Movie GFP mt

MT whole cell

Dynamic whole cell2 ludin5.mov

ANAT3231 - Cell Biology Lecture 6

School of Medical Sciences
The University of New South Wales

Dr Mark Hill
Cell Biology Laboratory
Room G20 Wallace Wurth Building
Email: m.hill@unsw.edu.au
http://cellbiology.med.unsw.edu.au

UNSW Copyright Notice

Movie GFP mt

MT whole cell

Dynamic whole cell2 ludin5.mov

Cytoskeleton

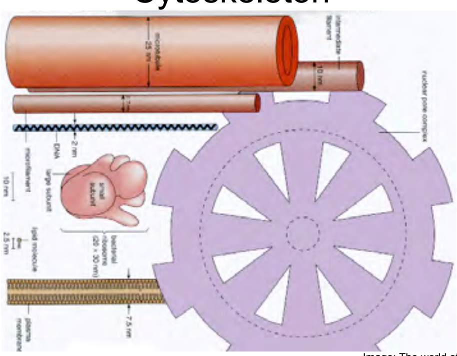
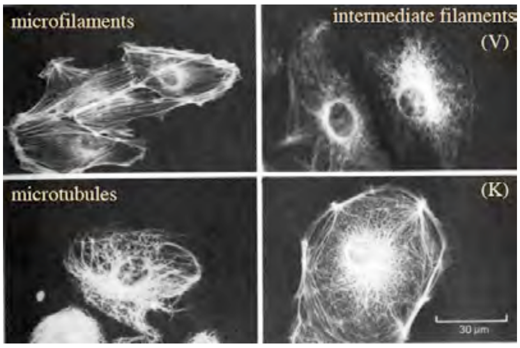


Image: The world of the Cell

Lecture Overview

- Microtubules (mt)
- Structure
- Formation
- Function
  - Will not cover mitosis today
- Polarity
- Turnover
- Associated Proteins (map)
- Motors
- Disorders
  - Alzheimers, cancer therapies

Cytoskeleton

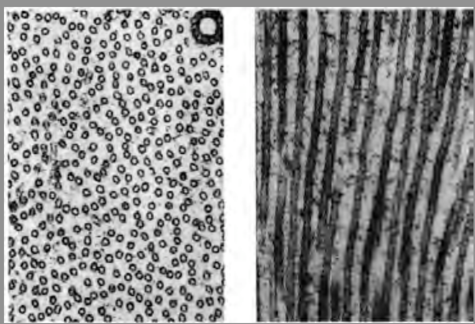


About Microtubules

- Cell organizing role
- Cytoskeleton
  - Largest fibre
  - 25 nm diameter
  - cytoplasmic
- All cells contain
  - Same core structure
  - Same motors
  - Different associated proteins
- Dynamic
  - Continuous remodelling
- Movement
  - Intracellular > cellular
  - Cell division

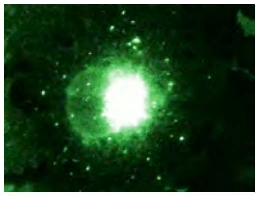
MBoC Fig 16-27 The interphase array of microtubules in a cultured fibroblast.

Microtubules (em)



Motility- Intracellular

- organelle movement
- vesicle transport
- mitosis & meiosis
- chromosome segregation
- gene expression
- transcription factor binding
- mRNA transport
- translation
- protein export
- transmitter release



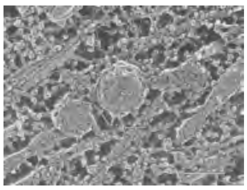
MBoC13_3.mov

Motility- Intracellular

MBoC13_3.mov

EM Axon transported vesicles

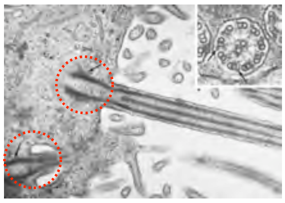
- Axonally transported vesicles and axonal cytoskeleton in longitudinal section
- Arrows point to rod shaped structures
  - appear as cross bridges between organelles and microtubules
    - bar 100 nm



Basic Neurochem Figure 28-7

EM: Basal Bodies of Cilia

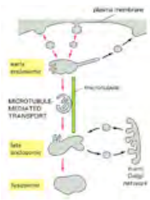
- Surface of ependymal cell
- contains basal bodies
  - red rings
- connected to cilia microtubules
  - longitudinal section
- Inset: cilia in transverse section
  - central doublet of microtubules
  - surrounded by nine pairs
  - one of each pair having a characteristic hook-like appendage (arrows) Å~100,000



Basic Neurochem Figure 1-17.

