mRNA, rRNA, RNA Interference, miRNA, siRNA

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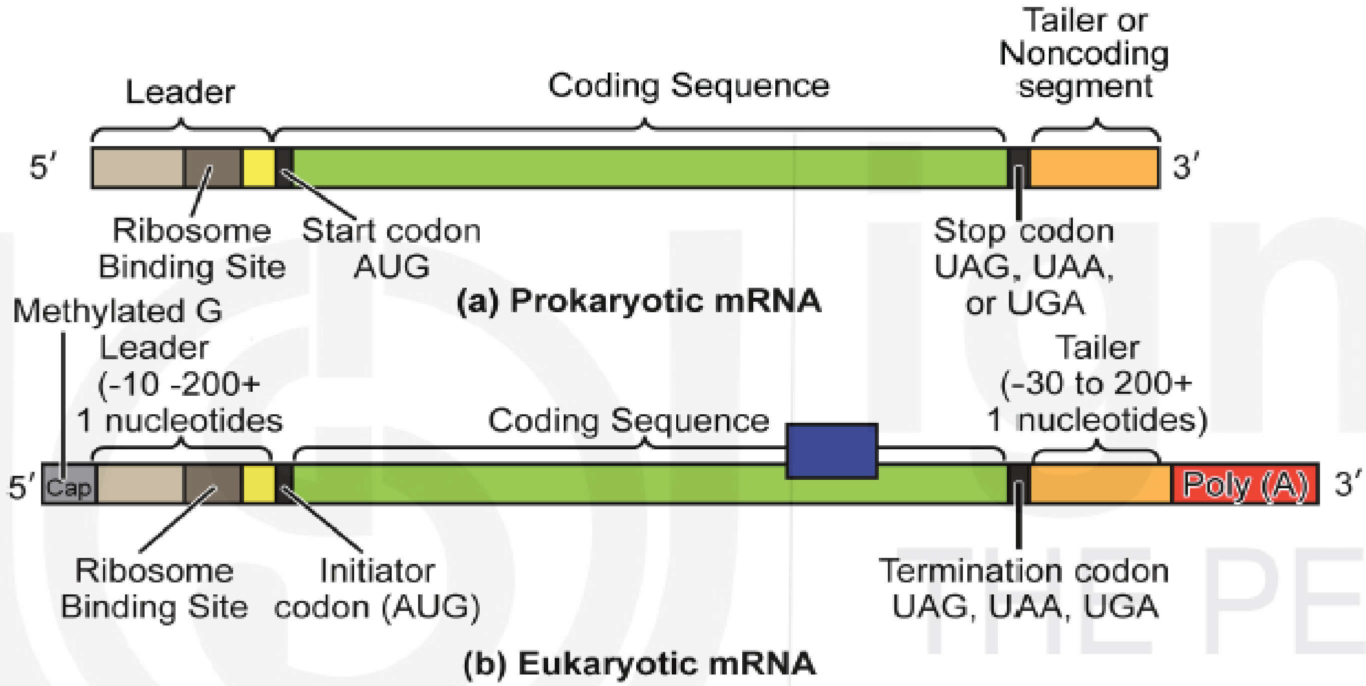
Messenger RNA

Messenger RNA carries genetic information from DNA (by transcription) and with the participation of ribosomes passes this genetic information to assemble amino acids in their correct order (translation). In bacteria, mRNA is transcribed directly from DNA but in eukaryotes, a pre-mRNA called primary transcript is first transcribed from DNA and then processed to yield mature mRNA. Both prokaryotic and eukaryotic mRNAs contain three primary regions (Fig. 12.3).



(a) Prokaryotic mRNA

(b) Eukaryotic mRNA

Fig. 12.3: a) Three primary regions of a mature (fully processed) mRNA --- 5´ - untranslated region, the protein- coding region, and the 3´ – untranslated region; b) Eukaryotic mRNA with its characteristic methylated G-cap and a Poly A tail.

