MOLECULAR BIOLOGY AND GENETIC ENGENEERING… (U5MB21MT)

DNA & RNA

DEPARTMENT OF MICROBIOLOGY, ADHIPARASAKTHI COLLEGE OF ARTS AND SCIENCE-KALAVAI(Ranipet District- TN)

DNA

- DNA is the GENETIC MATERIAL present in all living organism.
- DNA stands for De-oxy Ribo Nucleic Acid.
- It is in twisted ladder like structure.
- James Watson & Francis Crick proposed the double helical structure of DNA.
- It runs in an anti parallel structure.
- It has PENTOSE SUGAR, PHOSPHORIC ACID and NITROGENOUS BASE.

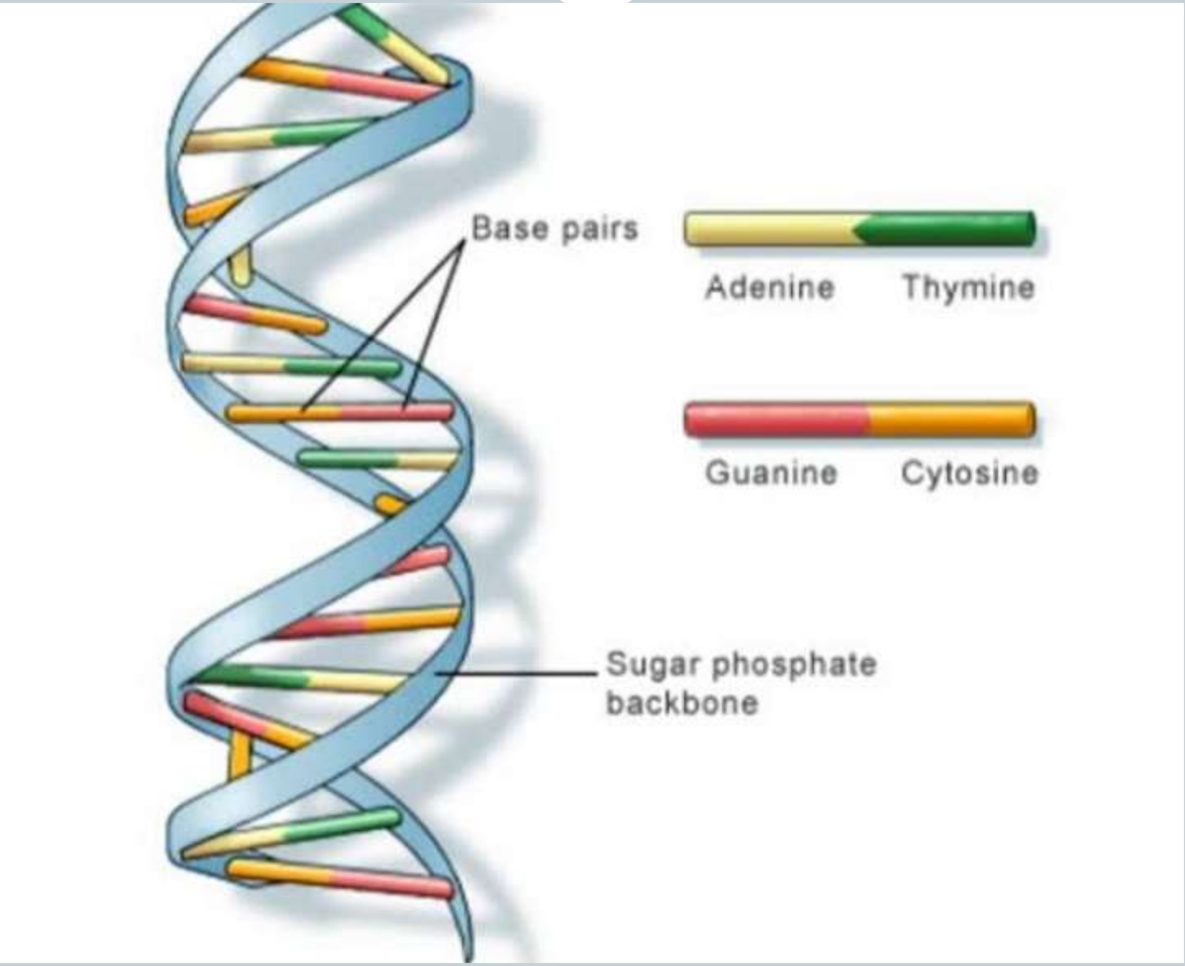
DNA

- It has 2 types of nitrogen bases,
  Purine,
  Prymidine.
- Purine has ADENINE and GUANINE,
- Prymidine has CYTOSINE and THYMINE.
- These Nitrogenous base pairs are linked with HYDROGEN BONDS.

DNA

- The ADENINE and THYMINE are linked by DOUBLE HYDROGEN BONDS.
- The CYTOSINE and GUANINE are linked by TRIPLE HYDROGEN BONDS.
- These base pairs are attached by CHARGOFF'S LAW RULE or CHARGOFF'S LAW OF EQUIVALENCE.
- TYPES:
  - **A- DNA,**
  - **B- DNA,**
  - **Z- DNA.**

