Answers **Paper Code: - AS-2237**

Department of Biotechnology
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End Semester Examination (2013-2014)
LBTM: 101 Molecular cell biology M.Sc. I Semester

Answer 1:- Multiple Choice Answers

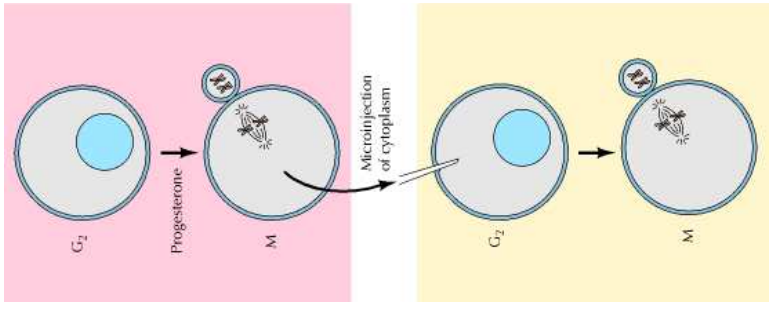
1. C
2. B
3. C
4. D
5. A
6. D
7. D
8. C
9. B
10. D

Answer 2:- <u>Discovery of Maturation promoting factor</u>

Maturation-promoting factor (MPF) is a heterodimeric protein composed of cyclin B and cyclin-dependent kinase (CDK1) that stimulates the mitotic and meiotic phases of the cell cycle. MPF promotes the entrance into mitosis (the M phase) from the G2 phase by phosphorylating multiple proteins needed during mitosis.

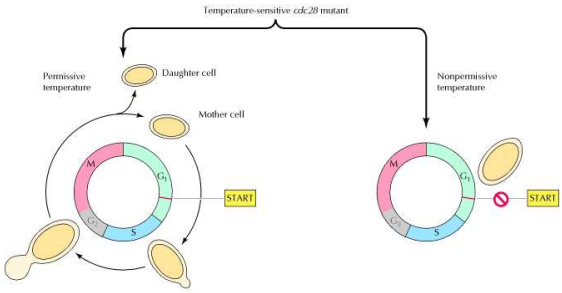
Three initially distinct experimental approaches contributed to identification of the key molecules responsible for cell cycle regulation.

1. **Studies of frog oocytes**:- These oocytes are arrested in the G2 phase of the cell cycle until hormonal stimulation triggers their entry into the M phase of meiosis. In 1971, two independent teams of researchers (Yoshio Masui and Clement Markert, as well as Dennis Smith and Robert Ecker) found that oocytes arrested in G2 could be induced to enter M phase by microinjection of cytoplasm from oocytes that had been hormonally stimulated. It thus appeared that a cytoplasmic factor present in hormone-treated oocytes was sufficient to trigger the transition from G2 to M in oocytes that had not been exposed to hormone. Because the entry of oocytes into meiosis is frequently referred to as oocyte maturation, this cytoplasmic factor was called maturation promoting factor (MPF).

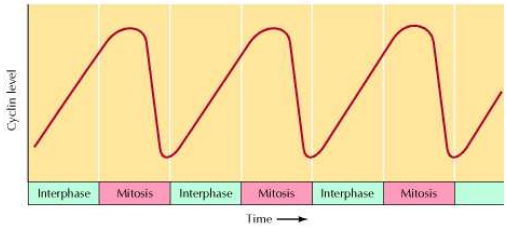


Like above explain the other two

2. **Genetic analysis of yeasts**



3. **Accumulation and degradation of cyclins in sea urchin embryos**



Answer 3:- <u>Upstream and downstream elements of adenylyl cyclase are</u>

Adenylyl cyclases are integral membrane proteins that consist of two bundles of six transmembrane segments.

Upstream: - G-Proteins

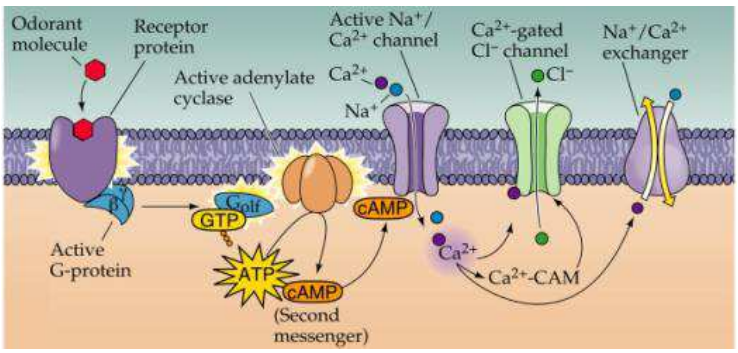
Downstream:- cAMP and other kinases

A soluble (non-membrane bound) form of adenylyl cyclase has recently been characterized in mammalian sperm. This form of the enzyme appears to be activated by bicarbonate ion.