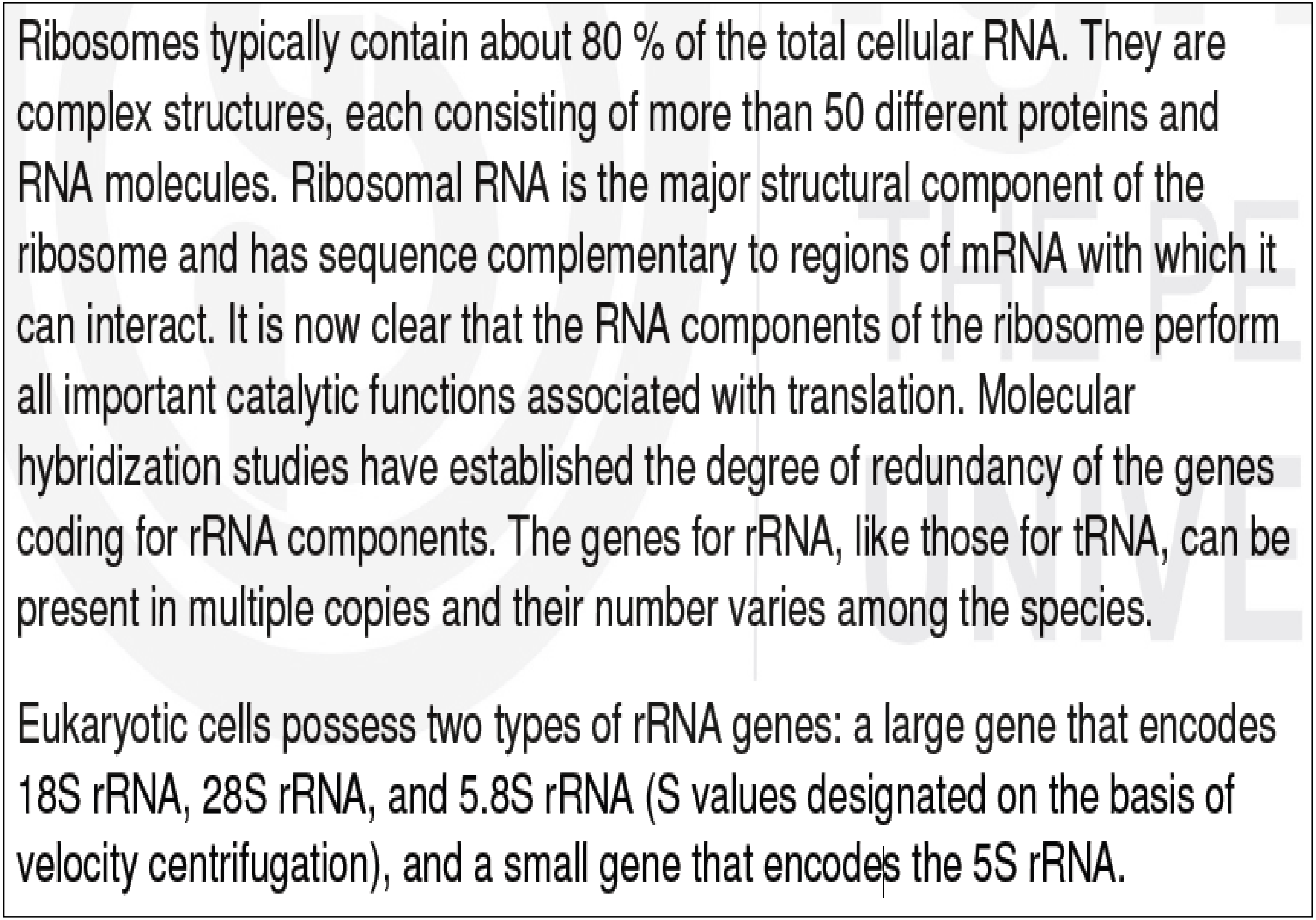
The short sequences of common nucleotides are called "**Consensus Sequences**". Consensus sequence simply means the sequences having considerable similarity. The presence of consensus in a set of nucleotides usually implies that the sequence is associated with an important function. Consensus sequences are conserved, i.e., identical or similar across different species.

