Chapter 3. HYALOPLASM & CYTOSKELETON

A. HYALOPLASM

I. DEFINITION
Hyaloplasm is a viscous gel also called cytosol, it is the medium in which bathe the cellular organelles and the cytoskeleton.

II. ULTRASTRUCTURE
Hyaloplasm contains particles not bounded by a membrane such as dense lipid inclusions, glycogen particles scattered or grouped in rosettes in the cell animal, starch particles in the plant cell and free ribosomal subunits.

III. CHEMICAL COMPOSITION
The last supernatant obtained by differential ultracentrifugation (UCD) (handout p.37) contains water (85%), enzymes, amino acids, ions, RNA, glucose and proteins.

IV. THE ROLES
The hyaloplasm is the crossroads of the metabolic reactions, which take place in the cell; it is the place of synthesis and degradation of proteins.

B. CYTOSKELETON

I. DEFINITION
It includes three types of elements: microtubules (MT), actin microfilaments (MF), and intermediate filaments (IF).

II. MICROTUBULES
MTs are unstable and polarized polymers present in the hyaloplasm; they are highlighted by the immunofluorescence technique.

1. Ultrastructure and Molecular Architecture
MTs consist of two types of globular proteins, alpha (α) tubulins and beta (β) tubulins that associate into dimers. The dimers polymerize into protofilaments, the polymerization takes place in the presence of GTP and Mg^{2+}. The association of 13 protofilaments gives a microtubule (hollow tube) 25 nm in diameter (handout p.67) (**Figure 1a**).
MTs have two ends that elongate at different speeds. The fast-polymerizing (+) end faces the plasma membrane (outward) while the slower-polymerizing (-) end faces the cell center.

2. Associated proteins
MTs are associated with proteins (MAP), some of which have a role in the stabilization of MTs; the others are specialized in the movement of vesicles and organelles along the MTs (**Figures 1b**, **2a** and **2b**).
These associated proteins are ATPases: the Kinesins, which transport towards the (+) end, located on the side of the plasma membrane and the Dyneins towards the (–) end facing towards the plasma membrane cell center.

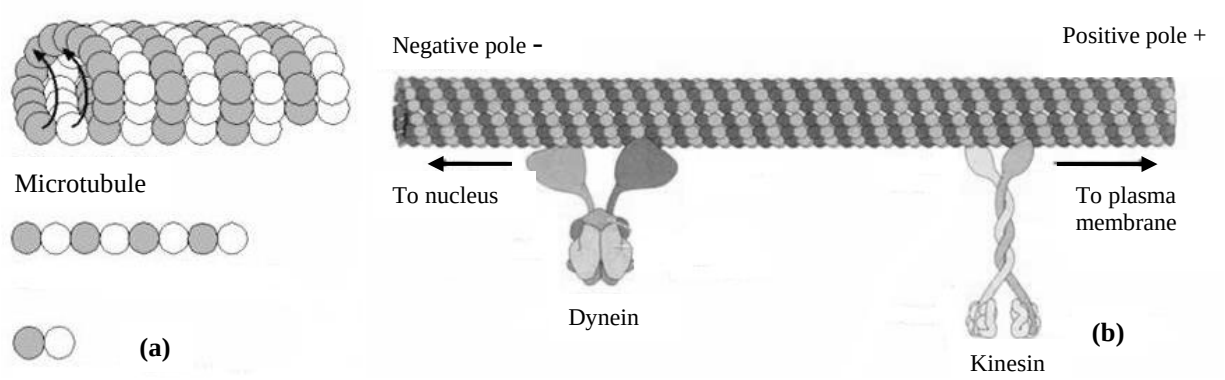


Figure 1. Molecular architecture of an MT (**a**) and associated proteins during transport (**b**).

