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Course Name	Course Code	Credits	System	Syllabus
CORE COURSE-11 CELL AND MOLECULAR BIOLOGY	**(BOT-A-CC-5-11-TH) THEORETICAL (Credits 4, Lectures 60)**	**THEORETICAL (Credits 4, Lectures 60)**	**CBCS**	**University of Calcutta**

SEMESTER V CORE COURSE- 11

CELL AND MOLECULAR BIOLOGY (BOT-A-CC-5-11-TH)

THEORETICAL (Credits 4, Lectures 60)

CELL BIOLOGY

1. Origin and Evolution of Cells: 1.1. Evolution of nucleic acid (from PNA to DNA), Concept of RNA world, Ribozymes, First cell, 1.2. Origin of eukaryotic cell (endosymbiotic theory), 1.3. Small RNA- riboswitch, RNA interference, si RNA, mi RNA- brief idea, 1.4.Organellar DNA (cp- and mt- DNA).

2. Nucleus and Chromosome: 2.1. Nuclear envelope, Nuclear lamina and Nuclear pore complex, 2.2. Nucleolus- ultrastructure and ribosome biogenesis, 2.3. Chromatin ultrastructure and DNA packaging in eukaryotic chromosome, 2.4. Centromere: types, structure and function.

3. Cell cycle and its regulation: 3.1. Kinetochore and spindle apparatus-structural organization and functions, 3.2.Microtubulesstructure, organization and function, 3.3. Mechanism of cell cycle control in Yeast (checkpoints and role of MPF), Apoptosis (Brief idea).

<u>EVOLUTION OF NUCLEIC ACID (RNA to DNA)</u>

The evolution of genetic material can be divided into at least three major phases:

1. first, genomes of "nucleic acid-like" molecules;
2. secondly, genomes of RNA; and
3. finally, double-stranded DNA genomes such as those present in all contemporary cells.

Using properties of nucleic acid molecules, the evolutionary transition from RNA alone as a cellular informational macromolecule prior to the evolution of cell systems based on double-stranded DNA can be explained.

The idea that ribonucleic acid-based cellular genomes preceded DNA is based on the following:

(1) protein synthesis can occur in the absence of DNA but not of RNA;

(2) RNA molecules have some catalytic properties;

(3) the ubiquity of purine and pyridine nucleotide coenzymes as well as other similar ribonucleotide cofactors in metabolic pathways; and

(4) the fact that the biosynthesis of deoxyribonucleotides always proceeds via the enzymatic reduction of ribonucleotides.

The “RNA prior to DNA” hypothesis can be further developed by understanding the selective pressures that led to the biosynthesis of deoxyribose, thymine, and proofreading DNA polymerases. Taken together these observations suggest that DNA was selected as an informational molecule in cells to stabilize earlier RNA-protein replicating systems.

These arguments include the facts that

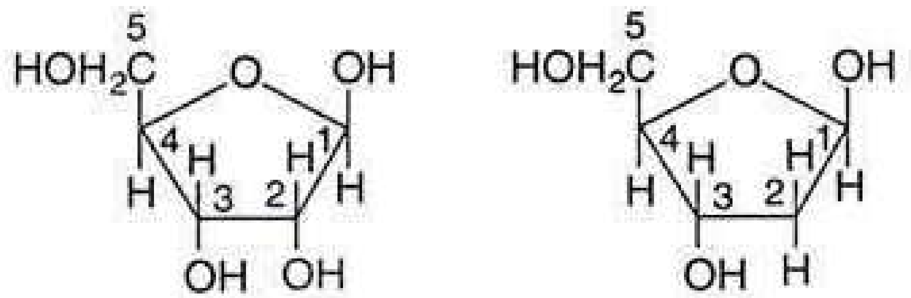
(1) the 2′-deoxy-containing phosphodiester backbone is more stable in aqueous conditions and in the presence of transition metal ions (such as Zn^{2+}) than its ribo-equivalents;

(2) the absence of proofreading activity in RNA polymerases leads to a higher rate of mutation in RNA genomes relative to DNA;

(3) information in RNA degrades because of the tendency of cytosine to deaminate to uracil and the lack of a correcting enzyme; and

(4) UV irradiation produces a larger number of photochemical changes in RNA molecules relative to double-stranded DNA. The absence of atmospheric UV attenuation during the early Earth environment (Hadean and early Archean) would have imposed an intense selection pressure favoring duplex DNA over other genetic information storage systems.

If RNA preceded DNA as a reservior of cellular genetic information, then an RNA-replicating oligopeptide must have been one of the earliest protoenzymes from which RNA polymerase presumably evolved. So it can be concluded that RNA polymerases are among the oldest classes of enzymes



D-Ribose D-2-Deoxyribose

***Fig. 2.4** : Structures of sugars present in nucleic acids (ribose is found in RNA and deoxyribose in DNA; Note the structural difference at C_2).*

The RNA World Hypothesis

- Proposed by Walter Gilbert, mid 1980s
- Concept of RNA catalyzing critical pre-biotic and early biological reactions
- RNA would give rise to RNA as an informational molecule
- RNA would give rise to protein
- RNA might bind amino acids proteins
- RNA might give rise to DNA
- Proteins might take over some functions
- DNA would take over informational functionality
- Eventually giving rise to the DNA → RNA → protein scheme found today

The first cells must have arisen from non-living material.

The origin of eukaryotic cells can be explained by the endosymbiotic theory.

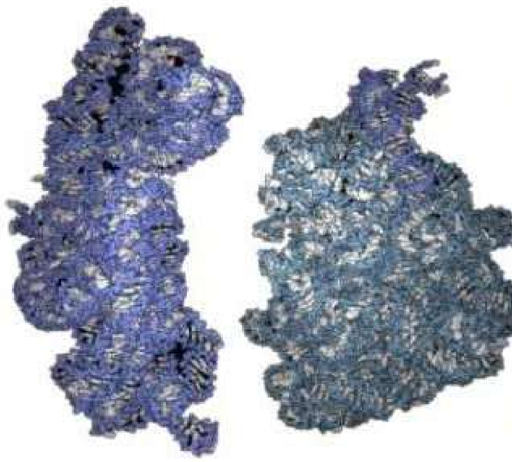
Evidence from Pasteur's experiments that spontaneous generation of cells and organisms does not now occur on Earth.

The first cell must have arisen from non living material

If we accept that there were times in the history of the Earth when cells did not exist then it is an obvious point that 'The first cells must have arisen from non-living material'. The only other possible explanation is that life, in the form of cells, was transported here from elsewhere in the universe. As illustrated above, it is extremely difficult (and given our level of technology currently impossible), to generate cells from anything but other cells. So how did the first cells arise?

Some of the key problems are:

1. Non-living synthesis of simple organic molecules, e.g. sugars and amino acids
2. Assembly of these organic molecules into polymers
3. Formation of polymers that can self-replicate (enabling inheritance)
4. Formation of membranes to package the organic molecules



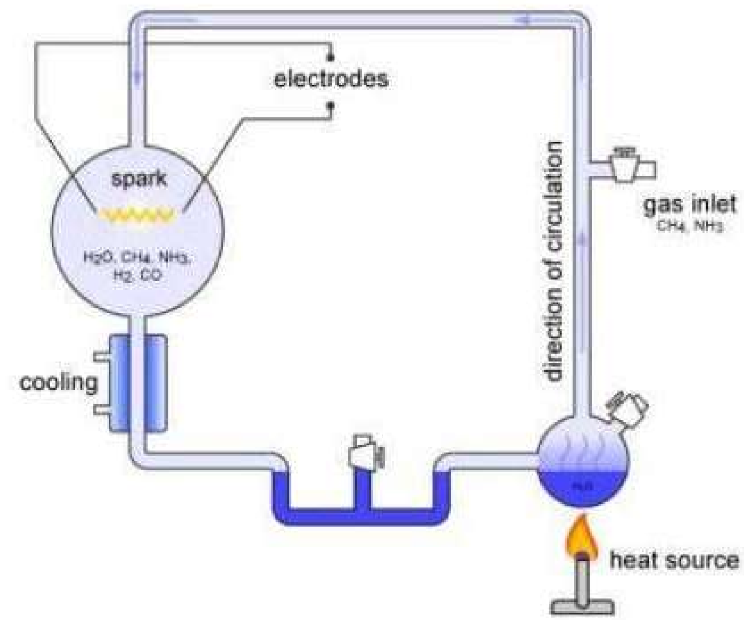
http://exploringorigins.org/resources.html

WATCH VIDEO OF FIRST CELL

1. Non-living synthesis of simple organic molecules:

Miller and Urey recreated the conditions of pre-biotic Earth in a closed system.