DNA STRUCTURE



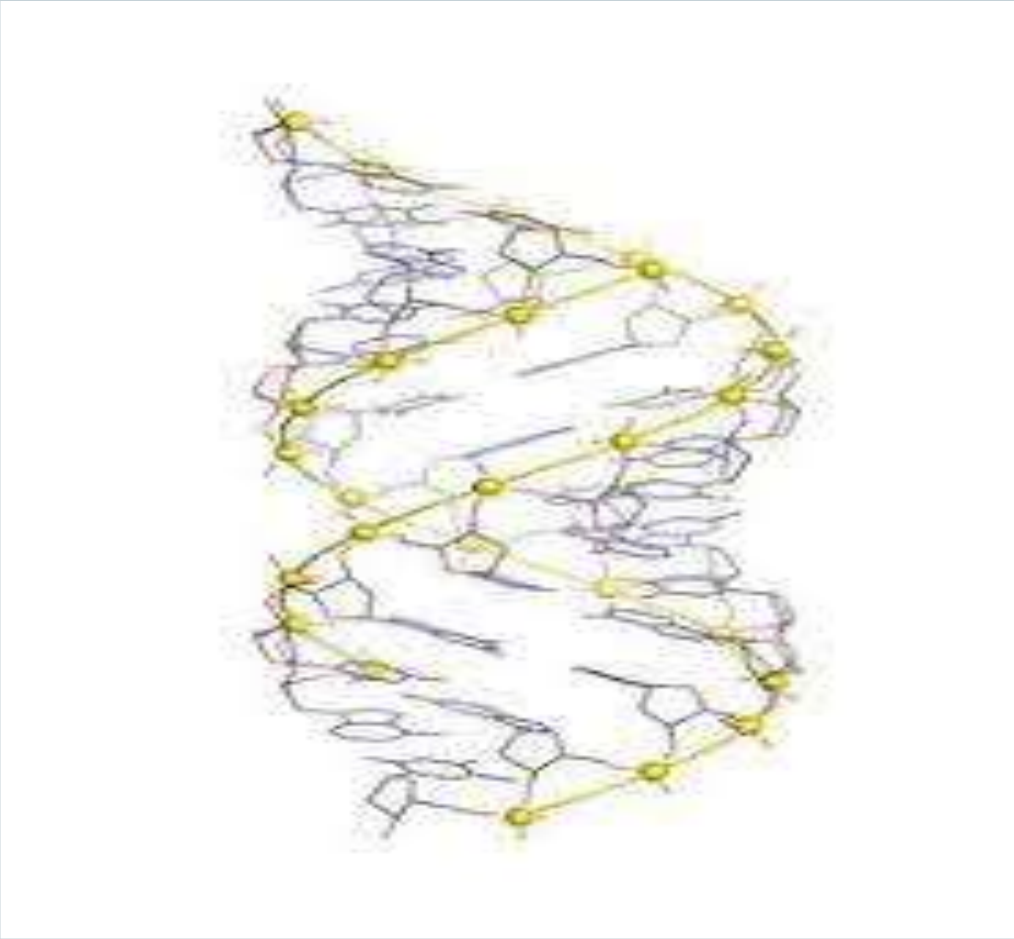
TYPES OF DNA

- **A- DNA:**
- **A-DNA** is one of the possible double helical structures which DNA can adopt.
- It is a right-handed double helix fairly similar to the more common B-DNA form, but with a shorter, more compact helical structure whose base pairs are not perpendicular to the helix-axis as in B-DNA.
- It was discovered by Rosalind Franklin, who also named the A and B forms.

TYPES OF DNA

- She showed that DNA is driven into the A form when under dehydrating conditions. Such conditions are commonly used to form crystals, and many DNA crystal structures are in the A form.
- Dehydration of DNA drives it into the A form, and this apparently protects DNA under conditions such as the extreme desiccation of bacteria.

TYPES OF DNA



TYPES OF DNA

- **B- DNA:**
- B-form DNA is **a right-handed double helix**, which was discovered by Watson and Crick based on the X-ray diffraction patterns. It is the common form of DNA exists under normal physiological condition. The double strands of B-DNA run in opposite directions.

TYPES OF DNA

- B-DNA is the Watson–Crick form of the double helix that most people are familiar with.
- They proposed two strands of DNA — each in a right-hand helix — wound around the same axis. The two strands are held together by H-bonding between the bases (in anti-conformation).
- The two strands of the duplex are antiparallel and plectonemically coiled. The nucleotides arrayed in a 5′ to 3′ orientation on one strand align with complementary nucleotides in the 3′ to 5′ orientation of the opposite strand.

TYPES OF DNA

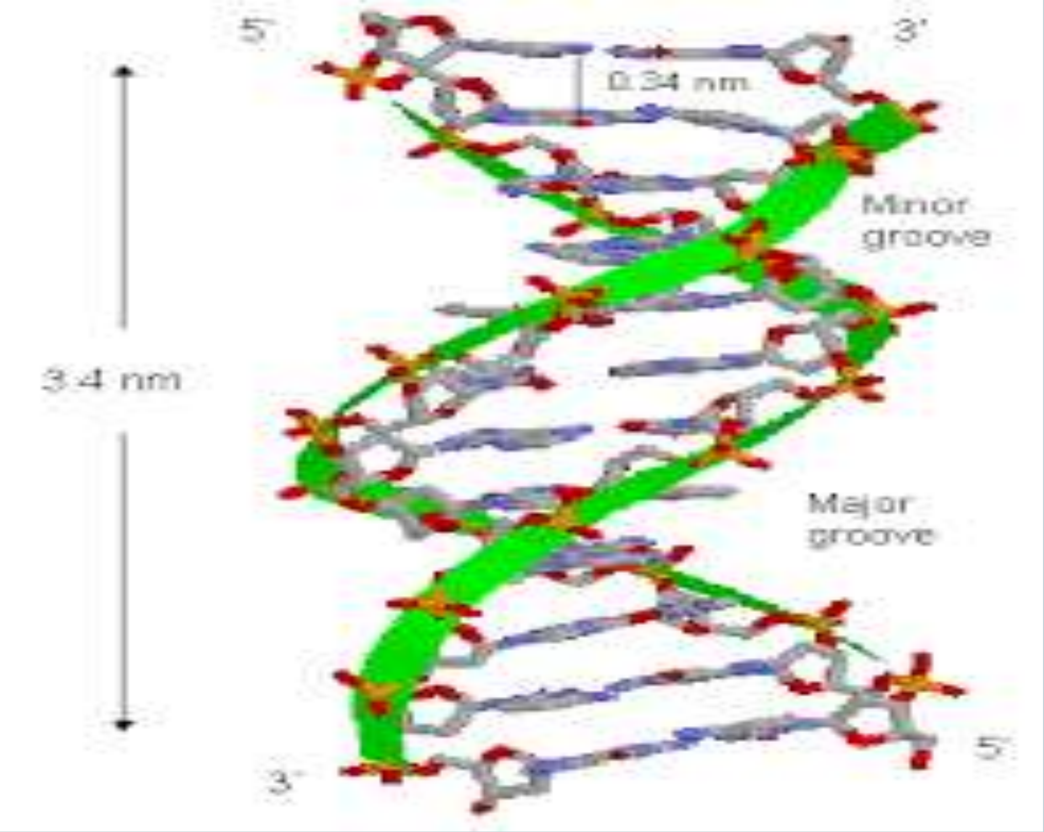
- Bases fit in the double helical model if pyrimidine on one strand is always paired with purine on the other. From Chargaff's rules, the two strands will pair A with T and G with C. This pairs a keto base with an amino base, a purine with a pyrimidine. Two H-bonds can form between A and T, and three can form between G and C.
- These are the complementary base pairs. The base-pairing scheme immediately suggests a way to replicate and copy the genetic information.

TYPES OF DNA

- 34 nm between bp, 3.4 nm per turn, about 10 bp per turn
- 9 nm (about 2.0 nm or 20 Angstroms) in diameter.
- 34^o helix pitch; -6^o base-pair tilt; 36^o twist angle

TYPES OF DNA

- **STRUCTURE OF B DNA:**



TYPES OF DNA

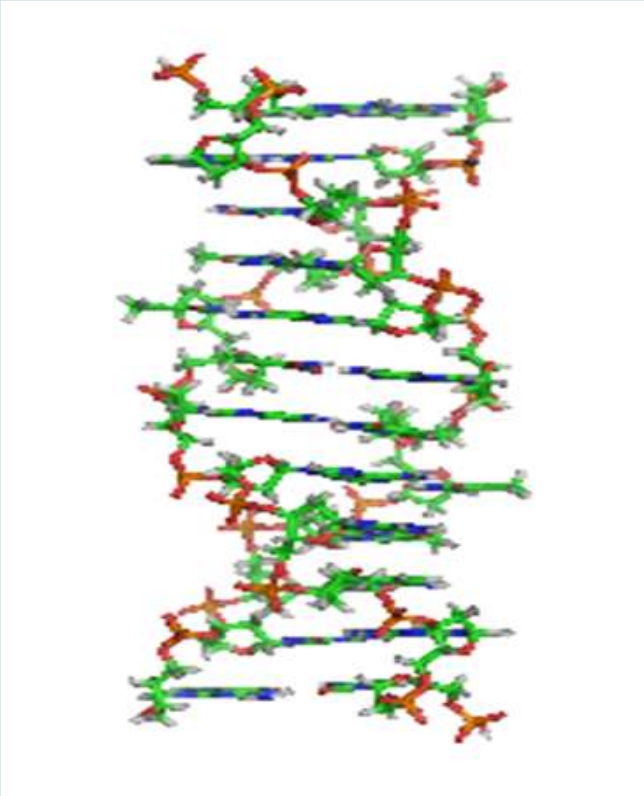
- **Z- DNA:**
- **Z-DNA** is one of the many possible double helical structures of DNA. It is a left-handed double helical structure in which the helix winds to the left in a zigzag pattern, instead of to the right, like the more common B-DNA form. Z-DNA is thought to be one of three biologically active double-helical structures along with A-DNA and B-DNA.