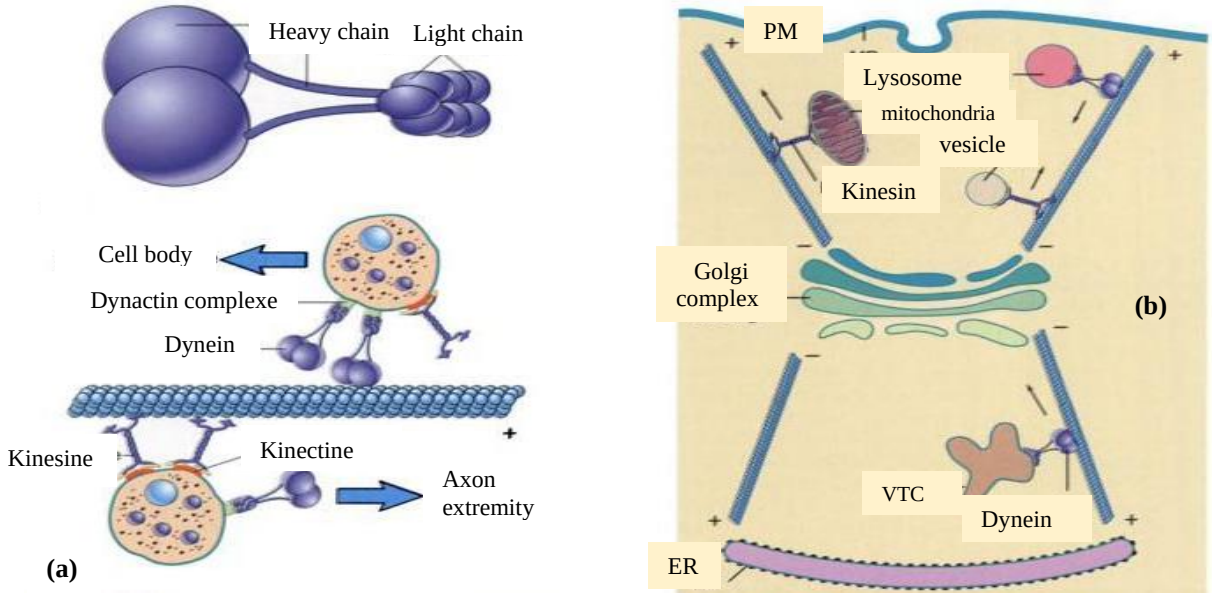


Figure 2. TM-Associated Proteins: Molecular Motors.

(**a**) Schematic of two vesicles (moving in opposite directions along the same microtubule, one driven by a kinesin moving towards the plus end of the pathway and the other by dynein cytoplasmic towards its minus end. In the model shown, each vesicle contains both types of motor proteins, but the kinesin molecules are inactivated in the upper vesicle and the dynein molecules are inactivated in the lower vesicle. Both motor proteins are attached to membrane of the vesicles by an intermediate, kinesin are attached by a membrane protein kinectin and dynein by a soluble protein complex, dynactin.
(**b**) Schematic representation of the transport of vesicles and organelles by kinesin and dynein, in a no polarized cell in culture.

3. Varieties of microtubules

There are two types of MT depending on the type of fixer and the fixing temperature.

a. Stable microtubules

They are preserved regardless of the type of fixative and regardless of the fixing temperature. Stable MTs are permanent cellular elements.

a_1. Centrioles
Two centrioles located near the nucleus (animal cell), arranged perpendicular to each other and surrounded by an amorphous matrix together constitute the centrosome. Only nine triplets of peripheral microtubules form the centriole, each triplet is made up of microtubules A, B and C.
The plant cell contains an amorphous matrix and does not contain centrioles (centrosome).

a_2. Basal body or kinetosome
It is found near the plasma membrane, at the base of cilia and flagella, and has a centriolar structure (9 peripheral triplets).

a_3. Cilia and flagella
They are expansions of the plasma membrane containing an axoneme whose organization is the same in cilia and flagella. The axoneme consists of 9 peripheral microtubule doublets and a central doublet surrounded by a protein sleeve. MT A and B form each peripheral doublet.

b. Labile microtubules
They are preserved by aldehyde fixatives (e.g. glutaraldehyde) and at a temperature above 4°C; this is the case for the MTs of the mitotic or achromatic spindle (handout p.135 to 139). Drugs (e.g. in the case of chemotherapy) can disrupt the polymerization or depolymerization of labile MTs, such as colchicine and vinblastine which prevent polymerization and Taxol which blocks depolymerization.

4. Functions
Stable and labile TMs are involved in:
- maintaining the shape of the cell,
- displacement of chromosomes in mitosis and meiosis,
- transport of endocytosis and exocytosis vesicles,
- displacement of intracellular organelles,
- axonal flow,
- movement of isolated cells (paramecium, spermatozoid, etc.), using cilia and flagella.

5. Biogenesis
MTs polymerize from organizing centers (MTOCs), these centers are:
- the kinetochore, organizing center of kinetochore TMs,
- the basal corpuscle or kinetosome, the organizing center of the axoneme of the cilia and flagella,
- the amorphous pericentriolar matrix (animal cell) or the amorphous mass (plant cell), organizing centers of the other MTs.

III. ACTIN MICROFILAMENTS
These are unstable and polarized polymers present in the hyaloplasm; they are highlighted by the immunofluorescence technique.

1. Ultrastructure and Molecular Architecture

MFs are about 5-7nm in diameter, G-actin (globular) polymerizes into F-actin (actin filament) (**Figure 3**), polymerization requires the presence of ATP and Mg^{2+} (handout p.67). Actin MFs exhibit two ends, a (+) end where polymerization is faster and a (-) end where actin polymerization is slower. There cytochalasin blocks the polymerization of actin MFs by attaching to their (+) ends.