Endocytic Pathway

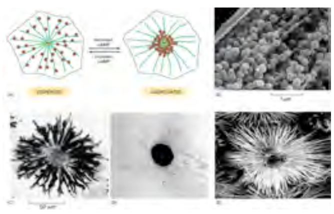
- movement occurs along microtubules and can be blocked with microtubule-depolymerizing drugs



MBoC Figure 13-34.

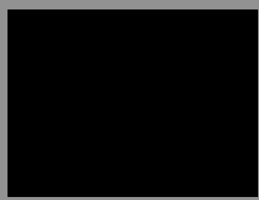
Fish pigment cells

- Changes in skin coloration in several species of fish
- Contain large pigment granules (brown), which change their location in response to neuronal or hormonal stimulus
- Dispersal and aggregation of pigment granules occur along microtubules



MBoC Figure 16-5.

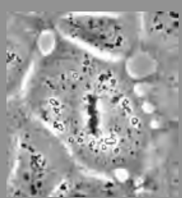
Movie: GFP-Tubulin



MT cell division

Mitosis brand1.mov

Movie: Microtubules and cell division



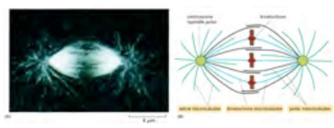
Mitosis

Mitosis mt spindle

Movies: Yu-li Wang's Laboratory
http://ylwang.umassmed.edu/video/index.htm

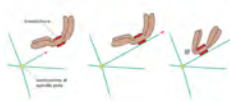
MT mitotic spindle

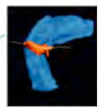
The three classes of microtubules of the fully formed mitotic spindle.



Confocal image of a mitotic spindle at metaphase from a *Drosophila* embryo, with the microtubules fluorescently labeled.

Diagram showing the three classes of microtubules in a spindle.



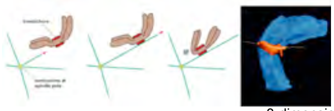


MBoC Figs 18-10, 18-19

MT Capture of kinetochores

Kinetochore binds to side of a growing microtubule and slides along it toward the spindle pole.
Left - *red arrow* shows direction of microtubule growth
gray arrow shows direction of chromosome sliding

Right - 3-dimensional reconstruction (from several thin-sections) of a prometaphase chromosome from a newt. Chromosome ***(blue)*** had started to move toward spindle pole after its kinetochore *(orange)* had attached to a single microtubule ***(white)***.

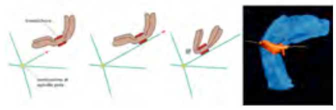


red arrow shows direction of microtubule growth
gray arrow shows direction of chromosome sliding

3-dimensional reconstructio
thin-sections) of a prometap
chromosome from a newt. C
(blue) had started to move

MT Capture of Kinetochores

Kinetochore binds to side of a growing microtubule and slides along it toward the spindle pole.

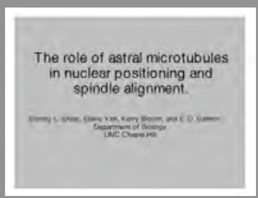


red arrow shows direction of microtubule growth
gray arrow shows direction of chromosome sliding

3-D reconstruction (from thin-sections) of a prometaphase chromosome from a newt. Chromosome ***(blue)*** had started to move toward spindle pole after its kinetochore *(orange)* had attached to a single microtubule ***(white)***.

Image: MBoC Figure 18-19

Movie mt and yeast

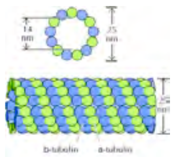


MT and yeast nucleus

Yeast tubes1.mov

MT Structure

- Long hollow tubes
  - 25 nm diameter
- Radiate from forming structure
  - Centrosome
  - Spindle pole
  - Basal Body
- Polarized
  - (+) plus and (-) minus ends
- Formed from Tubulin
  - 55 kD protein

