B. Sc (Honors) Zoology 2nd Semester [CBCS]

CORE COURSE IV

Cell Biology [CREDIT 4]

Theory

Unit 6: Cytoskeleton

Structure and Functions: Microtubules, Microfilaments and Intermediate filaments

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CYTOSKELETON:

STRUCTURE AND FUNCTION

Cytoskeleton can be defined as "*structural frame/ support of cells*" in simple terms (*Cyto* = Cell + *Skeleton* = structural support/ frame). Just like skeleton of vertebrates, so is cytoskeleton for Eukaryotic cells. These cytoskeletons are filamentous in nature made up of protein subunits that are held together by weak non-covalent bond.

They are categorized as:

1. Microtubules: hollow, long, unbranched and stiff. They are composed of protein tubulin.
2. Microfilaments: solid, thin, branched and stiff. They are composed of protein actin.
3. Intermediate filaments: rough, unbranched and robe like - flexible. They are composed of variety of proteins.

Irrespective of the types, cytoskeletons are polymers of protein subunits that elongate (increase in length) by polymerization. The process of polymerization is the addition of protein subunits to the existing subunits/ structure. Therefore, the increase in length occurs from one end - this end is termed '+' end and the opposite end as '–' end. The filament shortens by shedding of their subunits from the '–' end by the process called de-polymerization. The cytoskeletons are in a state of constant flux of polymerization (addition of subunits) in '+' end and de-polymerization (Shredding of subunits) in the '–'end. This phenomenon is often termed as 'state of dynamic instability'.

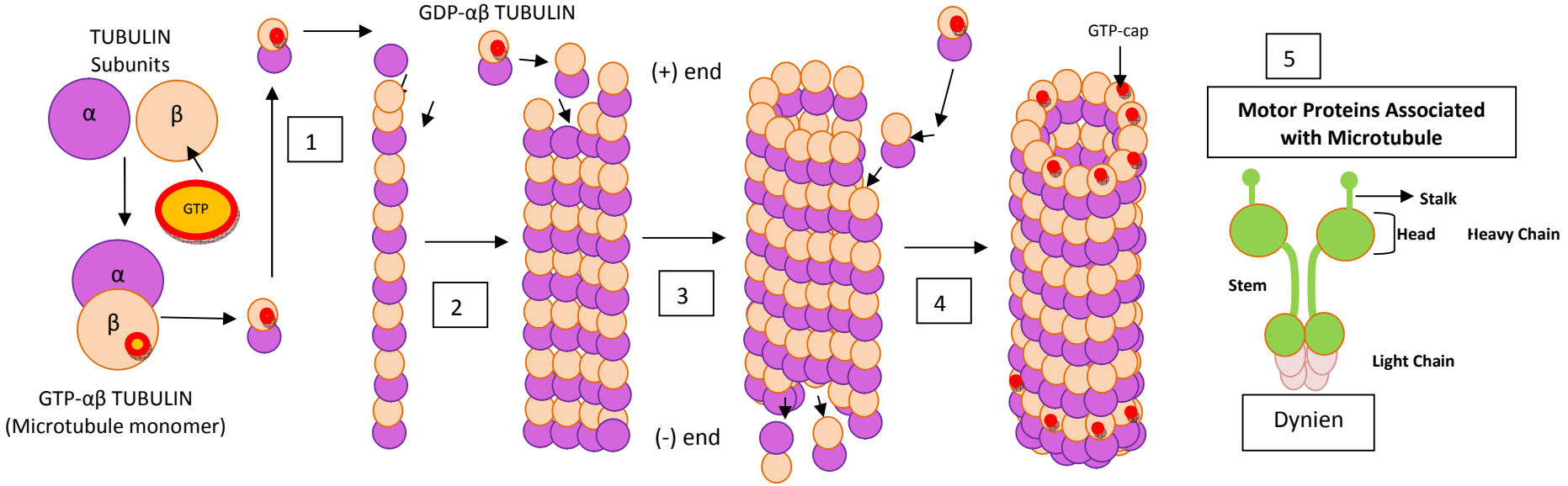
[**Tough it is important to know how they form and how they disintegrate through various process, we shall limit our understanding to the syllabus prescribed and discuss further in the future. So, for now we shall deal with them in brief, and, one can refer the suggested readings].

