NON-CODING RNAs

Types

Synthesis

Functions

Examples

Cells Produce Different Categories of RNA Molecules

Noncoding RNAs Are Also Synthesized and Processed in the Nucleus

TABLE 6–1 Principal Types of RNAs Produced in Cells

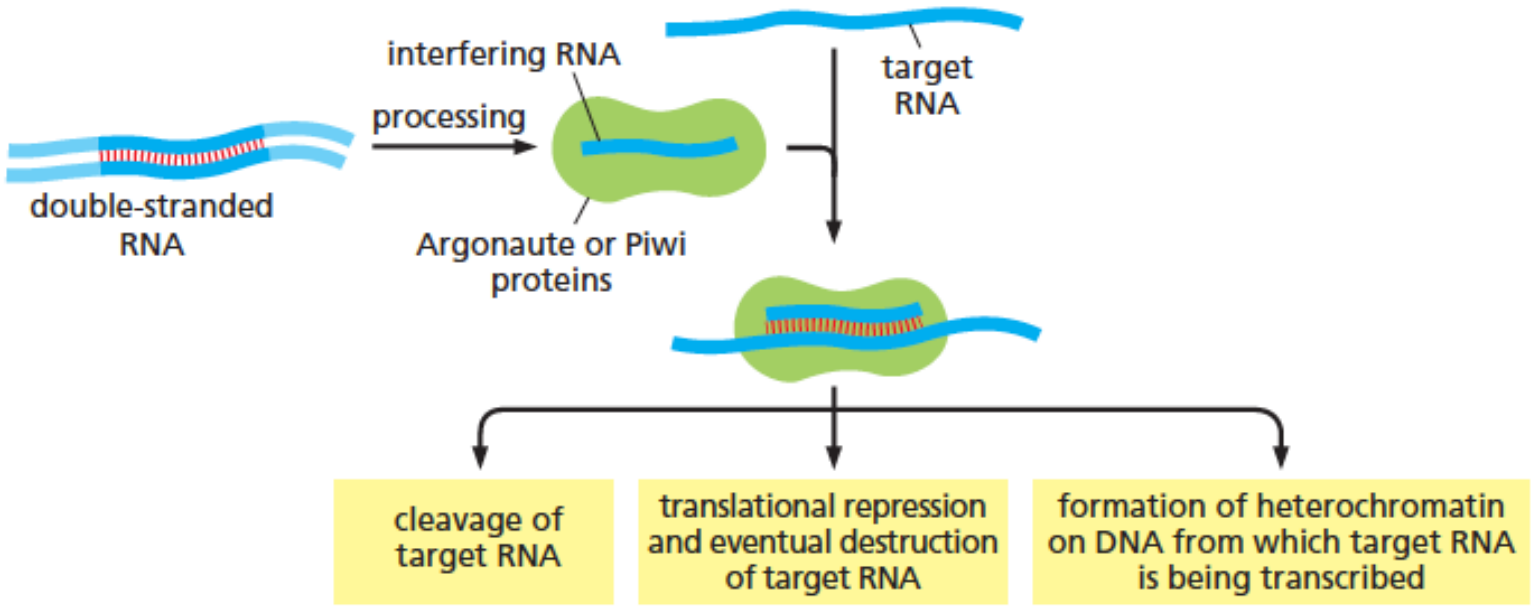
Type of RNA	Function
mRNAs	Messenger RNAs, code for proteins
rRNAs	Ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	Transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	Small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	Small nucleolar RNAs, help to process and chemically modify rRNAs
miRNAs	MicroRNAs, regulate gene expression by blocking translation of specific mRNAs and cause their degradation
siRNAs	Small interfering RNAs, turn off gene expression by directing the degradation of selective mRNAs and the establishment of compact chromatin structures
piRNAs	Piwi-interacting RNAs, bind to piwi proteins and protect the germ line from transposable elements
lncRNAs	Long noncoding RNAs, many of which serve as scaffolds; they regulate diverse cell processes, including X-chromosome inactivation

