CHAPTER 7

INTERACTIONS BETWEEN CELLS AND THEIR ENVIRONMENT

OBJECTIVES

- Define the general structure and function of the glycocalyx and extracellular matrix.
- Describe the function and structure of basement membranes (basal lamina).
- Describe the structures of the components of the extracellular matrix (collagen, GAGs, proteoglycans, glycoproteins) and differentiate between them.
- Clarify the steps involved in the adhesion of cells to a noncellular surface.
- Describe the membrane proteins involved in the adhesion of cells to noncellular surfaces.
- Compare and contrast the structures and functions of the different cell junctions in plants and animals.
- Describe the membrane proteins involved in cell-cell adhesion.
- Contrast the structures of plant and bacterial cell walls despite the similarities in their function.

LECTURE OUTLINE

Introduction

I. Materials present outside the plasma membrane play an important role in the cell's life
   A. Most cells in multicellular plants & animals are organized into clearly defined tissues in which component cells maintain a defined relationship with one another
      1. They also maintain a relationship with the extracellular materials that lie between the cells
      2. Even cells that are not found in solid tissue (white blood cells) must interact in highly specific ways with other cells & extracellular materials with which they come into contact
   B. These interactions regulate a number of diverse activities: cell migration, cell growth, cell differentiation
      1. They also determine the 3D organization of tissues & organs that emerges during embryonic development

II. Example of cell interactions in a tissue – human skin
   A. The skin's outer layer (epidermis) is a type of epithelial tissue (epithelia line spaces within the body)
      1. Epidermis consists largely of closely packed cells attached to one another & to an underlying noncellular layer by specialized contacts
      2. These contacts provide a mechanism for cells to adhere to & communicate with one another
   B. The deeper layer of the skin (dermis) is a type of connective tissue
      1. Like other connective tissues (tendons, cartilage), dermis consists largely of extracellular material, including a variety of distinct fibers that interact with each other in specific ways
      2. Cells (fibroblasts) are scattered throughout the dermis; the outer surface of their membranes contains receptors that mediate interactions between the cell & components of its environment
      3. The cell surface receptors interact not only with external surroundings, but are connected at their internal ends to various cytoplasmic proteins
      4. Such receptors exhibiting dual attachment are well suited to transmit messages between the cell & its environment

The Extracellular Space: Background

I. Glycocalyx (cell coat) - mediates cell-cell & cell-substratum interactions; mechanical protection for cells; barrier to particles moving toward plasma membrane
   A. Made of short sugar chains (oligosaccharides); project outward from virtually all integral proteins & some lipids in plasma membrane; closely applied to outer surface of plasma membrane
   B. Also contains additional extracellular materials secreted by cell into external space, where they stay closely associated with cell surface
   C. Very prominent in some types of cells like epithelial cells lining mammalian digestive tract

II. Extracellular matrix (ECM) - organized network of extracellular materials found beyond the immediate vicinity of membrane
   A. More than inert packing material or nonspecific glue that holds cells together; it plays a key regulatory role in determining cell shape & activities
      1. Experiment: digest ECM surrounding cultured cartilage or mammary gland epithelial cells with enzymes —> get decrease in secretory & synthetic activities of cells
      2. Add back ECM materials into culture —> restores differentiated state & cells produce usual products
   B. May consist of ill-defined, amorphous associations of proteins & polysaccharides (like loose connective tissue) or may be in the form of a distinct structure

III. ECM takes diverse forms in different tissues & organisms, but is composed of similar proteins
   A. Most proteins in cells are compact & globular; those of extracellular space are extended & fibrous
   B. Among their diverse functions, ECM proteins serve as scaffolds, girders, mortar & wire
   C. Alterations in amino acid sequence of extracellular proteins can lead to serious disorders

IV. ECM very prominent in connective tissues (cartilage, bones, tendons, corneal stroma)
   A. In connective tissue, cells occupy a small fraction of tissue volume
   B. ECM, not cells, gives tissues their identifiable properties: bone matrix hardness, cartilage matrix toughness & flexibility, tendon matrix tensile strength, corneal stroma matrix transparency