TYPES OF DNA

- It was resolved as a left-handed double helix with two antiparallel chains that were held together by Watson–Crick base pairs (see X-ray crystallography)
- It was solved by Andrew H. J. Wang, Alexander Rich, and coworkers in 1979 at MIT.
- The crystallisation of a B- to Z-DNA junction in 2005 provided a better understanding of the potential role Z-DNA plays in cells.

TYPES OF DNA

- Z- DNA:



TYPES OF DNA

	B form DNA	A form DNA	Z form DNA
Helical sense	Right handed	Right handed	Left handed
Major groove	Present	Present	Absent
bp/helical turn	10.5	11	12
Glycosyl bond conformation	Anti	Anti	Anti (for pyrimidines) and syn (for Purines)
Helix rise/bp	3.4Å	2.6Å	3.7Å
Base tilt	6°	20°	7°
Structure			

RNA

- **Ribonucleic acid** (**RNA**) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and deoxyribonucleic acid (DNA) are nucleic acids. Along with lipids, proteins, and carbohydrates, nucleic acids constitute one of the four major macromolecules essential for all known forms of life.

TYPES OF RNA

- Cellular organisms use messenger RNA (***mRNA***) to convey genetic information (using the nitrogenous bases of guanine, uracil, adenine, and cytosine, denoted by the letters G, U, A, and C) that directs synthesis of specific proteins. Many viruses encode their genetic information using an RNA genome.

TYPES OF RNA

- Synthesis of RNA is usually catalyzed by an enzyme—RNA polymerase—using DNA as a template, a process known as transcription. Initiation of transcription begins with the binding of the enzyme to a promoter sequence in the DNA (usually found "upstream" of a gene). The DNA double helix is unwound by the helicase activity of the enzyme. The enzyme then progresses along the template strand in the 3’ to 5’ direction, synthesizing a complementary RNA molecule with elongation occurring in the 5’ to 3’ direction. The DNA sequence also dictates where termination of RNA synthesis will occur.
-

TYPES OF RNA

- Primary transcript RNAs are often modified by enzymes after transcription. For example, a poly(A) tail and a 5' cap are added to eukaryotic pre-mRNA and introns are removed by the spliceosome.
- There are also a number of RNA-dependent RNA polymerases that use RNA as their template for synthesis of a new strand of RNA. For instance, a number of RNA viruses (such as poliovirus) use this type of enzyme to replicate their genetic material.
- Also, RNA-dependent RNA polymerase is part of the RNA interference pathway in many organisms.

TYPES OF RNA

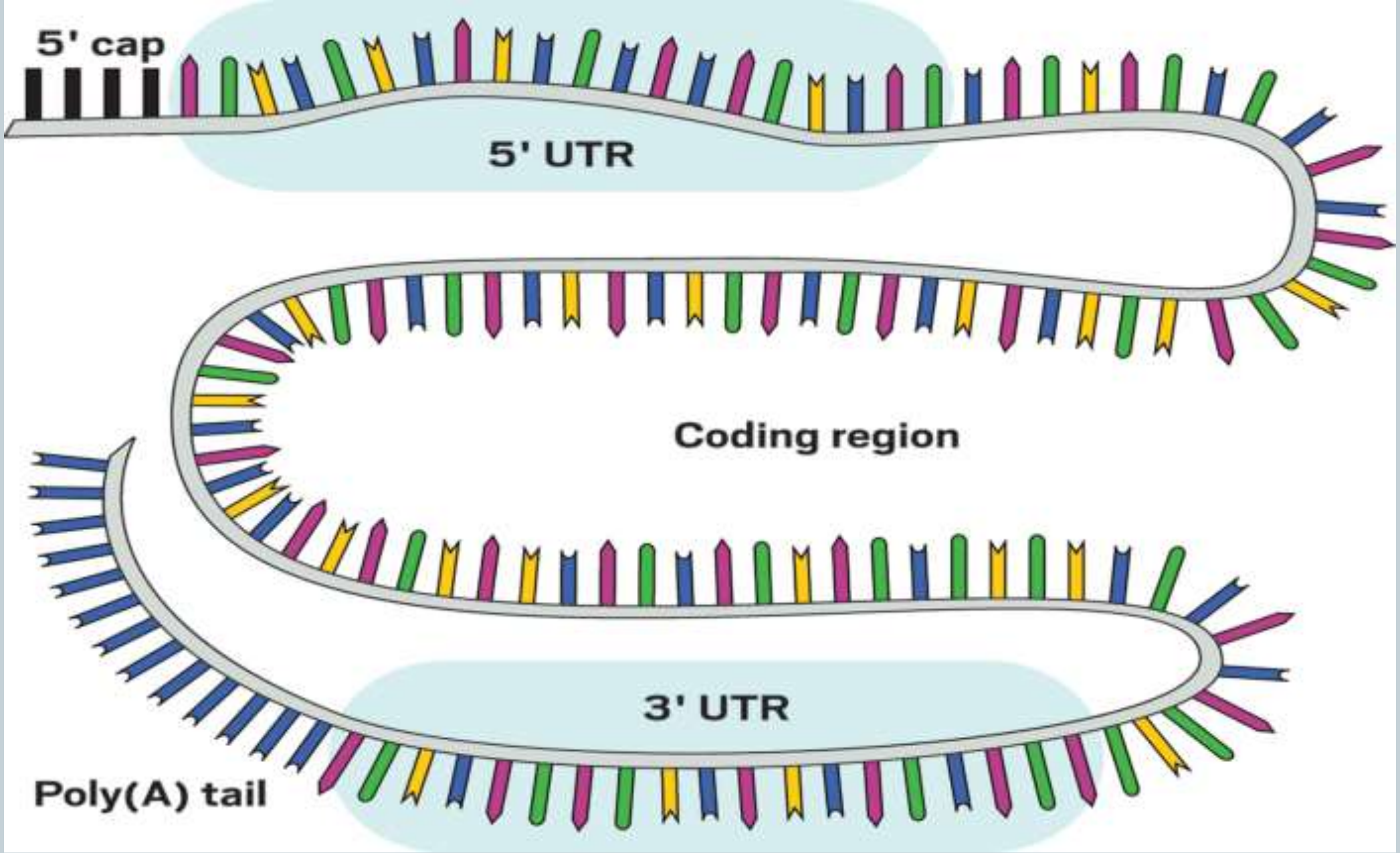
- TYPES:
  - M- RNA (MESSENGER RNA),
  - R- RNA (RIBOSOMAL RNA),
  - T- RNA (TRANSFER OR SOLUBLE RNA).

TYPES OF RNA

- **M-RNA:**
- It is along, single strandard.
- It consist of 500- 1500 nucleotides.
- It has the molecular weight about 5,00,000 daltons.
- It occupies about 5% of total RNA.
- If it codes for a single protein it is called as MONOCISTRONIC.
- If it codes for a more than one protein it is called as POLYCISTRONIC.

