2021

BOTANY — HONOURS

Paper : CC-11

(Cell and Molecular

Biology) Full Marks : 50

The figures in the margin indicate full marks.

Candidates are required to give their answers in their own words as far as practicable.

1. Answer any five questions from the following : 2×5

Explore

(a) A **ribozyme** is an RNA molecule capable of acting as an enzyme[1]. An example of a ribozyme is the RNA component of the RNase P complex from Escherichia coli, which is responsible for the catalytic activity[2].

(b) The **Wobble Hypothesis**, proposed by Francis Crick in 1966, provides an explanation for the degeneracy of the genetic code. It suggests that the precise pairing between the bases of the codon and the anticodon of tRNA occurs only for the first two bases of the codon. However, the pairing between the third base of the codon and the anticodon can exhibit some flexibility or "wobble"[3].

© **RNA interference (RNAi)** is a regulatory system within eukaryotic cells that controls the activity of genes. RNAi functions specifically to silence, or deactivate, genes[4].

(d) A **codon** is a sequence of three nucleotides which specifies one amino acid in the polypeptide chain[5]. An **anticodon** is a sequence of three nucleotides in tRNA (transfer RNA) that pairs with a complementary codon in mRNA during protein synthesis, ensuring the correct amino acid is incorporated[6].

(e) **Okazaki fragments** are short sequences of DNA nucleotides which are synthesized discontinuously and later linked together by the enzyme DNA ligase to create the lagging strand during DNA replication[7]. They are formed because DNA polymerase can only synthesize DNA in the 5' to 3' direction, which complicates the replication of the lagging strand[8].

(f) The **quiescent stage** in the cell cycle, also known as the G0 phase, is a stage where cells remain metabolically active but do not proliferate unless called to do so[9].

(g) The enzyme required for Polymerase Chain Reaction (PCR) is **Taq polymerase**. It is thermostable and is isolated from a heat-tolerant bacterium, Thermus aquaticus[10].

(h) **RNA editing** is a molecular process through which some cells can make discrete changes to specific nucleotide sequences within an RNA molecule after it has been generated by RNA polymerase[11].

2. Answer any two questions from the following : 5×2

(a) **Apoptosis**, also known as programmed cell death, is a biological process that occurs in all multicellular organisms, including plants and animals[12]. It is a form of cell death that is generally triggered by normal, healthy processes in the body[12].

The term apoptosis is derived from the Greek word meaning "dropping or falling off" and was first introduced by Kerr, Wyllie, and Currie[12]. It is a highly regulated and controlled process that confers advantages during an organism's life cycle[2].

Apoptosis plays a major role in the development of humans and in developing and maintaining a healthy immune system[1]. On average, 50 – 80 billion cells die every day in a human adult due to apoptosis[1]. During this biological process, infected cells, pre-cancerous cells, and other cancer cells are eliminated successfully and maintain the balance of cells in the human body[1].

Apoptosis can be initiated through one of two pathways[2]:

- **Extrinsic Pathway**: This pathway triggers apoptosis in response to external stimuli, like ligand binding at death receptors on the cell surface[1].
- **Intrinsic Pathway**: This pathway triggers apoptosis in response to internal stimuli such as biochemical stress, DNA damage, and lack of growth factors[1].

Apoptosis is significant for several reasons[1]:

- It helps to maintain homeostasis in multicellular organisms.
- Proper size of the body is maintained by apoptosis.
- Apoptosis maintains the constancy of cell number in an organism.
- The unwanted cells are eliminated from the body by apoptosis.

In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in a wide variety of diseases. Excessive apoptosis causes atrophy, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer[2].

(b) A **licensing factor** is a protein or complex of proteins that allows an origin of replication to begin DNA replication at that site[1]. Licensing factors primarily occur in eukaryotic cells, since bacteria use simpler systems to initiate replication[1].

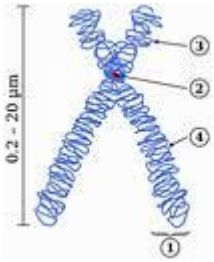
The origins of replication represent start sites for DNA replication and so their "firing" must be regulated to maintain the correct karyotype of the cell in question[1]. The origins are required to fire only once per cell cycle, an observation that led to the postulated existence of licensing factors by biologists in the first place[1]. If the origins were not carefully regulated then DNA replication could be restarted at that origin giving rise to multiple copies of a section of DNA[1]. This could be damaging to cells and could have detrimental effects on the organism as a whole[1].

