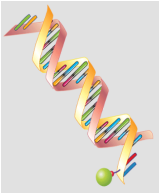
Gen susturma

- Gen ekspresyonunun kontrolü
  - Transkripsiyonel
    - mRNA sentezi artar/azalır
  - Post-transkripsiyonel
    - mRNA sentezi değişmez
    - sentez sonrası mRNA susturma

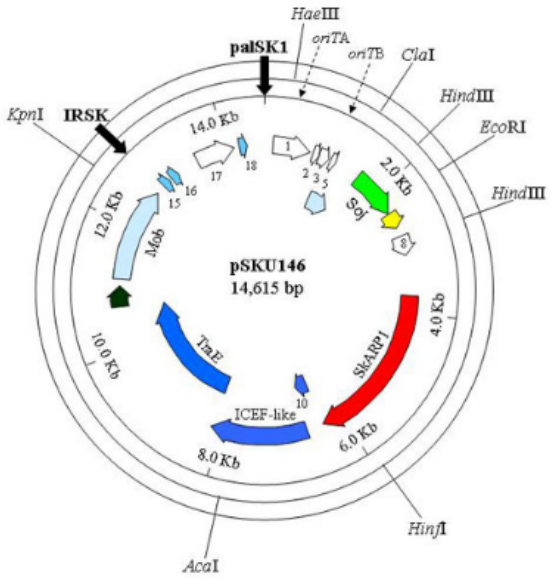
Tanımlar

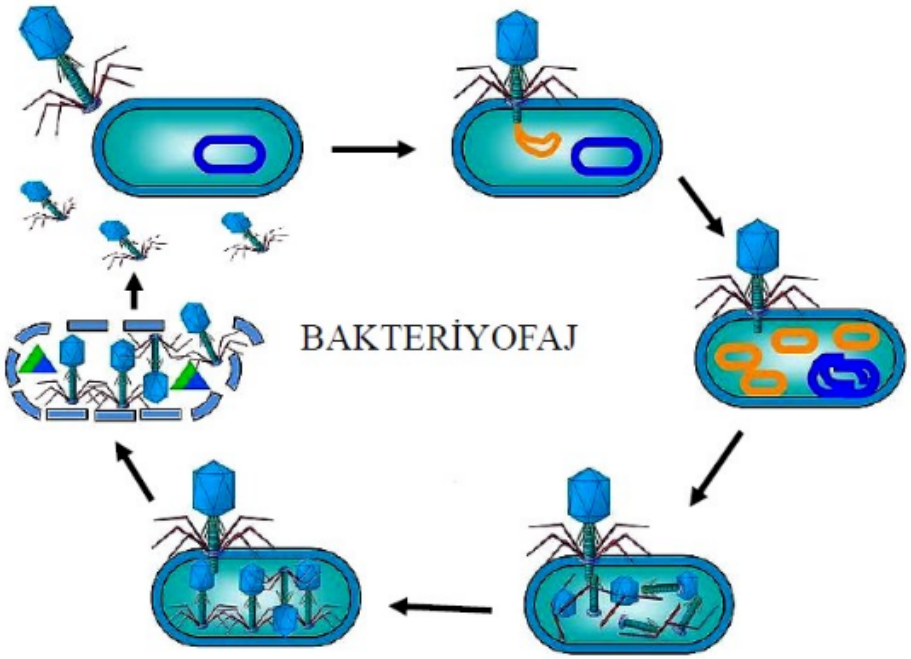
- PTGS
  - Post-transcriptional gene silencing
- Ribonükleikasit interferansı (RNAi)
  - dsRNA nın homolog mRNAları parçalaması
- RISC (RNA-induced silencing complex)
  - RNA aracılı susturma kompleksi
- *RITS (RNA-induced transcriptional Silencing complex)*



RNA interferans

- Hayvan, bitki, tek hücreli mantar
- Virüsler ve transpozonlar dsRNA üretir





RNAi yolakları

- siRNA (small interfering RNA)
- miRNA (mikroRNA)