TYPES OF RNA

- **M-RNA STRUCTURE:**



TYPES OF RNA

- **M- RNA:**
- Messenger RNA (mRNA) is a single-stranded RNA molecule that is complementary to one of the DNA strands of a gene. The mRNA is an RNA version of the gene that leaves the cell nucleus and moves to the cytoplasm where proteins are made. During protein synthesis, an organelle called a ribosome moves along the mRNA, reads its base sequence, and uses the genetic code to translate each three-base triplet, or codon, into its corresponding amino acid.

TYPES OF RNA

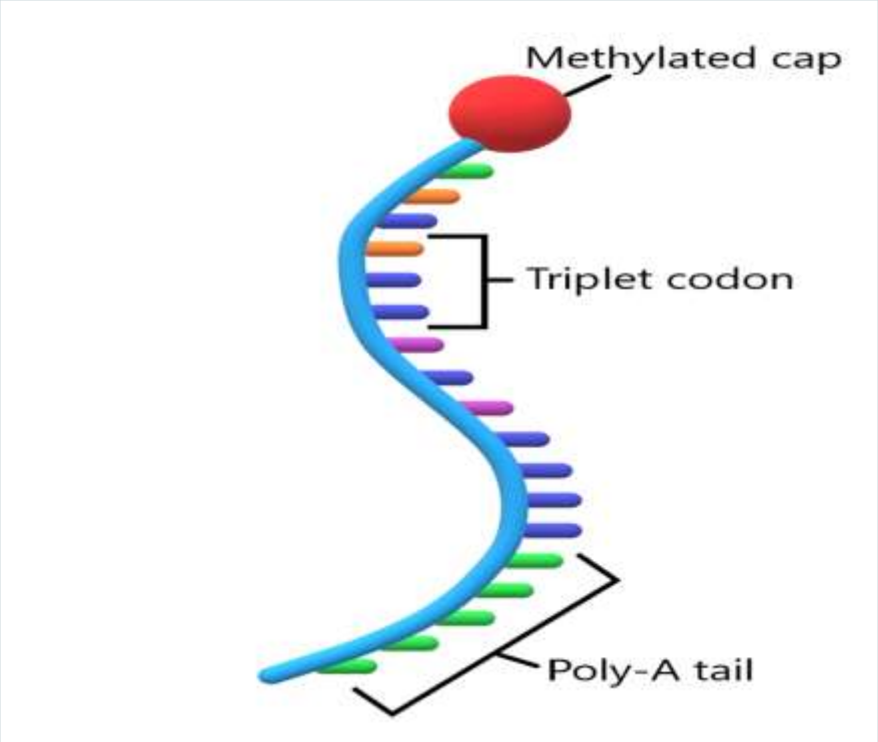
- Messenger RNAs, also known as mRNA, are one of the types of RNA that are found in the cell. This particular one, like most RNAs, are made in the nucleus and then exported to the cytoplasm where the translation machinery, the machinery that actually makes proteins, binds to these mRNA molecules and reads the code on the mRNA to make a specific protein. So in general, one gene, the DNA for one gene, can be transcribed into an mRNA molecule that will end up making one specific protein.

TYPES OF RNA

- It is other wise called as RIBOSOMAL RNA.
- It occupies about 80% of total RNA.
- It is found in ribonucleo protein.
- It helps in ribosome synthesis.

TYPES OF RNA

- **R-RNA:**



TYPES OF RNA

- **T- RNA:**
- It is otherwise called as TRANSFER RNA.
- It is soluble in NaCl.
- It has about 80 nucleotide.
- It is in 25,000 – 30,000 daltons weight.
- It was discovered by HOLLEY.
- It occupies about 15% of total RNA.
- It in clover leaf like structure.

TYPES OF RNA

- Transfer ribonucleic acid (tRNA) is a type of RNA molecule that **helps decode a messenger RNA (mRNA) sequence into a protein**. tRNAs function at specific sites in the ribosome during translation, which is a process that synthesizes a protein from an mRNA molecule.
- Function of tRNA. The job of tRNA is **to read the message of nucleic acids, or nucleotides, and translate it into proteins, or amino acids**. The process of making a protein from an mRNA template is called translation.