The Key functions of cytoskeleton are:

1. To provide structural support in maintaining shape of the cells and resilience to tension and stress.
2. Intracellular transport of vesicle and movement of mRNA (refer to vesicular transport: from ER to Golgi apparatus to Plasma membrane) and translocation of organelles (to position various organelles within the cell).
3. The cytoskeletons also functions as apparatus for cell motility by crawling movement (filopodia, lamellipodia) on substratum or swimming in aqueous medium through cilia or flagellar movement (microtubules) in single cell animals.
4. Motility: In multi-cellular organism, the contraction of muscles, movement of sperms, neurons, WBC and phagocytes are some mentions.
5. It forms the most essential component of cell division machinery. Cytoskeletons are responsible for the alignment and separation of Chromatids and subsequent cytokinesis to form daughter cells.

1. Microtubules:

Microtubules are stiff, hollow unbranched and inextensible tube found in all eukaryotes. Its function: to support cell structure and intracellular transport and cell organization. The diameter of the microtubule fibre is 25 nm with GTP-αβ tubulin heterodimers as protein subunits (monomers). The addition of tubulin incorporation is on the Beta tubulin + end. Tubulins are associated with MAPs and Kinesin and dyenin motor proteins.



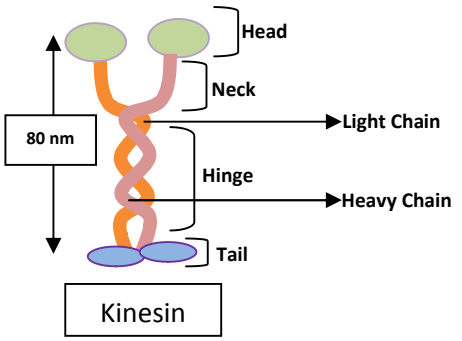


Figure 1: Structure and Assembly of Microtubules.

Microtubules form from the MTOC (Micro-Tubule Organizing Centre) nucleation centre near the centre of the cell and extents towards the periphery. The formation of microtubule in vitro occurs through 2 stages of nucleation and elongation in the MTOC.

1. Free αβ-tubulins dimmers aggregate to form short filaments – called protofilaments (this stage is also known as nucleation)
2. Proto-filament associates into lateral sheets with the addition of more tubulin dimer monomers.
3. The sheet conformation is unstable, hence, they wrap around to form circular tube with 13 protofilaments - microtubule
4. Free αβ-tubulins are GTP bounded in the β-subunit, which is hydrolyzed after incorporation.
5. Motor Proteins kinesin and dyneins are associated with tubulins. They are responsible for transport or translocation of organelles, vesicles on the microtubule. Kinesin moves from '- 'end to '+'end and dyenin from '+' end to '- 'end.

Microtubule subunits are in a state of constant flux, i.e., polymerization and depolymerisation are continuous - "*state of dynamic instability*". The stabilization of microtubule is effected by binding of GTP to the subunits at the ends which prevents depolymerisation. The average half-life of microtubule ranges from 10min in non-dividing cell to 20 sec in dividing cell.

Function of Microtubule:

