

The Cytoskeleton

- Microtubules
- Microfilaments
- Intermediate filaments
- Also motor proteins and other associated proteins that integrate system

Functions of the Cytoskeleton, Fig 9.1

- Structure and support
- Intracellular transport
- Contractility and motility
- Spatial organization

Microtubules, Figs 9.8, 9.9

- Hollow, long, tubular
- Found in mitotic spindle, cilia, flagella, cytoplasmic tracks, sub plasma membrane.
- Longitudinal rows are protofilaments (13/MT) alpha, beta subunits.
- Beta is + end-- fast growing, alpha is - end--slow growing
- “Grows” on + end
- MAPs give stability

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Microtubule-associated Proteins (MAPs)

Fig. 9.9

- Found in all cells with MT
- Different types, depending on cell type.
- (More is known about MAPs from nervous tissues because abundant.)
- MAPs can
 - interconnect MT**
 - increase stability of MT**
 - increase rate of assembly**

Microtubules as Structural Supports

Figs. 9.10, 9.12

- Tubular nature gives stiffness.
- Provides structural support like re-bar.
- Can be bundled together for longitudinal reinforcement, or looser for curved shapes.
- Polarity allows “directed mechanical activities” within cell.
- In plants, MTs act with cellulose for cell shape.

MTs for Intracellular Motility,

Figs 4.48, 9.11, 9.14

- Neurons are an example of a cell type whose function depends on MTs.
- Note structure of nerve cell.

- Axons can be many cm long; have no ribosomes, ER.
- Proteins and neurotransmitters are transported in vesicles up & down axon on MTs.
- Motor proteins generate movement along MT.

Motor Proteins

- Attach to MT or microfilaments and to cargo (vesicles, organelles, chromosomes, other cytoplasmic structures).
- Many different motor proteins, each with specialization (e.g., what they bind to).
- Motor proteins *transform* chemical energy (e.g., ATP, GTP) to mechanical energy to generate the *force* to perform the *work* of pulling or contracting.

The three families of motor proteins

- Kinesins (along microtubules)
- Dyneins (along microtubules)
- Myosins (along microfilaments)

Kinesins, fig 9.16

- Note structure (4 subunits)
- relatively small proteins, carries small vesicle loads.
- “walk” along MT toward + end using 1 ATP/step.
- 5 microns/sec tops (~ 900 x diffusion rate)
- moves in 8 nm steps (width of tubulin dimer)
- binding causes conform change, twisting other head forward to bind, then twist...etc.

Dynein Fig. 9.18 a,b

- First discovered in 1963 associated with cilia & flagella
- Cytosolic form purified ~ 1983.
- Ubiquitous in eukaryotic cells.
- Structure: 1.5 million daltons 2 heavy chains, other smaller chains
- Travels in - direction on MT
- Requires dynactin to bind to cargo

Motor Protein/MT model

Fig. 9.18 c

- Polarity of MT (alpha/beta) provides directionality for motors
- Recall: Kinesin +, dynein -
- Are MT organized in polar fashion or are they random in polarity, and motor proteins going + or - deliver cargo as needed on available MT?

How are MT formed?

Fig 9.19 a,c

- MTOC are sites where MT are formed.
- There are various types of MTOC.
- MTOC can determine number and orientation of MT.

- In animals centrosomes are major MTOC.
- Plants don't have centrosomes; have dispersed MTOC

Nucleation and *de novo* synthesis of MT, Fig 9.20

- Forming MT oriented + end out.
- Gamma tubulin associated at nucleation site (@ centrioles in animals), perhaps associate with nuclear membrane in plants (see Fig.9.21)
- Can "move" away as depolymerization occurs at - end, rapid addition to + end.

Dynamic Properties of MT

- Tubulin that forms MT are not covalently bonded
- Assemble and disassemble by free energy changes.
- Some MT are more labile (e.g., mitotic spindle); others very stable (flagella).
- MAPs affect stability.
- Low temp, high Ca⁺⁺, pressure, chemicals cause disassembly *in vivo*.

MTs of Plant Cells, Fig. 9.23

- During interphase (1) MTs distributed at periphery (cellulose synthesis)
- MTs form preprophase band.
- MTs form spindle from polar MTOCs during cell division
- After telophase spindle MTs disappear
- Phragmoplast MT direct cell plate (wall) deposition

MT dynamics *in vitro*.