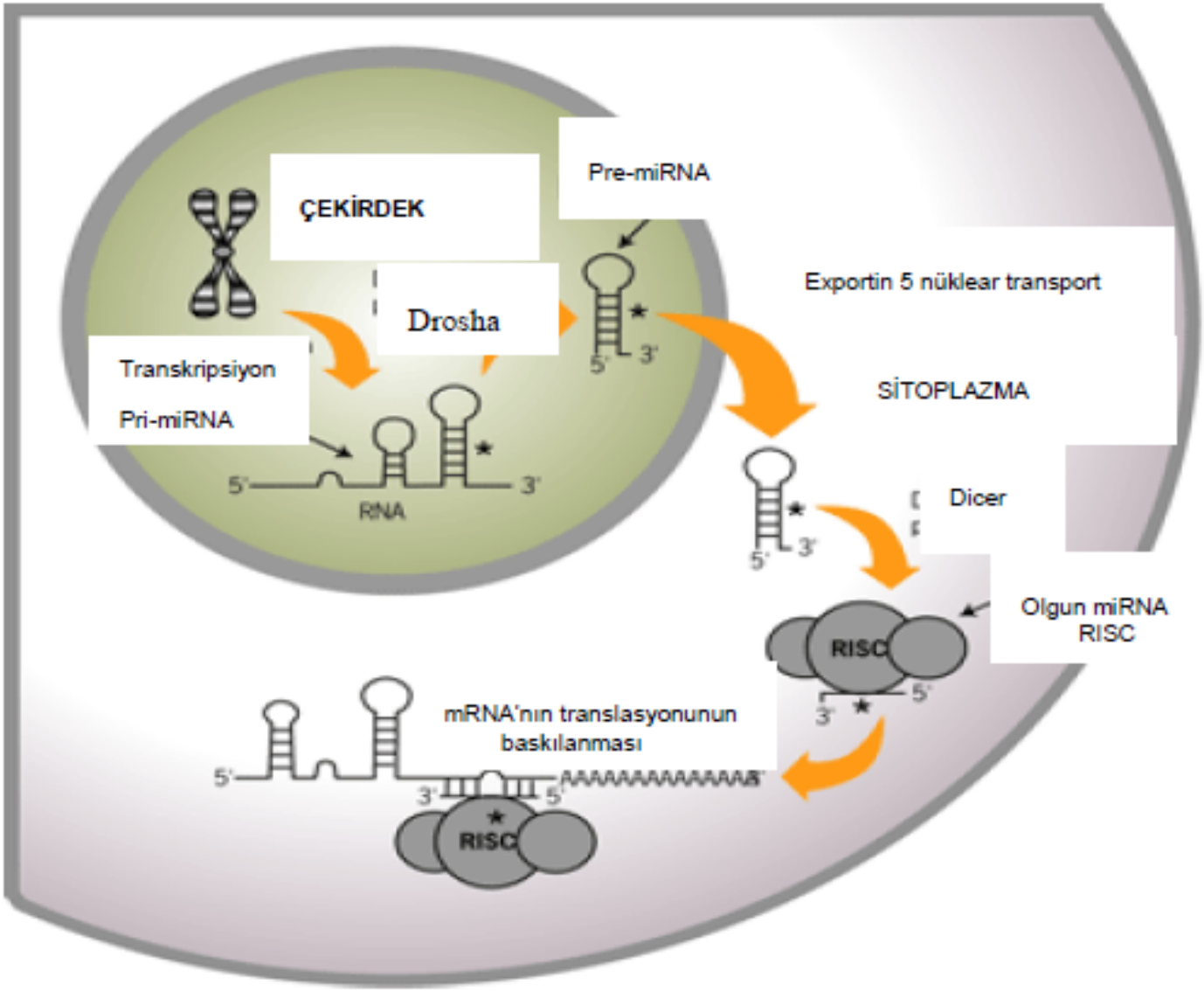
siRNA

- Small interfering RNA
- Çoğunlukla eksojen (viral)