The control that licensing factors exert over the cycle represents a flexible system, necessary so that different cell types in an organism can control the timing of DNA replication to their own cell cycles[1]. The factors themselves are found in different places in different organisms[1]. For example, in metazoan organisms, they are commonly synthesized in the cytoplasm of the cell to be imported into the nucleus when required[1]. The situation is different in yeast where the factors present are degraded and resynthesized throughout the cell cycle but are found in the nucleus for most of their existence[1].

In yeast, two of the proteins synthesized are called Cdc6 and Cdt1 and are only synthesized in G1 phase[1]. These two together bind to the origin recognition complex (ORC), which is already bound at the origin and in fact never leaves these sites throughout the cycle[1]. Now we have a so-called pre-replication complex, which then allows a heterohexameric protein complex of proteins MCM2 to 7 to bind[1]. This entire hexamer acts as a helicase unwinding the double-stranded DNA[1]. At this point, Cdc6 leaves the complex and is inactivated, by being degraded in yeast but by being exported from the nucleus in metazoans, triggered by CDK-dependent phosphorylation[1]. The next steps included the loading of a variety of other proteins like MCM10, a CDK, DDK, and Cdc45, the latter directly required for loading the DNA polymerase[1]. During this period, Cdt1 is released from the complex and the cell leaves G1 phase and enters S phase when replication starts[1]. From the above sequence, we can see that Cdc6 and Cdt1 fulfill the role of licensing factors[1].

© **Centromeres** are specialized DNA sequences in chromosomes that link or hold together the pair of sister chromatids[1]. They are involved in separating the

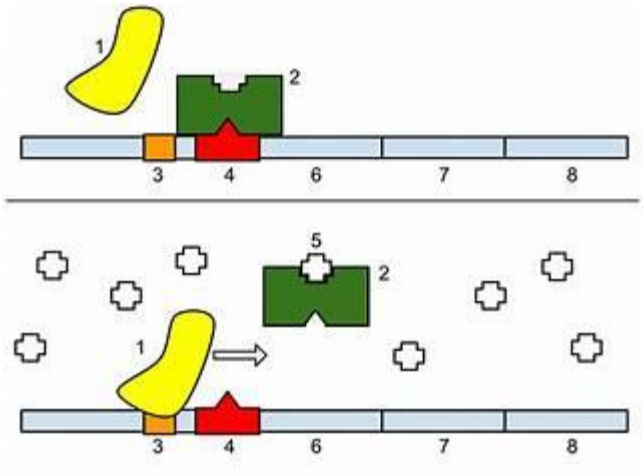
chromosome into a short arm (p) and a long arm (q)[1]. There are two main types of centromeres: point centromeres and regional centromeres[1].



1. **Point Centromeres**: These are centromeres where the mitotic spindle fibers are attracted to specific sequences of DNA[1]. In this case, the cell proteins bind to these particular DNA sequences and form the foundation for the binding of the mitotic spindle fibers[1]. The connection between protein and the DNA is present irrespective of its location and other factors[2].

2. **Regional Centromeres**: These are the most common form of centromeres and are larger than point centromeres, with their size ranging from several kilo to megabases[3]. Regional centromeres are determined during the mitotic spindle binding by a combination of characteristics working together to signal the location of a centromere and not by a precise sequence of DNA[1]. Humans and most eukaryotic cells have regional centromeres[1].

(d)



The positive control of the lac operon involves the catabolite activator protein (CAP), also known as the catabolite gene activator protein (CAP) or cyclic AMP receptor protein (CRP)[1].

When glucose levels are low, the concentration of cyclic AMP (cAMP) in the cell increases[1]. The cAMP binds to the CAP, and the cAMP-CAP complex then binds to the promoter region of the lac operon[1]. This binding enhances the binding of RNA polymerase to the promoter, thereby increasing the transcription of the lac operon[1].

The positive control of the lac operon allows the cell to respond to changes in the environment, specifically the availability of glucose and lactose[1]. When glucose levels are low (and cAMP levels are high), and lactose is available, the lac operon is transcribed and the enzymes necessary for lactose metabolism are produced[1].