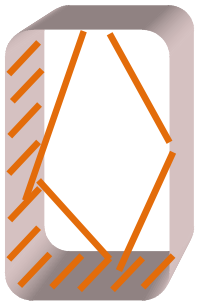


Fig 2. Structural Support

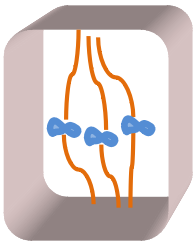


Fig 3. Separation of chromatids

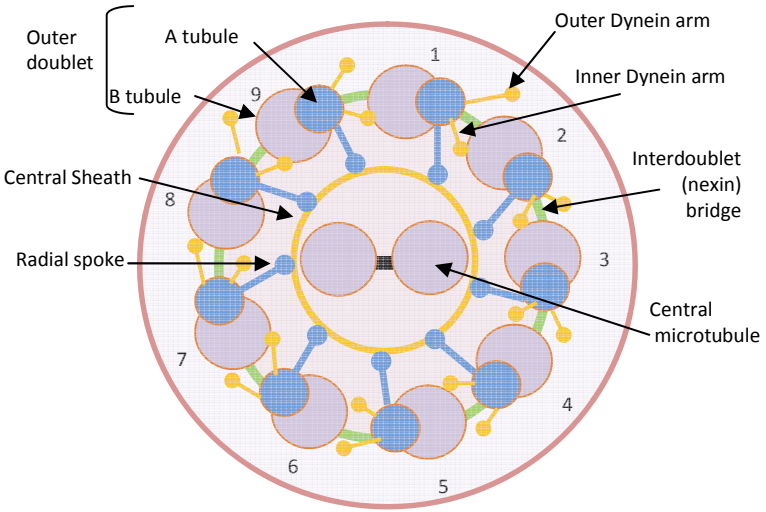


Fig 4: Structure of cilia or flagellar axenome cross section consist of i) 2 Central microtubule (Complete), ii) 9 peripheral microtubule (aka outer doublet: A tubule (complete), B tubule (incomplete), iii) 9 radial spokes from the incomplete microtubule, iv) outer and inner dynein arms from each A tubule. [Complete tubule = 13 tubulin molecules, incomplete = 10]. Note 9 microtubules in pairs + 2 microtubules in the centre.

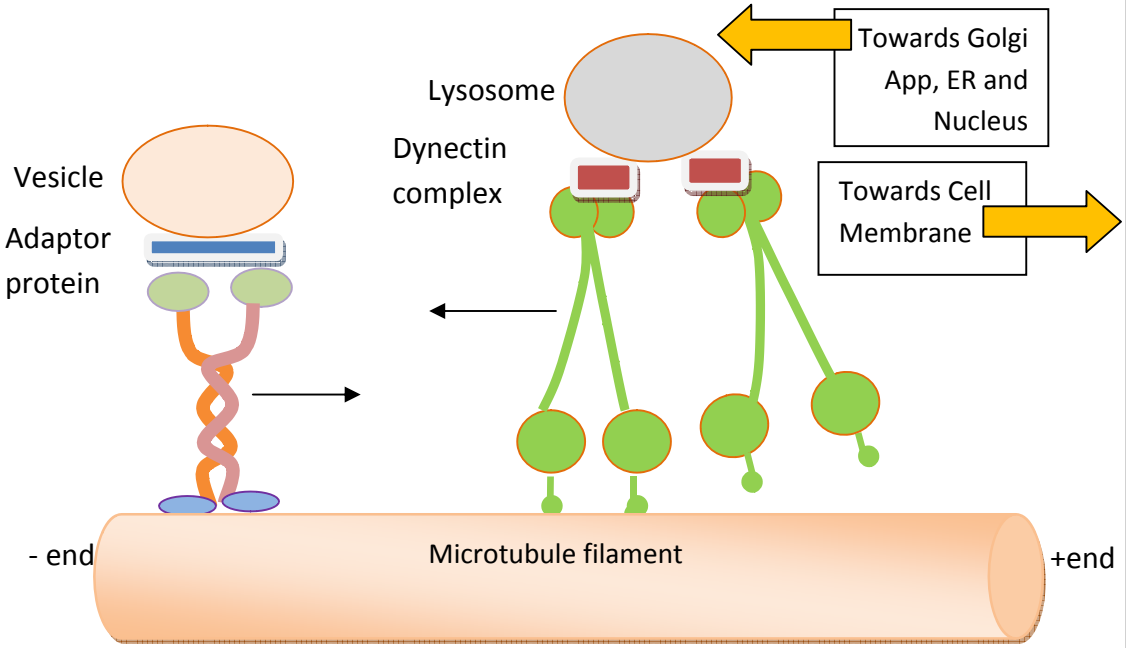


Fig 6: Transport of vesicles/ organelles to and fro Endoplasmic Reticulum-Golgi Apparatus-Plasma Membrane. Note;- kinesin moves from '-' to '+' end; In dynein from '+' to '-' end