miRNA

- Micro RNA
- Endojen

missed in analyses of the human genome



Şekil 4. miRNA yolağı

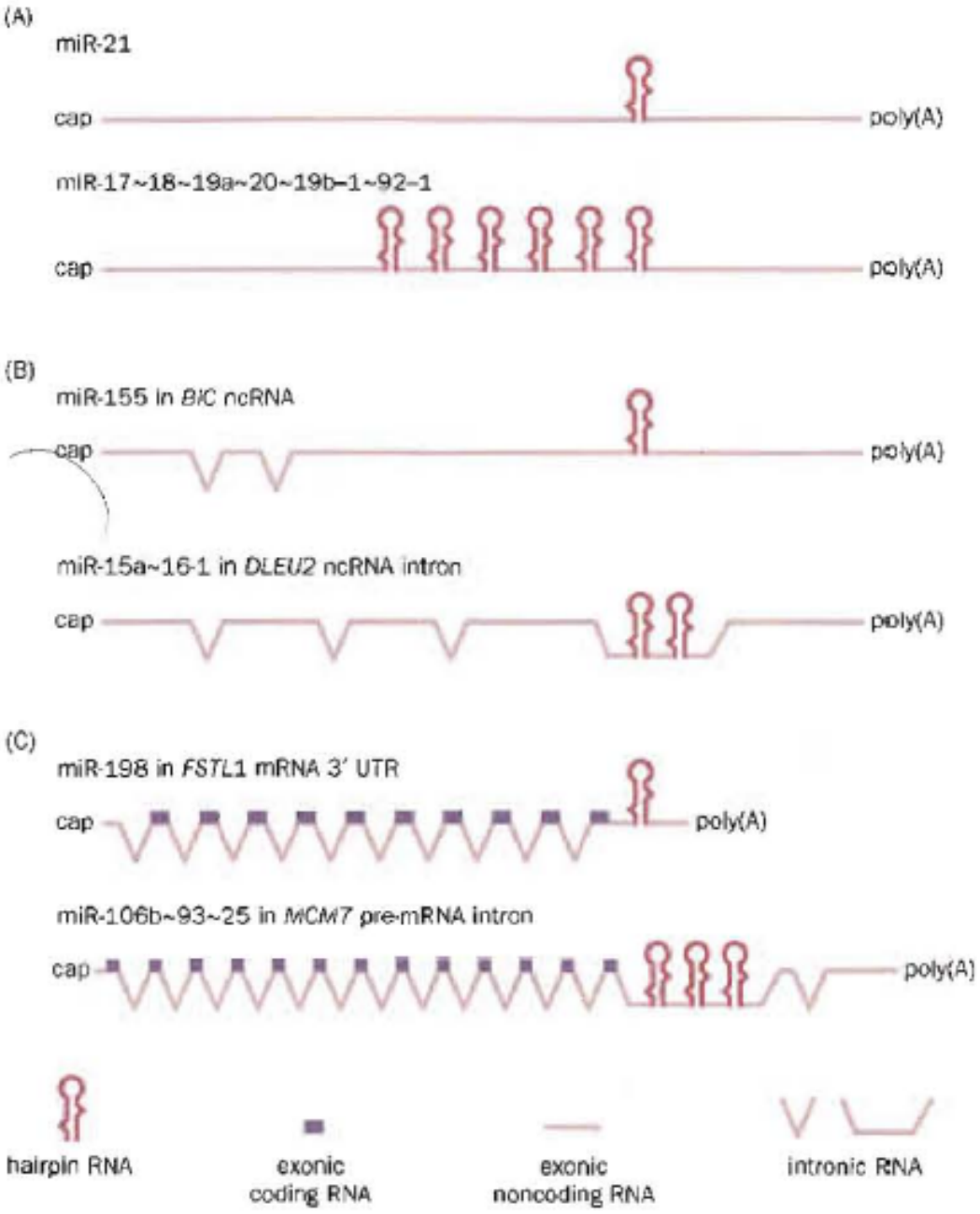


Figure 9.17 The structure of human pri-miRNAs. (A) Examples of transcripts that are used exclusively to make miRNAs: miR-21 is produced from a single hairpin within a dedicated primary transcript RNA; a single multigenic transcript with six hairpins that will eventually be cleaved to give six miRNAs, namely miR-17, miR-18, miR-19a, and so on. (B, C) Examples of miRNAs that are co-transcribed with a gene encoding either (B) a long noncoding RNA (ncRNA) or (C) a polypeptide. In