- The 5´end of mRNA begins with nucleotides which are not translated into proteins. This 5´ untranslated region (5´UTR) is called as the Leader and has no ribosome binding sites. In bacterial mRNA the end of 5´UTR has a consensus (in all cases) sequence that serves as a ribosome-binding site. This consensus sequence is a hexamer (six nucleotides) called the **Shine-Dalgarno sequence.** This sequence is located seven nucleotides upstream of the first codon (initiation/start codon) where the first amino acid is incorporated. In contrast, the eukaryotic mRNA lacks the equivalent consensus sequence in its 5´ – untranslated regions. In eukaryotic cells the ribosomes bind to a modified 5´ – end of mRNA.

- The next region of mRNA is the protein coding region which begins with the **start codon** and ends with the **stop codon**.

- The last region of the mRNA is the 3´ – untranslated region (3´ – UTR sometimes called **trailer**).The nucleotides at the 3´ – end of the mRNA are not translated into a protein. The 3´ – UTR provides stability to mRNA, and to the protein coding sequences for translation.

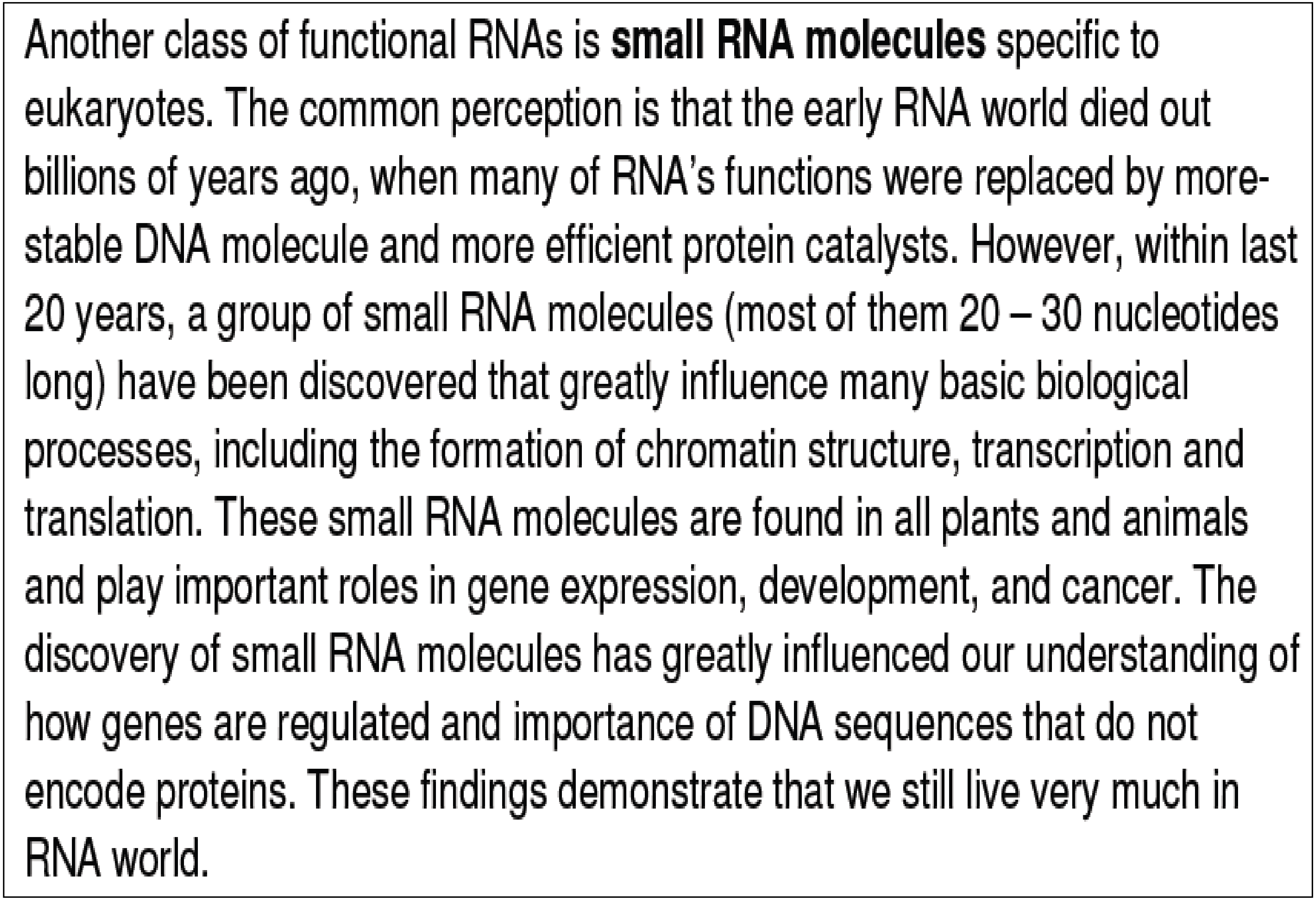
Ribosomal RNA



Ribosomes typically contain about 80 % of the total cellular RNA. They are complex structures, each consisting of more than 50 different proteins and RNA molecules. Ribosomal RNA is the major structural component of the ribosome and has sequence complementary to regions of mRNA with which it can interact. It is now clear that the RNA components of the ribosome perform all important catalytic functions associated with translation. Molecular hybridization studies have established the degree of redundancy of the genes coding for rRNA components. The genes for rRNA, like those for tRNA, can be present in multiple copies and their number varies among the species.

Eukaryotic cells possess two types of rRNA genes: a large gene that encodes 18S rRNA, 28S rRNA, and 5.8S rRNA (S values designated on the basis of velocity centrifugation), and a small gene that encodes the 5S rRNA.