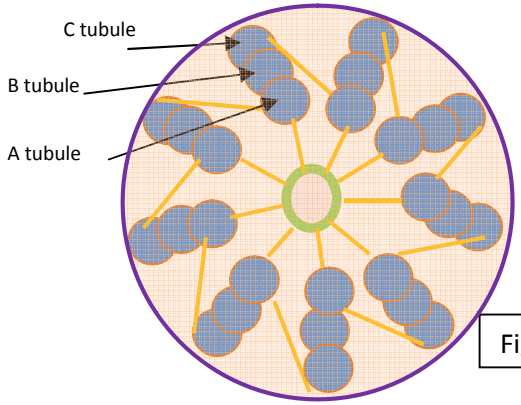


Fig 5A

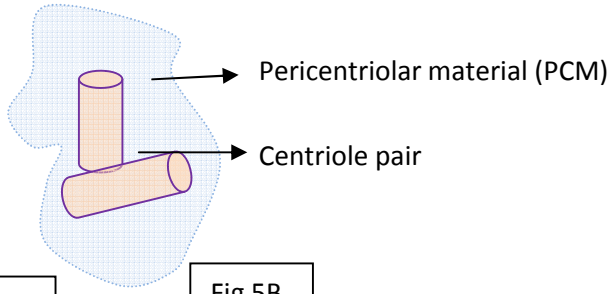


Fig 5B

Fig 5A) Centriole (note: 9 microtubule in triplets + 0 at the center). **Fig 5B)** Centrosome with pair of centrioles.

2. Microfilaments:

Microtubules are also known as actin filaments. They flexible branched and inextensible helical filaments found in all eukaryotes. Basically its function is for motility and contractility of the cell. The diameter of the microfilament or actin filament is 8 nm with ATP-Actin molecules as protein subunits (monomers). The actin molecules are incorporated on the + end. They are associated with Myosins and actin binding proteins.