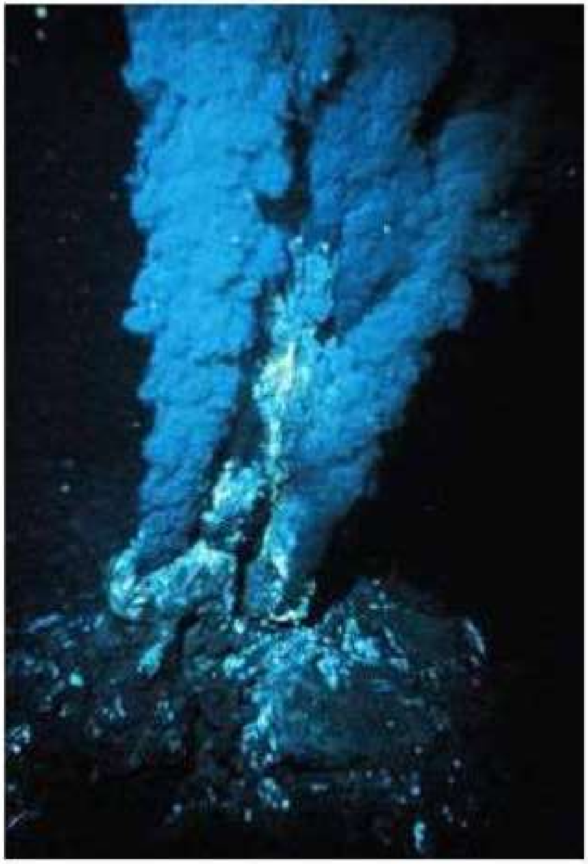
- These conditions included a reducing atmosphere (low oxygen), high radiation levels, high temperatures and electrical storms
- Water was boiled to form vapour and then was mixed with methane, ammonia and hydrogen
- The mixture of gases was exposed to an electrical discharge (sparks) to simulate lightning



- The mixture was then allowed to cool and after one week was found to contain some simple amino acids and complex oily hydrocarbons
- Based on these findings, it was concluded that under the hypothesised conditions of pre-biotic Earth, organic molecules could be formed

2. Assembly of these organic molecules into polymers:

Miller and Urey's experiments allowed for the formation of amino acids, **but** the conditions used also tended to hydrolyse bonds preventing polymers forming.



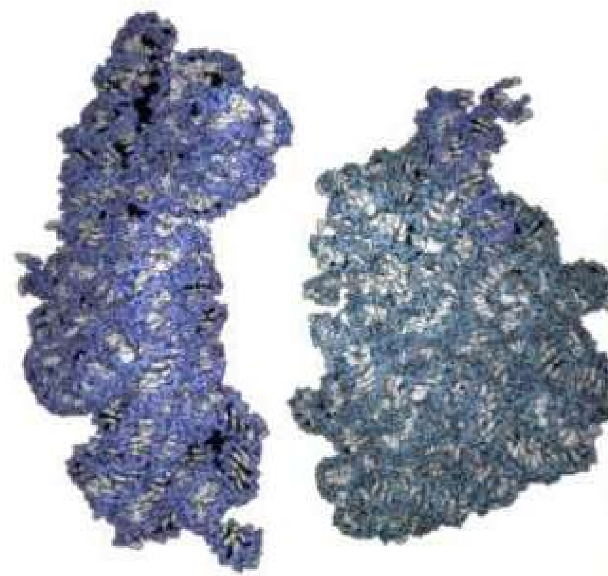
Deep-sea thermal vents

- Fissures in a planet's surface from which geothermally heated water issues. Vents are commonly found near in volcanically active areas)
- Along with heat energy the Vents issue a ready supply of reduced inorganic chemicals
- Vents provide the right conditions and chemicals to allow organic polymers to arise.

3. Formation of polymers that can self-replicate (enabling inheritance)

- DNA though very stable and effective at storing information is not able to self-replicate – enzymes are required
- However RNA can both store information and self-replicate - it can catalyse the formation of copies of itself.
- In ribosomes RNA is found in the catalytic site and plays a role in peptide bond formation

For more detail research the [RNA World Hypothesis]



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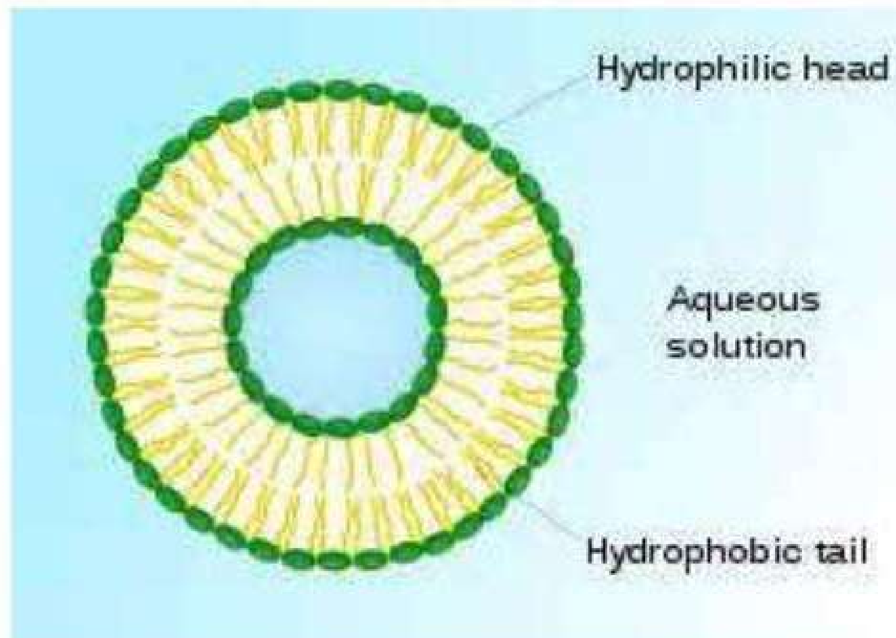
4. Formation of membranes to package the organic molecules

Experiments have shown that phospholipids natural assemble into bilayers, if conditions are correct.
Formation of the bilayer creates an isolated internal environment.
The formation of an internal environment means that optimal conditions, e.g. for replication or catalysis can be maintained.



Metabolism

- It is believed that cells initially used anaerobic respiration (in the absence of oxygen) in a process like glycolysis: the breakdown of glucose to lactic acid
- Then two things must have happened:
  - Origin of photosynthesis
  - Aerobic respiration

Photosynthesis

- Photosynthesis allows cells to use light and CO_2 to make glucose for energy
- The first photosynthetic bacteria, which evolved about3 billion years ago, probably used H_2S to convert CO_2 to organic molecules: a pathway still used by some bacteria today.
- H_2O as an electron donor and hydrogen for the conversion of CO_2 to organic compounds evolved later and led to the oxygenation of Earth's atmosphere.
- The use of H_2O in photosynthetic reactions produces the by-product free O_2; this mechanism is thought to have been responsible for making O_2 abundant in Earth's atmosphere.

Aerobic Respiration

- Once oxygen was abundant in the atmosphere, respiration began to use oxygen: aerobic respiration.
- O_2 is a highly reactive molecule, and oxidative metabolism, utilizing this reactivity, has provided a mechanism for generating energy from organic molecules that is much more efficient than anaerobic glycolysis.