REGULATION OF GENE EXPRESSION BY NONCODING RNAs

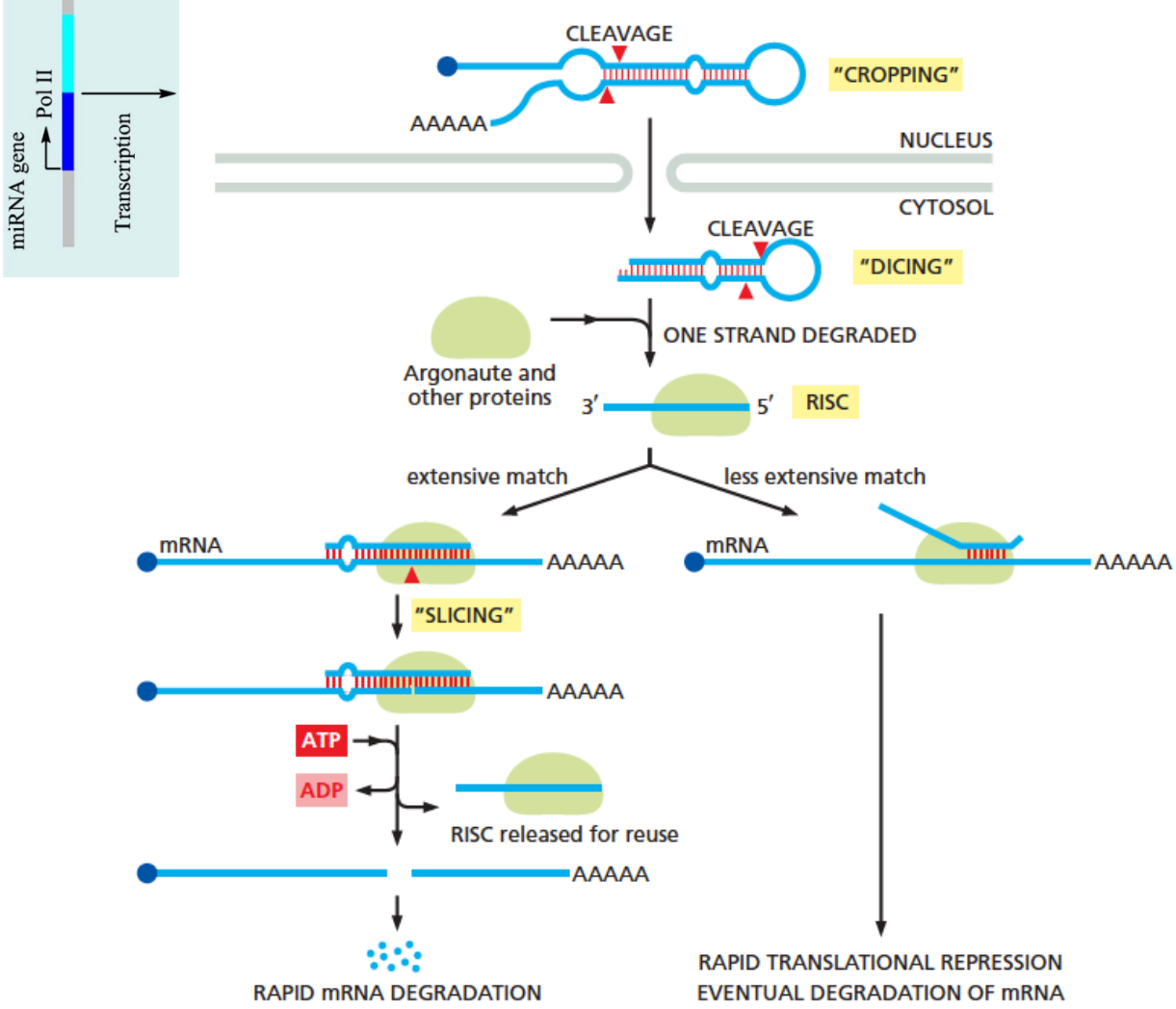
Small Noncoding RNA Transcripts Regulate Many Animal and Plant Genes Through RNA Interference

Three classes of small noncoding RNAs work in this way—microRNAs (miRNAs), small interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs)

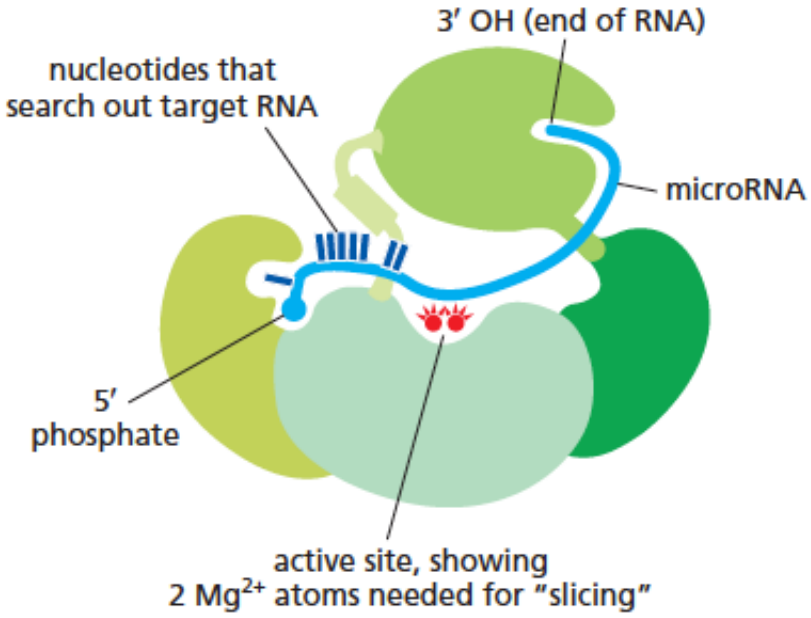
miRNAs Regulate mRNA Translation and Stability. Estimated Over 4000 different microRNAs (miRNAs) are produced from the human genome, and these appear to regulate at least one-third of all human protein-coding genes.



RNA interference in eukaryotes. Single-stranded interfering RNAs are generated from double-stranded RNA. They locate target RNAs through base-pairing and, at this point, several fates are possible, as shown. There are several types of RNA interference; the way the double-stranded RNA is produced and processed and the ultimate fate of the target RNA depends on the particular system.



