1 Cytoskeleton and Interactions Between the Cells and the Environments

1.1 Review

Microfilaments/Actin filaments are involved in cell motility, contractile activity and cytoknesis. The motor protein used is myosin.

1.2 Structure of Actin Filaments

Actin filaments are composed of actin monomer, with two protofilaments twisted in a right-handed helik. Actin filaments are polar due to the regular orientation of actin monomers in each protofilament.

1.3 Actin Monomers

- Free monomers are bound to ATP, itself constricted in the centre of the protein.
- Actin is an ATPase, and thus hydrolyses ATP. ADP remains bound after hydrolysis.
- ATP hydrolysis occurs more rapidly after actin monomers have been incorporated into the filament.
- Growth of the filament is faster at the plus end.
- Actin filaments have an ATP cap.
- ATP hydrolysis decreases the strength of binding between monomers in the filament.

1.4 Differences between Microtubules and Actin Filaments

Microtubules are heterodimers, actin filaments are monomers.

Microtubules have GTP cap, while actin filaments have ATP cap.

Microtubules bind to GTP, actin filaments to ATP.

Microtubular T and D form heterodimers, T and D of actin filaments form monomers.

In microtubules 13 parallel protofilaments forming a cylinder, in actin filaments 2 protofilaments twist around each other.

1.5 Actin Polymerisation In Vitro

Actin subunits in filaments all start as monomers. When salt is added, oligomers form and the lag phase is entered, while later an actin filament starts to grow in the growth phase. Then a steady state is reached, for which a continuous supply of ATP is required, with subunits of actin filaments coming on and off at the same rate.

The concentration of monomers at which the equilibrium phase persists is denoted as C_c.

Note that the plus end has a higher affinity for actin monomers than the minus end.

Suppose that a high concentration of radioactive monomers is added to a seed of actin filament.

There is a net addition on the plus and minus side, with more of it added on the plus end.

The actin filament gets longer, and thus the concentration of free monomers drops.

There is still a net addition on the plus end, while the minus end stays about the same.

The concetration drops again, and then the actin filament reaches the equilibrium phase.

At this point, treadmilling occurs, and thus, while the old monomers belonging to the seed are still removed, eventually all the old monomers are lost in the D-form.

1.6 Summary of Motor Proteins

- Actin motor protein is Myosin II, plus end directed (as in muscles)
- Microtubule motor proteins are dynein, minus end directed, and kinesin, plus end directed (as used for intracellular transport)

They all couple ATP hydrolysis with conformational changes to generate force.

All move in a specific direction along filaments that have polarity (plus/minus end).

1.7 Intermediate Filaments

Intermediate filaments are involved in structural support, they are tough, flexible and extensible.

However, they are not found in plants, and not all animal cells have them.

These filaments are prominent in cells under great mechanical stress.

1.7.1 Intermediate FilamentStructure

Coiled coil dumer forms a staggered antiparallel tetramer without polarity and without known motor proteins.

1.7.2 Kerating Filaments in Epithelial Cells

Epithelial line surfaces, cavities and organs.

Filaments in each cell are anchored at sites of cell-cell contact by desmosomes and provide mechanical strength.

1.8 Junctions

Cells interact with each other and the extracellular matrix to form tissues.

One of such interactions is via **junctions**:

- Anchoring junctions
- Occluding junctions
- Channel-forming junctions
- Single-relaying junctions (synapse)
- Tight junctions.

- Occluding junctions
- Adherence junctions
- Gap junctios
- Desmosomes

In polarised epithelial cells (most mature epithelial cells are polarised epithelial cells).

In polarised epithelial cells junctions are arrangad in a specific order. Other cells can also have junctions, but polarised epithelial cells have all of these junctions.

1.8.1 Tight Junctions

Tight junctions create a right seal bwtween cells by pveventiot of mixing with the extracellular envornments.

Tight junctions act as fences in the membrane.

1.8.2 Anchoring Junctions

- Cell-cell anchoring junctions and desmosomes
- Cell-matrix anchoring junctions

Adhesion and anchor proteins link cytoskeletal filaments of neighbouring cells.

Adhesion proteins are transmembrane proteins, with the extracellular domains interacting with adhesion proteins and extracellular matrix. Intracellular domains interact with anchor proteins.

Anchor proteins link the adhesion proteins to cytoskeletal filaments.

1.8.3 Adherens Junctions

Adherens junctions form the adhesion belt which encircles the inside of the plasma membrane. At adherens junctions cadherin proteins from neighbouring cells interact with each other.

Actin is tethered to cadherin by anchor proteins.