each part, the upper example shows single miRNAs located within (B) an exon of an ncRNA (miR-155) and (C) in the 3′ untranslated region (UTR) within a terminal exon of an mRNA (miR-198). The lower examples show multiple miRNAs located within intronic sequences of (B) an ncRNA (miR-15a and miR-16-1) and (C) a pre-mRNA (miR-106b, miR-93, and miR-25). Cap, $m^7G(5')ppp(5')G$. [Adapted from Du T & Zamore PD (2005) *Development* 132, 4645–4652. With permission from the Company of Biologists.]

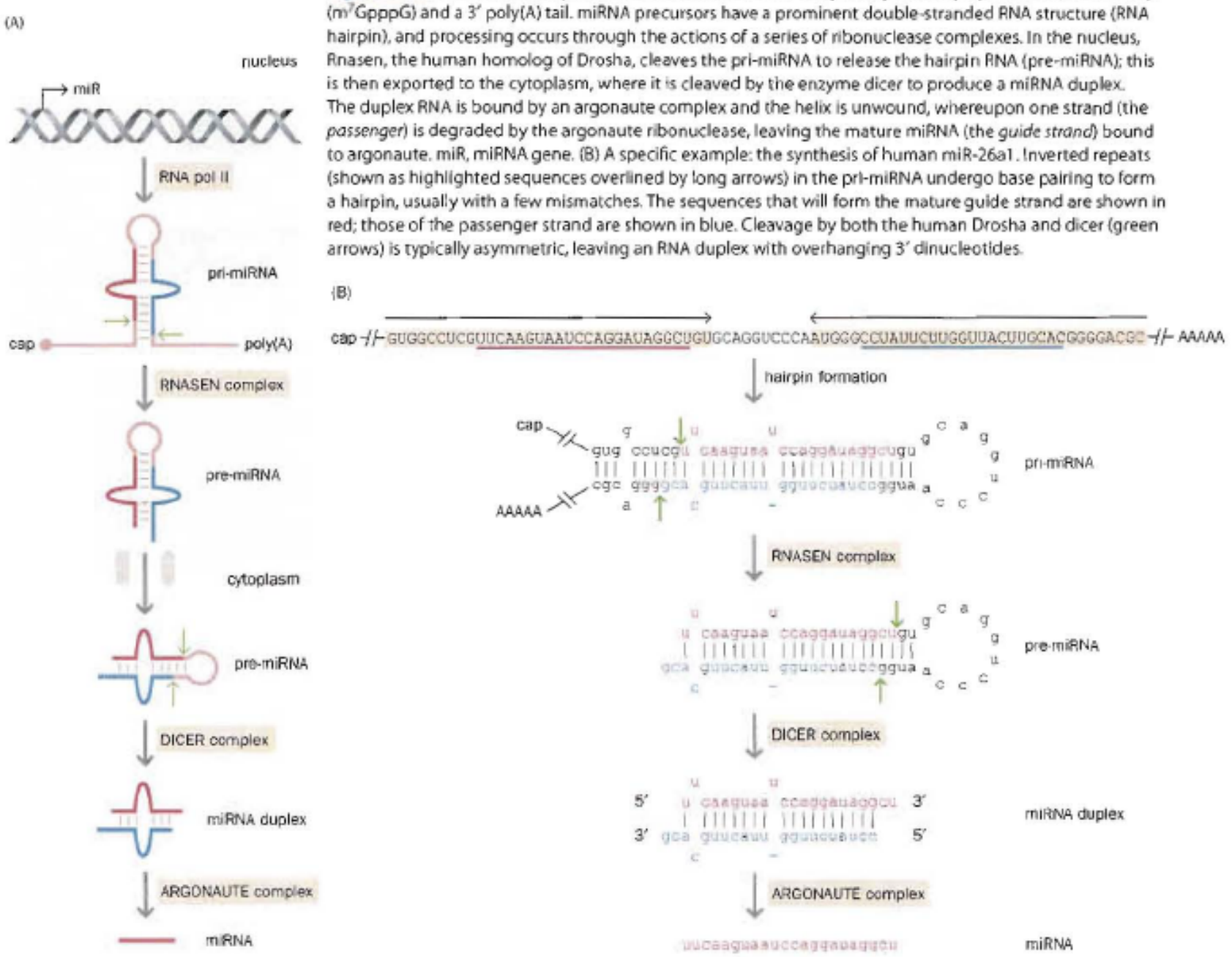
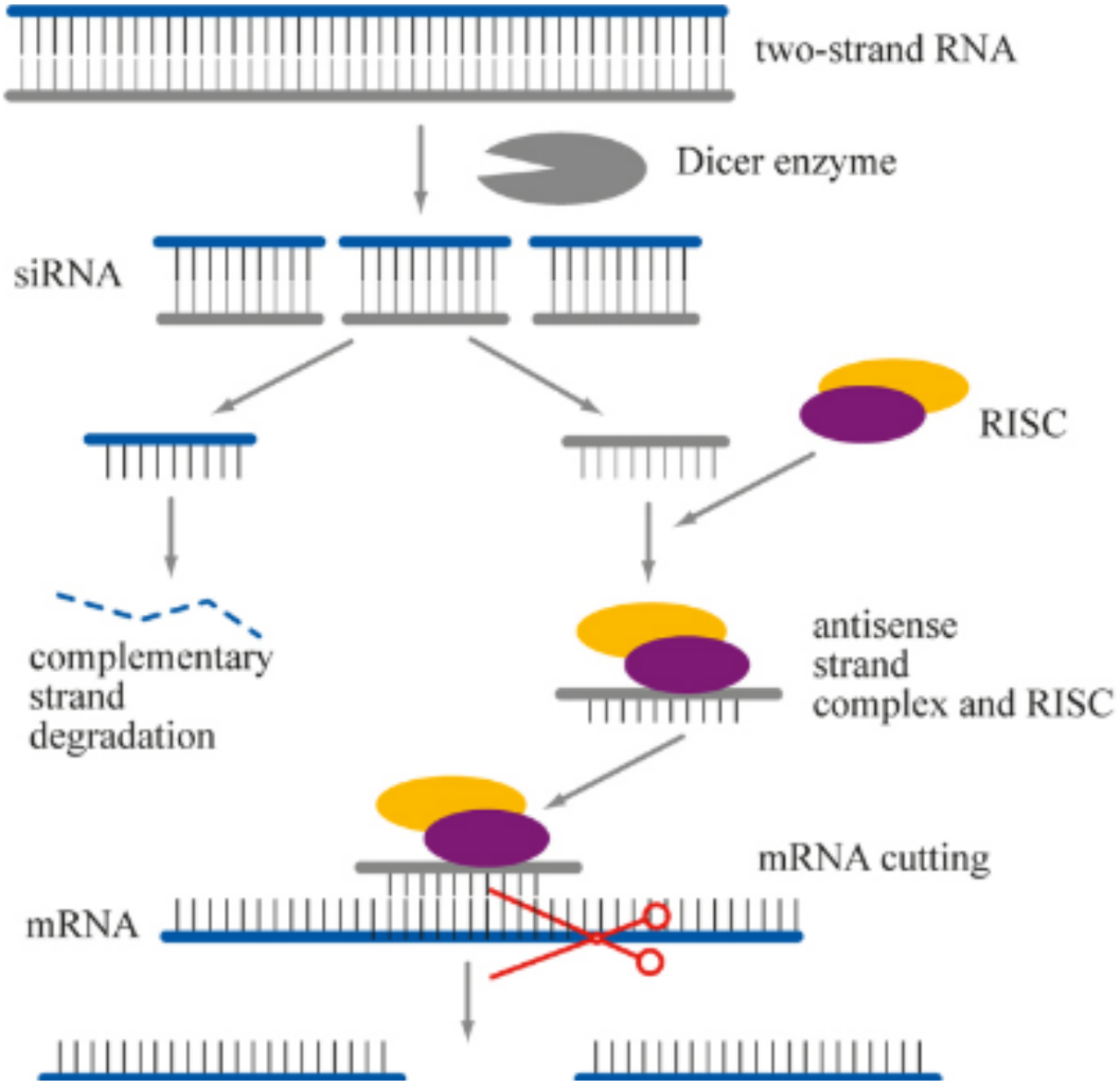
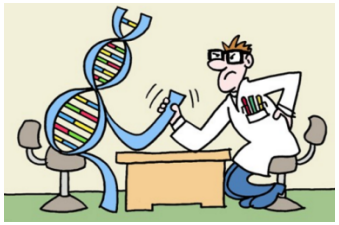