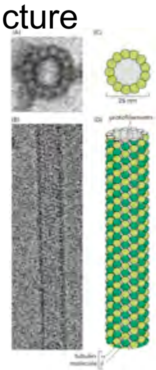


The Cell Fig 11.37. Structure of microtubules

Microtubule Structure

(A) EM of mt in cross-section
- ring of 13 distinct subunits
- each corresponds to a separate tubulin molecule
- an a/b heterodimer

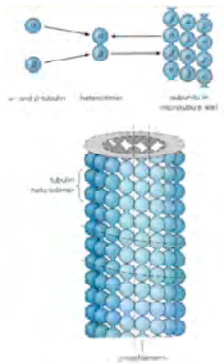
(B) EM of a mt assembled in vitro
(C) 13 molecules in cross-section
(D) side view of a mt
- tubulin molecules aligned into long parallel rows
- 13 Protofilaments
- Each is composed of a series of tubulin molecules, each an a/b heterodimer
  - mt is a polar structure with a different end of tubulin molecule (a or b) facing each end of microtubule



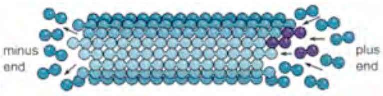
MBoC Fig 16-21. Microtubules

Tubulin Protofilaments

- dimers polymerize to form microtubules
- 13 linear protofilaments
  - head-to-tail arrays of tubulin dimers
  - arranged in parallel
  - assembled around hollow core

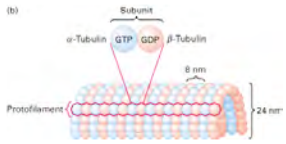


Microtubule Polarity



Tubulin subunits in a MT

- subunits aligned end to end into a protofilament
  - magenta highlight
- side-by-side packing of protofilaments forms wall of the microtubule
- slightly staggered so that a-tubulin in one protofilament contacts b-tubulin in the neighboring protofilaments



An alternative model: protofilaments are staggered by one-half subunit, forming a checkerboard pattern.
In either structure, mt has a structural polarity, addition of subunits occurs preferentially at one end, designated the (+) end.

Mol Cell Biol Fig 19-2. Microtubule structure.

Arrangement of Protofilaments

- Singlet
  - typical microtubule
  - tube built from 13 protofilaments
- doublet
  - additional set of 10 protofilaments
  - form a second tubule by fusing to the wall of a singlet
- Triplet
  - Attachment of another 10 protofilaments

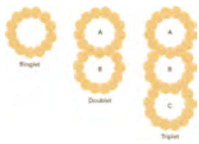


Image: Mol Cell Biol Figure 19-3

Tubulin

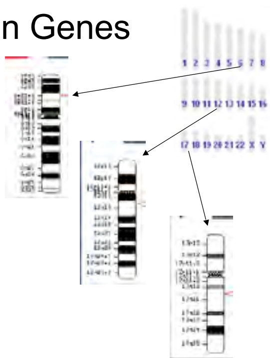
- dimer 55-kd polypeptides
  - α-tubulin (alpha-)
  - β-tubulin (beta-)
- encoded by related genes
- third type of tubulin
  - γ-tubulin (gamma-)
  - at centrosome
  - role in initiating mt assembly

1998

Ribbon diagram based on the 3.7 angstrom resolution model of tubulin developed at Berkeley Lab, shows a dimer consisting of two almost identical monomers. Each is formed by a core of two beta sheets (blue and green) surrounded by helices, and each binds to a guanine nucleotide (pink). In addition to a nucleotide binding site, each monomer also has two other binding sites, one for proteins, and one for the anti-cancer drug taxol.

Tubulin Genes

- human DNA contains about 14 copies per genome of both genes
  - Cleveland et al. (1980)
  - Beta β
    - 6p21.3
    - 15 to 20 members
  - Alpha α
    - mainly Chr.12
    - 15 to 20 dispersed genes
  - Gamma γ
    - 17q21
  - Also tubulin pseudogenes



Tubulin Synthesis Regulation

- autoregulation in animal cells
  - stability of polysome-bound tubulin messenger RNAs
  - beta-tubulin RNAs are selectively targeted as substrates for destabilization
    - not recognition of specific RNA sequences
  - co-translational recognition of amino-terminal beta-tubulin tetrapeptide after its emergence from ribosome
    - This motif could also be used in other systems where RNA degradation is coupled to ribosome attachment and translation

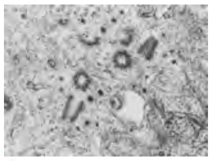
Nature 1988 Aug 18;334(6183):580-585

Tubulin Homology

- homology with a 40,000-MW bacterial GTPase, called FtsZ
  - bacterial protein has structural and functional similarities with tubulin
    - the ability to polymerize and a role in cell division
  - protein carrying out these ancestral functions in bacteria was modified during evolution to fulfill diverse roles of microtubules in eukaryotes?