TYPES OF RNA

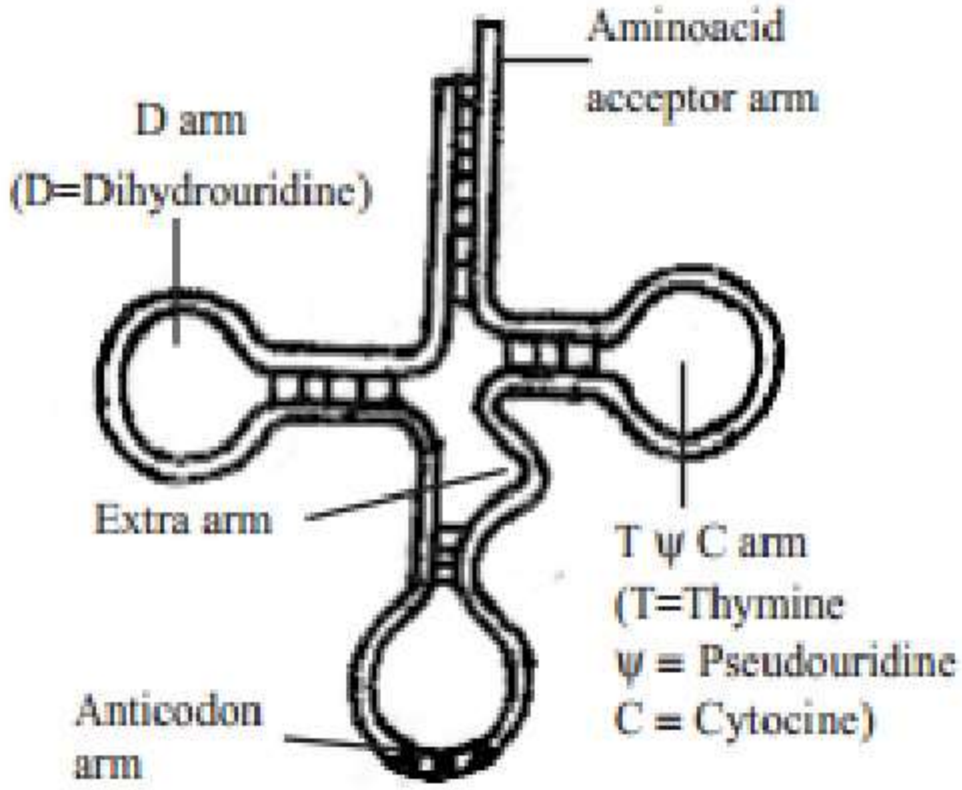
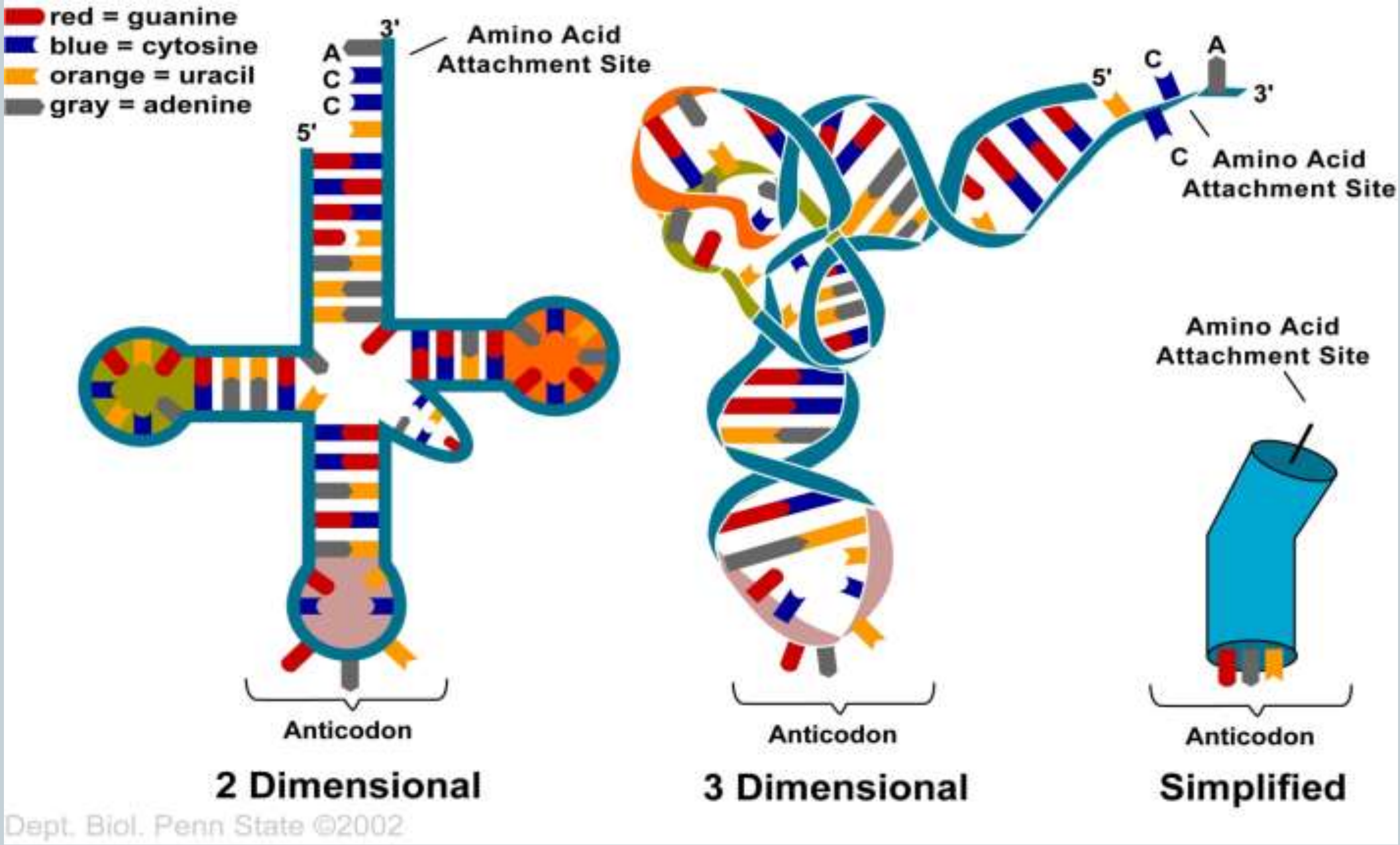


Fig. 3.10 Structure of tRNA

TYPES OF RNA

- **T-RNA:**



TYPES OF RNA

- **Arms in T- RNA:**
- **D- Arm:**
- The D loop contains the base dihydrouridine, for which the arm is named. The D loop's main function is that of **recognition**. It is widely believed that it acts as a recognition site for aminoacyl-tRNA synthetase, an enzyme involved in the aminoacylation of the tRNA molecule.
- **Amino acid acceptor Arm:**
- The acceptor stem codes **for amino acid sizes**; the bases of the anticodon code for amino acid polarities. Amino acid properties therefore dictate how tRNA bases are recognized by aminoacyl-tRNA synthetases. 3. tRNA codes are related to protein folding.

TYPES OF RNA

- **Thymine Pseudo Uridine Arm:**
- The T-arm or T-loop is a specialized region on the tRNA molecule which acts as a **special recognition site for the ribosome to** form a tRNA-ribosome complex during protein biosynthesis or translation.
- **Anticodon Arm:**
- An anticodon is found at one end of a transfer RNA (tRNA) molecule. During protein synthesis, each time an amino acid is added to the growing protein, a tRNA forms base pairs with its complementary sequence on the mRNA molecule, **ensuring that the appropriate amino acid is inserted into the protein**.

TYPES OF RNA

- **EXTRA ARM:**
- The long extra arms of human function as **major identify elements for serylation in an orientation-dependent, but not sequence-specific manner**.


Thank You!

MOLECULAR BIOLOGY AND GENETIC ENGENEERING…(U5MB21MT)

MONOCLONAL ANTIBODIES PRODUCTION…

DEPARTMENT OF MICROBIOLOGY,

ADHIPARASAKTHI COLLEGE OF ARTS AND SCIENCE-KALAVAI(Ranipet District- TN)

MONOCLONAL ANTIBODIES…

- An antibody is a protein used by the immune system to identify and neutralize foreign objects like bacteria and viruses.
- Each antibody recognizes a specific antigen unique to its target.
- **Monoclonal antibodies (mAb) are antibodies that are identical because they are produced by one type of immune cell, all clones of a single parent cell.**
- Polyclonal antibodies are antibodies that are derived from different cell lines. They differ in amino acid sequence.

MONOCLONAL ANTIBODIES…

- **HISTORY:**
- 1964 Littlefield developed a way to isolate hybrid cells from 2 parent cell lines using the hypoxanthine-aminopterin-thymidine (HAT) selection media.
- 1975 Kohler and Milstein provided the most outstanding proof of the clonal selection theory by fusion of normal and malignant cells.
- 1990 Milstein produced the first monoclonal antibodies.

MONOCLONAL ANTIBODIES…

- **HISTORY:**
- Paul Ehrlich at the beginning of the 20th century theorized that a cell under threat grew additional side-chains to bind the toxin, and that these additional side chains broke off to become the antibodies that are circulated through the body.
- It was these antibodies that Ehrlich first described as "magic bullets" in search of toxins.

MONOCLONAL ANTIBODIES…

- **Characters of Monoclonal Antibodies:**
- Monoclonal antibodies (mAb) are a single type of antibody that are identical and are directed against a specific epitope (antigen, antigenic determinant) and are produced by B-cell clones of a single parent or a single hybridoma cell line.
- A hybridoma cell line is formed by the fusion of a one B-cell lymphocyte with a myeloma cell.
- Some myeloma cells synthesize single mAb antibodies naturally

MONOCLONAL ANTIBODIES…

Monoclonal Antibody Production Method:

Monoclonal Antibody Production technology was developed in 1975.

Since its development it has been very important in the modern medical science with the diagnosis, therapy, research and even basic science today.