Here is a simplified diagram of the process:

```
5' ---|---P---O---Z---Y---A---|--- 3'
      |<----  lac operon  ---->|
      CAP
```

- P: Promoter, where RNA polymerase binds.
- O: Operator, where the lac repressor binds.
- Z, Y, A: Genes of the lac operon (lacZ, lacY, lacA).
- CAP: Catabolite Activator Protein binding site[1].

3. Answer any three of the following :

(a) **Gene cloning**, also known as DNA cloning, is a technique used to make identical or similar copies of a DNA or gene[1]. It involves copying the DNA sequence of a gene into a smaller, more easily manipulated piece of DNA, such as a plasmid[1]. This allows for the creation of multiple copies of a gene for various downstream applications[1].

An ideal gene cloning vector should possess the following properties[23456]:

- It must be small in size.
- It must possess multiple cloning sites or a unique restriction site for restriction endonuclease enzymes.
- It must be self-replicating inside the host cell.
- It must possess some marker gene such that it can be used for later identification of recombinant cell (usually an antibiotic resistance gene that is absent in the host cell).
- They should be easily isolated from the host cell.

Restriction endonucleases are enzymes that recognize specific sequences of DNA and cleave the phosphodiester bonds within the DNA molecule[7]. They are

classified into different types based on their structure and their mode of action[78]. Here are examples of each type:

- **Type I**: These enzymes cut DNA at random sites that are distant from their recognition sequences. An example is EcoKI[7].
- **Type II**: These enzymes cut DNA at defined positions close to or within their recognition sequences. Examples include EcoRI and BamHI[8].
- **Type III**: These enzymes cleave DNA about 25 base pairs from the recognition sequence. An example is EcoP15I[7].
- **Type IV**: These enzymes target modified DNA, such as methylated, hydroxymethylated, or glucosyl-hydroxymethylated DNA[7].

A **cDNA library** is a collection of cDNA (complementary DNA) fragments that have been cloned into a collection of host cells[91011]. cDNA is produced from fully transcribed mRNA found in the nucleus and therefore contains only the expressed genes of an organism[91011]. The cDNA library represents some portion of the transcriptome of the organism and is stored as a "library"[91011]. The information found within cDNA libraries can be a powerful and useful tool since gene products are easily identified[91011].

(b) **Aminoacylation of tRNA**, also known as tRNA charging or amino acid activation, is the process of adding an aminoacyl group to a tRNA molecule[123]. The amino acid is covalently attached to the end of the acceptor arm of the tRNA, which always ends with the base sequence 5′ CCA 3′[123]. A bond forms between the carboxyl group of the amino acid and the 3′-hydroxyl of the terminal adenine of the acceptor arm[123]. This process is catalyzed by enzymes called aminoacyl tRNA s.

Prokaryotic translation, the process of synthesizing proteins from mRNA, occurs in three stages: initiation, elongation, and termination[23].

1. **Initiation**: The mRNA-ribosome complex is formed and the first codon (always AUG) binds the first aminoacyl-tRNA (called initiator tRNA)[23]. Three initiation factors (IFs; IF-1, IF-2, and IF-3) help to assemble the initiation complex[23].
2. **Elongation**: The other codons are read sequentially and the polypeptide grows by addition of amino acids to its C-terminal end[23]. This process continues until a termination codon (Stop codon), which does not have a corresponding aminoacyl-tRNA with which to base pair, is reached[23].
3. **Termination**: Protein synthesis ceases and the finished polypeptide is released from the ribosome[23].

The factors involved in each step are as follows[23]:

- **Initiation Factors (IFs)**: IF-1, IF-2, and IF-3 are involved in the initiation of translation[23].
- **Elongation Factors (EFs)**: EF-TU, EF-TS, and EF-G are involved in the elongation of the polypeptide chain[23].
- **Release Factors (RFs)**: RF-1, RF-2, and RF-3 are involved in the termination of protein synthesis[2]

two key differences between prokaryotic and eukaryotic translation:

1. **Timing and Location**: In prokaryotes, translation occurs in the cytoplasm and is coupled with transcription, meaning both processes occur simultaneously[123]. In contrast, in eukaryotes, transcription occurs in the nucleus, and the mRNA is then exported to the cytoplasm where translation occurs[123].
2. **Ribosomes**: Prokaryotic ribosomes are 70S (composed of 50S and 30S subunits), while eukaryotic ribosomes are 80S (composed of 60S and 40S subunits)[123]. The difference in size and composition of the ribosomes affects the process of translation in both types of cells[123].