Small RNA Molecules



Another class of functional RNAs is **small RNA molecules** specific to eukaryotes. The common perception is that the early RNA world died out billions of years ago, when many of RNA's functions were replaced by more-stable DNA molecule and more efficient protein catalysts. However, within last 20 years, a group of small RNA molecules (most of them 20 – 30 nucleotides long) have been discovered that greatly influence many basic biological processes, including the formation of chromatin structure, transcription and translation. These small RNA molecules are found in all plants and animals and play important roles in gene expression, development, and cancer. The discovery of small RNA molecules has greatly influenced our understanding of how genes are regulated and importance of DNA sequences that do not encode proteins. These findings demonstrate that we still live very much in RNA world.

Classes of small RNA molecules:

i) **Small nuclear RNAs (snRNAs)** – They are small RNA molecules (90 to 400 nucleotides long) that associate with proteins to form RNP (ribonucleoprotein) particles known as snRNPs or *snurps*. This small RNA species also form ribonucleoprotein processing complex (*spliceosome*) that edits and removes introns from eukaryotic mRNAs.

A very large fraction of eukaryotic genome is transcribed into a group of functional RNAs that participate in the regulation of gene expression at various levels.

ii) **MicroRNAs (miRNAs) and Small interfering RNAs (siRNAs)** – These small RNAs found in many eukaryotes are responsible for a variety of different functions, including the regulation of gene expression, defense against viruses, suppression of transposons, and modification of chromatin structure. These two abundant classes of RNA molecules although differ in how they originate, they have a number of features in common and their functions overlap considerably as shown in Table 12.1.

Table 12.1: Location and functions of different classes of RNA molecules.