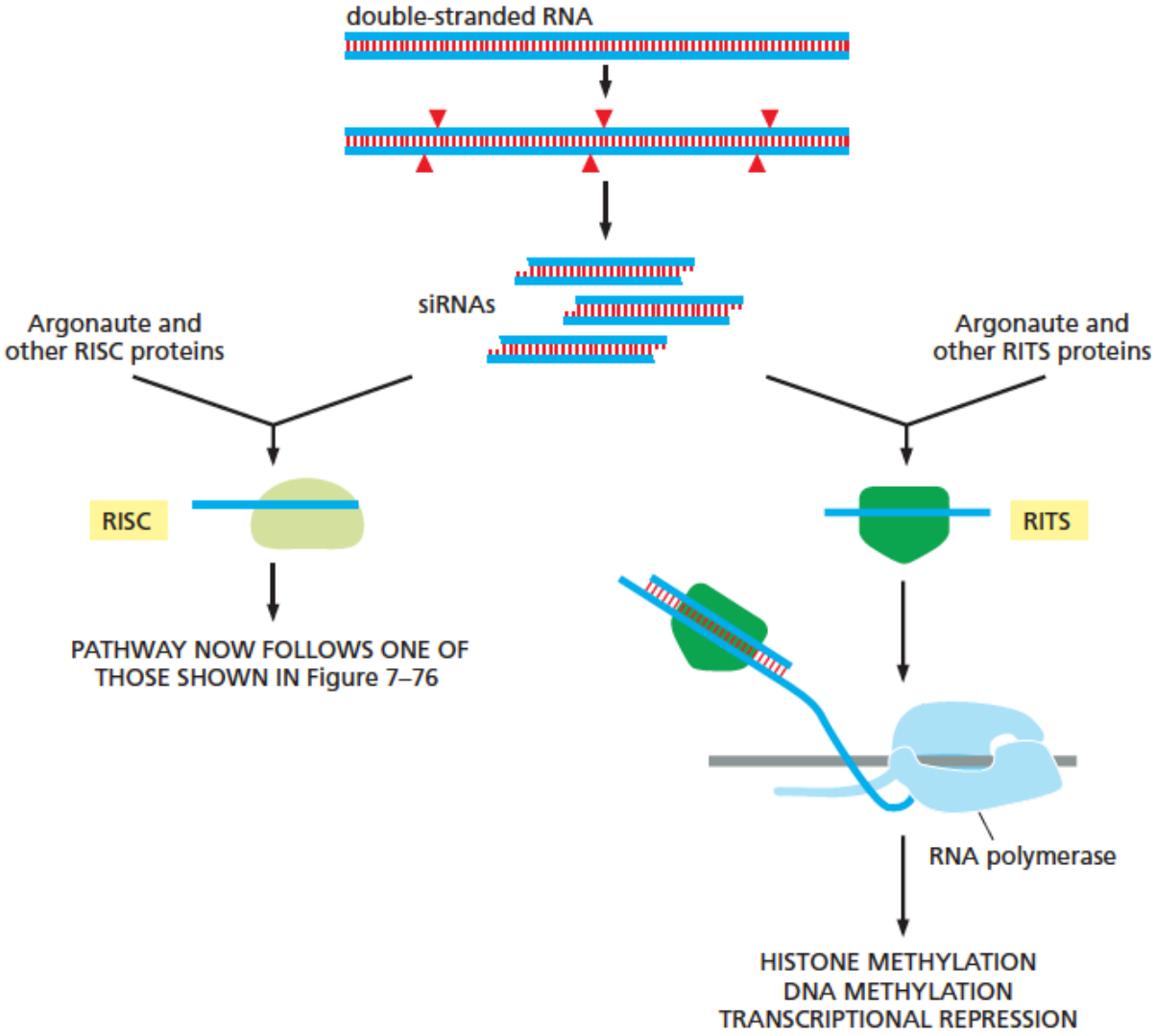
miRNA processing and mechanism of action. The precursor miRNA, through complementarity between one part of its sequence and another, forms a double-stranded structure. This RNA is cropped while still in the nucleus and then exported to the cytosol, where it is further cleaved by the Dicer enzyme to form the miRNA proper. Argonaute, in conjunction with other components of RISC, initially associates with both strands of the miRNA and then cleaves and discards one of them. The other strand guides RISC to specific mRNAs through base-pairing. If the RNA–RNA match is extensive, as is commonly seen in plants, Argonaute cleaves the target mRNA, causing its rapid degradation. In mammals, the miRNA–mRNA match often does not extend beyond a short seven-nucleotide “seed” region near the 5' end of the miRNA. This less extensive base-pairing leads to inhibition of translation, mRNA destabilization, and transfer of the mRNA to P-bodies, where it is eventually degraded.