It is still largely dependent upon animal testing however. Because it requires immunization of mice in order for them to create the antibodies to be grown.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- Monoclonal Antibody Production or mAb is produced by cell lines or clones obtained from the immunized animals with the substance to be studied.
- Cell lines are produced by fusing B cells from the immunized animal with myeloma cells.
- To produce the desired mAb, the cells must be grown in either of two ways: by injection into the peritoneal cavity of a suitably prepared mouse (the in vivo, or mouse ascites, method) or by in vitro tissue culture.
- The vitro tissue culture is the method used when the cells are places in culture outside the mouse's body in a flask.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- A mouse should be immunized against a target cell "X".
- This will allow the mouse to produce antibodies for that will target against the "X" antigen.
- Once the mouse has formed antibodies to the "X" antigen the cells are then isolated in the mouse's spleen.
- Monoclonal antibodies are produced by fusing single antibody-forming cells to tumor cells grown in culture.
- The resulting cell is called a hybridoma.
- Hybridoma cells are continuously growing cell line generated by the fusion of a myeloma cell and a normal cell that are capable of producing antibodies.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- Each hybridoma will produce relatively large quantities of identical antibody molecules.
- It is Because the hybridoma is multiplying in culture, it is possible to produce a population of cells, each is producing identical antibody molecules.
- These antibodies are called "monoclonal antibodies" because they are produced by the identical offspring of a single, cloned antibody producing cell.

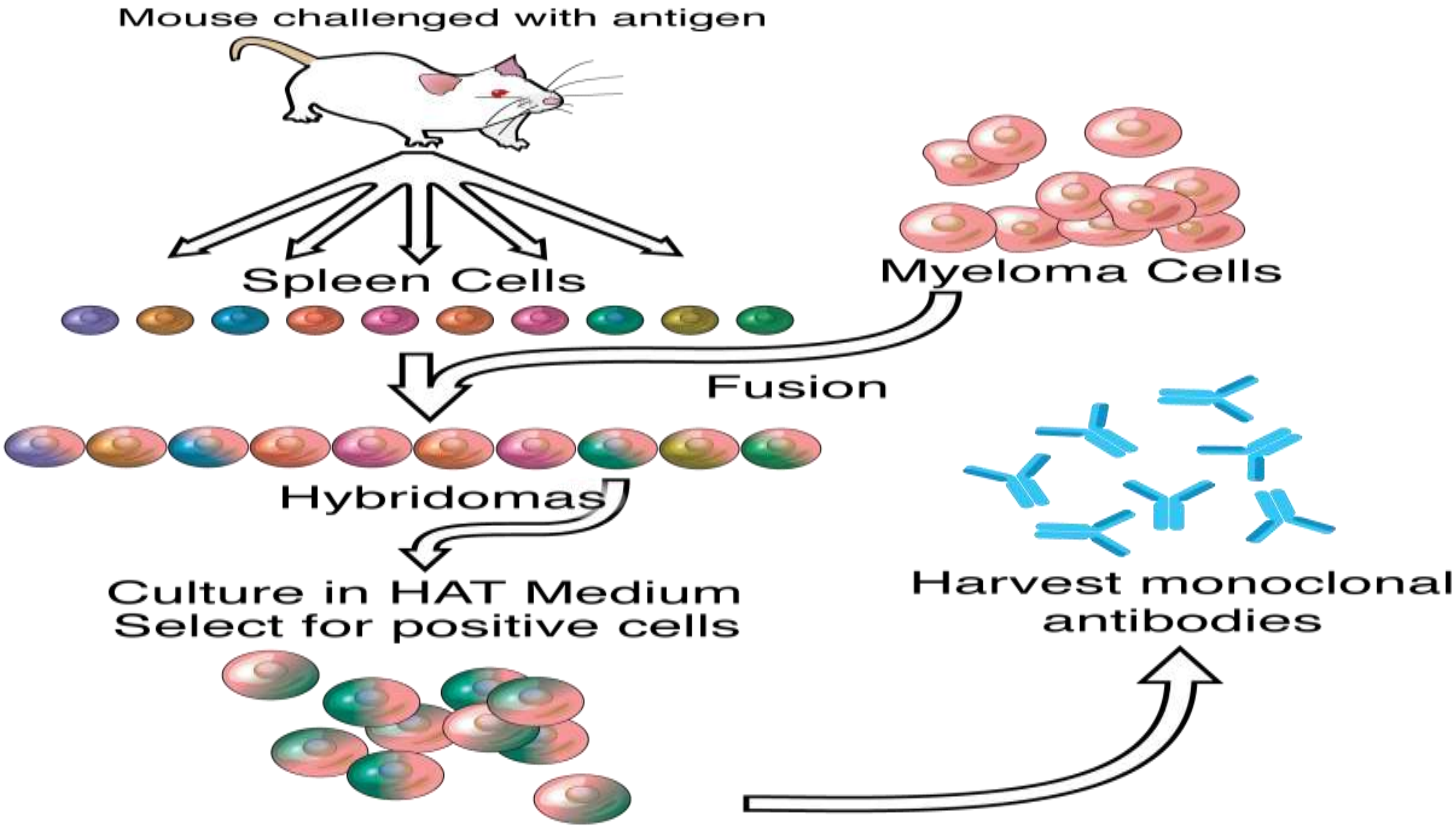
MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- **Practical steps in monoclonal antibody production:**
- 1) Immunize animal.
- 2) Isolate spleen cells (containing antibody-producing B cells)
- 3) Fuse spleen cells with myeloma cells (e.g. using PEG - polyethylene glycol)
- 4) Allow unfused B cells to die.
- 5) Add aminopterin to culture to kill unfused myeloma cells.