Class of RNA	Cell Type	Location in Eukaryotic Cells*	Function
Ribosomal RNA (rRNA)	Bacterial and eukaryotic	Cytoplasm	Structural and functional components of the ribosome
Messenger RNA (mRNA)	Bacterial and eukaryotic	Nucleus and cytoplasm	Carries genetic code for proteins
Transfer RNA (tRNA)	Bacterial and eukaryotic	Cytoplasm	Helps incorporate amino acids into polypeptide chain
Small nuclear RNA (snRNA)	Eukaryotic	Nucleus	Processing of pre-mrRNA
Small nucleolar RNA (snoRNA)	Eukaryotic	Nucleus	Processing and assembly of rRNA
Small cytoplasmic RNA (scRNA)	Eukaryotic	Cytoplasm	Variable
MicroRNA (miRNA)	Eukaryotic	Cytoplasm	Inhibits translation of mRNA
Small interfering RNA (siRNA)	Eukaryotic	Cytoplasm	Triggers degradation of other RNA molecules
Piwi-interacting RNA (piRNA)	Eukaryotic	Cytoplasm	Thought to regulate gametogenesis, but function poorly defined

*All eukaryotic RNAs are transcribed in the nucleus

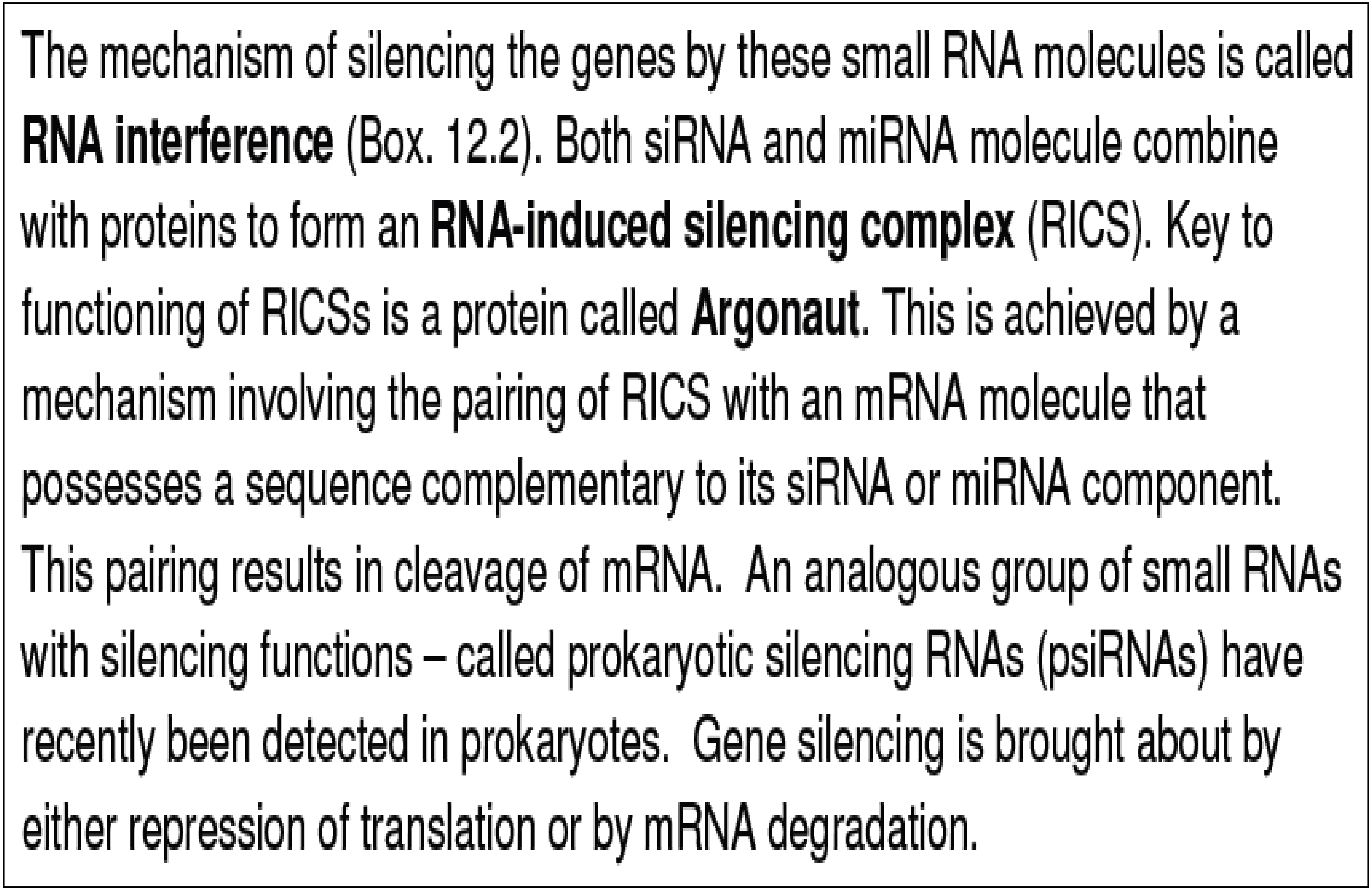
- Both miRNAs and siRNAs are about 22 nucleotides long. Small interfering RNAs arise from cleavage of mRNAs, RNA transposons and RNA viruses. Some miRNAs are cleaved from RNA molecules transcribed from sequences that encode miRNA only, but others are encoded in the introns and exons (a segment that codes for proteins) of mRNAs.