In the cell, adenylyl cyclases and G-proteins interact to catalyze the formation of adenosine 3'-5'-monophosphate (cAMP) from 5'ATP.

There are nine identified isoforms of adenylyl cyclases. All of the isoforms are activated by G-alpha subunits, however some can be activated by other molecules such calcium ions.

When adenylyl cyclase is activated, it catalyses the conversion of ATP to cyclic AMP, which leads to an increase in intracellular levels of cyclic AMP.

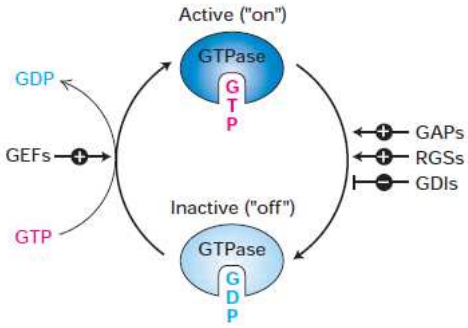


Answer 4:-

a. **<u>Role of Ran protein in nuclear import</u>**

Ran is a monomeric **G protein** that exists in two conformations, one when complexed with GTP and an alternative one when the GTP is hydrolyzed to GDP. The two **importins** form a heterodimeric *nuclear-import receptor:* the α- subunit binds to a basic

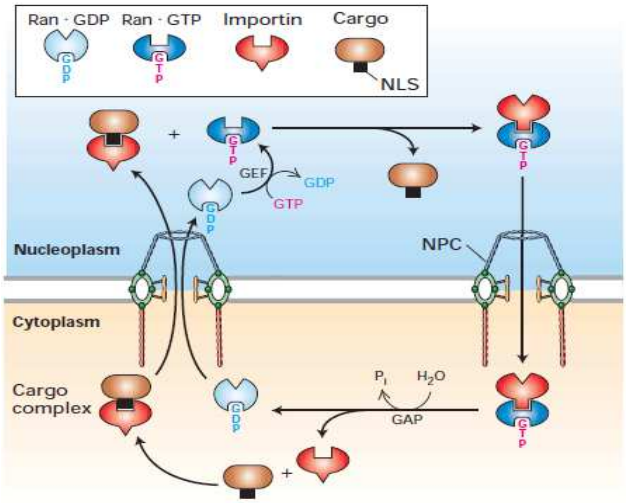
NLS in a "cargo" protein to be transported into the nucleus, and the β subunit interacts with a class of nucleoporins called *FG-nucleoporins*



In the nucleoplasm, interaction of Ran·GTP with the importin causes a conformational change that decreases its affinity for the NLS, releasing the cargo. To support another cycle of import, the importin-Ran·GTP complex is transported back to the cytoplasm. A GTPase accelerating protein (GAP) associated with the cytoplasmic filaments of the NPC stimulates Ran to hydrolyze the bound GTP. This generates a conformational change causing dissociation from the importin, which can then initiate another round of import. Ran·GDP is bound by NTF2 and returned to the nucleoplasm, where a guanine nucleotide–exchange factor (GEF) causes release of GDP and rebinding of GTP.

b. <u>Involvement of nuclear localization signal</u>

A nuclear localization signal or sequence (NLS) is an amino acid sequence which 'tags' a protein for import into the cell nucleus by nuclear transport. Typically, this signal consists of one or more short sequences of positively charged lysines or arginines exposed on the protein surface. Different nuclear localized proteins may share the same NLS. An NLS has the opposite function of a nuclear export signal, which targets proteins out of the nucleus.



c. <u>Functions of Peroxisomes</u>

Peroxisomes are ubiquitous organelles in eukaryotes that participate in the metabolism of fatty acids and other metabolites. Peroxisomes have enzymes that rid the cell of toxic peroxides. They have a single lipid bilayer membrane that separates their contents from the cytosol. The principal

function of peroxisomes is to house many metabolic pathways that are involved in various aspects of lipid metabolism. These include the following:
1. Enzymes involved in the degradative oxidation (e.g., β-oxidation of very long chain fatty acids, 2-methyl-branched fatty acids, dicarboxylic acids, leukotrienes, bile acid intermediates and cholesterol side chains, and both α- and β-oxidation of 3-methyl branched chain fatty acids);
2. The early steps in the synthesis of ether glycerolipids or plasmalogens;
3. The formation of bile acids, dolichol, and cholesterol; and
4. The catabolism of purines, polyamines, and amino acids, and the detoxification of reactive oxygen species such as hydrogen peroxide, superoxide anions, and epoxides. In methylotrophic yeasts, peroxisomes are also involved in the metabolism of methanol and methyl amines.