There are many types of cadherin proteins, and one type of cadherin protein will bind to the same type of cadherin proteins.

1.8.4 Adherens junction formation

Cadherin proteins become concentrated on touch.

Interactions of cells with each other are not simply structural.

Cells sort themeselvels into layers because difeferent cadherin proteins are expressed in each cell type. Cadherin form homotypic junctions, as shown by the classical experiment of *sorting out*, when mesoderm and ectoderm were mixed and then observed to self-assemble into layers.

1.8.5 Desmosomes and Hemidesmosomes

Link intermediate filaments provide the most sturctural strength. Desmosomes, in turn, provide the link to a neighbouring cells.

The structure of desmosomes isdetermined by desmoglein and desmocollin adhesion proteins.

Anchor proteins (plakoglobin, desmoplakin) link the adhesion proteins to intermediate filaments.

1.9 Channel Forming Junctions: Gap Junctions

These types of junctions allow for communcation between cells and are composed of connexin proteins.

A gap junction is made up of connexin proteins, of which 6 come together to form connexons, of which there are two in the junction.

Gap junctions often form plaques.

Gap junctions electrically and metabolically allow the passage of ions and metabolites at more than 1000 daltons, which involves the passage of cAMP, nucleotides, glucose and amino acids. Macromolecules and proteisns, however, do not go through.

Gap junctions are gate, and can be in an open or closed state. One connexon on its own is usually closed. Adramatic increase in cytosolic Ca^{2+} will close gap junctions.

Why does Ca^{2+} have such an effect?

Suppose there are two cells with open gap junctions. Assume one cell membrane is destroyed, and thus the calcium ions from cytosole get in and thus its concentration becomes abnormally high, which make metabolites leak – better close the gate.

1.10 Plant Cell Wall

Plant cells produce and deposit their cell wall. It is composed of cellulose and pectin, and more rigid than the animal tissues.

Basal lamina (also called basemement membrane) is a specialised extracellular matrix, beneath which there is connective tissue.

Epidermis is the epithelial tissue of a skin.

Dermis is the connective tissue of a skin.

1.10.1 Epithelial vs Connective Tissue

In epithelial tissue there is a thin basal lamina, while there is plentiful extracellular membrane in connective tissue.

Epithelial cells are attached to each other, while connective tissue cells are attached to the matrix.

1.10.2 Extracellular Matrix

- Connective tissues
- Not a static structure
- Different composition of ECM in tissues give them specific properties

Transmembrane proteins help cells interact with ECM.

1.10.3 Collagen

- Resists pulling forces
- Secreted by fibroblasts, epithelial and smooth muscle cells

1.10.4 Proteoglycans and Glycoproteins

In proteoglycans there is at least one sugar side ghan which is a GAG.

Glycoproteins have any sugar attached.

Thus, all proteoglycans are glycoproteins, but not all glycoproteins are proteoglycans.

Example 1.1

Aggrecan and decorin are proteoglycans of the ECM.

Ribonuclease is a secreted glycoprotein.

Proteoglycans are space filling.

Glycosaminoglycans (GAG) is a long, linear, chains of a repeating disaccharide (at least one aminosugar) which are highly negatively charged (and thus attract Na^+ and water).

An example of GAG is hyaluronan.

It is relatively simple, with a long chain of disaccharide subunits (up to 25000). They can be linked to aprotein but often they are not. Most GAG are synthesised inside the cell on proteins and then exocytosed. Hyaluronan is spun directly from the cell surface.

Another example is elastin, networks of which stretch and relax like a rubber band. Other components (cross-links, which are often modified glyin) of the ECM provide strength preventing the spontaneous stretching.

Fibronectin binds collagen and cell surface receptors. It is a dimer linked by disulfide bonds, with the RGD site being the cell binding site.

Each protein is composed of several linked domains, ecah domain having a different structure.

The basement membrane is a special type of ECM, and underlies all epithelia. It is thin, but the tissue of the basal lamina secretes ECW and influences cell polarity.

Basal lamina surrounds muscles, fat cells and Schwann cells, playing the structural role. In the kidney it divides two cell sheets.

The basement membrane is an attachment site for epithelial cells.

Laminin organizes the basement membrane by interacting with other components of the ECM and integrin in the plasma membrane.

A laminin protein is often in ECM and is involved in cell migration. It is p rimer of α-laminin, β-laminin and γ-laminin.

Hemidesmosomes spot-weld epithelial cells to the basal lamin, with keratin filaments inside the cell linked to laminin in the ECM through interaction with the transmembrane protein integrin.

Integrins interact with laminin to organize the basement membrane.