- Each miRNA is cleaved from a single-stranded RNA precursor that forms small hairpins, whereas multiple siRNAs are produced from the cleavage of an RNA duplex consisting of two different RNA molecules.

- Usually siRNA have exact complementarity (a relationship between two molecules following lock and key principle) with their target mRNA or DNA sequences and suppresses gene expression by degrading mRNA or inhibiting transcription whereas miRNAs have limited complementarities with their target mRNAs and often suppresses gene expression by inhibiting translation.

- Finally, miRNAs usually silence the genes that are distinct from those from which the miRNA were transcribed, whereas siRNAs typically silence the gene from which the siRNAs were transcribed.

RNA Interference

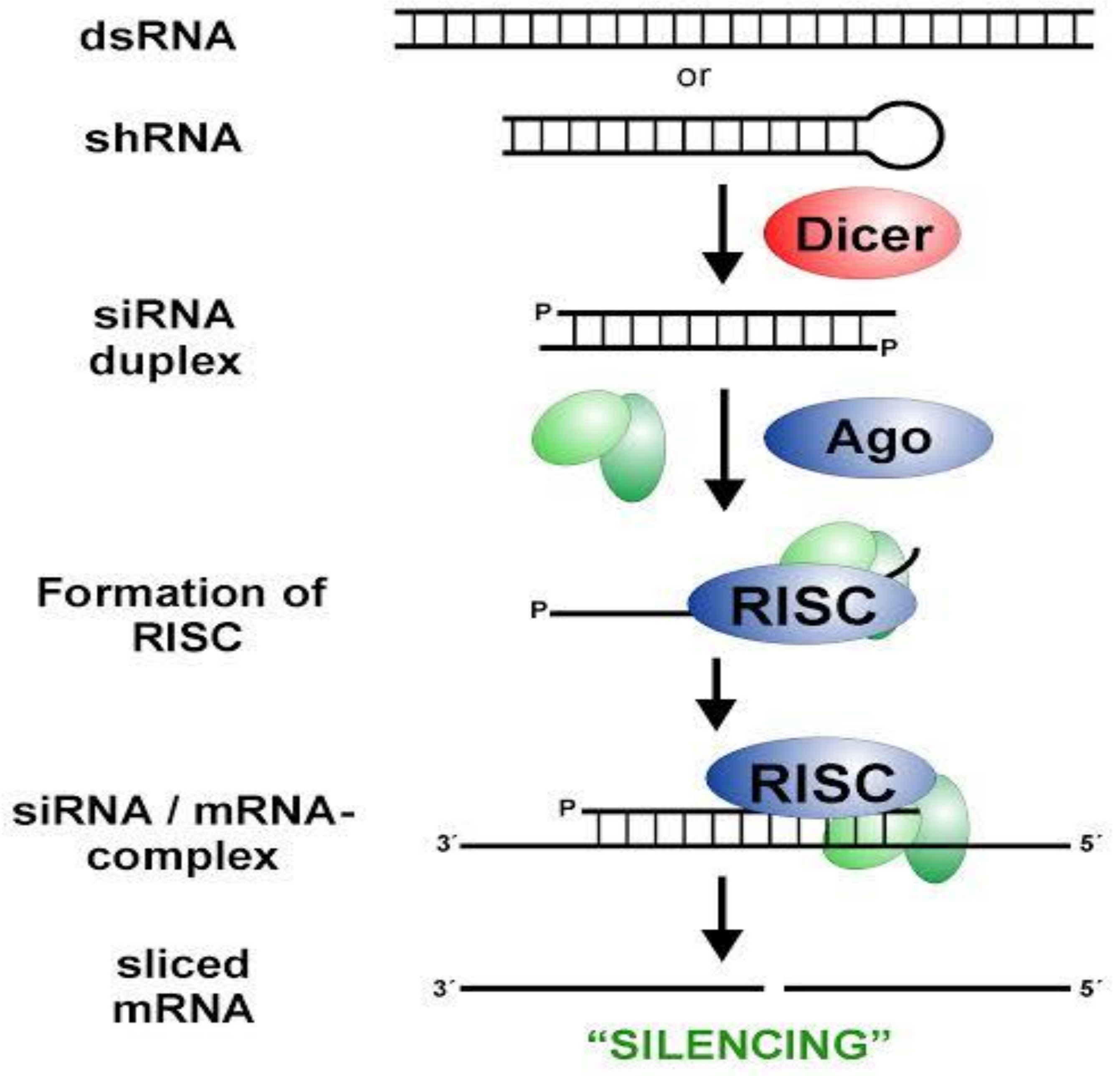


The mechanism of silencing the genes by these small RNA molecules is called **RNA interference** (Box. 12.2). Both siRNA and miRNA molecule combine with proteins to form an **RNA-induced silencing complex** (RICS). Key to functioning of RICSs is a protein called **Argonaut**. This is achieved by a mechanism involving the pairing of RICS with an mRNA molecule that possesses a sequence complementary to its siRNA or miRNA component. This pairing results in cleavage of mRNA. An analogous group of small RNAs with silencing functions – called prokaryotic silencing RNAs (psiRNAs) have recently been detected in prokaryotes. Gene silencing is brought about by either repression of translation or by mRNA degradation.