V. Components of the ECM - members of a small number of molecular families
   A. Collagens – one of most important & ubiquitous ECM molecules; fibrous glycoprotein family
      1. Functions only as part of ECM & only found there
   B. Proteoglycans - protein-polysaccharide complex
   C. Fibronectin, Laminin, ECM Proteins

The Extracellular Space: Basement Membrane as an Example of ECM

I. Basement membrane (basal lamina); a continuous ~50 - 200 nm thick sheet; one of best defined examples; found in the following places:
   A. It surrounds muscle & fat cells
   B. It underlies basal surface of epithelial tissues (skin epidermis, digestive & respiratory tract linings)
   C. It underlies the inner endothelial lining of blood vessels

II. Functions of basement membrane
   A. Provides mechanical support for the attached cells
   B. Generates signals that maintain cell survival
   C. Maintains epithelial cell polarity
   D. Serves as a substratum for cell migration & determines cell migration path

E. Separates adjacent tissues within an organ (compartmentalization)
F. Acts as barrier to passage of macromolecules & errant cancer cells - prevents passage of proteins out of blood as it flows through porous-walled body capillaries (kidney – good example)
   1. Kidney glomerulus - blood filtered under high pressure through double-layered basal lamina separating glomerular capillaries from kidney tubule wall
   2. Basal lamina around glomeruli may thicken abnormally in long-term diabetics —> kidney failure

Components of the Extracellular Matrix: Collagens

I. Comprise a fibrous glycoprotein family; present only in ECMs; found throughout animal kingdom
   A. Noted for high tensile strength (resistance to pulling forces); it is estimated that a 1 mm dia collagen fiber can suspend a 10 kg [22 lb] weight without breaking
   B. It is the single most abundant protein in human body (constitutes >25% of all protein); reflects widespread occurrence of extracellular materials
   C. Collagen molecules provide the insoluble framework that determines many ECM mechanical properties

II. Made mostly by fibroblasts (found in various connective tissue types), smooth muscle & epithelial cells
   A. >20 distinct types identified; each restricted to particular sites in body; 2 or more can be present together in same ECM; get functional complexity by mixing several types in same fiber (heterotypic)
      1. Heterotypic fibers are biological equivalent of metal alloys
      2. Different structural & mechanical properties result from different mixtures of collagens in fibers
   B. Many differences among collagen family members, but all share 2 important structural features:
      1. All collagen molecules are trimers consisting of 3 polypeptide [α] chains - may be identical or 2 or 3 different chains
      2. Along at least part of length, the 3 chains wind around each other; form unique, rodlike triple helix
   C. Some are fibrillar collagens (I, II, II) - assemble into rigid, cable-like fibrils; then into thicker fibers visible in light microscope
      1. Individual collagen molecules of a fibril are not in register but are staggered ~1/4 length relative to their neighbors
      2. The staggered arrangement adds to the mechanical strength of the complex & causes banding patterns characteristic of collagen fibers
   D. Fibrils are strengthened further by covalent cross-links between lysine & hydroxylysine residues on adjacent collagen molecules - if disrupted weakened
      1. Cross-linking process continues through life
      2. May contribute to decreased skin elasticity & increased brittleness of bones among elderly

III. Collagen provides insoluble framework that determines many of the ECM mechanical properties & 3D organization of collagen molecules often correlates with properties of tissue in which it is found
   A. Tendons (connect muscles to bones) must resist large pulling forces during muscle contraction - collagen fibers aligned parallel to long axis of tendon & parallel to direction of pulling force
   B. Cornea – serves as durable, transparent (so light can pass through), protective layer at eyeball surface
      1. Stroma (thick middle layer) – fibrils are relatively short, in orthogonal layers (fibers in layer are parallel to each other, but perpendicular to those in adjoining layers) like plywood (gives strength)
      2. Fiber size uniformity & ordered packing promote transparency (minimizes light scattering)
   C. Basement membrane (very thin, mechanically supportive sheets); type IV collagen (only seen here)
      1. Type IV collagen is nonfibrillar collagen & is organized in flattened network; it provides mechanical support & serves as a lattice for deposition of other ECM material
      2. Type IV collagen trimer has some interspersed nonhelical segments, not a long, uninterrupted triple helix like Type I (gives flexibility)