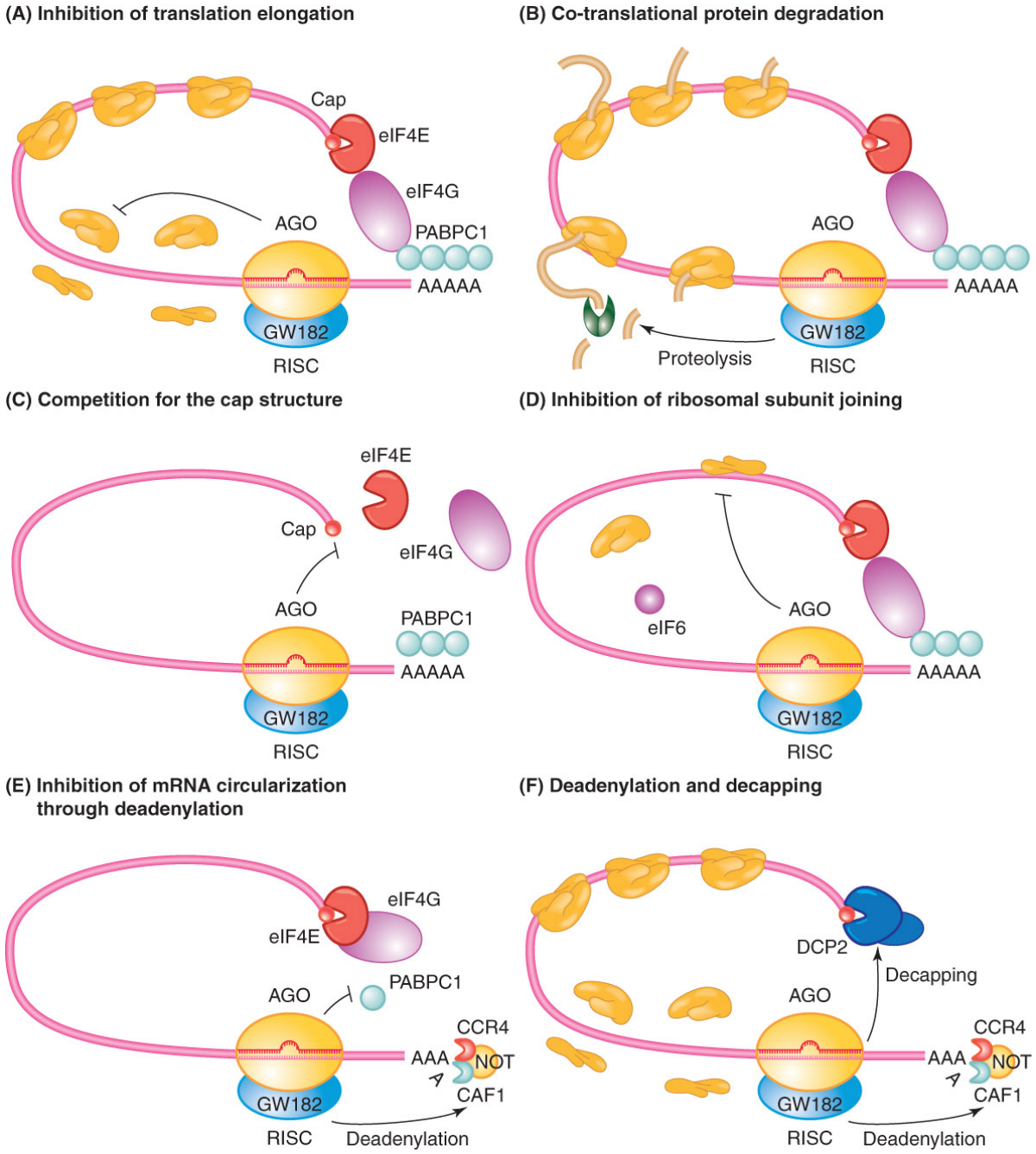
Human Argonaute protein carrying an miRNA. The protein is folded into four structural domains, each indicated by a different color.
The miRNA is held in an extended form that is optimal for forming RNA–RNA base pairs.

The active site of Argonaute that "slices" a target RNA, when it is extensively base-paired with the miRNA, is indicated in red.

Many Argonaute proteins (three out of the four human proteins, for example) lack the catalytic site and therefore bind target RNAs without slicing them



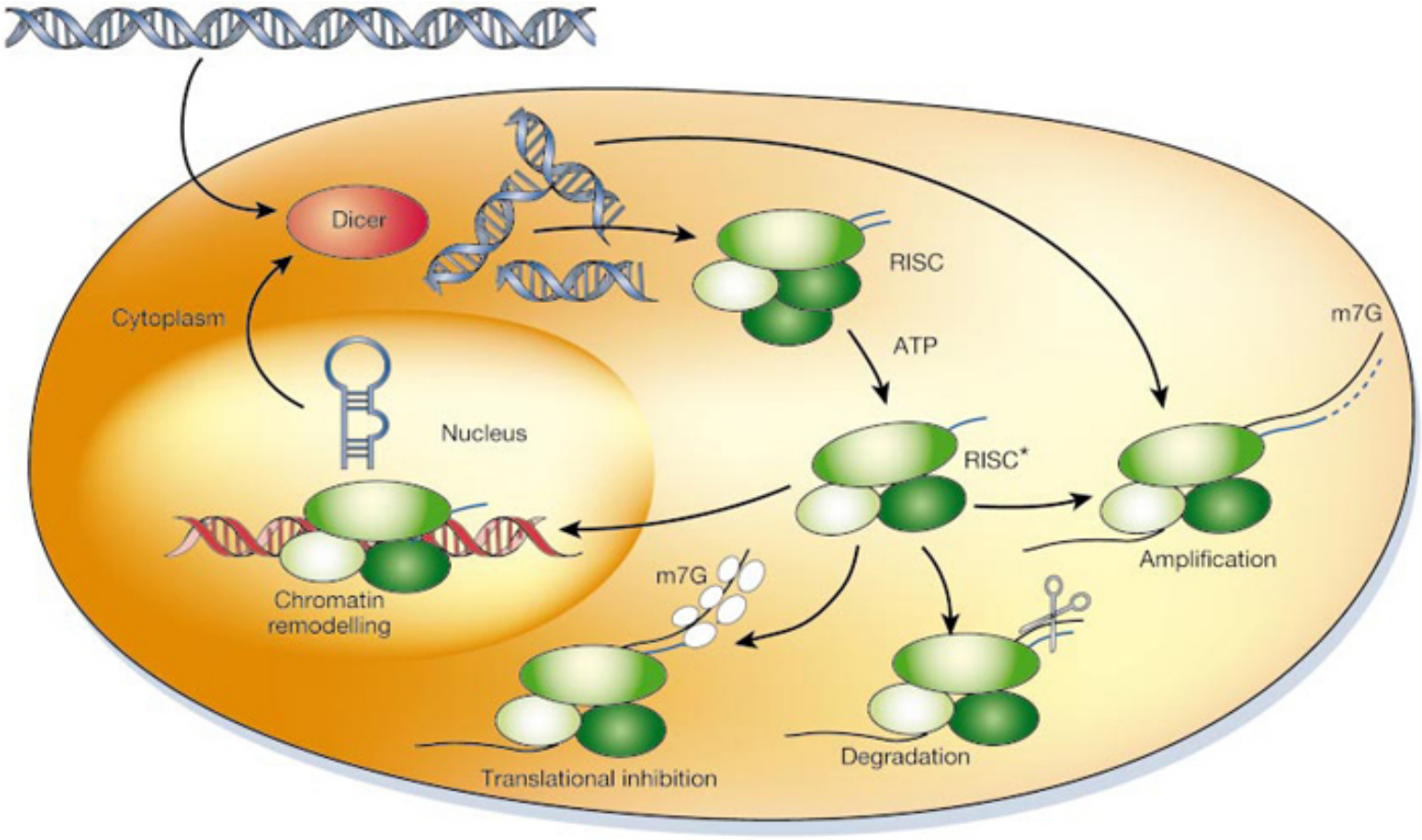
RNA interference directed by siRNAs. In many organisms, double-stranded RNA can trigger both the destruction of complementary mRNAs (left) and transcriptional silencing (right). The change in chromatin structure induced by the bound RITS (RNA-induced transcriptional silencing) complex