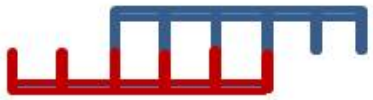
Figure 9.16 Human miRNA synthesis. (A) General scheme. The primary transcript, pri-miRNA, has a 5′ cap (m^7GpppG) and a 3′ poly(A) tail. miRNA precursors have a prominent double-stranded RNA structure (RNA hairpin), and processing occurs through the actions of a series of ribonuclease complexes. In the nucleus, Rnasen, the human homolog of Drosha, cleaves the pri-miRNA to release the hairpin RNA (pre-miRNA); this is then exported to the cytoplasm, where it is cleaved by the enzyme dicer to produce a miRNA duplex. The duplex RNA is bound by an argonaute complex and the helix is unwound, whereupon one strand (the *passenger*) is degraded by the argonaute ribonuclease, leaving the mature miRNA (the *guide strand*) bound to argonaute. miR, miRNA gene. (B) A specific example: the synthesis of human miR-26a1. Inverted repeats (shown as highlighted sequences overlined by long arrows) in the pri-miRNA undergo base pairing to form a hairpin, usually with a few mismatches. The sequences that will form the mature guide strand are shown in red; those of the passenger strand are shown in blue. Cleavage by both the human Drosha and dicer (green arrows) is typically asymmetric, leaving an RNA duplex with overhanging 3′ dinucleotides.


two-strand RNA
Dicer enzyme
siRNA
RISC
complementary
strand
degradation
antisense
strand
complex and RISC
mRNA cutting
mRNA