3. Also have globular heads on each end that serve as interaction sites between molecules & give the complex its lattice-like character

IV. Given their abundance & widespread distribution, serious disorders can be caused by abnormalities in fibrillar collagen formation
   A. Burns or traumatic injuries to internal organs can cause scar tissue buildup (largely fibrillar collagen)
   B. Type I collagen mutations - osteogenesis imperfecta, potentially lethal condition characterized by extremely fragile bones, thin skin & weak tendons
   C. Type II collagen mutations - alter cartilage properties; causes dwarfism & skeletal deformities
   D. A number of collagen gene mutations - cause various distinct but related collagen matrix structure defects (Ehler-Danlos syndromes) – one causes hyperextendable joints & highly extensible skin
   E. Type IV collagen gene mutations - Alport syndrome, an inherited kidney disease in which the glomerular basement membrane is disrupted

Components of the Extracellular Matrix: Proteoglycans

I. Basement membranes & other ECMs contain large amounts of distinctive type of protein-polysaccharide complex called a **proteoglycan**
   A. Consist of core protein to which glycosaminoglycan (GAG) chains are covalently attached
   B. GAGs - repeating disaccharides (2 different sugars; -A-B-A-B-); very acidic due to both carboxyl & sulfate groups on their component sugar rings
   C. ECM proteoglycans may assemble into gigantic complexes by linking core proteins to hyaluronic acid (a nonsulfated GAG); can occupy very large volumes (equivalent to that of bacterial cell)

II. Due to sulfated GAG negative charges, proteoglycans bind large numbers of cations, which, in turn, attract lots of H_2O
   A. They form porous hydrated gel; that fills extracellular space & acts like packing material to resist crushing (compression) forces
   B. This complements adjacent collagens, which resist pulling forces & provide scaffold for proteoglycans
   C. Together they give cartilage & other ECMs strength & resistance to deformation (wiggle your ears)
   D. The ECM of bone is also made of collagen & proteoglycans but it is hardened by impregnation with calcium phosphate salts

Components of the Extracellular Matrix: Fibronectin, Laminin & Other ECM Proteins

I. Matrix implies a structure made up of a network of interacting components; this is apt for ECM
   A. It contains a number of proteins, in addition to collagen & proteoglycans that interact with one another in highly specific ways
   B. Many ECM proteins occur in families (more than 1 form; each formed by alternate mRNA splicing)
      1. Different family members made in different tissues & at different times during development
      2. Different protein forms may have different properties (characteristics may not apply to all forms)

II. Fibronectin (fibrous) - one of best studied ECM proteins; has features common to most other matrix components & ECM proteins; consists of linear array of distinct building blocks (a modular construction)
   A. Each fibronectin polypeptide is constructed from a sequence of ~30 independently folding Fn modules of 3 distinct types (FnI, FnII & FnIII)
      1. Fn modules were first found in fibronectin, but they are part of many other proteins
      2. Found in proteins from blood clotting factors to membrane receptors & other ECM proteins

3. Presence of shared segments among diverse proteins suggests that many present-day genes have arisen during evolution by fusion of parts of separate ancestral genes
4. In fibronectin, the 30 or so structural modules combine to form 5 or 6 larger functional domains

B. Each of the two polypeptide chains making up fibronectin contains:
1. Binding sites for other ECM components (collagen, proteoglycans, etc.); link these molecules into stable, interconnected networks
2. Binding sites for cell surface receptors (form stable ECM-cell attachments); endothelial cell will adopt shape unlike it does in body when it spreads over a square surface coated with fibronectin

C. Fibronectin & other ECM proteins are important when tissues are involved in dynamic activities (embryonic development)
1. Development involves waves of cell migration over pathways containing ECM proteins; different cells follow different routes from one part of embryo to another
2. Migrating cells guided by proteins like fibronectin contained in landscape over which they pass
3. Neural crest cells follow fibronectin pathways from early nervous system throughout embryo; antibodies to fibronectin bind & block recognition sites on fibronectin —> inhibit cell movements
4. Mice lacking functional fibronectin gene —> abnormal; do not survive past early development