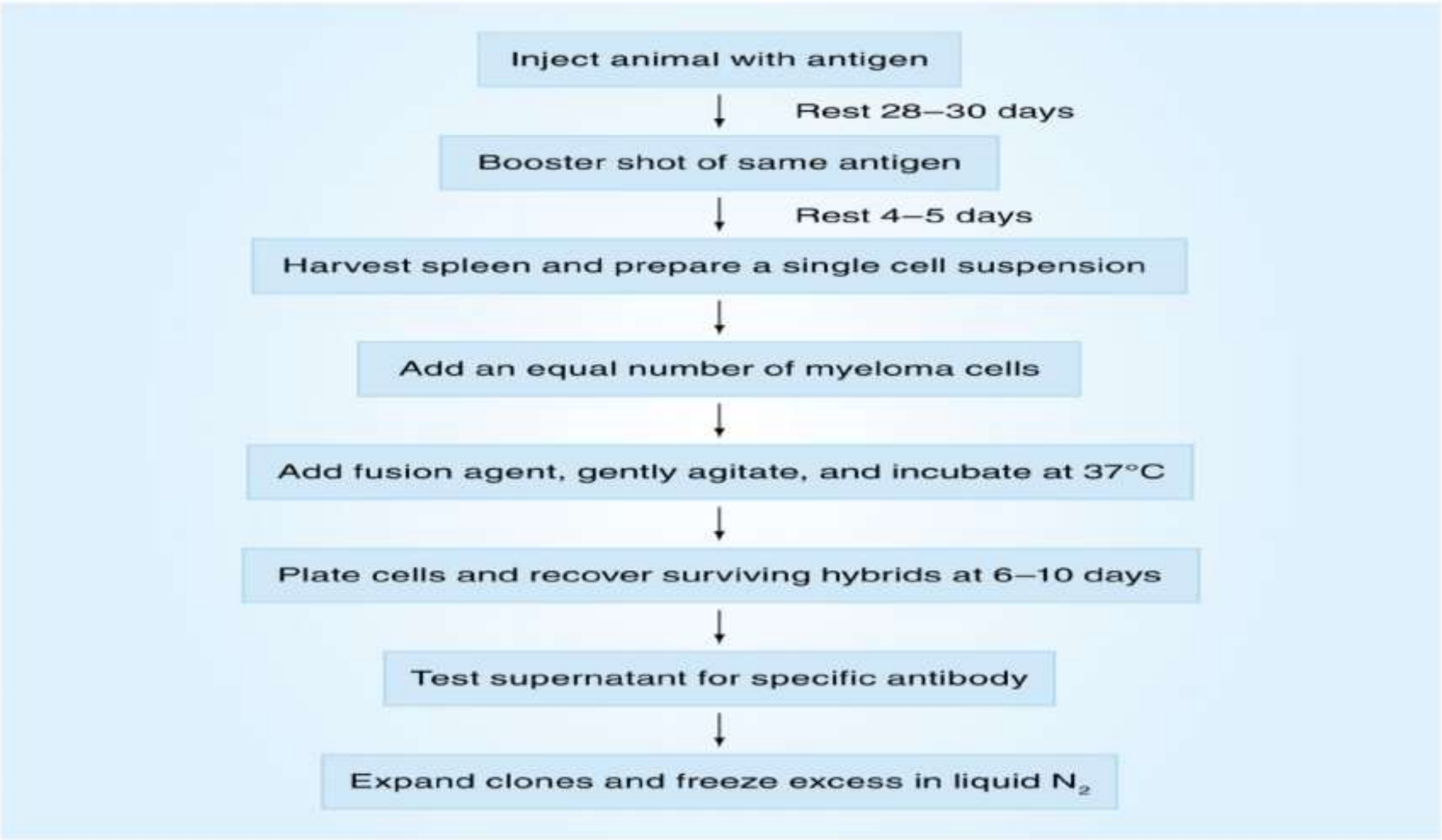
MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- 6) Clone remaining cells (place 1 cell/well and allow each cell to grow into a clone of cells)
- 7) Screen supernatant of each clone for presence of the desired antibody.
- 8) Grow the chosen clone of cells in tissue culture indefinitely.
- 9) Harvest antibody from the culture supernatant.