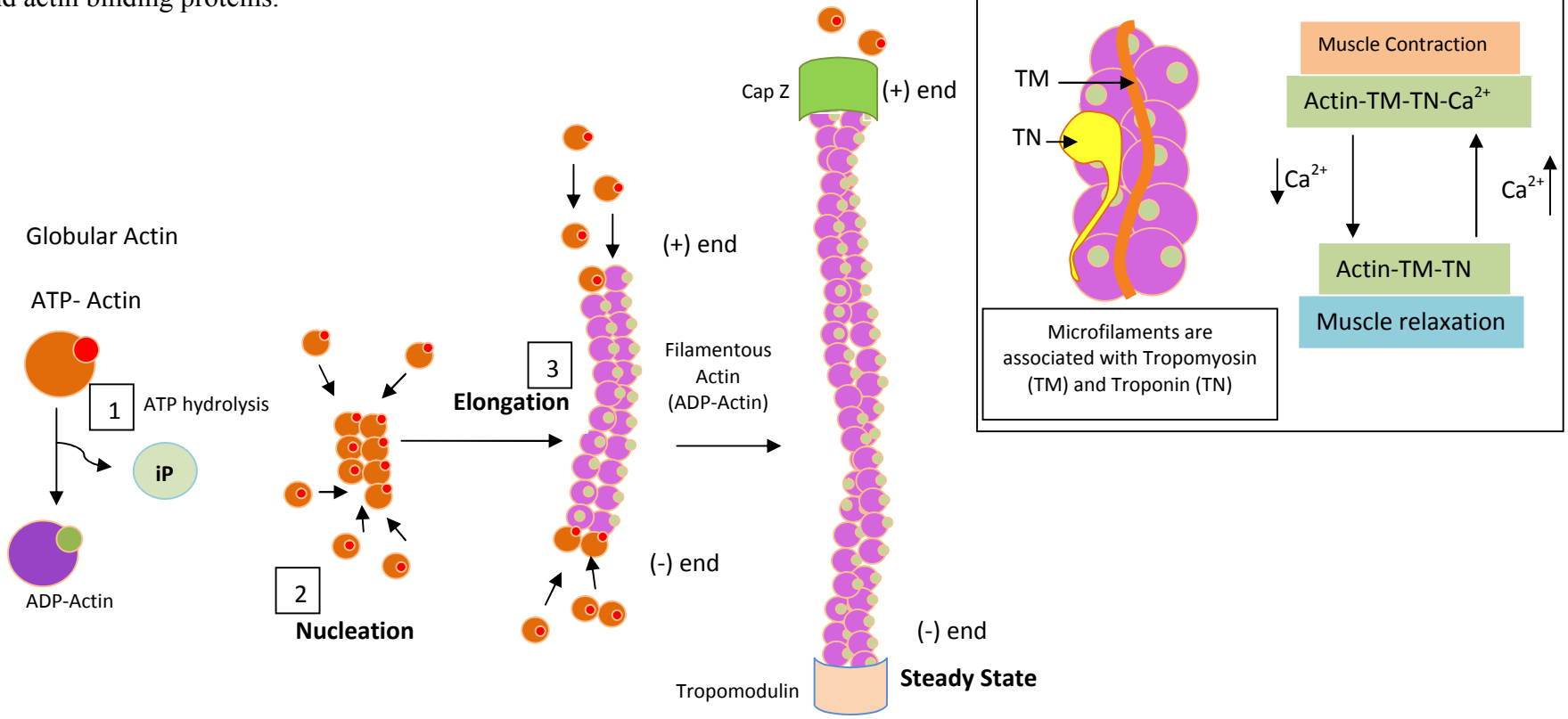


Fig 7. Structure and Actin Polymerization *in vitro*: It occurs in 3 stages:
Nucleation: initial phase, ATP- bound Actin monomers aggregate into short oligomer called NUCLEI. The nuclei maturation occurs after the hydrolysis of bounded GTP to GDP (colour change from brown with red to blue with green). The actin in nucleation stage with GTP is also called G-actin.
Elongation: the nuclei elongates rapidly by adding ATP-Actin monomers from both ends.
Steady state: after the formation of nuclei, the ATP bounded in the actin molecule hydrolyze to form ADP-Actin and elongates to become stable (F-actin). The de-polymerization in the '-' end is stabilized by protein capping (tropomodulin). In the '+'end cap protein *CapZ* prevents addition of loss of actin molecules in a steady state.

Functions of Actin filaments/ microfilaments:
1. Membrane endocytosis during phagocytosis
2. Vesicle transport along ER - GA – PM axis
3. Locomotion for single cell organism: endoplasmic streaming
4. Muscle Contraction: filament sliding
5. Cytokinesis during cell division

3. Intermediate filaments:

Intermediate filaments are flexible, tough and extensible filaments found only in animal cells. Its function is primarily for structural support of the cell. The diameter of the intermediate filaments is 10-12 nm with about 70 different protein subunits (monomers). The addition of protein subunit monomers is internal. Tubulins are associated with plakins (cell junction proteins).

The monomers or intermediate filaments proteins are classified according to their distribution in specific tissues they are: Nucleus (Nuclear Lamin A, B, C), skin epithelium (Acidic and Basic keratins), Neurons (Neurofilament – FF-L, M, H and internexin), Cell Junctions Type-III (vimentin, desmin, periferin).