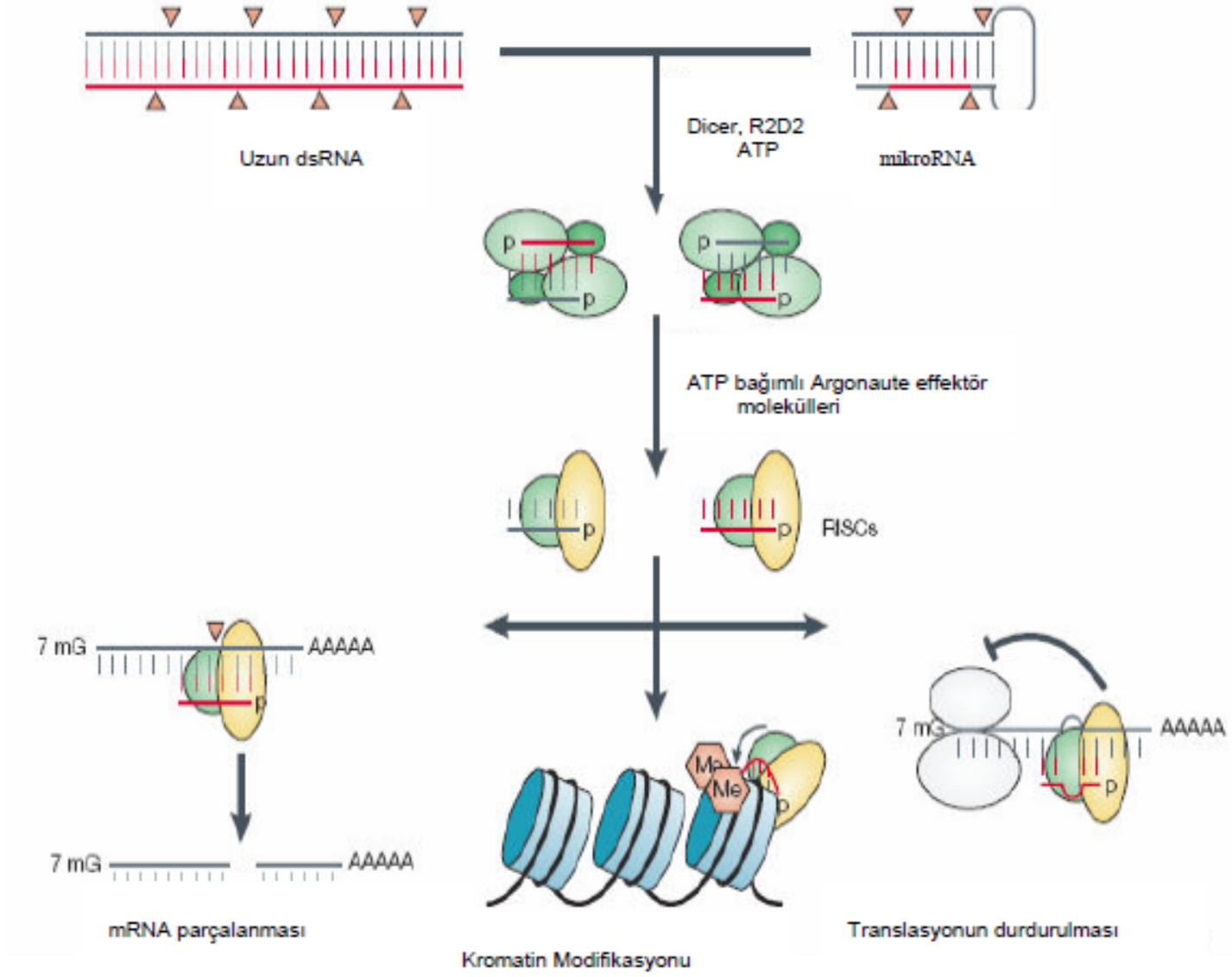
siRNA



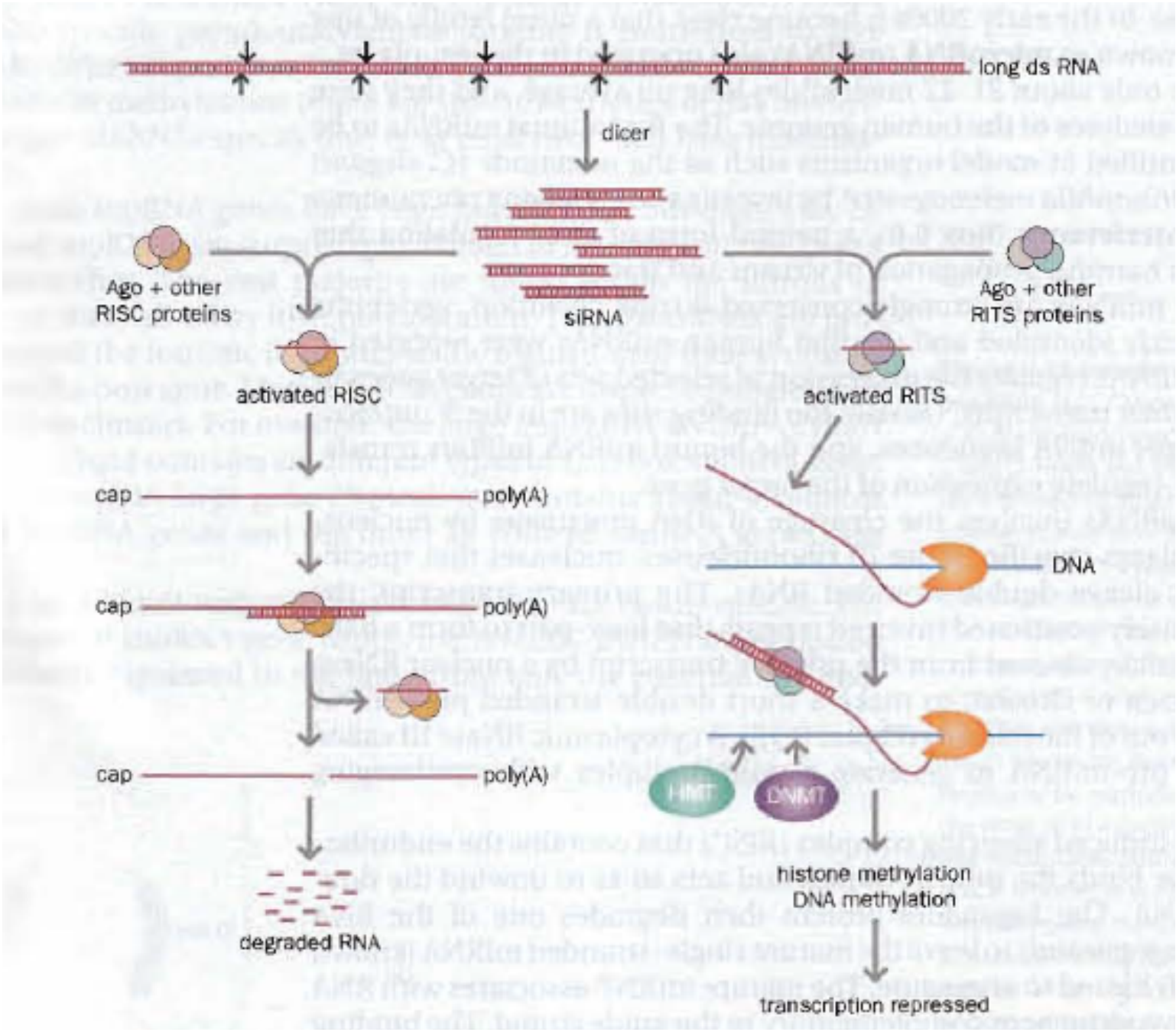
- Memeliler için çoğunlukla ekzojen
  - Virüs kaynaklı
- 70-80 bç dsRNA lardan kesilerek oluşur
  - Endonükleaz olan RNAIII enzimi dicer
- 3' uçlarda 2nt lik çıkıntılar olan 20-21bç lik siRNA lar



siRNA duplex



Şekil 3. siRNA'nın gen ifadesini engellemesi


long ds RNA
dicer
siRNA
Ago + other
RISC proteins
activated RISC
Ago + other
RITS proteins
activated RITS
cap
poly(A)
cap
poly(A)
cap
poly(A)
degraded RNA
DNA
HMT
DNMT
histone methylation
DNA methylation
transcription repressed

Konular

- siRNA aracılı tedavi
  - Zorlukları
  - Geliştirilmesi
- Son klinik çalışmalar
- Gelecekte yapılması olası ilaçlar

ASO sınıfları

- Rnase H-bağımlı ASO
- Exon-skipping ASO
- siRNA
- Anti-miR ve miRNA taklitleri

ASO (antisens oligonükleotit)

- Uygun mRNA ile baz eşleşmesi
- Tek zincirli
  - Translasyon inhibisyonu
  - Splicing modifikasyonu

- Vücut sıvılarında nükleazlar
- Doğal immün yanıtı tetikleme
- Hücre membranı sorunu