Answers 5

Metastasis and cancer cell invasion

Metastasis: - Metastasis is a complex process that involves the spread of a tumor or cancer to distant parts of the body from its original site. However, this is a difficult process. To successfully colonize a distant area in the body a cancer cell must complete a series of steps before it becomes a clinically detectable lesion.
Write down the different steps digramaticaly

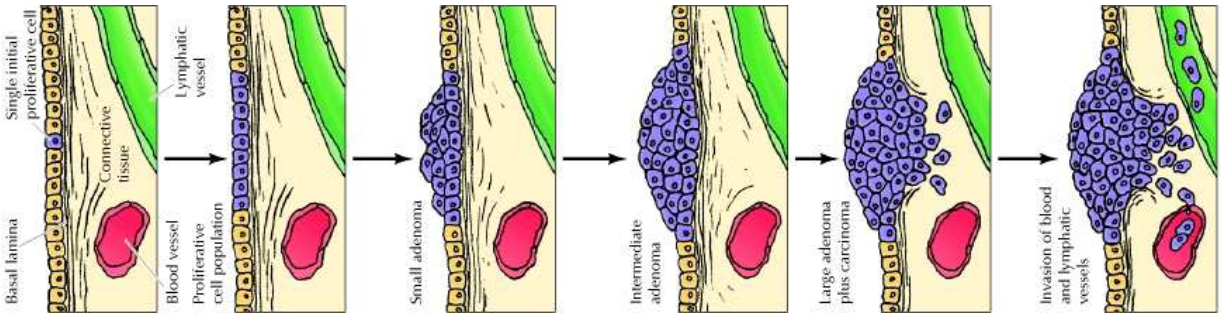


Figure:- Malignant cells generally secrete proteases that digest extracellular matrix components, allowing the cancer cells to invade adjacent normal tissues. Secretion of collagenase, for example, appears to be an important determinant of the ability of carcinomas to digest and penetrate through basal laminae to invade underlying connective tissue.

Answer 6

a. **Mass spectroscopy (MS)**
   Mass spectrometry is an analytical tool used for measuring the **molecular mass** of a sample.

Explain the different steps of MS i.e.

1. Ionisation
2. Acceleration
3. Deflection
4. Detection

Use of MS

i. **Biotechnology:** *the analysis of proteins, peptides, oligonucleotides*
ii. **Pharmaceutical:** *drug discovery, combinatorial chemistry, pharmacokinetics, drug metabolism*
iii. **Clinical:** *neonatal screening, haemoglobin analysis, drug testing*

iv. **Environmental:** *PAHs, PCBs, water quality, food contamination*
v. **Geological:** *oil composition*

How can mass spectrometry help biochemists?

- Accurate molecular weight measurements.
- Reaction monitoring.
- Amino acid sequencing.
- Oligonucleotide sequencing.
- Protein structure.

b. <u>SDS-PAGE</u>

- **It** is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility On the basis of their size, and no other physical feature.
- **SDS** (sodium dodecyl sulfate) is a detergent (soap) that can dissolve hydrophobic molecules but also has a negative charge (sulfate) attached to it. So If the proteins are denatured and put into an electric field (only), they will all move towards the positive pole at the same rate, with no separation by size.
- However, if the proteins are put into an environment that will allow different sized proteins to move at different rates. The environment is polyacrylamide. The entire process is called **polyacrylamide gel electrophoresis** (PAGE).
- Small molecules move through the polyacrylamide forest faster than big molecules. Big molecules stays near the well.
- The end result of SDS- PAGE has two important features:
  1) all proteins contain only primary structure &
  2) all proteins have a large negative charge which means they will all migrate towards the positive pole when placed in an electric field.

