Lecture Overview

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

References

- ANAT3231 Lecture
  - http://cellbiology.med.unsw.edu.au/units/science/lecture06.htm
- Essential Cell Biology
  - Ch16, p518-527
- Molecular Biology of the Cell
  - Ch16, p803-820
- The Cell
  - Ch8
- Molecular Cell Biology
  - Ch19
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

Microtubules (em)

Motility- Intracellular

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

EM Axon transported vesicles

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

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)
  - 100,000 Å

Endocytic Pathway

- Endocytic movement occurs along microtubules
  - can be blocked with mt-depolymerizing drugs
Fish Pigment Cells

- Changes in skin coloration in fish
  - Contain large pigment granules (brown)
  - Change location in response to neuronal or hormonal stimulus
  - Dispersal and aggregation of pigment granules occur along MTs

Movie: Microtubules and Cell Division

MT Mitotic Spindle

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

MT Capture of Kinetochores

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

Movie: MT and Yeast

The role of astral microtubules in nuclear positioning and spindle alignment.

MT Structure

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

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Microtubule Structure

(A) EM of mt in cross-section
- ring of 13 distinct subunits
- Each a separate tubulin molecule
- an alpha/beta 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

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

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

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

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

Image credit: Nature 1998
**Tubulin Genes**

- human DNA contains about 14 copies per genome of both genes
  - Cleveland et al. (1980)
    - Beta β
      - 6q21.3
      - 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 mRNAs
  - beta-tubulin RNAs
    - selectively targeted as substrates for destabilization
    - not recognition of specific RNA sequences
  - co-translational recognition of amino-terminal beta-tubulin tetrapeptide after emergence from ribosome
    - Motif could be used in other systems where RNA degradation is coupled to ribosome attachment and translation

**Tubulin Homology**

- FtsZ
  - bacterial GTPase (40,000 Mr)
  - bacterial protein has structural and functional similarities with tubulin
  - 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 MT embedded in centrosome matrix surrounding a pair of centrioles
- matrix determines number of MTs in a cell
  - By nucleating growth of new MTs

**Microtubule Organization**

**Movie: Microtubules and Mitochondria**

MT and mitochondria
Movie: Microtubules and Endoplasmic Reticulum

MT and ER

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

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 enters 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.

Neuron- Axon and Dendrites

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 αβ-tubulin subunits

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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)

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

Movie: GFP mt

MT dynamics

Movie: GFP mt

Movie: GFP mt

Movie: GFP mt

Microtubule Movement

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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

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

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.)

MAPs-MAP2

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

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

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

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

Motor-mediated Transport

- 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

Microtubule Motors

- The Cell Fig 11.45. Microtubule motor proteins

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Movie: Kinesin on Microtubule

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

Movie: Microtubules in vitro

Movie of in vitro microtubule gliding: kinesin, expressed in bacteria, is adsorbed onto the surface of a glass slide. Kinesin motility experiments are performed with an introduction of ATP and rhodamine-labelled microtubules. Motors are randomly adsorbed, but only motors that are oriented properly with respect to the microtubule axis are able to produce motion.

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

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
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

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

Online References

- Medline
  - 23,653 Refs, 121 Refs (review, 1 year)
- OMIM
  - Beta Tubulin
  - Gamma Tubulin
  - Alpha Tubulin
  - Map2
  - Tau
- Tau
  - http://bioinformatics.weizmann.ac.il/hotmolecbase/entries/tau.htm