MT Formation- Centrosome

- slow-growing minus end of each MT is embedded in the centrosome matrix surrounding a pair of centrioles
- matrix determines the number of MTs in a cell
  - By nucleating growth of new microtubules



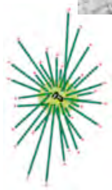
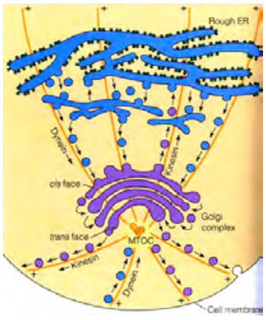
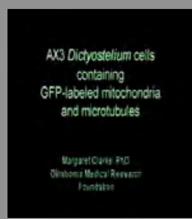


Image: MBoC Fig 16-3. Centrosome with attached microtubules

Microtubule Organization



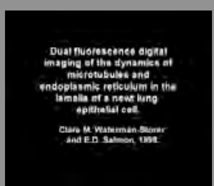
Movie: Microtubules and Mitochondria



MT and mitochondria

mitochondria in dicto.mov

Movie: Microtubules and Endoplasmic Reticulum



MT and ER

mk1280757005.mov

Centrosome Cycle

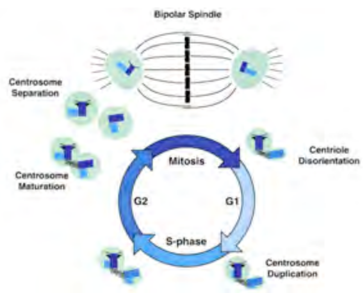
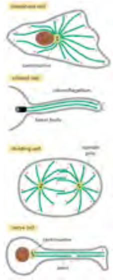


Image: Meraldi & Nigg, The centrosome cycle.FEBS Lett. 2002 19;521(1-3):9-13

Orientation of MTs in Cells

- (-) Minus ends of MTs generally embedded in a microtubule-organizing center (mtoc)
  - alpha
- (+) plus ends often located near the plasma membrane
  - beta

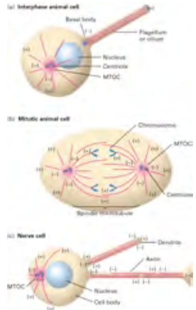


MBoC Fig 16-26. Orientation of microtubules in cells.

Orientation of cellular mt

(a) **Interphase animal cells**: (-) ends of most mts are proximal to the MTOC. MTs in flagella and cilia have their (-) ends continuous with the basal body, acts as the MTOC in these structures.
(b) **Cell enter mitosis**: microtubule network rearranges, forming a mitotic spindle. (-) ends of all spindle mts point toward one of the two MTOCs, or poles, as they are called in mitotic cells.
(c) **Nerve cells**: (-) ends of axonal microtubules are oriented toward the base of the axon.
- dendritic mts have mixed polarities.

- **Plant cells**, contain numerous MTOCs, microtubules line the cell cortex. Webs of microtubules cap growing ends of a plant cell.



Mol Cell Biol Fig 19-7. Orientation of cellular microtubules

Neuron- Axon and Dendrites

•**axon and dendrite cytoskeletons differ in composition and organization**
• cytoskeletal proteins are synthesized on free polysomes in cell body
  • then transported to their different cellular compartments
• except MAP2
  • some synthesized in cell body and MAP2 mRNA is transpoirted into dendrites to be synthesized locally
• MTs of cell bodies, dendrites and axons nucleated at microtubule-organizing center *(MTOC)*
  •then released and delivered to either dendrites or axon
• dendrites
  • microtubules have mixed polarities

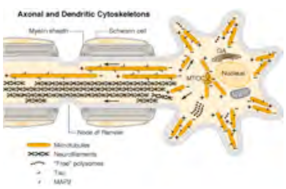
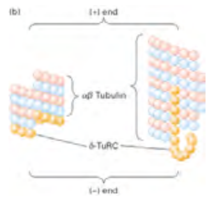


Image: Basic Neurochem Figure 8-3

Gamma-Tubulin-mediated assembly of microtubules

- models for g-tubulin-mediated assembly of microtubules
- alternative models
  - gamma -TuRC nucleates microtubule assembly either by
  - left- presenting a row of gamma -tubulin subunits
  - right- forming a protofilament, which can directly bind a/b-tubulin subunits
    - modified from C. Wiese and Y. Zheng, 1999, Curr. Opin. Struc. Biol. 9:250–259.