RNA Interference was discovered by Andrew Fire and Craig Mello in 2006 who were awarded the Nobel Prize.

RNA interference (RNAi) is a precise mechanism operative in eukaryotic cells which helps a cell to check the invasion of foreign genes (from viruses and transposons and also to check the expression of their own genes). RNA interference is triggered by double stranded RNA molecules, which may arise in several ways: i) by the transcription of inverted repeats into an RNA molecules that then base pairs with itself to form double- stranded RNA, ii) by simultaneous transcription of two different RNA molecules that are complementary to one another that pair, forming double-stranded RNA, or iii) by infection of viruses that make double stranded RNA. These double-stranded RNA molecules are chopped up by an enzyme appropriately called DICER, resulting in tiny RNA molecules that are unwound to produce siRNAs and miRNAs.

Geneticists speculate that RNA interference evolved as a defense against RNA viruses and transposable elements that move through RNA intermediate. However, RNA interference is also responsible for number of key genetic and developmental processes including changes in chromatin structure, translation, cell fate and proliferation, and cell death. Geneticists also use RNAi machinery as an effective tool for blocking the expression of specific genes and hence in gene silencing.


dsRNA
or
shRNA
Dicer
siRNA duplex
P
P
Ago
Formation of RISC
P
RISC
RISC
siRNA / mRNA-complex
P
3´
5´
sliced mRNA
3´
5´
"SILENCING"