miRNA, siRNA suppression of translation

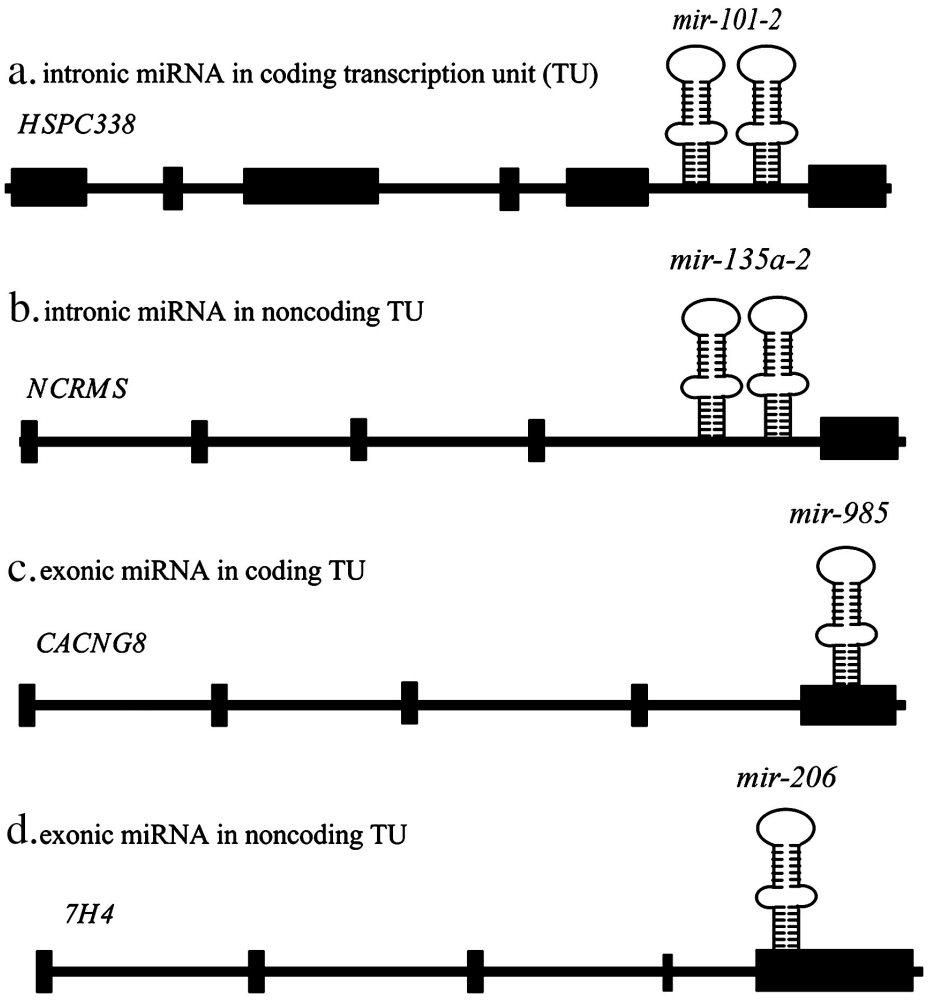
RNA Interference Is Also Used as a Cell Defense Mechanism