c. <u>Fluorescence Resonance Energy Transfer (FRET)</u>

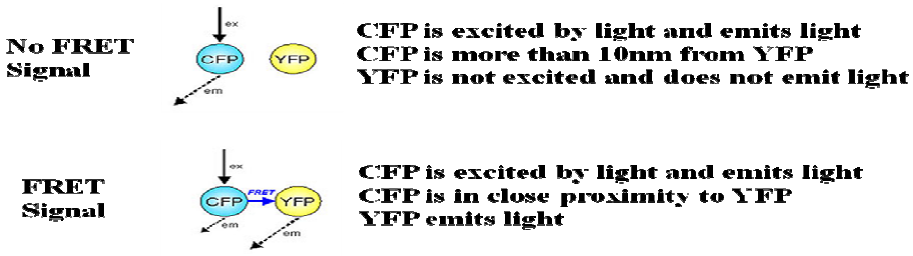
FRET is the non-radioactive transfer of photon energy from an excited fluorophore (the donor) to another fluorophore (the acceptor) when both are located within close proximity (1-10nm).

Using FRET one can resolve the relative proximity of molecules beyond the optical limit of a light microscope to reveal

(1) Molecular interactions between two protein partners,

(2)Structural changes within one molecule (eg. enzymatic activity or DNA/RNA conformation),

(3) Ion concentrations using special FRET-tools like the CFP-YFP cameleon



Answer 7:-

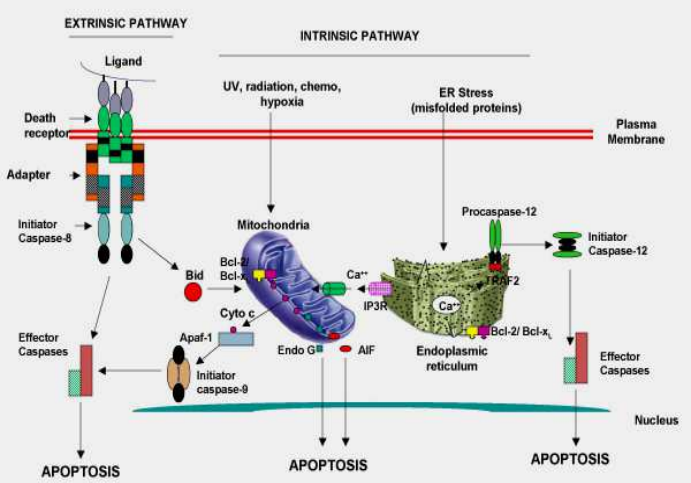
a. Programmed Cell Death

Apoptosis or programmed cell death is an essential physiological process that plays a critical role in development and tissue homeostasis. Apoptotic cells may be characterized by specific morphological and biochemical changes, including cell shrinkage, chromatin condensation, and internucleosomal cleavage of genomic DNA.

Steps

1. Cytoplasm shrinks
2. Chromosomes condense and fragment
3. Nuclear membrane breaks down
4. Apoptotic body formation
5. Engulfment of the cell corpse

- It is an important process of cell death
- Can be initiated extrinsically through death ligands (e.g. TRAIL, FasL) activating initiator caspase 8 through induced proximity.
- Can be initiated intrinsically through DNA damage (via cytochrome c) activating initiator caspase 9 through oligomerization.
- Initiator caspases 8 and 9 cleave and activate effector caspase 3, which leads to cell death.



b. Role of oncogene in cancer development

Oncogenes are genes whose presence can contribute to uncontrolled cell proliferation and cancer.

Explain how cellular oncogenes arise

Oncogenes can arise inside cells in two fundamentally different ways.

1. A mechanism involves the participation of *viruses* that introduce oncogenes into the cells they infect.
2. A series of mechanisms that convert normal cellular genes into oncogenes, often as a result of exposure to *carcinogenic agents*.

Cellular oncogenes arise from Proto-oncogenes

Proto-oncogenes are normal cellular genes

- can be converted into oncogenes
- contribute to the regulation of cell *proliferation* and *survival*

The *mutations* of proto-oncogenes can cause cancer.