III. Laminin also has specific domains – family of extracellular glycoproteins; consist of 3 different polypeptide chains linked by disulfide bonds; organized into a cross with 3 short arms & 1 long arm

A. Extracellularly, it greatly influences cell's potential for migration, growth & differentiation
1. Guides embryonic axon tips as grow outward from central nervous system to distant targets

2. Critical role in primordial germ cell (PGC) migration – PGCs follow laminin paths from yolk sac (outside embryo) through blood & embryonic tissues to developing gonad, become eggs or sperm
   a. During migration, PGCs traverse surfaces particularly rich in laminin
   b. PGCs possess cell surface protein that adheres strongly to one of laminin's subunits

B. Certain cells migrate over laminin-containing matrix that they secrete (keratinocytes – skin cells)
1. Isolate keratinocytes from mice genetically engineered to lack genes for this type of laminin
2. Their migratory ability is vastly diminished

C. Also binds tightly to other laminins, proteoglycans, basal lamina components, cell surface receptors
1. Basal lamina type IV collagens & laminin may form separate, but interconnected, networks
2. These interwoven networks give basal lamina both strength & flexibility
3. Basal lamina with this structure are not restricted to vertebrates; seen throughout animal kingdom
4. Laminin, proteoglycan, type IV collagen genes found in invertebrates (fruit flies, nematodes)

D. ECM exhibits dynamic properties, both in space & over time
1. ECM fibrils stretch several times normal length as they are pulled on by cells; they contract when tension is relieved
2. Fibronectin thought to elongate as β-sheets making up Fn domains become reversibly unfolded in response to applied tension
3. Temporally, ECM components are subject to continual degradation & reconstruction; renews the matrix & allows it to be remodeled during embryonic development or after tissue injury
4. Even the calcified matrix of bone is subject to continual restoration

E. ECM degradation, along with that of cell surface receptors, accomplished mostly by a zinc-containing enzyme family (matrix metalloproteinases [MMPs])
1. MMPs are either secreted into extracellular space or anchored to plasma membrane
2. As a group, MMPs can digest all of the diverse ECM components, but individual family members are limited as to the types of extracellular proteins they can attack
3. Excessive or inappropriate activity of MMPs is likely to cause disease; implicated in a number of pathological conditions (arthritis, hepatitis, atherosclerosis, tooth/gum disease, tumor progression)

IV. Other ECM molecules
   A. Tenascin - large, oligomeric glycoprotein; on many cell types & a variety of cancer cell surfaces depending on cell type, can promote or discourage cell adhesion
   B. Entactin - component of basement membranes; thought to play role in adhesion & penetration of early mammalian embryo into uterine lining
   C. Thrombospondin - secreted into ECM by many cells; prominent in matrix surrounding lining of mature blood vessels; inhibits formation of new blood vessels (angiogenesis)

V. Interactions among ECM materials can be very complex
   A. Some may contain binding sites with opposite activities (one may promote & another inhibit cell adhesion); thus effects on cell behavior may change from time to time
   B. Spatial arrangement of various components may ultimately determine their effect on cell behavior
   C. ECM components like fibronectin, laminin, proteoglycans & collagen are capable of binding to receptors situated on the cell surface

Interactions of Cells with Noncellular Substrates: Integrins

I. Integrins - integral membrane protein family; composed of 2 membrane spanning chains (α & β; linked noncovalently); thought to be on surface of virtually all vertebrate cell types (found only in animals)
   A. Most important family of receptors that attach cells to their extracellular microenvironment; they bind to specific substances (ligands) in the extracellular environment
   B. Many (18) different α & 8 different β subunits identified on cell surfaces; thus many heterodimers (only ~24 integrins identified on cell surfaces), each with specific distribution within body
      1. >100 possible α & β pairings are possible theoretically
      2. Most cells have a variety of different integrins; most integrins are present on a variety of cell types
   C. EM pictures (late 1980s) suggest that the 2 subunits are oriented to form a globular extracellular head connected to the membrane by a pair of elongated "legs"
      1. The legs of each subunit cross bilayer as a single transmembrane helix & end in a small cytoplasmic domain of ~20 – 70 amino acids
      2. Exception to this is β_4 chain, which has an extra 1000 or so amino acids as part of its cytoplasmic domain; this huge addition makes β_4 integrins able to extend much more deeply into cytoplasm
   D. In 2001, the first X-ray crystallographic structure of the extracellular portion displayed a highly unexpected feature
      1. Rather than "standing upright", the integrin $\alpha_v\beta_3$ was bent dramatically at the "knees" so that the head faces the outer plasma membrane surface rather than the extracellular space
      2. Electron micrographs provided corroborating evidence that the structure was not an artifact of crystallization procedure as had been suspected