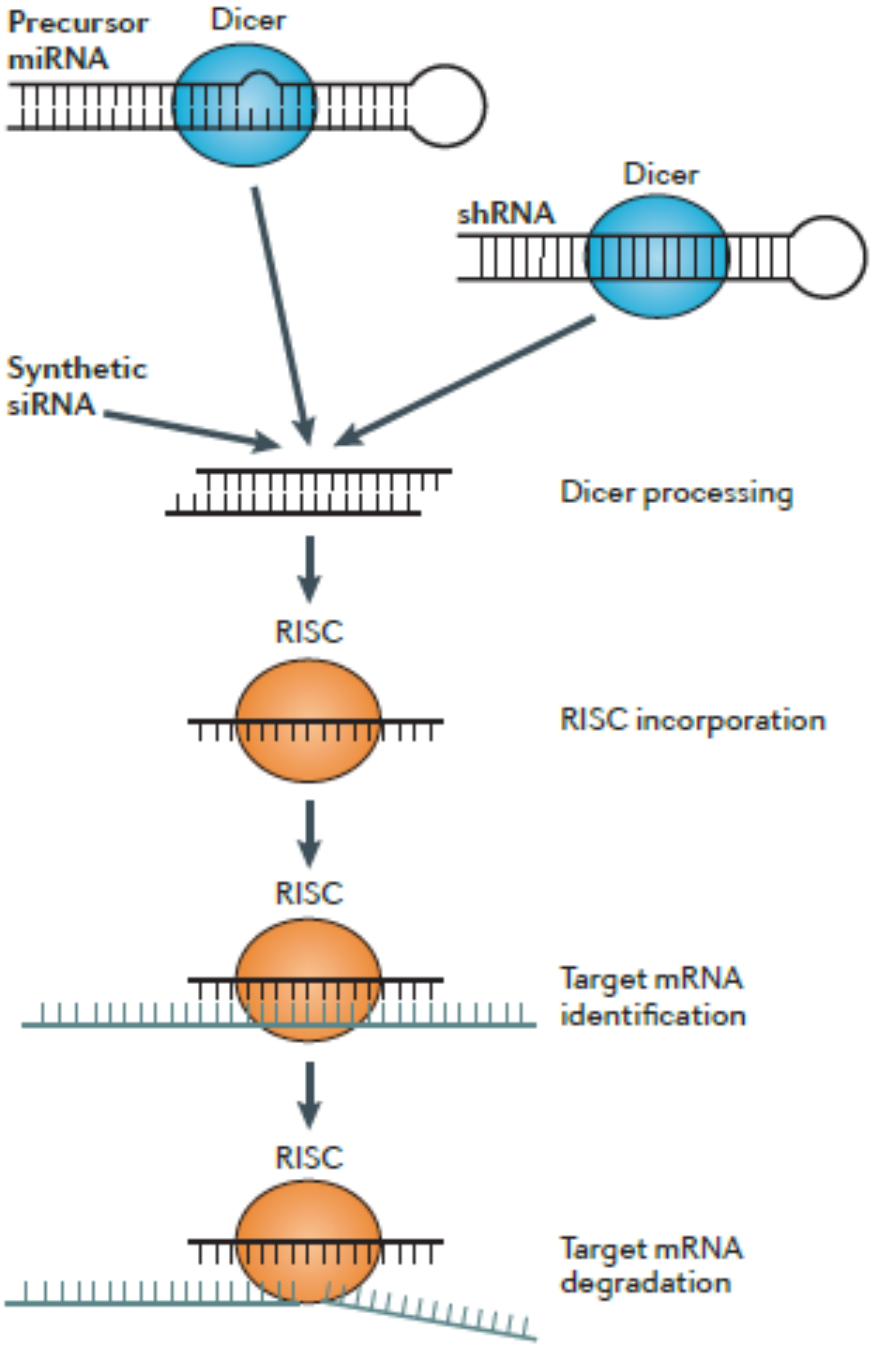
ASO

- Hedefe özgü
- Daha stabil
- Hücre penetrasyonunu yapabilen

  - İlk ASO fomivirsen
    - CMV retiniti 1998
  - Mipomersen
    - Ailesel hiperkolesterolemi

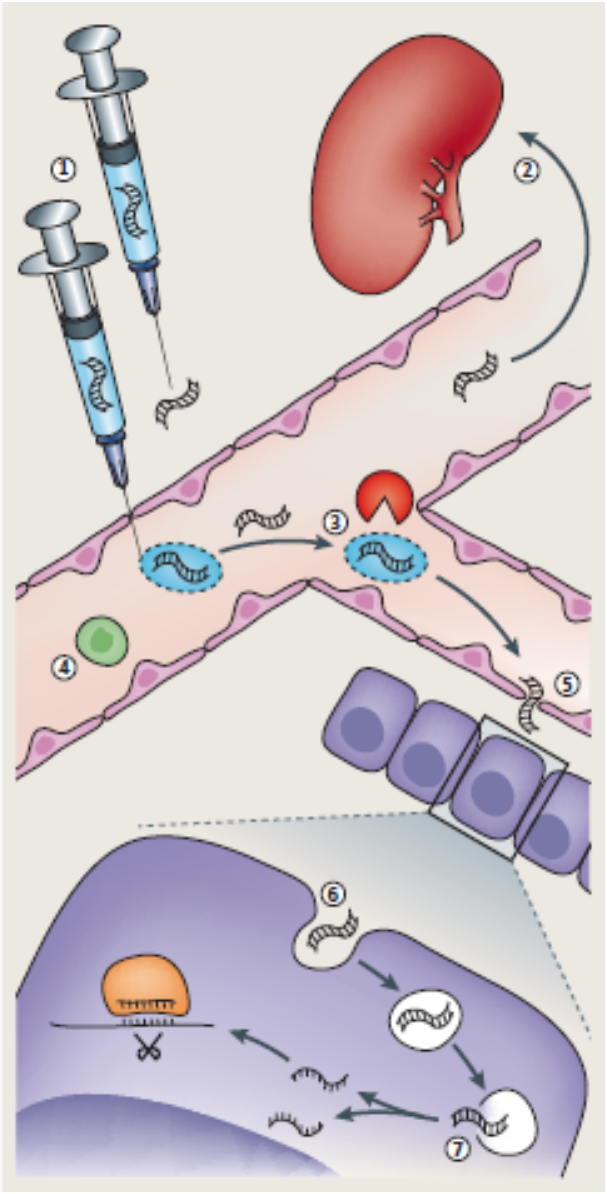
siRNA

- İlk kez 13 yıl önce Fas SİRNA enjekte edilen fareler otoimmün hepatitten korunmuş
- 2 yıldır birçok Faz I ve Faz II siRNA ilaçları
  - Potent (%98)
  - Uzun süreli (haftalar)
  - Klinik iyileşme
  - Kabul edilebilir toksisite
- İki çalışma Faz III aşamasında
  - Ailesel nörodejeneratif hast.
  - Ailesel kardiomyopati



- Büyük ve negatif yüklü
- Endosome aracılı geçiş
- Endosome membranından sitozole

- Aktif siRNA haftalarca RISC içinde kalabilir
- Aynı siRNA farklı transkriptleri hedef alabilir
- Her bölünmede azalır
- Katalitik aktivitesi yüksek
- Sitozoldeki birkaç yüz siRNA yeterli