Endosymbiotic Theory

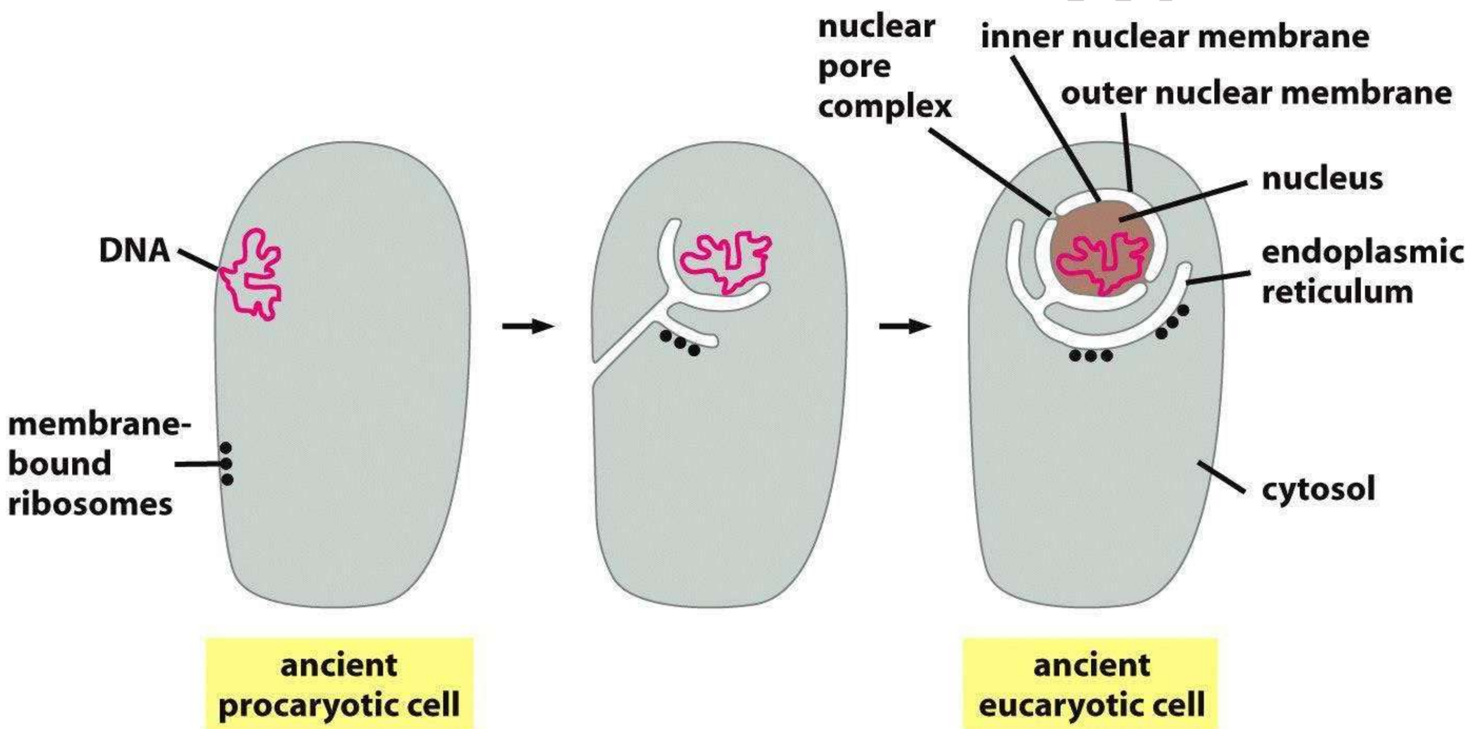
- Photosynthesis and respiration initially took place in the cytoplasm.
- However, when we look at eukaryotic cells today (like our own), we see that many have chloroplasts and mitochondria: organelles where these reactions take place.
- They also have a nuclear membrane encasing the DNA
- The organelles are thought to have been acquired as a result of the association of prokaryotic cells with the ancestor of eukaryotes.

ENDOSYMBIOSIS

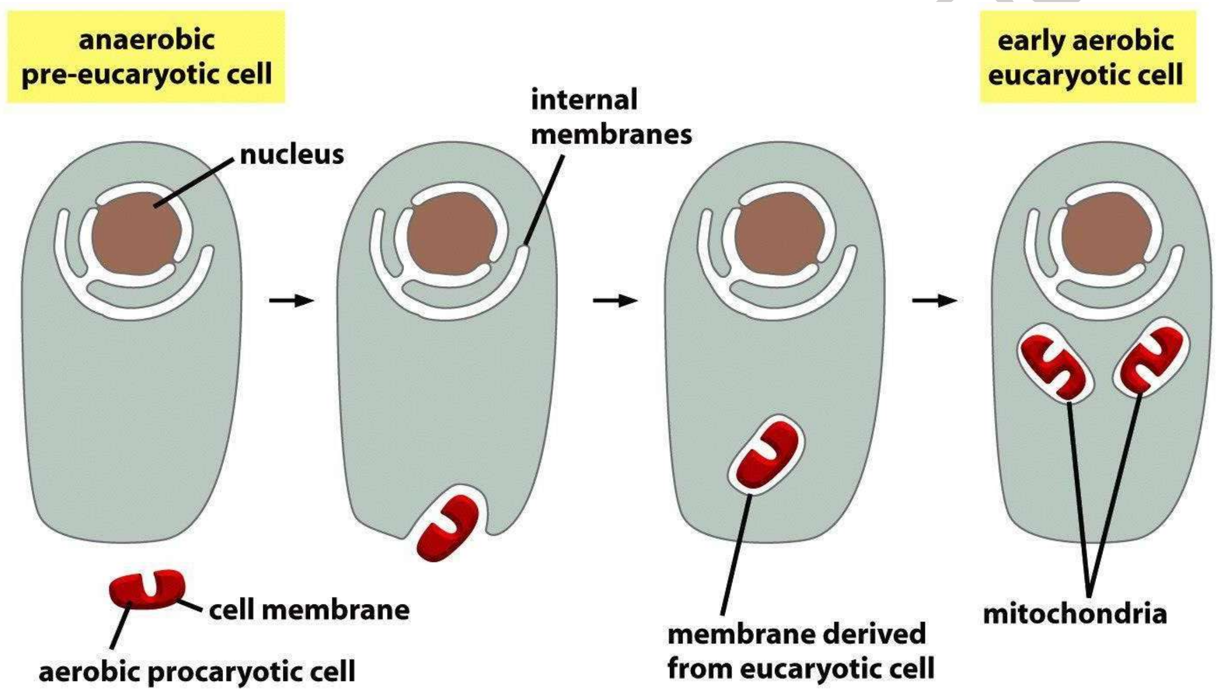
What is endosymbiosis?

- The theory of endosymbiosis helps explain the evolution of eukaryotic cells by looking at the origin of mitochondria and chloroplasts
- The key points to the theory are:
  - About 2 billion years ago a bacterial cell took up residence inside a eukaryotic cell
  - The eukaryotic cell acted as a ‘predator’, bringing the bacterial cell inside (by endocytosis)
  - The eukaryotic cell and the bacterial cell formed a symbiotic relationship, in which both organisms lived in contact with one another
  - The bacterial cell then went through a series of changes to ultimately become a mitochondrion
- In this process the eukaryote helped the bacteria by providing protection and carbon compounds. The bacteria, after a series of changes, became specialised in providing the eukaryote with ATP.
- It is thought that mitochondria were originally aerobic heterotrophic bacteria and chloroplasts were originally cyanobacteria

Evolution of nuclear membrane and ER

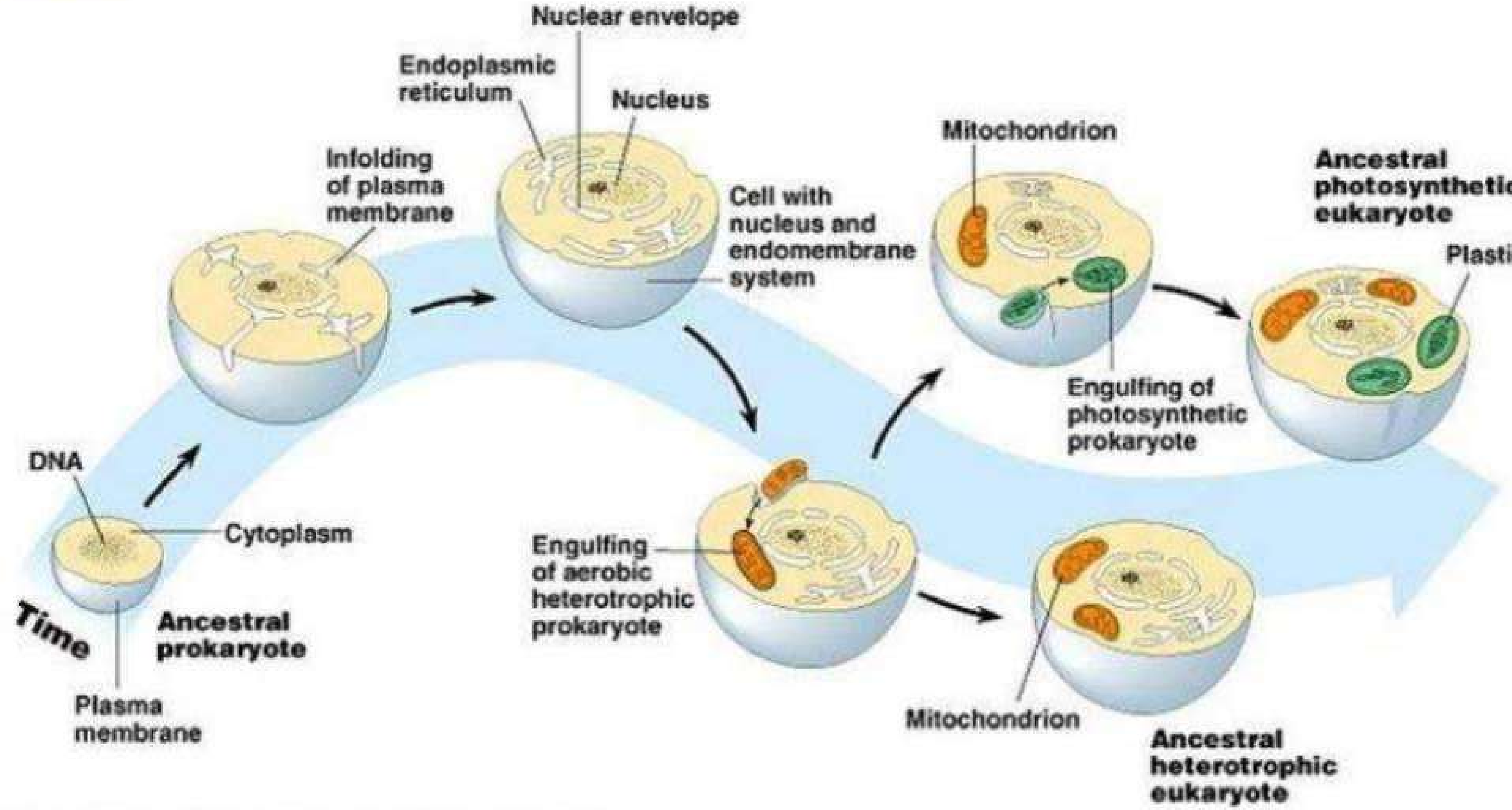


Mitochondria evolved from engulfed bacteria



It is virtually certain that mitochondria originate from bacteria that were engulfed by an ancestral pre-eukaryotic cell and survived inside it, living in ***symbiosis (endosymbiosis)*** with their host. Note that the double membrane of present-day mitochondria is thought to have been derived from the plasma membrane and the membrane of the engulfed bacterium.