Mol Cell Biol Fig 19-8. Gamma-Tubulin-mediated assembly of microtubules

MT Dynamic Instability

- continual and rapid MT turnover
  – half-lives of only several minutes
- this rapid turnover critical for remodeling of the cytoskeleton during mitosis
  - Tim Mitchison and Marc Kirschner (1984)

Movie: GFP mt

MT dynamics

Dynamics ludin1.mov

Movie: GFP mt

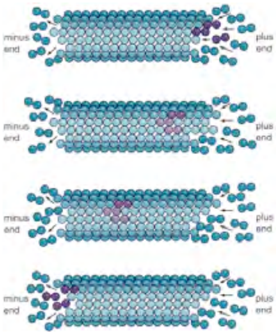
MT leading edge

MT severing

MT Treadmilling

- Treadmilling
  - dynamic behavior when tubulin bound to GDP continually lost from minus end
  - replaced by the addition of tubulin bound to GTP to plus end of same microtubule
  - GTP hydrolysis also results in dynamic instability
    - individual microtubules alternate between cycles of growth and shrinkage

Microtubule Movement



GTP hydrolysis destabilizes MTs

- Addition of tubulin adds GTP to end of protofilament
  - grows in linear conformation readily packed into MT wall
  - becoming stabilized
- Hydrolysis of GTP
  - changes subunits conformation
  - force protofilament a curved shape
  - less able to pack into the MT wall
  - protofilaments with GDP-containing subunits forced linear conformation by lateral bonds within MT wall, mainly in stable cap of GTP-containing subunits

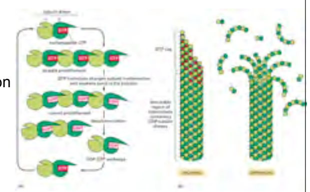
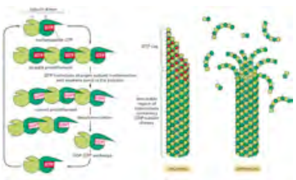


Image: MBoC Fig 16-33

GTP hydrolysis destabilizes MTs

- GTP cap loss
  - GDP-containing protofilaments relax to curved conformation
  - Leads to progressive disruption of MT
  - eventual disassembly of protofilaments
  - into free tubulin dimers



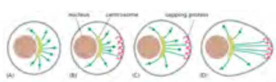
MBoC Fig 16-33

MT stability and cell polarity

Model

A newly formed MT will persist only if both of its ends are protected from depolymerizing.

Minus ends of MTs are protected by organizing centers from which these filaments grow. **Plus ends** are initially free but can be stabilized by other proteins.



(A) nonpolarized cell with new MTs growing and shrinking from a centrosome in all directions randomly.

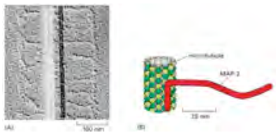
(B) The array of MTs encounters structures in a specific region of the cell cortex that can cap (stabilize) the free plus end of the MTs.

(C and D) The selective stabilization of those MTs that happen by chance to encounter these structures will lead to a rapid redistribution of the arrays and convert the cell to a polarized form

Image: MboC Fig 16-34

Microtubule Associated Proteins

- Many different
- Neurons
  - MAPs
  - Tau



(A) EM shows regularly spaced side arms formed on a MT by a large MAP-2 from brain. Portions of the protein project away from the MT, as shown in (B).
(EM courtesy of William Voter and Harold Erickson.)

MBoC Fig 16-35 microtubule-associated protein.