Membrandan geçemeyecek kadar büyük
fakat böbrekten süzülecek kadar küçük

Endosome ile h. İçine alınma

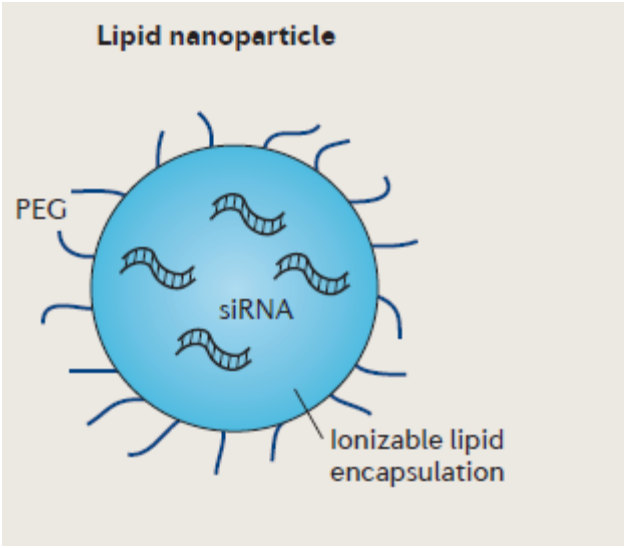
Hedefe yönelik siRNA

- ➢Yüksek afiniteli antikorlar
- ➢Aptamerler
- ➢Reseptör ligandlar

- ✓Düşük dozda etkin
- ✓Azalmış toksisite

Lipid nanopartiküller içinde siRNA – LNP

- 50-100nm
- PEGylated (polietilen-glikol konjuge) lipidler, kolesterol, nükleik asitler
- Apolipoprotein reseptörleri görünce endositozla içeri alıyor, lipidler endosomun içindeki düşük pH nedeniyle protonize oluyor ve endosom membranını parçalanmasını tetikliyor



- Karaciğere ve dalakta filtreleniyor
  - Diğer hedeflere etkisi azalıyor
  - Bu yüzden karaciğer en büyük hedef organ
  - Topikal hedefler; göz, mukoza, cilt
- LNP ler pencereli kapilleri olan bölgelere rahat erişir
  - Solid tümörler

GalNac-siRNA bileşimleri

- ❖N-asetilgalaktozamin
- ❖non kovalent
- ❖Directly conjugated

- ✓En etkin, Faz III
- ✓LNP lere göre daha iyi tolare ediliyor
- ✓Subkutanöz uygulanabilir

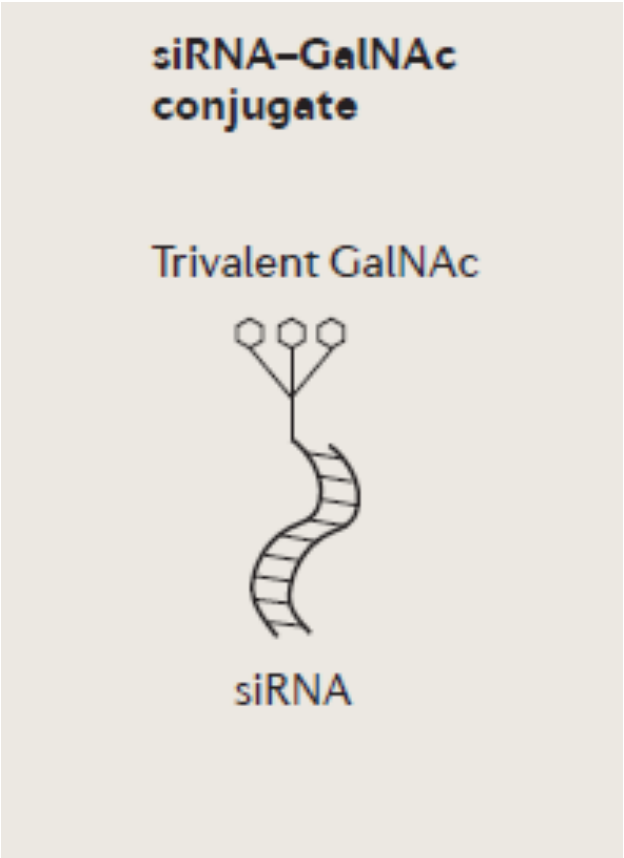


Table 1 | **RNAi therapeutics with demonstrated gene knockdown or efficacy in humans***

Company	Drug	Target	Disease	Developmental phase	Mechanism	Formulation or conjugation	Efficacy	Refs
Alnylam Pharmaceuticals	ALN-TTRsc (also known as revusiran)	TTR	TTR-mediated amyloidosis	Phase III	siRNA	GalNAc conjugate	>90% serum TTR protein reduction	67
Alnylam Pharmaceuticals	ALN-PCS02	PCSK9	Hypercholesterolaemia	Phase I	siRNA	Second-generation LNP	~70% reduction of plasma PCSK9 protein	65
Alnylam Pharmaceuticals	ALN-TTR02	TTR	TTR-mediated amyloidosis	Phase III	siRNA	Second-generation LNP	>80% serum TTR protein reduction	62
Alnylam Pharmaceuticals	ALN-AT3	AT3	Haemophilia A and B, and rare bleeding disorders	Phase I	siRNA	GalNAc conjugate	>60% AT3 serum protein reduction	68
Alnylam Pharmaceuticals	ALN-TTR01	TTR	TTR-mediated amyloidosis	Phase I (terminated)	siRNA	First-generation LNP	~40% serum TTR protein reduction	62
Arrowhead Research	ARC-520	HBV	HCV	Phase II	siRNA	Dynamic PolyConjugate	~50% viral load reduction	71
Regulus Therapeutics	RG-101	miR-122	HCV	Phase II	Anti-miR	GalNAc conjugate	Viral load reductions of up to 4.8 log10	90
Santaris Pharma/Roche	SPC3649 (also known as miravirsen)	miR-122	HCV	Phase II	Anti-miR	Naked	Viral load reductions of up to 3.0 log10	89

AT3, anti-thrombin III; GalNAc, *N*-acetylgalactosamine; HBV, hepatitis B virus; HCV, hepatitis C virus; LNP, lipid nanoparticle; PCSK9, proprotein convertase subtilisin/kexin type 9; RNAi, RNA interference; siRNA, small interfering RNA; TTR, transthyretin. *This table summarizes RNAi therapeutics for which there is quantitative evidence of gene knockdown or inhibition.