Explanation of endosymbiosis



Evolution of photosynthetic eukaryotic cells like algae and plant cells

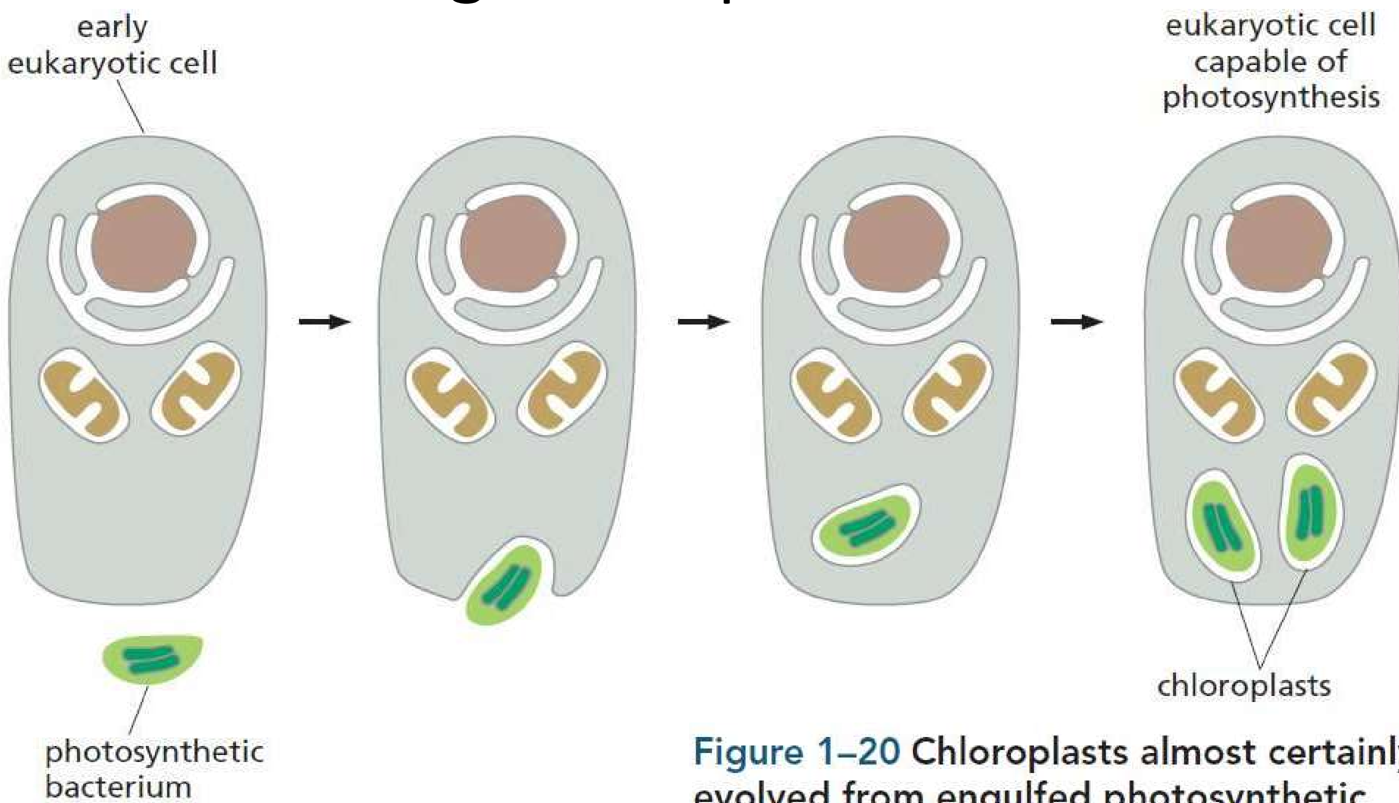


Figure 1–20 Chloroplasts almost certainly evolved from engulfed photosynthetic bacteria. The bacteria are thought to have been taken up by early eukaryotic cells that already contained mitochondria.

EVIDENCE FOR ENDOSYMBIOSIS

- Mitochondria and chloroplasts are similar in size and structure to bacteria
- Both organelles are bound by a double membrane - the outer membrane may be derived from the engulfing vesicle, and the inner one may be derived from the plasma membrane of the original prokaryote
- Mitochondria and chloroplasts contain a limited amount of genetic material and divide by fission (independently of the host cell). Their DNA is a circular loop like that of prokaryotes.
- Although most of the proteins within mitochondria and chloroplasts are now produced by the eukaryotic host, they do have their own ribosomes and they do produce some proteins. Their ribosomes resemble those of prokaryotes.

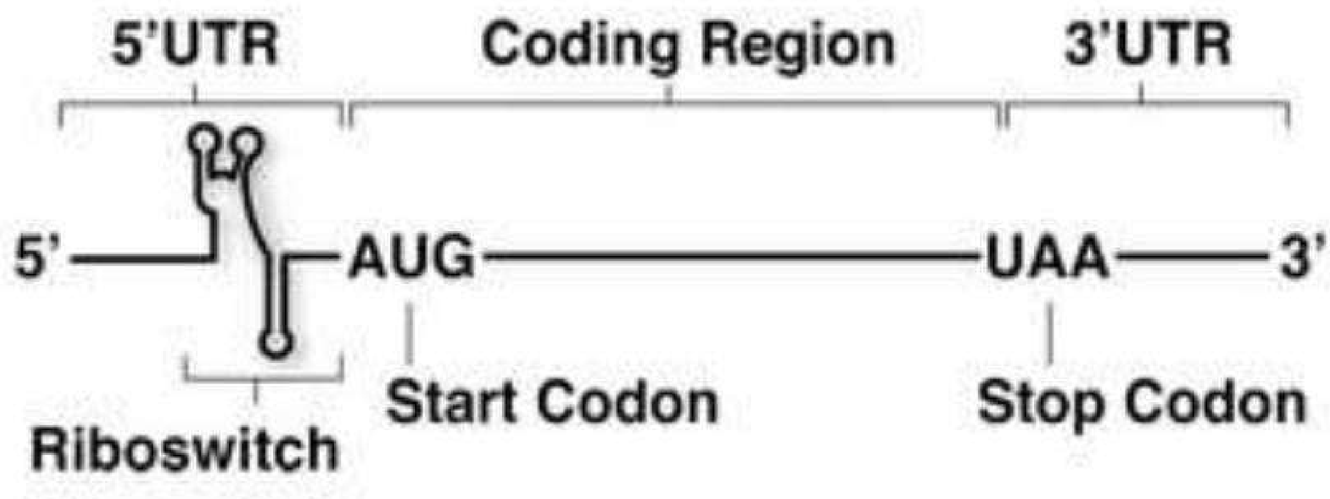
EVIDENCE FOR ENDOSYMBIOSIS

(TAKEN FROM PEARSON)

- "The final bit of evidence for endosymbiotic theory is DNA. DNA provides a code made up of 64 different 'words'. Interestingly, this code has the same meaning in nearly all organisms on Earth and is said to be 'universal'. There are only slight variations, which can be explained by changes since the common origin of life on our planet. As mentioned, the mitochondria of eukaryotic cells have a DNA code that more closely resembles bacteria than eukaryotic cells. Most scientists believe that the more DNA two organisms have in common, the more closely related they are to one another."

riboswitches

- RNA elements
- Binding to small metabolite/ligand- bringing conformational changes.
- Locates in the 5' untranslated region.
- Regulate gene expression
- Very sensitive and ligand specific.



A typical bacterial mRNA transcript controlled by a riboregulatory element such as a riboswitch is composed of three sections: the 5' untranslated region (5' UTR), the protein-coding region beginning with the start codon (AUG) and ending with a stop codon (UAA), and the 3' untranslated region (3' UTR).