RNA Interference Can Direct Heterochromatin Formation



RNA interference mechanism

Organization of miRNA Genes in the Genome

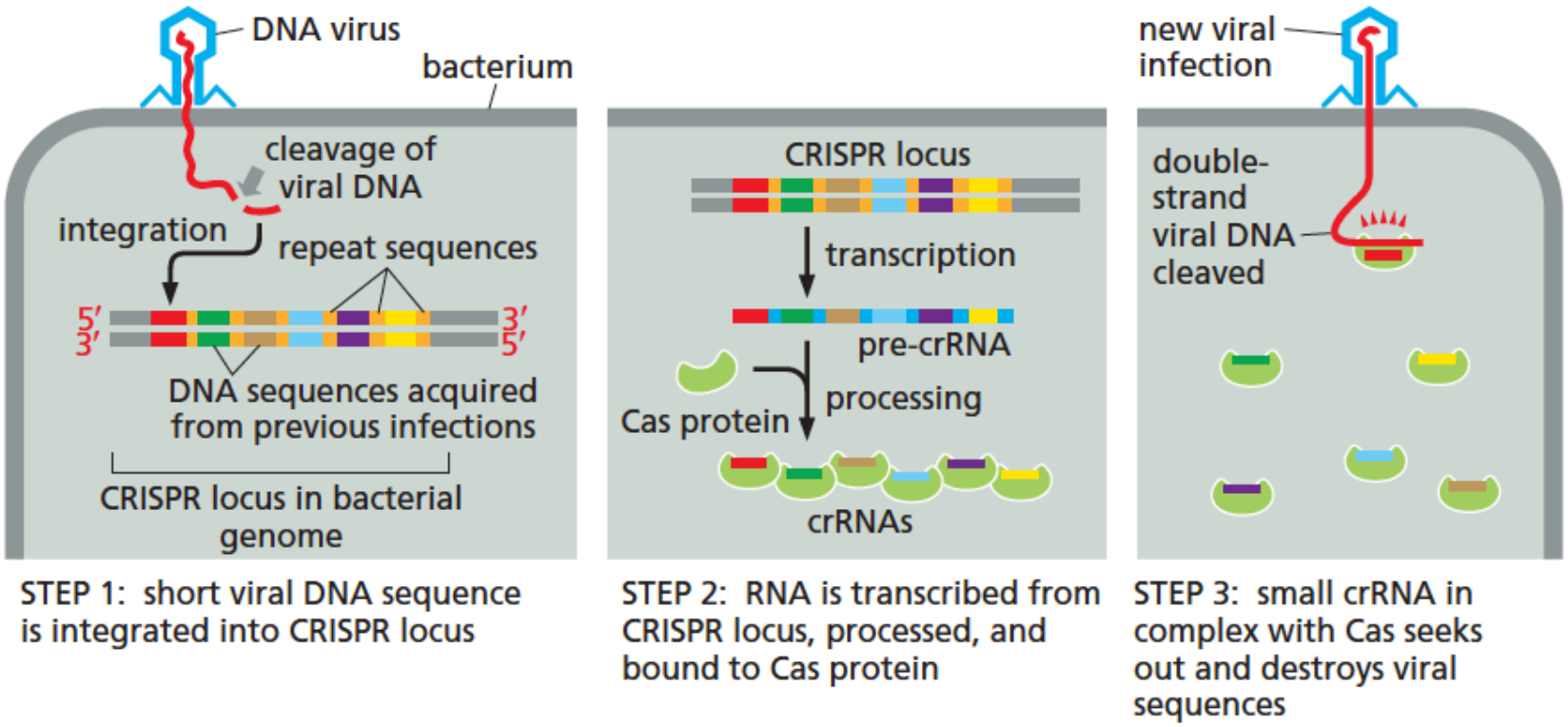


RNA Interference Has Become a Powerful Experimental Tool

Although it likely arose as a defense mechanism against viruses and transposable elements, RNA interference, as we have seen, has become thoroughly integrated into many aspects of normal cell biology, ranging from the control of gene expression to the structure of chromosomes. It has also been developed by scientists into a powerful experimental tool that allows almost any gene to be inactivated by evoking an RNAi response to it.

Bacteria Use Small Noncoding RNAs to Protect Themselves from Viruses

These viruses generally have DNA genomes. A recent discovery revealed that many species of bacteria (and almost all species of archaebacteria) use a repository of small noncoding RNA molecules to seek out and destroy the DNA of the invading viruses. Many features of this defense mechanism, known as the CRISPR system.



CRISPR-mediated immunity in bacteria and archaebacteria. After infection by a virus (left panel), a small bit of DNA from the viral genome is inserted into the CRISPR locus. For this to happen, a small fraction of infected cells must survive the initial viral infection. The surviving cells, or more generally their descendants, transcribe the CRISPR locus and process the transcript into crRNAs (middle panel). Upon reinfection with a virus that the population has already been "vaccinated" against, the incoming viral DNA is destroyed by a complementary crRNA (right panel). For a CRISPR system to be effective, the crRNAs must not destroy the CRISPR locus itself, even though the crRNAs are complementary in sequence to it. In many species, in order for crRNAs to attack an invading DNA molecule, there must be additional short nucleotide sequences that are carried by the target molecule. Because these sequences, known as PAMs (protospacer adjacent motifs), lie outside the crRNA sequences, the host CRISPR locus is spared (see Figure 8–55).

piRNAs Protect the Germ Line from Transposable Elements

One system of RNA interference relies on piRNAs (piwi-interacting RNAs, named for Piwi, a class of proteins related to Argonaute).

piRNAs are made specifically in the germ line, they block the movement of transposable elements.