II. Many integrins can exist on surface of cell in an inactive conformation - they can be activated rapidly by events within the cell that alter the conformation of cytoplasmic domains of the integrin's subunits
   A. These changes are propagated through the molecule, increasing the integrin's affinity for an extracellular ligand
   B. Example – platelet aggregation during blood clotting occurs only after cytoplasmic activation of $\alpha_{IIb}\beta_3$ integrins, which increases their affinity for fibrinogen
      1. This type of alteration in integrin affinity is triggered by changes occurring inside the cell & is called "inside-out" signaling
      2. Without the inside-out signal, the integrin remains inactive protecting the body against the formation of an inappropriate blood clot
   C. Evidence suggests strongly that the bent conformation of an integrin corresponds to the inactive state that cannot bind a ligand

1. An $\alpha_v\beta_3$ integrin containing a bound ligand when analyzed by X-ray diffraction, no longer exhibits the bent structure but is present instead in the upright conformation
2. The ligand is bound to the integrin's head in a region where the α & β subunits come together
3. If these conformations respectively represent the active & inactive forms, what triggers the transformation?

D. One can prepare the extracellular portion of an integrin as a soluble α/β heterodimer lacking the associated transmembrane & cytoplasmic domains normally present as part of molecule
1. If the α & β subunits of these extracellular fragments are experimentally bound to one another at the bases of their legs, the molecules are unable to bind a ligand
2. When the linkage between the 2 legs is cleaved, the integrin fragment can now bind ligand
3. Thus, the ligand-binding ability of the integrin head, which projects from cell's outer membrane surface, depends on spatial arrangement of the α & β cytoplasmic tails on inner side of membrane
4. This idea is supported by lots of evidence that supports the occurrence of inside-out signaling as exemplified by platelet $\alpha_{IIb}\beta_3$ binding to fibrinogen
5. Cytoplasmic domains of integrins bind a wide array of proteins, including molecules (like talin) that cause separation of α & β subunits
6. Separation of cytoplasmic ends of integrins is thought to send a change in conformation through the integrin legs
7. This leads the integrin to assume an upright position in which the protein head can interact specifically with the appropriate ligand

III. The ligand-binding site of an activated integrin molecule forms at a crevice in the integrin's head where surfaces of the α & β subunits contact each other

A. The binding site on the β subunit consists of a spherical-shaped segment called the I domain

B. The binding site on the α subunit is situated on the upper surface of a protein motif known as a β-propeller (so-named because it is composed of a number of flattened blades)
1. In nearly half of all integrins, the α subunit contains an extra domain, an I domain (very similar to the I domain found in all of the β subunits)
2. In those α subunits with an I domain, the ligand-binding site is found on this part of the subunit rather than on the β-propeller

C. Calcium ions are required to maintain the proper integrin structure & facilitate ligand binding
1. There is a site in the I domain (MIDAS) that binds a divalent cation & a negatively charged residue (e.g., glutamic or aspartic acid) in the ligand

IV. Integrins have been implicated in 2 major types of cell activities

A. Adhesion of cells to their substratum (or to other cells)

B. Signal transmission from external environment to cell interior ("outside-in" signaling)
1. Signals transmitted across membrane via conformational changes
2. Binding of integrin extracellular domain to ligand (fibronectin, laminin) induces a conformational change at the cytoplasmic end of the integrin
3. Changes at cytoplasmic end can, in turn, alter the way the integrin interacts with nearby cytoplasmic proteins, like the enzyme focal adhesion kinase [FAK]
4. As integrins bind extracellular ligand, they can activate cytoplasmic protein kinases, which can then phosphorylate other proteins; starts a chain reaction (may even activate specific genes in nucleus)