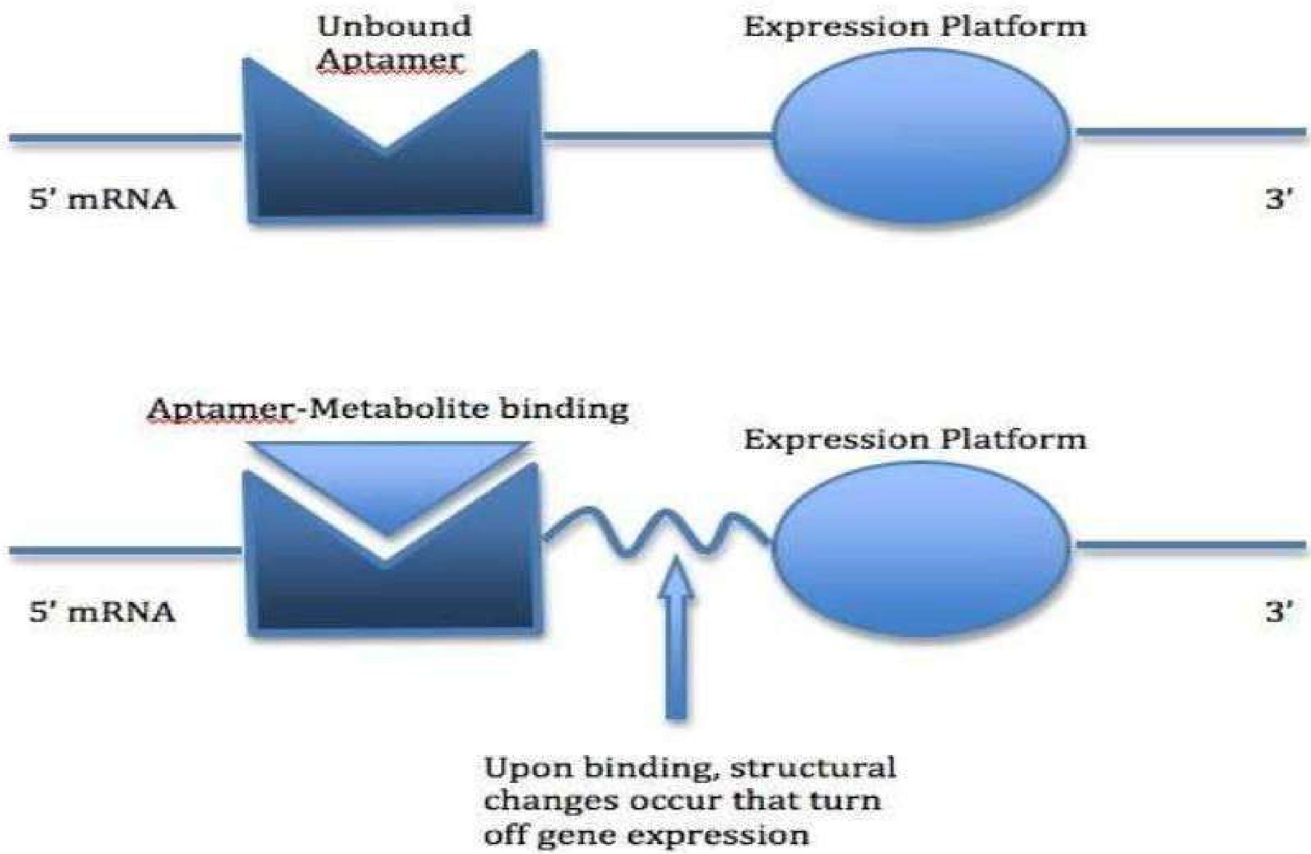
STRUCTURE

- Two domains

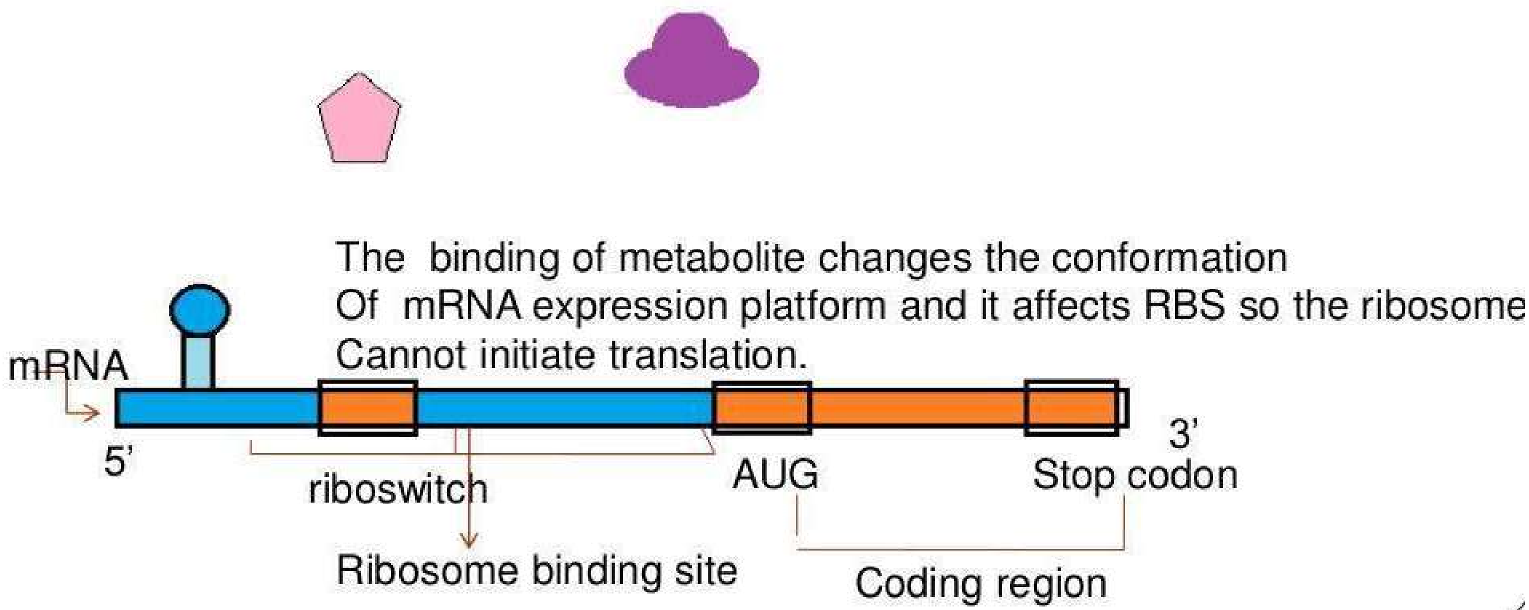
  - Aptamer-(Ligand binding domain)
  - Ligand recognation and binding.
  - Highly conserved.

  - Expression platform.
  - Less conserved
  - Adopts two mutually exclusive conformation.
  - Shine-dalgano sequence locates in this domain.

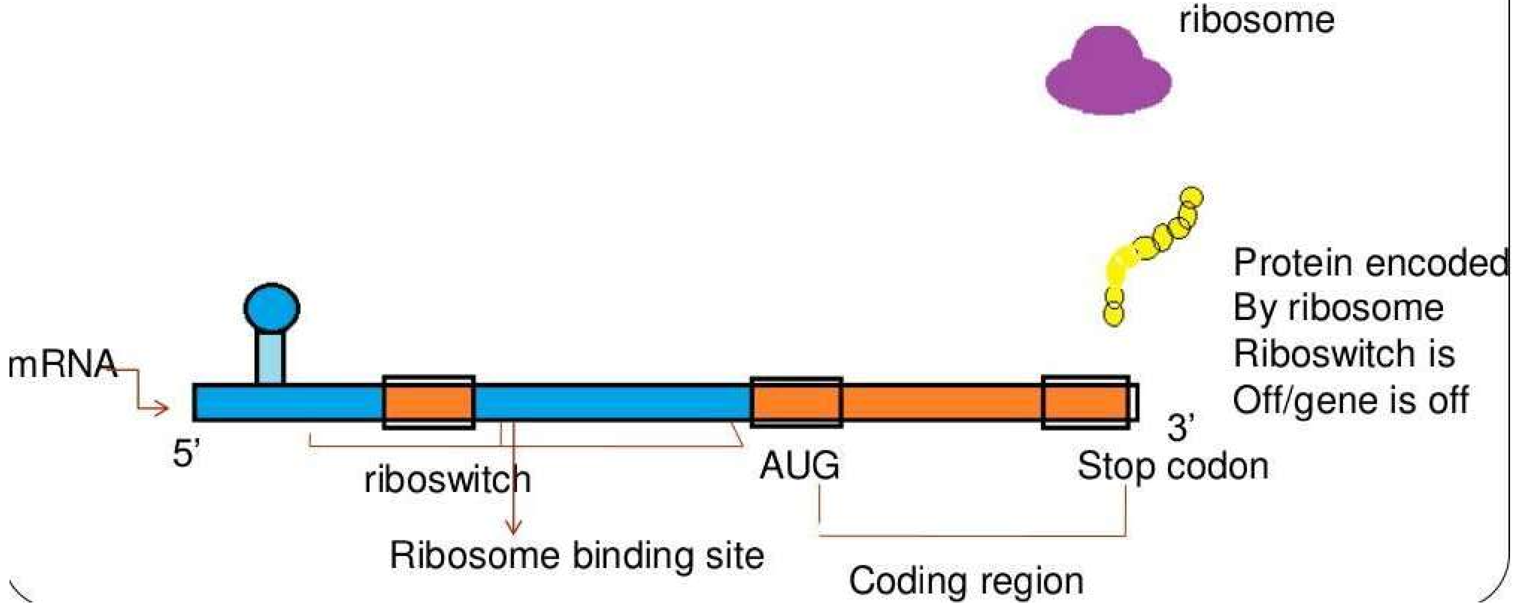
structure


Unbound
Aptamer
Expression Platform
5' mRNA
3'
Aptamer-Metabolite binding
Expression Platform
5' mRNA
3'
Upon binding, structural
changes occur that turn
off gene expression