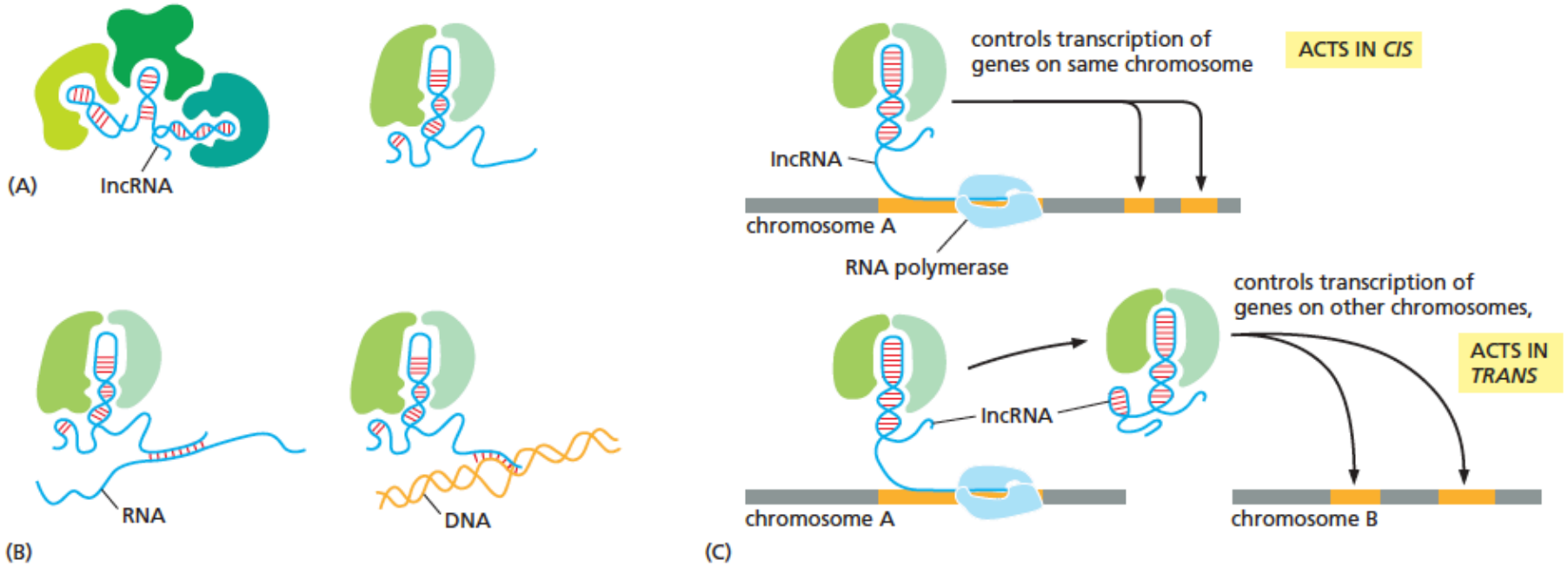
Found in many organisms, including humans, genes coding for piRNAs consist largely of sequence fragments of transposable elements. These clusters of fragments are transcribed and broken up into short, single-stranded piRNAs. The processing differs from that for miRNAs and siRNAs (for one thing, the Dicer enzyme is not involved), and the resulting piRNAs are slightly longer than miRNAs and siRNAs; moreover, they are complexed with Piwi rather than Argonaute proteins.

Once formed, the piRNAs seek out RNA targets by base-pairing and, much like siRNAs, transcriptionally silence intact transposon genes and destroy any RNA (including mRNAs) produced by them.

Many mysteries surround piRNAs. Over a million piRNA species are coded in the genomes of many mammals and expressed in the testes, yet only a small fraction seem to be directed against the transposons present in those genomes. Are the piRNAs remnants of past invaders? Do they cover so much “sequence space” that they are broadly protective for any foreign DNA? Another curious feature of piRNAs is that many of them (particularly if base-pairing does not have to be perfect) should, in principle, attack the normal mRNAs made by the organism, yet they do not. It has been proposed that these large numbers of piRNAs may form a system to distinguish “self” RNAs from “foreign” RNAs and attack only the latter. If this is the case, there must be a special way for the cell to spare its own RNAs.

Long Noncoding RNAs Have Diverse Functions in the Cell

- Noncoding RNA molecules have many functions in the cell.
- Yet, as is the case with proteins, there remain many noncoding RNAs whose function is still unknown.
- Many RNAs of unknown function belong to a group known as long noncoding RNA (lncRNA).
- Arbitrarily defined as RNAs longer than 200 nucleotides
- Number of lncRNAs (an estimated 8000 for the human genome, for example) came as a surprise to scientists.
- Most lncRNAs are transcribed by RNA polymerase II and have 5' caps and poly-A tails, and, in many cases, they are spliced.



Roles of long noncoding RNA (lncRNA). (A) lncRNAs can serve as scaffolds, bringing together proteins that function in the same process. RNAs can fold into specific three-dimensional structures that are often recognized by proteins. (B) In addition to serving as scaffolds, lncRNAs can, through formation of complementary base pairs, localize proteins to specific sequences on RNA or DNA molecules. (C) In some cases, lncRNAs act only in cis, for example, when the RNA is held in place by RNA polymerase (top). Other lncRNAs, however, diffuse from their sites of synthesis and therefore act in trans.

Animal miRNAs and their biological functions.

miRNAs	Target gene	Biological functions	Species
bantam	HID	Cell death and proliferation	*D. melanogaster*
let-7	*lin-41*, HBL-1	Regulation of developmental timing	*C. elegans*
lin-4	*lin-14*, lin-28	Physiological condition and developmental timing	*C. elegans*
lsy-6	COG-1	Neuronal cell fate and developmental timing	*C. elegans*
miR-1	HAND 2	Cardiomyocyte differentiation and proliferation	*Mus musculus*
miR-7	Notch targets	Notch signaling	*D. melanogaster*
miR-14	Caspase?	Cell death and proliferation	*D. melanogaster*
miR-15a, miR-16-1	Bcl_2	Down-regulated in B cell chronic lymphocyte leukemia	
miR-16	Several	AU-rich element mediated mRNA instability	*Homo sapiens*
miR-17-92	c-Myc, E2F1	Upregulated in B-cell lymphoma	*H. sapiens*
miR-32	Retrovirus PFV1	Antiviral defense	*H. sapiens*
miR-143	ERK5	Adipocyte differentiation	
miR-143, miR-145	Unknown	Downregulated in colonic adenocarcinoma	*H. sapiens*
miR-146	c-Myc, ROCK1	Development and function of immune system	*H. sapiens*
miR-155	PU-1, c-Maf	T-cell development and in innate immunity	Mouse
miR-181	unknown	Regulation of hematopoietic cell fate	*M. musculus*
miR-196	HOXA7, HOXB8, HOXC8, HOXD8	Development?	*M. musculus*
miR-223	NFI-A, Mef2c	Regulation of granulocytic maturation	*H. sapiens*
miR-273	DIE-1	Neuronal cell fate and developmental timing	*C. elegans*
miR-372, miR-373	LATS2		
miR-375	Myotrophin	Insulin secretions	*M. musculus*
miR-430	?	Brain morphogenesis	*D. rerio*
SVmiRNAs	SV40 viral mRNAs	Susceptibility to cytotoxic T cells	

Table 3. Recent preclinical and clinical trials based on miRNA therapeutics.