MAPs-MAP2

- 2q34-q35
- Neuron expression
- MAP2
  - a 280-kD protein
  - concentrated in neuronal soma and dendrites
  - Developmentally regulated expression

OMIM http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?157130

MAP2 Developmental Expression

(rat)

- MAP2B
  - present throughout brain development
- MAP2A
  - appears during end of second week of postnatal life
- MAP2C
  - present during early brain development
  - disappears from the mature brain
    - except for the retina, olfactory bulb, and cerebellum
  - MAP2A and MAP2B
    - encoded by 9-kb mRNAs
  - MAP2C
    - encoded by a 6-kb mRNA

MAPs- Tau

- Gene 17q21.1
- Mr 45-60 kDa
- Neuron Expression
  - Enriched in axons
  - phosphorylated

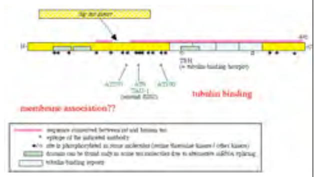


Image: HotMolecbase

Tau- Alzheimer Disease

- neuronal cytoskeleton is progressively disrupted and replaced by tangles of paired helical filaments (PHFs)
- PHFs are composed mainly of hyperphosphorylated forms of tau

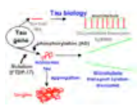


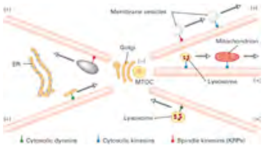
Image: Nature July 2003
Image: www.alzheimers.org/pubs/conv09n4.html
Image: www.medsch.wisc.edu/path703/slide/lectslides/cnsdegen.html

Tau- Alzheimer disease

- Elevated tau inhibit intracellular transport
  - mainly plus-directed transport by kinesin motors
    - from center of cell body to neuronal processes
  - organelles are unable to penetrate the neurites
    - peroxisomes, mitochondria, and transport vesicles carrying supplies for the growth cone
  - Leads to
    - stunted growth
    - increased susceptibility to oxidative stress
    - pathologic aggregation of proteins such as amyloid precursor protein (APP)
  - tau:tubulin ratio is normally low
    - increased levels of tau become detrimental to the cell

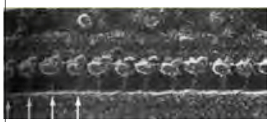
From OMIM: Stamer et al. (2002)

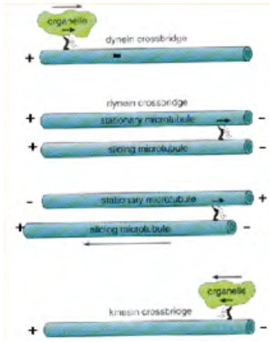
Motor-mediated Transport



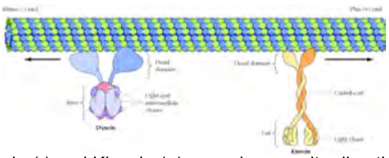
Mol Cell Biol Figure 19-26.

Microtubule Motors





Microtubule Motor Proteins



- Dynein (-) and Kinesin (+) move in opposite directions
  - globular heads of heavy chains bind mts
  - motor domains
- Dynein
  - 2 or 3 heavy chains (two are shown here)
  - multiple light and intermediate chains
- Kinesin
  - 2 heavy chains, wound around each other in a coiled-coil structure
  - 2 light chains

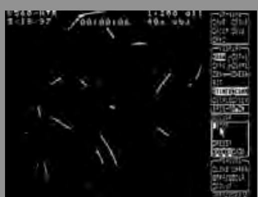
The Cell Fig 11.45. Microtubule motor proteins

Movie: Kinesin on Microtubule

Animated model for processive motion by conventional kinesin based upon atomic structures representing different nucleotide states.

Vale, R.D., Milligan, R.A. (2000) Science 288: 88-95

Movie: Microtubules in vitro

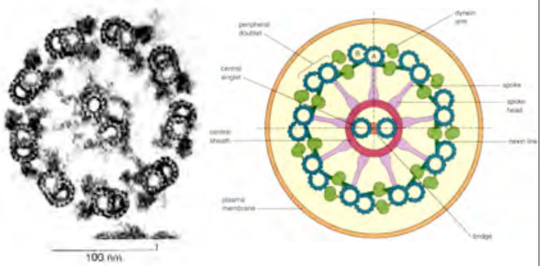


Movie of in vitro microtubule gliding Kinesin, expressed in bacteria, is absorbed onto the surface of a glass slide. Rhodamine-labelled microtubules and ATP (1mM) are introduced, and the adsorbed motors transport the microtubules across the surface. Motors are randomly adsorbed, but only motors that are oriented properly with respect to the microtubule axis are able to produce motion.