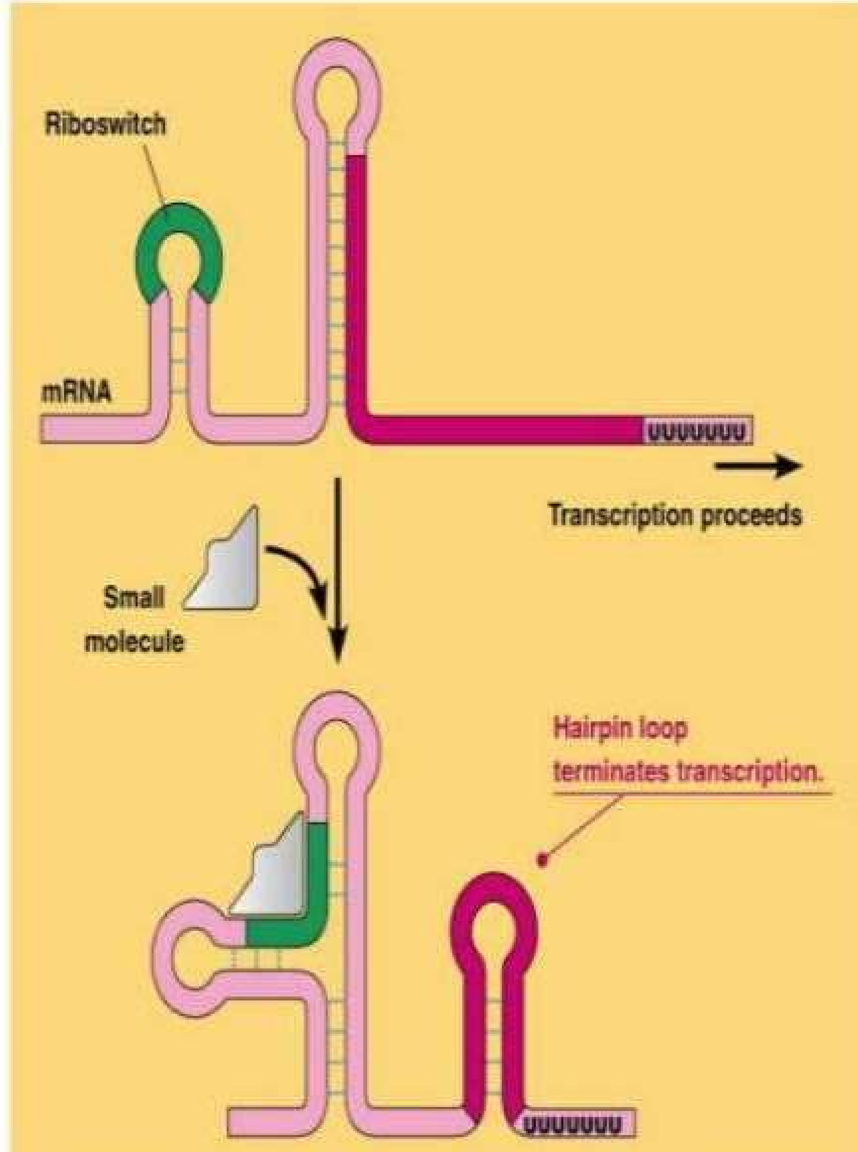
Riboswitch is on..in presence of metabolite



REPRESSION OF TRANLATION INITIATION



Transcription termination. Binding of a small molecule to a riboswitch in the leader sequence of some mRNAs triggers the formation of a hairpin loop that terminates transcription. FMN binding to the leader sequence of the mRNA transcribed from the *rib* operon of *B. subtilis* works in this way.



Examples..

- TPP riboswitch : this riboswitch binds TPP (thiamin pyrophosphate in order to regulate the transport and synthesis of thiamin as well as other metabolites with similar properties.
- Lysine riboswitch : binds to lysine and regulates its biosynthesis, catabolism, and transport.
- Glycine riboswitch : this riboswitch regulates glycine metabolism. This is the only riboswitch known currently to be able to perform cooperative binding.
- FMN riboswitch : this riboswitch binds FMN (flavin mononucleotide) in order to regulate the transport and synthesis of riboflavin.
- Purine riboswitch : binds purines to regulate its transport and metabolism. Different forms of this riboswitch are able to bind either guanine or adenine depending on the pyrimidine in the riboswitch.
- Cobalamin riboswitch : this riboswitch binds adenosylcobalamin, the coenzyme form of B12 vitamin, in order to moderate the synthesis and transport of cobalamin and other similar metabolites.
- as well as many others such as SAM riboswitch, PreQ1 riboswitch, SAH riboswitch, glmS riboswitch, and cyclic di-GMP riboswitch

RNA INTERFERENCE

In 2006, Andrew Fire and Craig C. Mello shared the Nobel Prize in Physiology or Medicine for their work on RNA interference in the nematode worm C. elegans, which they published in 1998.

Andrew Fire

Craig C. Mellow

What is RNAi?

- **RNA interference (RNAi):** Cellular process by which an mRNA is targeted for degradation by a dsRNA with a strand complementary to a fragment of such mRNA.
- A selective gene knock-down phenomenon.

Specific terms for gene silencing

- ✓ Post-transcriptional gene silencing (**PTGS**) - Plants
- ✓ **Quelling** - Fungi
- ✓ RNA interference (**RNAi**) – Animals
- ❖ RNAi operates and its natural **role for virus defence** and **endogenous gene regulation** in plants

In Interference

- RNA
  - siRNA: dsRNA 21-22 nt.
  - miRNA: ssRNA 19-25nt. Encoded by non protein coding genome
- RISC:
  - RNA induced Silencing Complex, that cleaves mRNA
- Enzymes
  - Dicer : produces 20-21 nt cleavages that initiate RNAi
  - Drosha : cleaves base hairpin in to form pre miRNA; which is later processed by Dicer

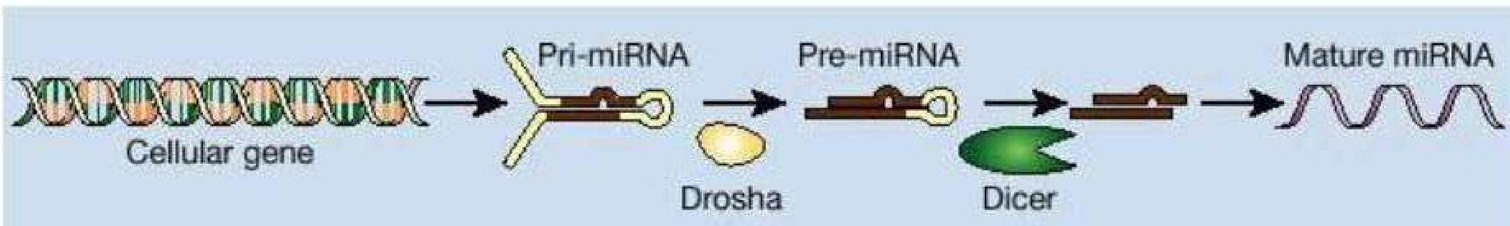
Multiple pathways for RNAi

There are two main RNAi pathway:

1. small interfering RNAs (siRNAs) generated via processing of longer dsRNA and
2. microRNAs (miRNAs) that are generated via processing of stem loop precursors

miRNA Biogenesis

- ✓ Transcribed from endogenous gene as pri-miRNA
  - Primary miRNA: long with multiple hairpins
  - Imperfect internal sequence complementarity
- ✓ Cleaved by Drosha into pre-miRNA
  - Precursor miRNA: ~70nt imperfect hairpins
  - Exported from nucleus
- ✓ Cleaved by Dicer into mature miRNA
  - 21-25nt
  - Symmetric 2nt 3' overhangs, 5' phosphate groups