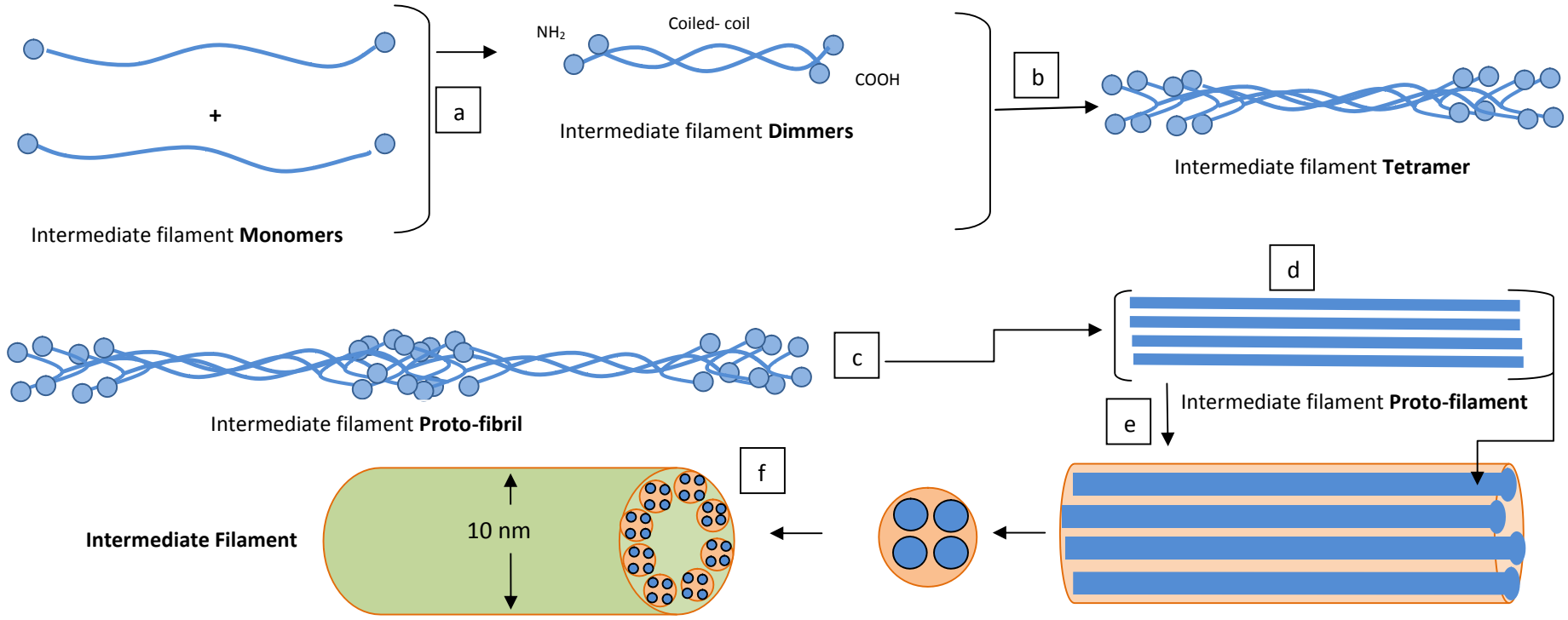


Fig 7. Hierarchy of Intermediate filament (IF) assembly:

a. IF proteins forms parallel dimmers with highly conserved coiled-coil core domain with globular head and tail

b. Two identical dimmers bind to form tetramers side-by-side, arranged anti-parallel and staggered.

c. Tetramers in pairs assemble end to end to elongate and form **proto-fibril** (elongation of fibres)

d. 4-Proto-fibrils are helically twisted against each other to form **Proto-filament**.

e.4-Protofilament are helically twisted to form one matured unit

f. 8 proto-filaments units forms a 10 nm **Intermediate filament (IF)**

Functions of Intermediate filaments:

1. Membrane mechanical support (nucleus inner membrane lined with Lamin A and C) and organizes nuclear content.
2. In cytosol, they form internal framework that supports the cell and add resilience of the cell.
3. They form the connecting network for cell attachment to their extra cellular matrix through hemidesmosomes and cell-cell adhesion through desmosomes.
4. They form the interconnecting link between cytoskeletons.