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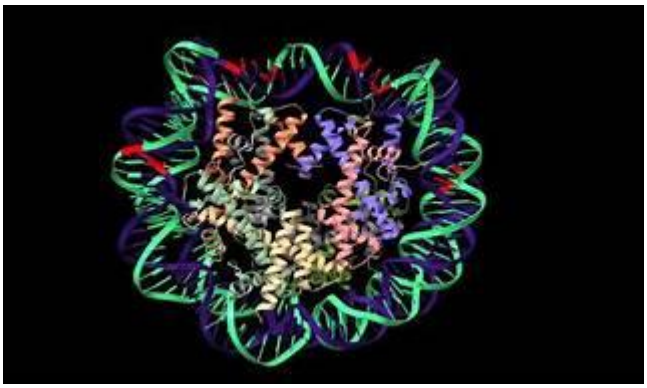
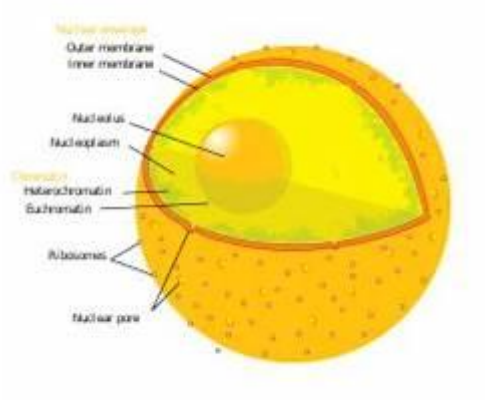


The packaging of DNA is a fascinating process that allows nearly 2 meters of DNA to fit into a microscopic nucleus. This is achieved through a structure known as the nucleosome[12].
Nucleosomes are the basic repeating subunits of chromatin, which is the material that makes up chromosomes[13]. Each nucleosome is composed of DNA wound 1.65 times around a group of eight histone proteins[1]. Histones are small, positively charged proteins that bind tightly to the negatively charged DNA due to the phosphate groups in its phosphate-sugar backbone[12].
The histone proteins involved in the formation of a nucleosome are H2A, H2B, H3, and H4[2]. Two molecules each of these four histones form an octamer, or an eight-part structure[2]. The DNA helix wraps around this octamer to form the nucleosome[12].
The nucleosomes are further coiled to form a 30-nanometer chromatin fiber[1]. These fibers are then compressed and folded to produce a 250 nm-wide fiber, which is tightly coiled into the chromatid of a chromosome[1].
This hierarchical packaging of DNA into nucleosomes and then into more compact structures allows the long DNA molecules to fit into the tiny space

of the nucleus[12]. It's a remarkable feat of biological engineering that enables the storage and retrieval of genetic information in a highly efficient manner. Please note that this is a simplified explanation of a very complex process that involves many other proteins and mechanisms[12].

(ii)



The Nuclear Pore Complex (NPC) is a large protein complex found in the nuclear envelope in eukaryotic cells[1]. It serves as a channel that facilitates the selective transport of various molecules across the nuclear envelope[1].

Here's a detailed description of the ultrastructure of the NPC:

1. **Composition**: The NPC is composed of multiple copies of ~30 different proteins called nucleoporins[2]. Each NPC comprises at least 456 individual protein molecules, and 34 distinct nucleoporin proteins[1].
2. **Structure**: The NPC is octagonally symmetrical around its cylindrical axis[2]. Each NPC comprises eight protein subunits encircling the actual pore, forming the outer ring[1]. Additionally, these subunits project a spoke-shaped protein over the pore channel[1].
3. **Size**: The plant NPC (1150×640 Å) is larger than its yeast counterpart (960×350 Å), but smaller than that in vertebrates (1450×800 Å)[2].
4. **Distribution**: NPCs are non-randomly distributed over the nuclear envelope[2]. The density of the NPCs is largely unchanged during cell growth, but NPCs become predominantly organized into rows in stationary phase cells[2].
5. **Function**: The principal function of nuclear pore complexes is to facilitate selective membrane transportation of various molecules across the nuclear envelope[1]. This includes the transportation of RNA and ribosomal proteins from the nucleus to the cytoplasm, in addition to proteins (such as DNA

polymerase and lamins), carbohydrates, signaling molecules, and lipids moving into the nucleus[1].