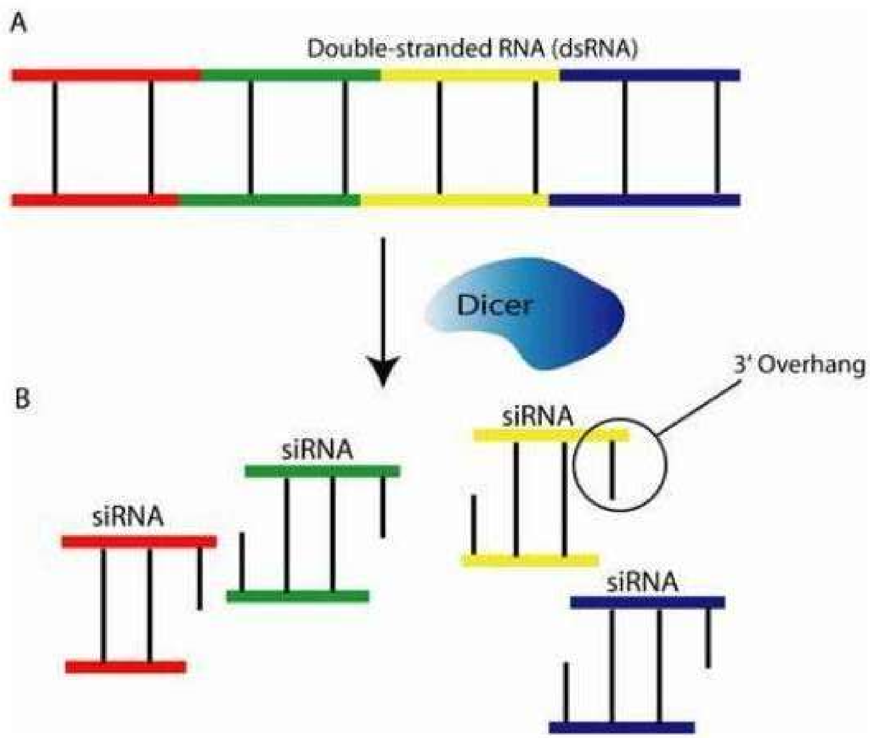
Novina and Sharp, 2004

Mechanism of RNAi

- Initiation phase
- Maintenance phase
- Signal amplification and spreading phase