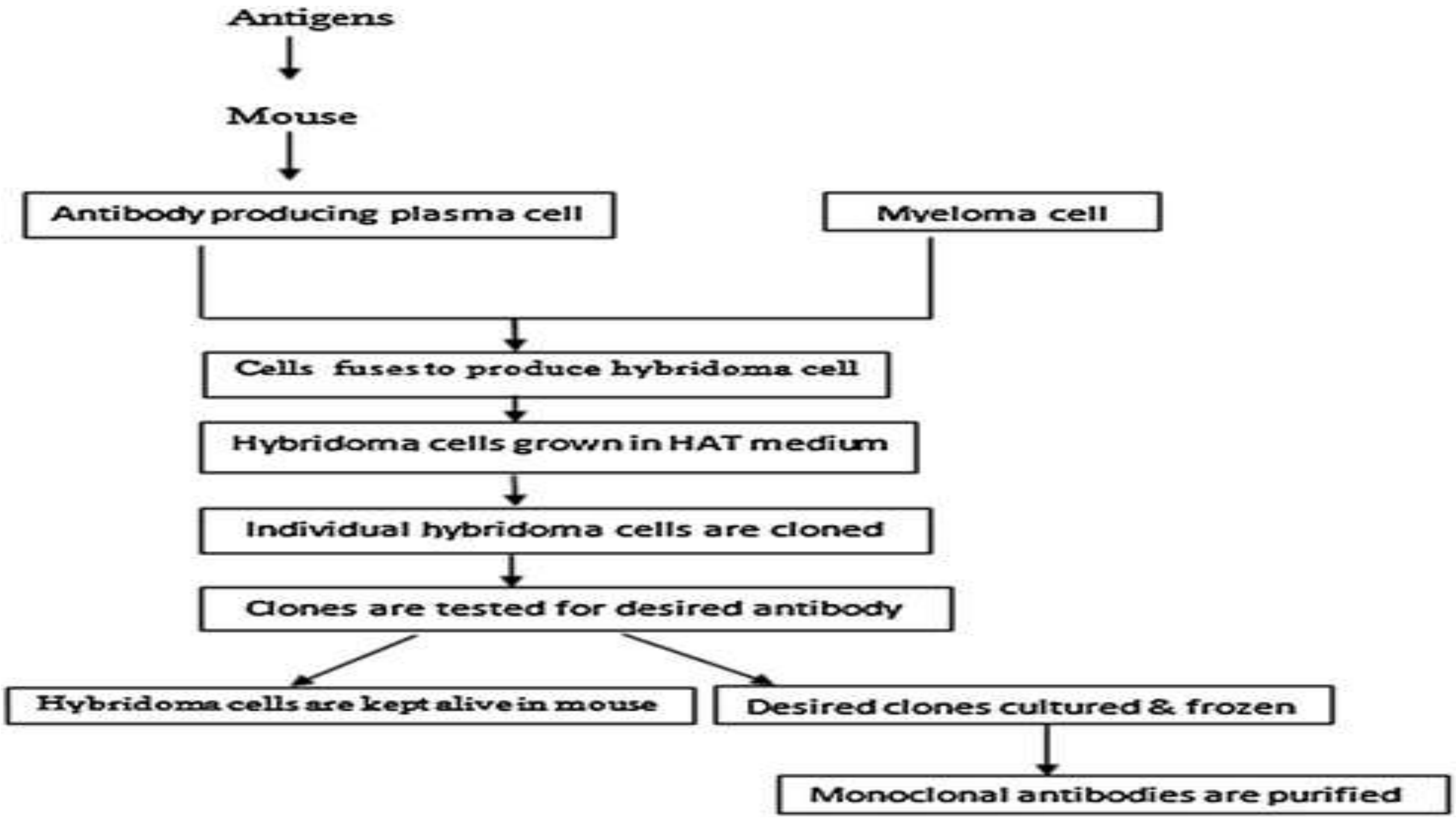
MONOCLONAL ANTIBODIES…



MONOCLONAL ANTIBODIES…

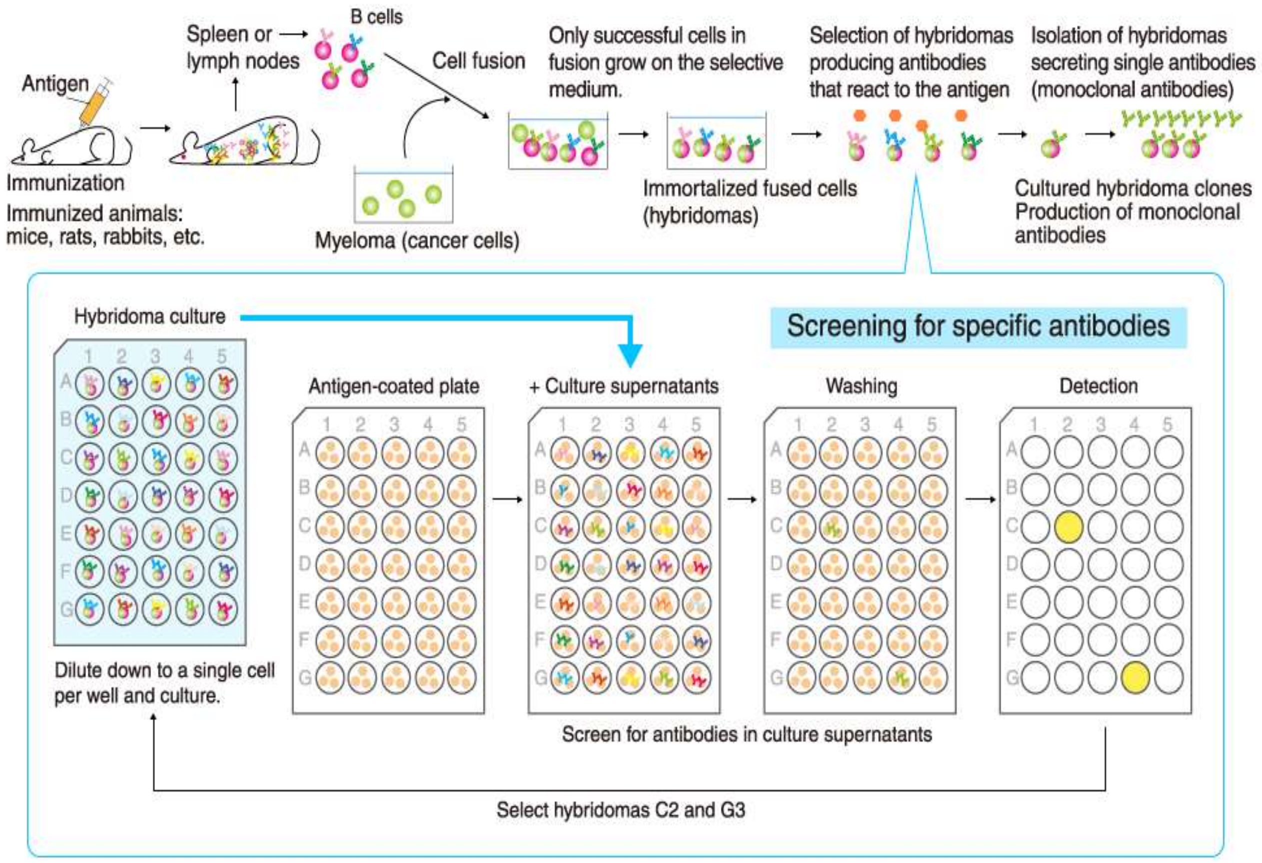


MONOCLONAL ANTIBODIES…



MONOCLONAL ANTIBODIES…

- **SELECTION OF ANTIBODIES:**



MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- Types of Monoclonal Antibodies Hybridoma creates Monoclonal antibodies Monoclonal antibodies are typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen.
- However, recent advances have allowed the use of rabbit B-cells.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- This method is used because antibodies must be formed from the immunization of the substance being studied. So antibodies must be produced.
- Once the antibodies are produced the animal aspect of the study can be eliminated and tissue culture can then be used.
- When using live mice researchers have found that it is the better option because in vitro doesn't always produce adequate cell lines that are adaptive to tissue culture

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- Protein denaturation can occur from purification techniques and antibody activity is decreased with normal activity not represented.
- Also cell lines could possibly become contaminated when using in vitro technique.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- **Monoclonal – Diagnostic use:**
- A monoclonal antibody can be used to detect pregnancy only 14 days after conception.
- Other monoclonal antibodies allow rapid diagnosis of hepatitis, influenza, herpes, streptococcal, and Chlamydia infections.
- They can be used to detect for the presence and quantity of this substance, for instance in a Western blot test (to detect a substance in a solution) or an immunofluorescence test.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- Monoclonal antibodies can also be used to purify a substance with techniques called immunoprecipitation and affinity chromatography.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- **Limitations with Mouse Monoclonals:**
- Problem in medical applications is that the standard procedure of producing monoclonal antibodies yields mouse antibodies, and these are rejected by the human immune system

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- **Conjugated monoclonal antibody therapy:**
- Toxins or radioactive isotopes are bound to the constant region of the MAbs.
- When the MAb binds to the surface cells of a tumor the toxin or radioactivity will kill the cancer cells and all cells within a certain radius (a killing zone).
- In this way cancer cells within the tumor will be killed.

MONOCLONAL ANTIBODIES…

- **Monoclonal Antibody Production Method:**
- **Monoclonal antibodies for cancer treatment:**
- Possible treatment for cancer involves monoclonal antibodies that bind only to cancer cells specific antigen and induce immunological response on the target cancer cell (naked antibodies).
- The mAb can be modificated for delivery of [toxin], radioisotope, cytokine.

MONOCLONAL ANTIBODIES…

- **Possible side effects of monoclonal antibodies:**
- Monoclonal antibodies are given intravenously (injected into a vein).
- Compared with the side effects of chemotherapy, the side effects of naked mAbs are like an allergic reaction.
- These are more common while the drug is first being given.
- Some mAbs can also have other side effects that are related to the antigens they target.
- For example, bevacizumab (Avastin®), an mAb that targets tumor blood vessel growth, can cause side effects such as high blood pressure, bleeding, poor wound healing, blood clots, and kidney damage.

MONOCLONAL ANTIBODIES…

- Fever,
- Chills,
- Weakness,
- Headache,
- Nausea,
- Vomiting,
- Diarrhea,
- Low blood pressure,
- Rashes.

MONOCLONAL ANTIBODIES…

- In 1975, Kohler and Milstein first fused lymphocytes to produce a cell line which was both immortal and a producer of specific antibodies.
- The two scientists were awarded the Nobel Prize for Medicine in 1984 for the development of this "hybridoma."
- The value of hybridomas to the field was not truly appreciated until about 1987, when MAbs were regularly produced in rodents for diagnostics.

MONOCLONAL ANTIBODIES…

- Hybridoma Selection The “HAT Trick”
- Myeloma cells have been genetically engineered such that they can not use hypoxanthine, aminopterin, and thymidine (HAT medium) as a source for nucleic acid biosynthesis and will die in culture.
- Only B cells that have fused with the engineered myeloma cells will survive in culture when grown in HAT medium.

MONOCLONAL ANTIBODIES…

- **SOME OF THE COMMONLY USED MONOCLONAL ANTIBODIES NAME:**
- abciximab (Reopro)
- adalimumab (Humira, Amjevita)
- alefacept (Amevive)
- alemtuzumab (Campath)
- basiliximab (Simulect)
- belimumab (Benlysta)
- bezlotoxumab (Zinplava)
- ipilimumab (Yervoy)

MONOCLONAL ANTIBODIES…

- canakinumab (Ilaris)
- certolizumab pegol (Cimzia)
- cetuximab (Erbitux)
- daclizumab (Zenapax, Zinbryta)
- denosumab (Prolia, Xgeva)
- efalizumab (Raptiva)
- golimumab (Simponi, Simponi Aria)
- inflectra (Remicade)
- ixekizumab (Taltz)
- secukinumab (Cosentyx)
- ustekinumab (Stelara)

MONOCLONAL ANTIBODIES…

- natalizumab (Tysabri)
- nivolumab (Opdivo)
- olaratumab (Lartruvo)
- omalizumab (Xolair)
- palivizumab (Synagis)
- panitumumab (Vectibix)
- pembrolizumab (Keytruda)
- rituximab (Rituxan)
- tocilizumab (Actemra)
- trastuzumab (Herceptin)


THANK YOU