Please note that this is a simplified explanation of a very complex structure that involves many other proteins and mechanisms[21].

Fidelity of DNA replication refers to the accuracy with which the DNA sequence is copied during the process of replication[12]. This is crucial for cell reproduction and the production of accurate daughter DNA using the parental DNA as a template[2].

Here are some key points about the fidelity of DNA replication:

1. **DNA Polymerases**: The fidelity of DNA replication is maintained by the action of DNA polymerases[3]. These enzymes are responsible for adding nucleotides to the new DNA strand[3].
2. **Error Rate**: Given that the genome of E.coli comprises about 4 x 10^6 base pairs, the admissible rate of error for a totally correct replication is one erroneous base for every 10^6, or even 10^7, base pairs[1].
3. **Correction Mechanism**: The correction mechanism is based on the fact that the DNA polymerases need both a primer and a template[1]. If a base in its enol form has been incorporated, its return to the keto form results in a non-pairing[1]. Therefore, the DNA polymerase can no longer catalyze the formation of a phosphodiester bond with the following nucleotide[1]. Its 3′ → 5′ exonuclease activity eliminates the ill-paired nucleotide, thus liberating a 3'OH utilizable by the DNA polymerase[1].
4. **Proofreading Activity**: The other major mechanism responsible for the fidelity of DNA replication is the proofreading activity of DNA polymerases[2]. The 3′ → 5′ exonucleases of these polymerases precisely cut the mismatched bases that have been incorporated at the end of a growing DNA chain, thereby increasing the accuracy of replication[2].

Post-transcriptional modifications are crucial steps in the gene expression process in eukaryotic cells[12]. These modifications transform the primary RNA transcript into a mature, functional mRNA molecule that can be translated into a protein[12]. Here are the key post-transcriptional modifications observed in eukaryotic mRNA:

1. **5' Capping**: During the early stages of transcription, a 7-methyl guanosine cap is added to the 5' end of the mRNA[1]. This cap protects the mRNA from degradation, aids in the export of the mRNA from the nucleus, and is involved in the initiation of translation[1].

2. **3' Polyadenylation**: At the 3' end of the mRNA, a poly-A tail consisting of approximately 200-300 adenine residues is added[1]. This tail also protects the mRNA from degradation, aids in the export of the mRNA from the nucleus, and helps in the initiation of translation[1].

3. **Splicing**: Splicing is the process of removing introns (non-coding sequences) from the pre-mRNA and joining the exons (coding sequences) to form a mature mRNA[1]. This process is carried out by the spliceosome, a large complex of small nuclear ribonucleoproteins (snRNPs)[1].

4. **RNA Editing**: In some cases, the mRNA sequence is altered post-transcriptionally in a process known as RNA editing[2]. This can involve the insertion, deletion, or substitution of nucleotides[2].

5. **Transport of RNA to Cytoplasm**: The mature mRNA is then transported out of the nucleus and into the cytoplasm for translation[3].

6. **Stabilization/Destabilization of mRNA**: The stability of mRNA in the cytoplasm can be influenced by various factors, including the length of the poly-A tail and the presence of certain sequences in the mRNA[3].

These modifications not only protect the mRNA molecule, but also enhance the efficiency of protein synthesis[123].

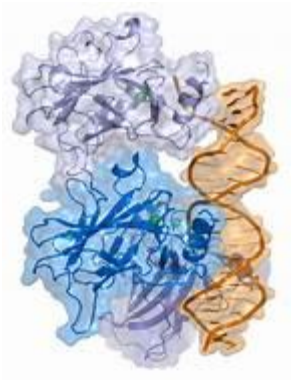
e. (i) Tumor suppressor genes are a type of gene that regulates cell division and prevents the formation of tumors[12]. They produce proteins that inhibit cell growth, repair DNA damage, and induce programmed cell death (apoptosis)[12]. When these genes mutate, they can lose their ability to control cell growth, leading to the development of cancer[12].