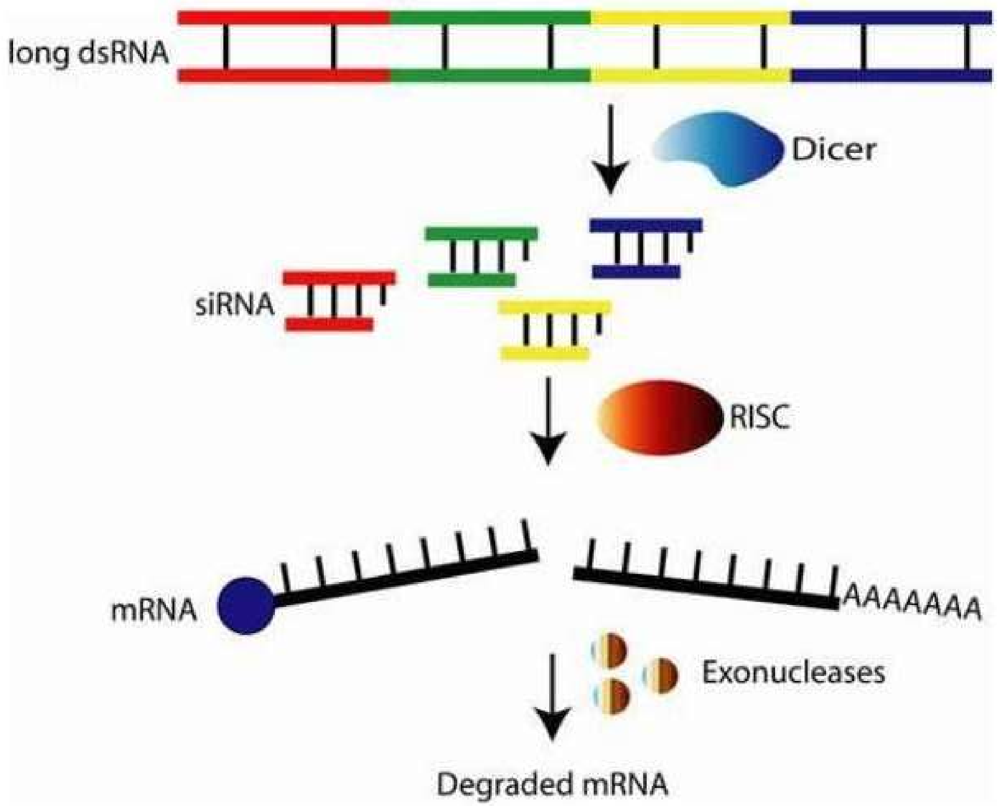
Initiation phase

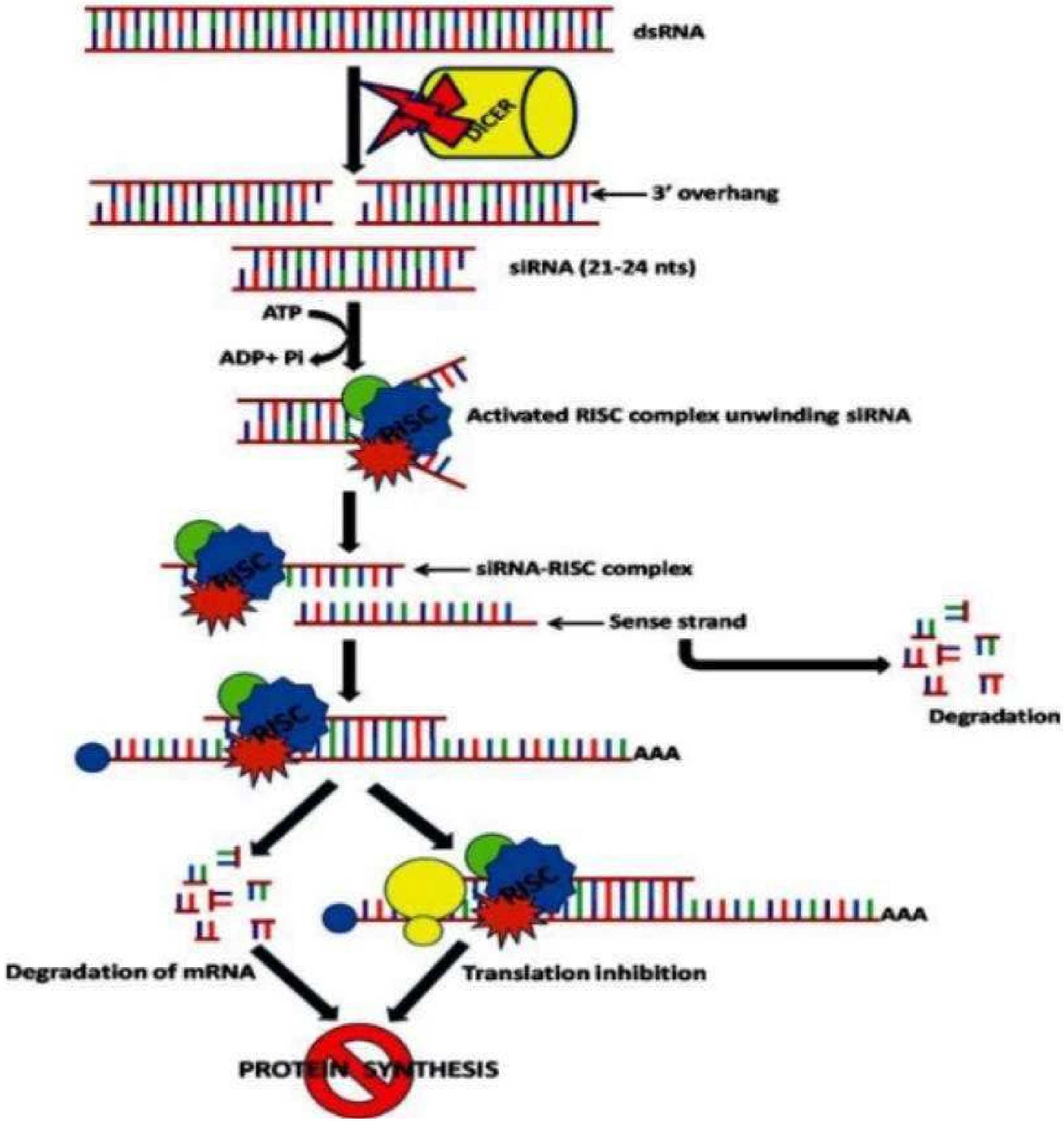
dsRNA cut by Dicer



Maintenance phase

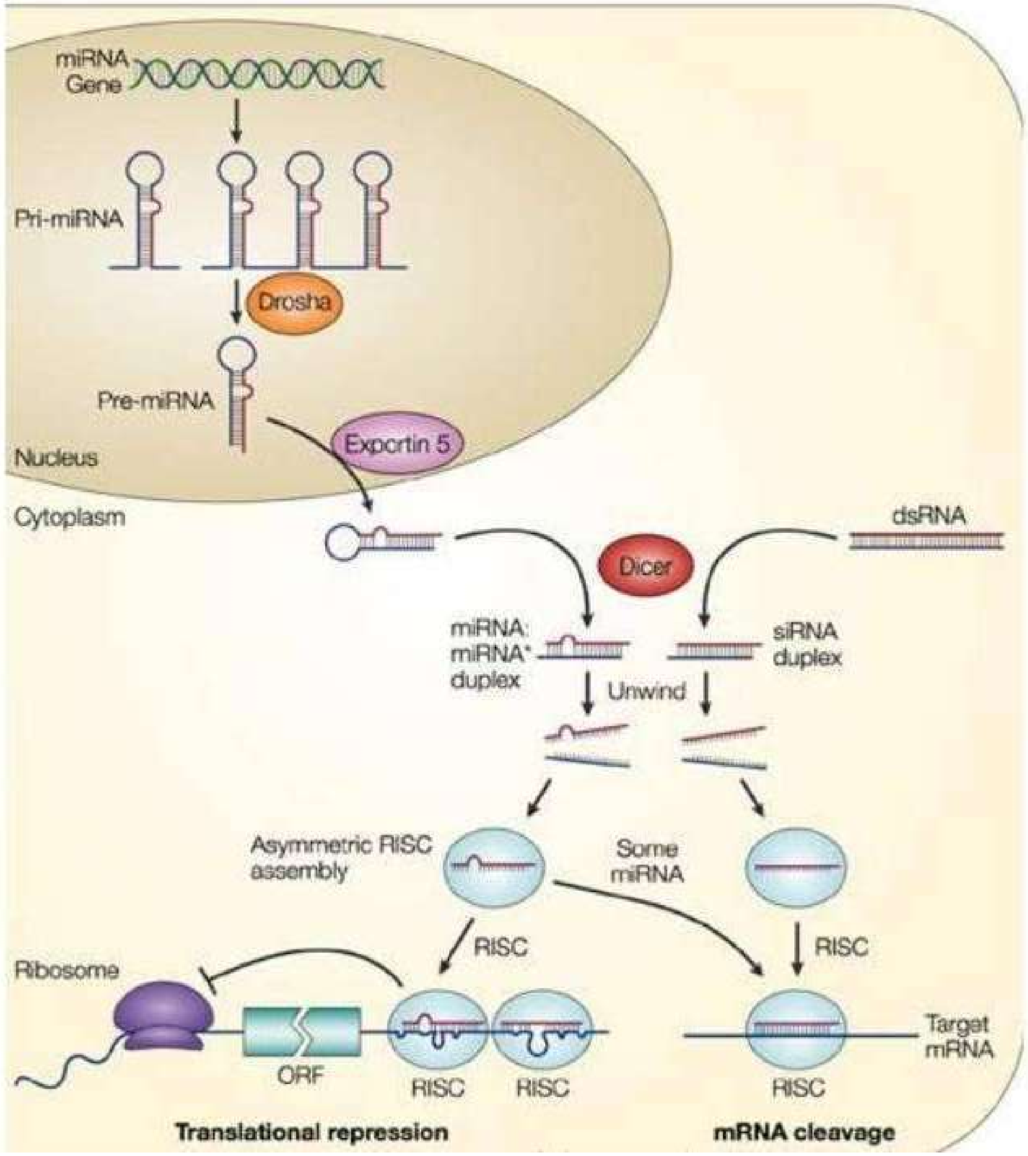
siRNA incorporated into RISC





Signal amplification and spreading phase

Jagtap *et. al.*, 2011



Initiation

Execution

He and Hannon, 2004

siRNA

- also as short interfering RNA or silencing RNA.
- It is a class of double - stranded RNA molecules.
- It is 20-25 bp in length.
- It is similar to miRNA.
- Operating withing the RNA interference RNAi pathway by the enzyme Dicer.
- It interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, resulting no translation.

Micro RNA or mi RNA

- Micro RNA is involved in regulation of gene expression.
- In a cell, miRNA is transcribed from DNA but not translated into proteins.
- Micro RNAs are non-coding molecules of approximately 21-23 nucleotides.
- Micro RNAs inhibit the expression of mRNA molecule.
- Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules.
- The main function of miRNAs is to down regulate gene expression.
- It has been reported that a typical mammalian cell contains as many as 50,000 different miRNAs.
- Micro RNAs were first described nor the worm C. elegans in 1993.
- The term micro RNA was only introduced in 2001.
- Only one strand of DNA can function as templates to give rise to miRNA

TABLE 20.1. Comparison of small interference RNA (siRNA) and microRNA (miRNA)

S.No.	*Particulars*	*siRNA*	*miRNA*
1.	Function	Gene regulation	Gene regulation
2.	Size in nucleotides	20-25	21-23
3.	Strands	Two complementary	Single
4.	Attachment to mRNA	Coding region	Non-Coding region
5.	Effect on translation	Translation is blocked	Translation is blocked

	Occurrence	Configuration	Length	Complementarity to target mRNA	Biogenesis	Action	Function
miRNA	In plants and animals	Single stranded	19–25 nt	Not exact - a single miRNA may target hundreds of mRNAs	Expressed by genes whose purpose is to make miRNAs, but regulate genes (mRNAs) other than the ones that expressed them	Inhibit translation of mRNA	Regulators (inhibitors) of genes
siRNA	In plants and lower animals In mammals??	Double stranded	21–22 nt	100% perfect match, and therefore siRNAs knock down specific genes, with rare exceptions	Regulate the same genes that express them	Cleave mRNA	Gene-silencing in plants and animals that do not have antibody-or cell-mediated immunity

Advantages of RNA interference

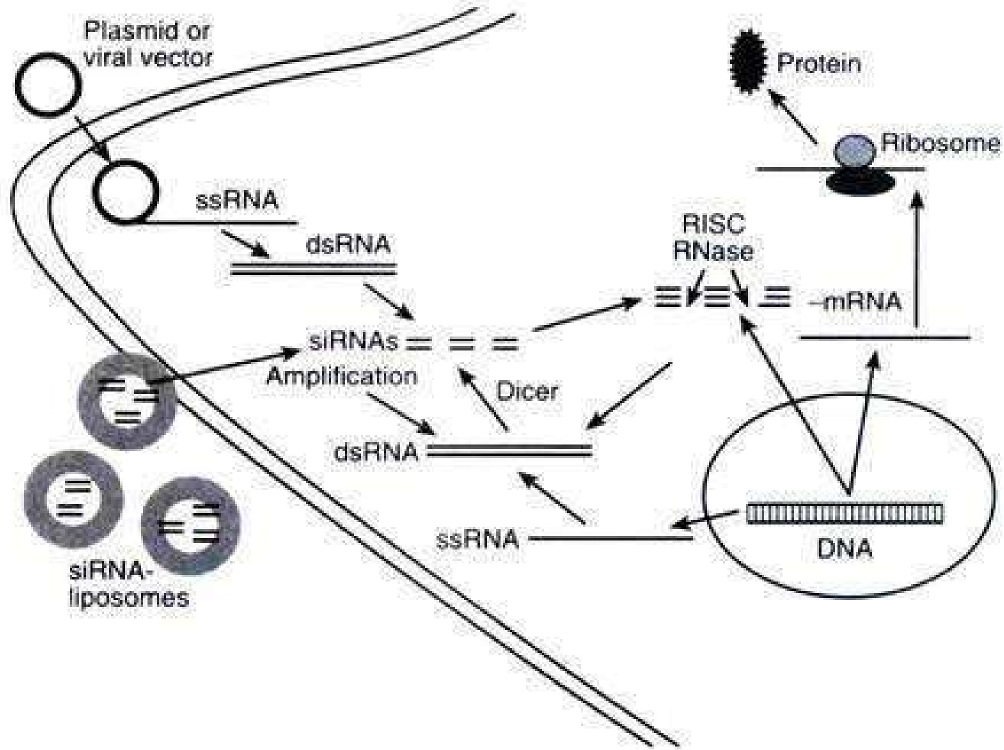


Fig. 17.1: Pathways of RNAi. Cells produce single-stranded RNA (ssRNA), which provides a template for the formation of dsRNA, which involves the activity of RNA-dependent RNA polymerases. The dsRNA is then cleaved by a protein called Dicer to form small 21-23 nucleotide siRNAs. The siRNAs (blue) then associate with the specific mRNA targeted by their nucleotide sequence (red) in a nucleic acid-protein complex called RISC, which includes RNase activity that degrades the mRNA at sites not bound by the siRNAs. The synthesis of the protein encoded by the mRNA targeted by the siRNAs is prevented, and that protein is selectively depleted from the cell. RNAi-mediated silencing can be induced experimentally by introducing synthetic siRNAs into cells using various transfection methods including liposomes (bottom left). Viral vectors can also be used to express dsRNAs against a specific gene, which are then acted upon by Dicer.