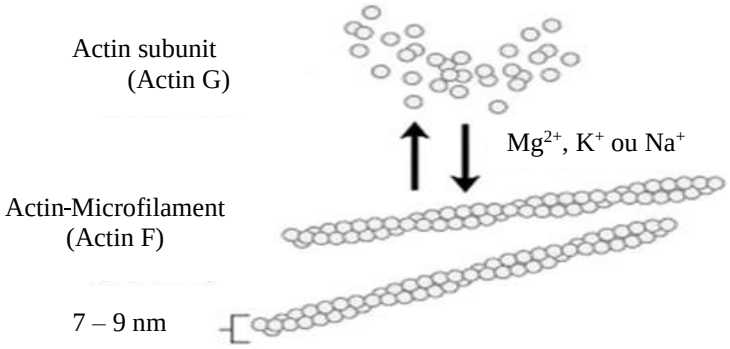


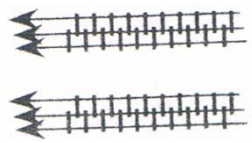
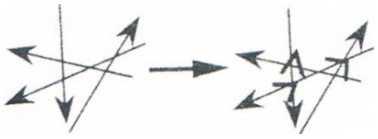
Figure 3. Molecular architecture of actin MFs.

2. Associated proteins

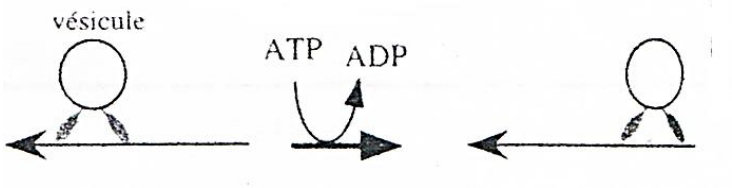
Several types of proteins associate with actin microfilaments and are involved in different functions:

a. Control of polymerization and depolymerization of MFs: profilin promotes polymerization and caldesmon prevents depolymerization.

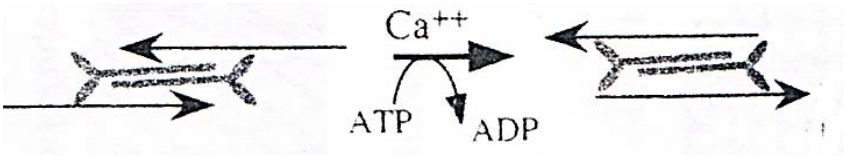
b. Organization of MFs: α-actinin, fimbrin, villin and filamim are involved in the organization of MFs into bundles or networks (table below).

MF organisation	Associated protéins	Schematic representation	
Wide bundles	α-actinin		Fixing at the positive (+) end
Tight bundles	Fimbrin Villin		Microvillosity
Networks	Filamin **V**		MF Stabilisation

c. Movements of vesicles and organelles: myosin I, a motor protein in the presence of ATP, makes it possible to move vesicles and organelles all along the actin MFs from the (-) end to the (-) end. +).



d. Muscle contraction: During muscle contraction, ATPase-active myosin II in the presence of Ca++ binds to actin MF to form an actomyosin complex.



3. Function

Actin microfilaments are involved in several functions:
- the shape and maintenance of cell polarity (e.g. microvilli),
- the intracellular movements of the organelles (cyclosis movements of the chloroplasts in the plant cell,
- the movements of intracellular vesicles,
- cell movements using pseudopodia (e.g. displacement: amoeba and leukocytes),
- cytodieresis, during cell division,
- the formation of cell junctions.

IV. INTERMEDIATE FILAMENTS

1. Ultrastructure and Molecular Architecture

The intermediate filaments are stable polymers present in the hyaloplasm and in the nucleoplasm, their diameter is between 8 and 10 nm (**Figure 4**). They are made up of proteins fibrous.

2. Families of fibrous proteins

a. Lamins: form a network at the periphery of the nucleus (nucleoplasmic face) of eukaryotic cells; they have a supporting role of the nuclear envelope and an attachment point to the dense chromatin. At the time of cell division, the network of lamins is destructured and the nuclear envelope fragmented into vesicles.

b. Cytokeratins: fibrous proteins specific to epithelial cells, they form a network that anchors in the desmosome and the hemidesmosome. This network of keratin is in continuity with the neighboring cells, they participate in the resistance to tensile forces.

c. Vimentin: cell-specific fibrous protein of connective and cartilage cells, found in hyaloplasm.

d. Desmin: it is specific to muscle cells, it consolidates the myofibrils and gives the muscle its striated appearance in light microscopy.

e. Neurofilament proteins: are proteins specific to neurons.