Here are some examples of tumor suppressor genes:

- **BRCA1 and BRCA2**: These genes are involved in repairing damaged DNA and, in their normal state, can prevent the development of certain cancers, such as breast and ovarian cancer[3].
- **p53 or TP53**: This gene produces a protein that regulates the cell cycle and functions as a tumor suppressor[45].
- **Rb**: This gene is involved in controlling cell division[5].
- **APC**: This gene controls the availability of a transcription factor[5].
- **PTEN**: This gene is involved in cell cycle regulation and apoptosis[6].
- **JAK2, NPM1, IL2, TCF3**: These are also examples of tumor suppressor genes[6].

Please note that mutations in these genes can increase the risk of developing certain types of cancer[12].

(ii)



Explore

The p53 gene, also known as TP53, is often referred to as the "guardian of the genome" because of its critical role in preserving genomic integrity[1]. When functioning normally, the p53 protein inhibits the phenotypic and genomic alterations associated with cancer development[1].

However, if the p53 gene is mutated, it can have significant effects on a cell[21345]:

1. **Loss of Tumor Suppression**: The p53 protein's main function is to repair DNA to prevent altered DNA from being passed on to daughter cells[1]. If the p53 protein is mutated, the cell cycle becomes unrestricted, and the damaged DNA is replicated, resulting in uncontrolled cell proliferation and potentially leading to the formation of cancer tumors[1].
2. **Gain of Oncogenic Functions**: Mutations in p53 not only lead to the loss of wild-type p53 functions but can also result in a dominant negative effect[4]. These mutations can lead to new functions that help sustain the growth of a cancer[2]. Some of these "gain-of-functions" can include inducing resistance to cancer drugs[2].
3. **Increased Proliferation and Tumorigenicity**: Mutations in p53 have been found in up to 50% of all human cancers and cause an increase in oncogenic phenotypes such as proliferation and tumorigenicity[3].
4. **Impact on Various Cancers**: The TP53 gene is mutated in approximately half of all human malignancies, including those of the breast, colon, lung, liver, prostate, bladder, and skin[1].

In summary, a mutation in the p53 gene can lead to a loss of its tumor-suppressing function, potentially resulting in uncontrolled cell growth and the development of cancer[21345].

(iii) Benign and malignant tumors are two different types of growths that can occur in the body. Here's how they differ:

Benign Tumors[12]:

- Benign tumors are not cancerous[12].

- They do not invade surrounding tissues or spread to other parts of the body[12].
- They are usually harmless unless they are pressing on nearby tissues, nerves, or blood vessels, or taking up space in the brain[12].
- Common benign tumors include fibroids, lipomas, adenomas, and hemangiomas[2].
- They usually respond well to treatment and can be surgically removed[2].

Malignant Tumors[12]:

- Malignant tumors are cancerous[12].
- They can grow uncontrollably and invade nearby tissues[12].
- The cancer cells in a malignant tumor can spread (metastasize) to other parts of the body[12].
- Common malignant tumors include breast cancer, lung cancer, colorectal cancer, prostate cancer, and stomach cancer[1].
- They require more aggressive treatment, often involving a combination of surgery, radiation therapy, and chemotherapy[12].

In summary, the main difference between benign and malignant tumors is that benign tumors are noncancerous and do not spread to other parts of the body, while malignant tumors are cancerous and can invade nearby tissues and spread to other parts of the body[12].

(iv) **Metastasis of Cancer**[1234]: Metastasis refers to the process where cancer cells break off from the primary tumor, enter the bloodstream or lymphatic system, and spread to other parts of the body[1]. This spread can occur via the circulation or lymphatic system and impact distant organs or tissues[1]. Metastatic cancer is often more difficult to cure than localized cancer[1]. The treatment often consists of a mix of medicines, such as chemotherapy, radiation, targeted therapies, and, in some cases, surgery to treat symptoms, delay the disease's progression, and improve the patient's quality of life[1].

v-oncogene[56789]: A v-oncogene is a viral gene that has the potential to cause cancer[5]. It is found in certain viruses and can trigger uncontrolled growth in abnormal host cells[7]. The term "v-" prefix indicates that the oncogene is of viral origin[9]. For example, v-Src is a gene found in Rous sarcoma virus (RSV) that encodes a tyrosine kinase that causes a type of cancer in chickens[7]. It was the first retroviral oncogene to be discovered[7]. Oncogenes can be activated in cells in different ways, such as through gene mutations, epigenetic changes, or chromosome rearrangements[6].