V. Outside-in signals transmitted by integrins & other cell-surface molecules can influence many aspects of cell behavior including differentiation, motility, growth & even cell survival

A. Influence of integrins on cell survival is best illustrated by comparing normal & malignant cells
1. Most malignant cells can grow suspended in liquid culture medium; normal cells, in contrast, only grow & divide if cultured on a solid substratum, they die in suspension cultures

2. Normal cells may die in suspension culture since their integrins cannot interact with extracellular substrates &, as a result, cannot transmit life-saving signals to the cell interior
3. On the other hand, malignant cell survival no longer depends on integrin binding

B. Linkage between integrins & their ligands mediates adhesions between cells & their environment
1. Individual cells may express a variety of different integrins on their cell surface; thus, such cells can bind to a variety of different extracellular components
2. Despite the apparent overlap, most integrins appear to have unique functions – knockout mice that lack different integrin subunits exhibit distinct phenotypes
3. For example, α_8 knockouts show kidney defects; α_4 knockouts exhibit heart defects; α_5 knockouts have vascular defects

VI. Most extracellular proteins binding integrins have a tripeptide sequence arginine-glycine-aspartic acid (RGD); found in cell-binding sites of proteoglycans, fibronectin, collagen, laminin, other ECM proteins
A. Often RGD sequence is on an extended loop of these proteins
B. The binding site for the loop is thought to reside on the I domain of β subunit of specific integrins

VII. Applications & examples of integrin involvements – discovery of RGD sequences' importance has opened the door to new treatments of medical conditions involving receptor-ligand interactions
A. Blood clot (thrombus) forming in diseased artery can block blood flow to major organs & cause heart attack or stroke; clot starts with blood platelet (nonnucleated cells circulating in blood) aggregation
1. Platelet aggregation requires interaction of platelet-specific integrin ($\alpha_{Iib}\beta_3$) with soluble RGD-containing blood proteins (fibrinogen, von Willebrand factor) which link platelets together
2. RGD-containing peptides can inhibit blood clot formation; stop platelet aggregation by preventing platelet integrin from binding to blood proteins
3. Led to design of new class of non-peptide, anti-thrombotic agents (like Aggrastat) that resemble RGD structure but bind only to platelet integrin
4. Other studies – also, highly specific antibodies (ReoPro) directed against $\alpha_{IIB}\beta_3$ integrin can prevent clots in patients undergoing high-risk vascular surgeries

B. Integrin cytoplasmic domains contain binding sites for a variety of cytoplasmic proteins, including several that act as adaptors to link the integrin to actin filaments of the cytoskeleton
1. The role of integrins in connecting the ECM & the cytoskeleton is best seen in 2 specialized structures: focal adhesions & hemidesmosomes

Interactions of Cells with Noncellular Substrates: Focal Adhesions & Hemidesmosomes

I. Focal contacts anchor cells to their substratum – it is much easier to study cell adhesion to a surface *in vitro* (in a culture dish) than with an extracellular matrix inside an animal
A. Much of our knowledge of cell-matrix interactions is derived from studies of cells adhering to various substrates *in vitro*

B. Steps in cell adhesion to culture dish
1. Cell initially has rounded morphology like most animal cells suspended in aqueous medium
2. As cell contacts substratum, it sends out projections that make increasingly stable attachments
3. Over time, cell flattens & spreads itself out on substratum with rearrangement of cytoskeleton (may be mediated by transmembrane signaling)
4. Bottom surface of fibroblasts or epithelial cells is not pressed uniformly against substratum, but is anchored to surface (as close as 10 nm) at scattered, discrete sites (**focal contacts** or **focal adhesions**)

C. Focal adhesions – dynamic structures; can be rapidly disassembled if the adherent cell is stimulated to move or enter mitosis

1. Integrin clusters (often $\alpha_V\beta_3$) are seen in membrane focal adhesion region; integrin cytoplasmic domains are connected by various adaptors to actin filaments of cytoskeleton connect dish ECM & cytoskeleton (actin); cytoskeleton attachment seems necessary for cell adhesion
2. Actin filaments along with myosin molecules are part of cell's contractile machinery, which can create or respond to mechanical forces
3. Attach cultured cell to gelated surface with uniform grid pattern that is deformed by local forces -> surface distorted by traction (pulling) forces generated by focal adhesions on cell undersurface
4. Binding of extracellular ligand (fibronectin, laminin) can activate protein kinases (like FAK) that transmit signals throughout cell, including cell nucleus