V. FUNCTIONS OF THE CYTOSKELETON

The cytoskeleton performs several functions such as:
- The structure and support of the cell
- The transport of vesicles and organelle inside the cell
- Contractility and motility
- Spatial organization in the cell

To know more

1. Cau P. and Seite R. 2002- Cell Biology Course. Ed. Ellipses.
2. Campbell N.A. and Reece J.B. 2004- Biology. Ed. DeBoeck.
3. Rescott L., Harley J.P. and Klein D.A. 1995- Microbiology, translated from English by Bacq-calberg CM., Coyette J., Hohet P. and Nguyen-Distèche M. Ed. DeBoeck, 1014p.
4. Raven PH and Johnson 2000- Biology. Ed.Mc Graw.
5. Albert, Bray, Johnson, Lewis, Raif, Roberts and Walter 1999 The Essentials of Cell Biology: An Introduction to Molecular biology of the cell. Ed. Flammarion Medicine Sciences.

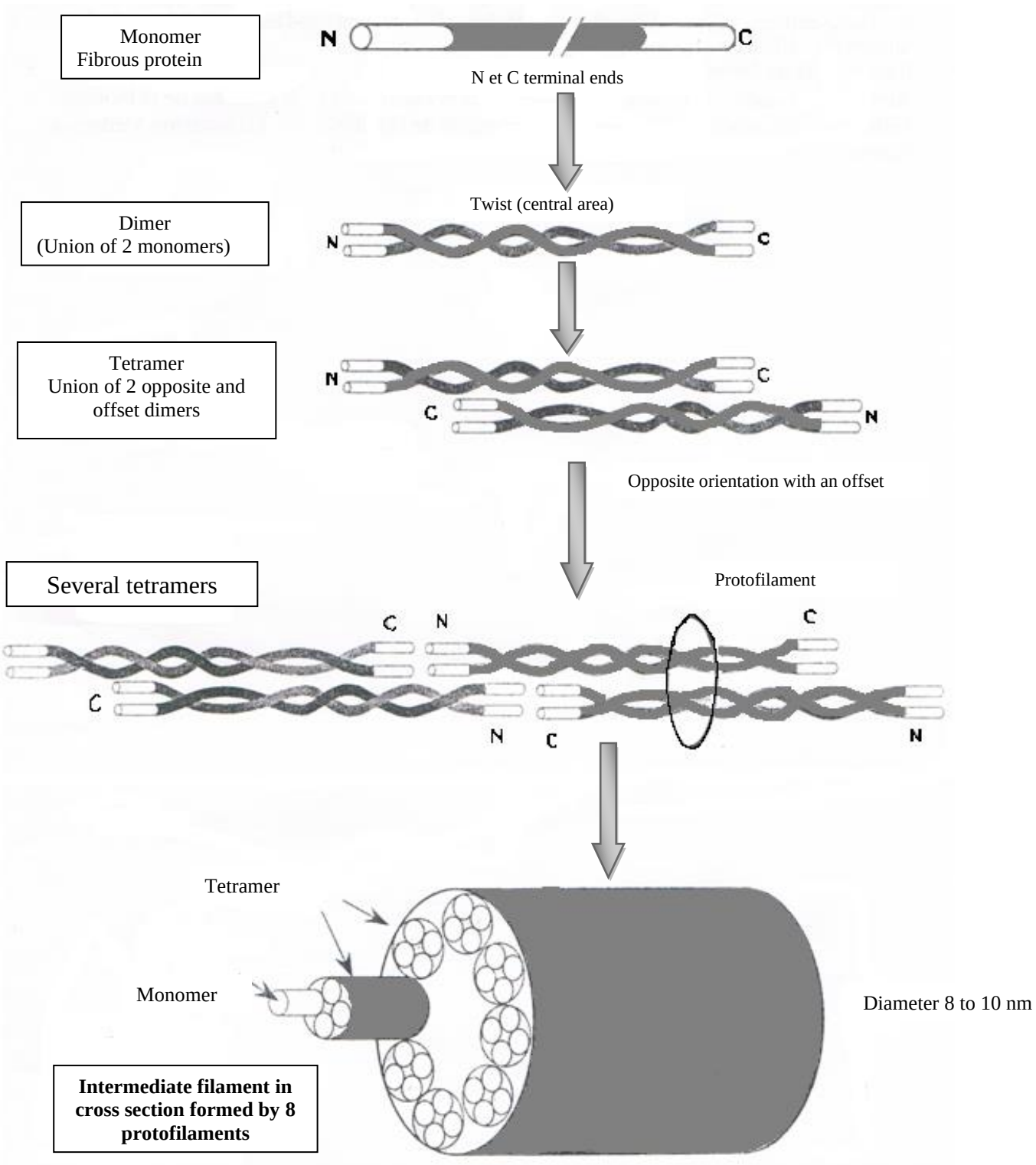


Figure 4. Biogenesis and molecular architecture of an intermediate filament.