Fig 9.25

- Cell homogenate with GTP, Mg⁺⁺, EGTA, MTs assemble/disassemble by temp changes.
- Can also assemble in cell free systems with pieces of MTs to provide nucleation sites.

MT-based cilia and flagella, Figs. 9.29b

- Not found in most fungi, nematodes, bacteria.
- Cilia and flagella are related structures.
- Cilia shorter, flexes in one direction
- Flagella longer, waveform motion.

Structure of Cilia and Flagella, Fig. 9.31

- Enclosed by plasma membrane
- Core is array of 9 doublet MTs encircling 2 central MTs (9/2)
- Note A and B tubules
- Ciliary dynein extends from As attaching by base to neighboring Bs.
- Originate from basal bodies with 9 triplet tubules (centriole-like)
- Transition zone, 9 doublets, no central tubules

Microfilaments, Figs 9.44, 9.46

- Actin monomers associate in staggered way and may appear as a twisted double helix, but is just one strand.

- Note barbed (+) and pointed (-) ends of monomers; gives polarity.
- Actin-ATP monomers added to plus end faster.
- Gradually dephosphorylated leading to greatest instability at minus end --disassembles there.

Actin is an ATPase. Why?

- G Actin is monomer state; when assembled into microfilaments, then is called F actin.
- Nucleotide state controls assembly
- ATP-actin assembles faster at + or barbed end
- The actin cytoskeleton alone can perform mechanical work because
 - **G-ATP-actin forms F-ATP-actin forms F-ADP-actin**
- This process is exergonic
- Growing at one end and disassembly at the other end is called treadmilling. Filament can appear to move through space.

How does a cell control microfilament growth?

- By sequestering actin monomers --- *monomer binding proteins*
- By capping + (barbed) ends -- + *capping proteins*
- By capping - (pointed) ends -- - *capping proteins*
- By increasing stability-- *microfilament binding proteins*
- By cutting filaments -- *severing proteins*
- By regulating ATP/ADP exchange ($< [ATP]$)

The Actin Cytoskeleton is organized into Bundles and Networks of filaments. Figs. not in text

- In this figure of a cell with filopodia projections, the plasma memb. has been dissolved, the cell dried down onto a surface then processed for scanning electron microscopy by carbon shadowing.

Actin filaments in a migrating fibroblast cell

- Drawing (not in text) shows projections from leading edge of migrating cell. The insets show arrangement of microfilaments in three regions of the cell.
- The arrowhead point toward the plus ends of the filaments.
- Note plus ends are on the leading edge of projections, but have mixed orientation in gel-like cortex.
- Nucleating regions on plasma memb are sites for projections
- Microfilament gel lattice supports opposing force.

Myosin, Fig 9.48

- All motor proteins interacting with actin filaments are in myosin superfamily.
- Motor requires ATP hydrolysis
- Myosin II-muscle, Myosin I-other.
- Most myosins move toward + end of microf.
- Can take big steps with two heads see Fig 9.52.

Muscle contraction from myosin II/ microfilament interaction. (re: Fig 9.61

- If restrained (e.g., in muscle, or when attached) then microfilament may move.

- One headed myosin can move microfilament by rotation at neck upon conformational change driven by ATP hydrolysis.
- Provides “power stroke” due to amplification by large head.
- Supports sliding filament theory of muscle contraction. Note filament length stays constant.

Evolution of the cytoskeleton

- MT and motility are basic structures of all eukaryotic organisms
- There are no distinct intermediates between prokaryotes and eukaryotes
- Three domains or two?

–**Archaea, Eubacteria, Eukarya or**

–**Bacteria/Eukarya (a chimera of Archaea and Eubacteria)**

Lynn Margulis et al promotes two domains. She says: look at cytoskeleton and motor proteins, not ribosome DNA. She says Eukarya is a chimera of prokaryotes not just mitochondria and chloroplast endophytes. One was a transport/motility specialist in addition to the oxygen metabolist that brought mitochondria, and the reducing power specialist that brought plastids.

Deciphering Protein Evolution Re: Barry A. Palevitz paper , 2001

- Endosymbiont events of prokaryotes provided mitochondria and plastids.
- But how was cytoskeleton (compartmentalization and intracellular motility) acquired?
- Found that tubulins share a common ancestor with FtsZ protein (bacterial cell division protein)
- FtsZ protein self assembles into filaments in vitro with added GTP

–**Related in shape to MT but not overall aa sequence**

–**Domains show homology however**

End