- gain-of-function mutations can induce tumor formation

Activation

1. Point mutation
2. Gene amplification
3. Chromosomal translocation
4. DNA rearrangement
5. Insertional mutagenesis

Proteins produced by Oncogenes

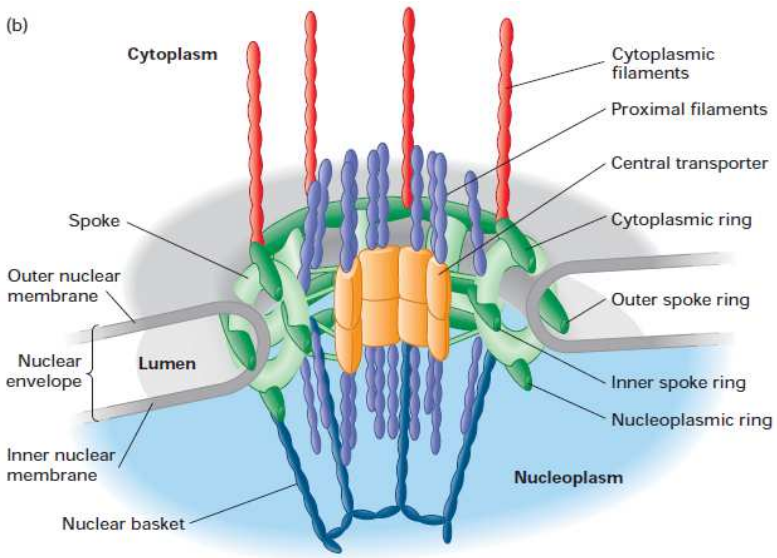
1) Growth factors
2) Receptors
3) Enzymes that catalyze protein phosphorylation
4) Proteins that bind to and regulate the activity of DNA or other proteins

Answer 8:-

a. <u>Systemic Lupus Erythematosus (SLE)</u>

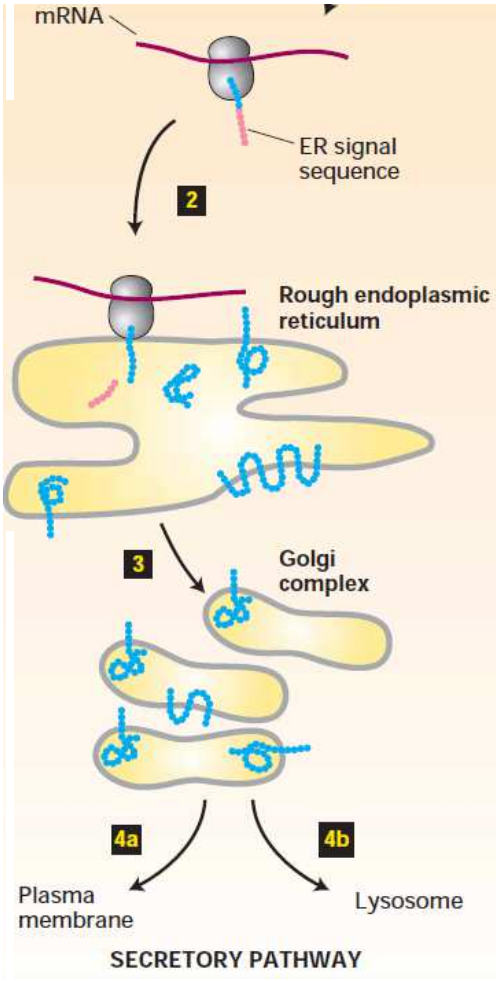
Systemic Lupus Erythematosus is a chronic autoimmune disease in which patients produce antibodies against their own normal cell constituents, particularly nuclear antigen. The disease affects women about ten times more frequently then it affects men. The disease is characterized by the formation of large amounts of antigen-antibody complexes that are deposited in tissues and blood vessels throughout the body. The tissue damage and inflammation resulting from deposition of these immune complexes produces a variety of clinical manifestation, including skin rashes, arthritis and kidney disease. Damage to the kidney is particulary common and can be severe enough to cause kidney failure and death. There is no cure for SLE. It is treated with immunosuppression, mainly with cyclophosphamide, corticosteroids and other immunosuppressants. SLE can be fatal. The leading cause of death is from cardiovascular disease due to accelerated atherosclerosis. SLE cannot be prevented, but the consequences can be prevented.

b. <u>Structure of nuclear envelope</u>



c. Secretory Pathways

The secretory pathway is a series of steps a cell uses to move proteins out of the cell; a process known as secretion. The path of a protein destined for secretion has its origins in the rough endoplasmic reticulum, a membrane-bound compartment in the cell. The protein then proceeds through the many compartments of the Golgi apparatus and finally ends up in a vesicle that transiently fuses at the cell plasma membrane via permanent plasma membrane structures called porosomes, depositing the proteins outside of the cell.



Ribosomes synthesizing nascent proteins in the secretory pathway are directed to the rough endoplasmic reticulum (ER) by an ER signal sequence (step 2). After translation is completed on the ER, these proteins can move via transport vesicles to the Golgi complex (step 3). Further sorting delivers proteins either to the plasma membrane or to lysosomes (steps 4a, 4b).