http://valelab.ucsf.edu/images/mov-invitmtglid.mov

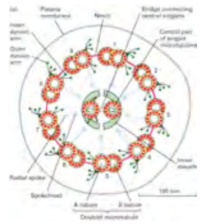
Flagella

Flagella



Ciliary and Flagellar Axonemes

- 9 + 2 MT arrangement
- dynein arms and radial spokes with attached heads occur at intervals along the longitudinal axis
- central microtubules, C1 and C2

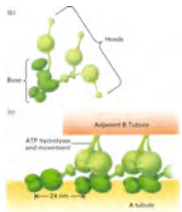


Cross-sectional of a typical flagellum

Mol Cell Biol Figure 19-28

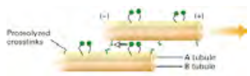
Axonemal Dynein

- arrangement of globular domains and short stalks
  - attachment of outer dynein arm to the A tubule of one doublet and cross-bridges to B tubule of an adjacent doublet
  - attachment to A tubule is stable
- In presence of ATP
  - successive formation and breakage of cross-bridges to adjacent B tubule leads to movement of one doublet relative to the other



Mol Cell Biol Figure 19-32.

Dynein-mediated sliding of axonemal mt

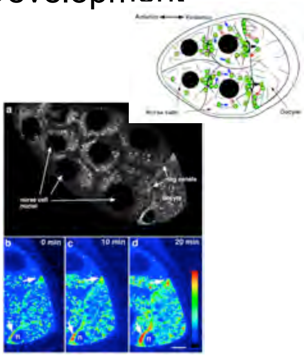


- Dynein arms attached to A subfiber of one MT walk along B subfiber of adjacent doublet toward its (-) end (small arrow), moving this microtubule in the opposite direction (large arrow)
- When nexin cross-links are broken, sliding can continue unimpeded

Image: The Cell Fig 19-31

MTs In Development

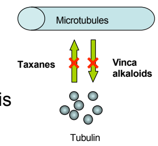
- nurse cells in insect ovarioles
  - supply developing oocytes with cellular components
    - mRNAs, proteins
  - pass from one cell to another through intercellular bridges traversed by microtubules
    - mRNAs encode axis-determining factors in Drosophila embryos
  - mRNAs are further translocated and localized within oocyte to sites where products of their translation will function



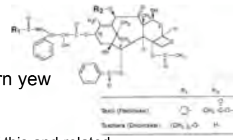
From: Development 125, 3655-3666 (1998)

MT Drugs and Cancer

- drugs affect microtubule assembly
  - experimental tools in cell biology
  - treatment of cancer
- Colchicine and Colcemid
  - bind tubulin
  - inhibit mt polymerization, blocks mitosis
- Vincristine and Vinblastine
  - cancer chemotherapy
  - selectively inhibit rapidly dividing cells
- Taxol
  - stabilizes microtubules rather than inhibiting their assembly
  - also blocks cell division



Taxol (Paclitaxel)



- 1971 from the bark of the Western yew
  - Taxus brevifolia Nut (Taxaceae)
  - antitumor and antileukemic activity
  - found in roots, leaves, and stems of this and related members of yew family
- complex ester
  - an oxetan ring attached to a derivative of taxane
- tool for investigating MT function
- clinical trials in a variety of cancers
  - Initial development limited by low abundance in yew trees
  - now novel synthetic methods
  - identification of new sources of taxanes

References

- ANAT3231 Lecture
  - http://cellbiology.med.unsw.edu.au/units/science/lecture0506.htm
- Essential Cell Biology
  - Ch16, p518-527
- Molecular Biology of the Cell
  - Ch16, p803-820
- The Cell
  - Ch8
- Molecular Cell Biology
  - Ch19

Online References

- Medline
  - 23,653 Refs, 121 Refs (review, 1 year)
- OMIM
  - Beta Tubulin
    - http://www.ncbi.nlm.nih.gov:80/entrez/dispomim.cgi?id=301850
  - Gamma Tubulin
    - http://www.ncbi.nlm.nih.gov:80/entrez/dispomim.cgi?id=191135
  - Alpha Tubulin
    - http://www.ncbi.nlm.nih.gov:80/entrez/dispomim.cgi?id=602529
  - Map2
    - http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=157130
  - Tau
    - http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=157140
- Tau
  - http://bioinformatics.weizmann.ac.il/hotmolecbase/entries/tau.htm