Cytoskeleton network in a Eukaryotic cell: Microtubule – Intermediate filaments-

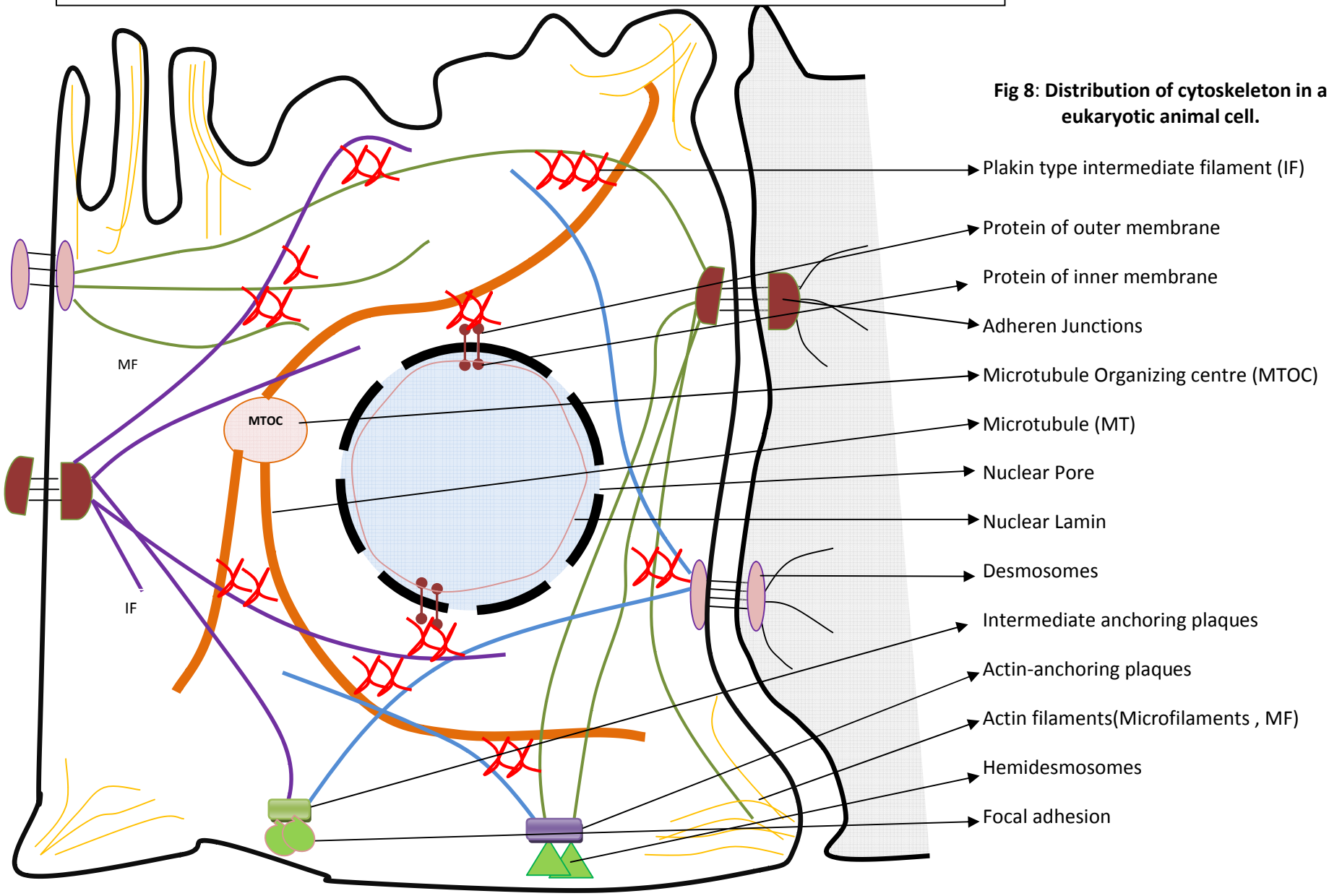


Fig 8: Distribution of cytoskeleton in a eukaryotic animal cell.

Suggested readings:

1. Karp, G. (2010). *Cell and Molecular Biology: Concepts and Experiments*. VI Edition. John Wiley and Sons. Inc.
2. Becker, W.M., Kleinsmith, L.J., Hardin. J. and Bertoni, G. P. (2009). *The World of theCell*. VII Edition. Pearson Benjamin Cummings Publishing, San Francisco.
3. Lodish, H. Molecular Cell Biology 5th ed, freeman, 2003. (ISBN 0716743663/c/967/s)