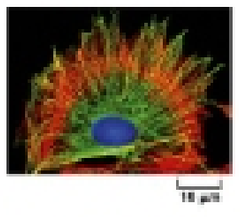
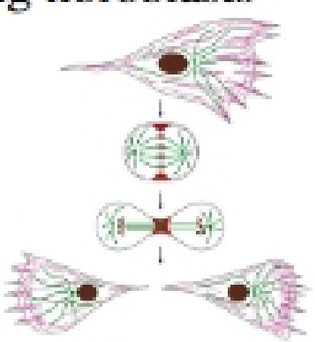
Part 3 (final exam)

The cytoskeleton- assembly and dynamic structure

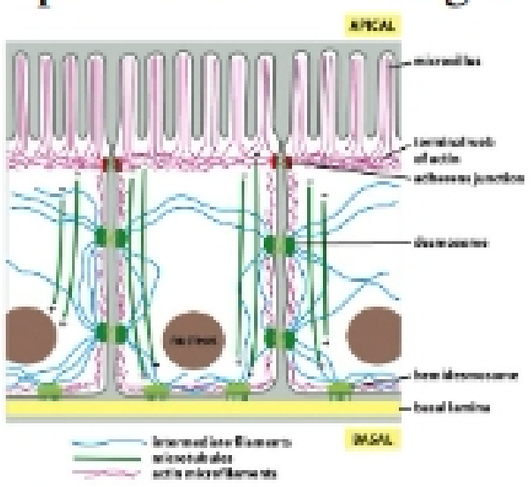
- Functions (movement and structure)
    - 1. Maintaining and directing cell structure
    - 2. Intracellular support
        - Organelle movement
        - Vesicle movement
    - 3. Spatial organization of cell
    - 4. Contractility and Mobility
        - Actin/myosin contraction
        - Movement of cilia and flagella
        - Chromosome movement
        - Cell migration- amoeboid movement
- Studying the cytoskeleton
    - **Fluorescence Microscopy**- to see the fixed structure
        - Immunofluorescence: use of antibodies against cytoskeleton proteins
            - Stain a fixed section of the cell with antibodies
                - The ones tagged with red proteins are directed against actin
                - Green- tubulin
                - Blue- nucleus
        - 
        - Use of fluorescently-tagged drugs that bind to cytoskeleton proteins
            - E.g. phalloidin- binds to actin filaments
    - **Video-enhanced light microscopy**- can detect presence of tubules and filament bundles, and follow movement of vesicles in living cells (but can't resolve details of structure)
    - **Electron Microscopy**- especially of freeze-etched surfaces
    - **Genetic engineering**- to study the FUNCTION of the cytoskeleton
        - The development of genetically engineered organisms or cell lines that lack a cytoskeletal or motor protein (knockout mutants) has been enormously productive
            - Mouse embryos that lack a motor protein called cytoplasmic dynein fail to develop beyond 8 days sue to dispersion and fragmentation of the Golgi complex, suggesting a role for dynein in maintaining the structural integrity of the Golgi
                - Golgi breaks into vesicles in the absence of dynein

- Cell lines or organisms can be engineered to over-express an artificially-constructed "dominant negative" form of a cytoskeletal protein.
    - "**Dominant-negative**" proteins are non-functional mutant proteins that compete with the native protein and inhibit its function
        - Interferes with the function of a normal protein
        - If you are able to develop and express this protein, you can knock out the function of the protein you are targeting without knocking out the gene of the protein you are targeting
    - Cells expressing the dominant negative protein show defects in the specific functions of the protein similar to those of knockout mutants
- Cells that are engineered to express "small interfering" RNA sequences (**siRNA's**) targeted to be complementary to the mRNA's of specific cytoskeletal proteins may also show defect in functions associated with those proteins
    - The siRNA binds to the specific mRNA that has the complementary sequence, promotes its destruction and silences the translation of the protein that the mRNA specifically codes for
    - siRNAs can trigger the destruction of mRNA

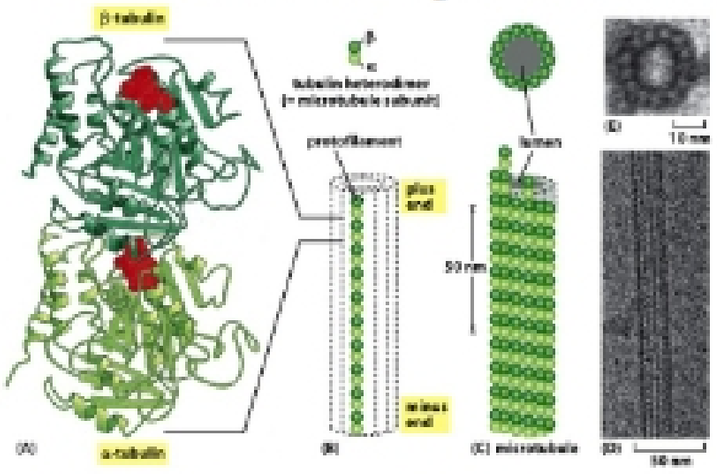
- Cytoskeleton is formed from:
    - 1. **Microtubules**
    - 2. **Intermediate filaments**
    - 3. **Microfilaments**
- Cytoskeleton can be dynamic (changing) or stable:
    - Dividing fibroblasts



- Stable- Epithelial cells in the gut



- **Mictotubules**
    - Large arrays of rigid microtubule bundles help maintain the overall shape of many cell types

- Maintain the internal organization of cells
  - Treatment with microtubule-disorganizing drugs can seriously disrupt organelle distribution
    - i.e. the Golgi is dispersed throughout the cell, instead of being localized just outside the nucleus
    - In plant cells, microtubule bundles at the edge of the cytoplasm direct cellulose deposition in the neighboring cell wall by influencing the positioning of cellulose-synthesizing enzymes
- Important in maintaining the shape of very elongated cell extensions or processes
  - i.e. Axons of nerve cells- treatment with microtubule-disorganizing drugs causes collapse of growing axons in the developing nervous system
- Form the:
  - Mitotic spindles of dividing cells
  - The core of cilia and flagella
  - A network of rigid tubules that radiate through the cytoplasm of all eukaryote cells
- Formed from alpha and beta tubulin
  - Globular proteins- approx. 50kDa M.W.
- Structure:
  - A rigid tube, whose wall is made from approx. 13 protofilaments, composed of alternating alpha and beta tubulins
  - Approx. 25 nm in diameter and up to a micron or more in length
  - The filament is polarized, with a (+) and a (-) end
    - (+) end is composed of a row of beta tubulins; grows more rapidly
    - (-) end is composed of a row of alpha tubulins
  - 
- Proteins that bind to microtubules:
  - 1. Motor proteins- kinesins and dyneins
  - 2. MAPS (microtubule associated proteins)- proteins that cross-link MT's or regulate their assembly
    - MAP2- its long projecting arm creates bundles of widely-spaced microtubule arrays
    - Binding of MAPS to microtubules is often regulated by their phosphorylation