Disease or condition	Trial title	Targeted status
Asthma	miRNA analysis in premenstrual asthma	Unspecified
Barrett's esophagus, esophageal adenocarcinoma	miRNA expression in upper gastroin-testinal mucosal tissue	Unspecified
Cancer and liver infection	miR-34a mimetics	miRNA 34a and tumor p53 protei
Cancer, acute leukemia myelogenous	AML miRNA therapy	Unspecified
Epstein–Barr virus and herpes simplex virus infection	Herpes virus therapy	Unspecified
HCV infection	Hepatitis C therapy	Unspecified
HCV infection	Anti-mir-122 oligo	miRNA122
HCV infection, hypercholesterolemia	SPC-3649	miRNA122
Hepatitis C	miRNA-122 clinical course of patients with chronic HCV infection	Unspecified
Healthy	Safety study of SPC3649 in healthy men	Unspecified
Heart failure	miRNA inhibitors	miRNA 208a
Heart failure	miRNA mimetics	Unspecified
HIV/AIDS infection	HIV therapy	Unspecified
Inflammatory bowel disease	miRNA in inflammatory bowel disease	Unspecified
Leukemia	Studying biomarkers in cell samples from patients with acute myeloid leukemia	Unspecified
Lungs and non-small cell cancer	Osolo miRNA therapy	miRNA let-7a-1
Melanoma	miRNA expression and function in cutaneous malignant melanoma	Unspecified
Naevi malignant melanoma	Expression patterns of miRNA processing enzyme Dicer	Unspecified
Pregnant women	miRNA profile in umbilical cord blood NK cells	Unspecified
Prostate cancer	Prostate cancer miRNA	Unspecified
Pulmonary arterial hypertension	Expression and significance of miRNA	Unspecified
Renal cell carcinoma	miRNA expression in renal cell carcinoma	Unspecified
Sepsis	Circulating miRNAs as biomarkers of sepsis	Unspecified
Skin Cancer	Expression levels of miRNA processing enzymes Dicer and Drosha in epithelial skin cancer	Unspecified
Transplant	CMV miRNA expression *in vivo* and Immune Evasion	Unspecified
Unspecified	Antagomirs	Unspecified
Unspecified	Anti-inflammatory miRNA	Unspecified
Unspecified	Anticancer miRNA	Unspecified

[a] Source: www.clinicaltrials.gov.

Type	Description	Length	Characteristics	Function
miRNA	Micro RNA	20-24 nt	Pri-miRNA produced in the nucleus as capped and polyadenylated ssRNA with a imperfectly paired stem-loop structure Processing by Drosha and Dicer lead to a production of mature dsRNA with exact ends	Perfect complementarity: Ago2-mediated cleavage of mRNA Non-perfect complementarity: Suppression of translation or mRNA degradation (deadenylation, decapping, and exonucleocytic degradation)

Type	Description	Length	Characteristics	Function
siRNA	Small interfering RNA	20-24 nt	Canonical form long, linear, perfectly base-paired dsRNA Processed by Dicer into mature siRNA with heterogenous end composition Effector functions occur primarily in the cytoplasm supported by Ago proteins	Perfect match: endonucleocytic cleavage Non-perfect match or endonuclease-inactive RISC: translational repression or exonucleocytic degradationInduction of heterochromatin formation Silencing of the same locus from which they are derived

Type	Description	Length	Characteristics	Function
piRNA	PIWI-interacting RNA	24-31 nt	Effector phase occurs primarily in the cytoplasm mediated by Ago proteins, dicer does not act on processing, hence they are longer Precursor ssRNA, which is modified to contain 3′-terminal 2′-O-methyl Strong preference for uridine at the 5′ end	Silencing of transposable elements in the germline Minor functions in transcriptional silencing and translational activation

Name	Description	Length	Characteristics	Functions
lncRNA	Long non-coding RNA	>200 nt	Precursor ssRNA Many lncRNAs are subject to splicing, polyadenylation, and other post-transcriptional modifications Mostly nuclear RNAs but a subset also located in the cytoplasm Not evolutionary conserved with exceptions	Chromatin remodelling Transcriptional regulation Post-transcriptional regulation (splicing, TF localization) Precursors for siRNAs Component of nuclear organelles

Summary

Although we have encountered noncoding RNAs (tRNAs, rRNAs, snoRNAs), the sheer number of other noncoding RNAs produced by cells has surprised scientists.

One well understood use of noncoding RNAs occurs in RNA interference, where guide RNAs (miRNAs, siRNAs, piRNAs) base-pair with mRNAs.

RNA interference can cause mRNAs to be either destroyed or translationally repressed.

It can also cause specific genes to be packaged into heterochromatin suppressing their transcription.

In bacteria and archaebacteria, RNA interference is used as an adaptive immune response to destroy viruses that infect them.

A large family of large noncoding RNAs (lncRNAs) has recently been discovered.

Although the function of most of these RNAs is unknown, some serve as RNA scaffolds to bring specific proteins and RNA molecules together to speed up needed reactions.