III. Focal adhesions form in cells grown *in vitro*, but similar types of adhesive contacts are found in certain tissues, like muscle & tendon
   A. In body, the tightest attachment between a cell & ECM is at epithelial cell basal surface where they are anchored to underlying basement membrane by specialized adhesive structure (hemidesmosome)
   B. They contain dense plaque on membrane inner surface with filaments coursing outward into cytoplasm
      1. Filaments are thicker than actin of focal adhesions & made of keratin (intermediate filaments)
      2. Primarily supportive rather than contractile; keratin-containing filaments of hemidesmosome are linked to ECM by membrane-spanning integrins ($\alpha_6\beta_4$)
      3. These integrins, like focal adhesion integrins, also transmit signals from the ECM that affect the shape & activities of attached epithelial cells
   C. Bullous pemphigoid – rare autoimmune disease (people make antibodies against hemidesmosome plaque proteins, **bullous pemphigoid antigens**); demonstrates importance of hemidesmosomes
      1. Autoimmune disorders are caused by production of antibodies (autoantibodies) directed against one's own tissues; responsible for a wide variety of conditions
      2. Presence of autoantibodies causes lower epidermal layer to lose attachment to underlying basement membrane & thus to underlying connective tissue layer of dermis
      3. Causes severe blistering of skin when fluid leaks into space under epidermis
   D. Epidermolysis bullosa (a similar inherited blistering disease) - found in patients with genetic alterations in any one of a number of hemidesmosomal proteins (α_6 or β_4 integrin subunit or laminin)
      1. Other epithelia in body (gastrointestinal & urinary tract linings) may also be affected

Interactions of Cells with Other Cells – Experimental Approaches

I. Little is known about the mechanisms that generate complex 3-D cell arrangements in developing organs – it is presumed to depend heavily on selective interactions between cells of same & different types
   A. Evidence indicates that cells can recognize the surfaces of other cells, interacting with some & ignoring others

II. Experiments demonstrating cell adhesion – difficult to study cell interactions occurring in small organs of a developing embryo
   A. Early experiments involved removal of developing organ from chick or amphibian embryo, dissociating its tissues to single cells & observing their ability to reaggregate in culture
   B. If one disaggregates cells from 2 different developing organs & mixes them —> they form mixed clump then sort themselves out; each cell adheres long term only to cells of the same type
      1. Once separated into a homogeneous cluster, these cells often differentiate into many of the structures they would have formed within an intact embryo
      2. Add labeled cells to dishes with monolayers of varied cell types —> cells stick to own type better
   C. Little was known about nature of cell-cell adhesion molecules until techniques developed for purifying integral membrane proteins & recently for the isolation/cloning of genes encoding these proteins
      1. Dozens of proteins involved in cell adhesion have now been identified
      2. Different arrays of these proteins on different cells are responsible for specific interactions between cells within complex tissues

III. Four distinct families of integral membrane proteins play a major role in mediating cell-cell adhesion
   A. Selectins
   B. Certain members of immunoglobulin superfamily (IgSF)
   C. Certain members of the integrin family
   D. Cadherins

Interactions of Cells with Other Cells – Families of Integral Membrane Proteins

I. Selectins - integral membrane glycoprotein family; binds to specific sugar arrangement in oligosaccharides that project from other cells' surfaces
   A. The name of this class of cell-surface receptors comes from lectin, a term for a compound that binds to specific carbohydrate groups
   B. During 1960s - remove lymphocytes from peripheral lymph nodes, label them radioactively & reinject —> go back to site of origin from which they were derived (**lymphocyte homing**)
      1. Homing also studied *in vitro* by allowing lymphocytes to adhere to frozen tissue sections of lymphoid organ —> selectively adhere to venule endothelial lining of peripheral lymph nodes
      2. Binding could be blocked by antibodies against specific glycoprotein on lymphocyte surface (glycoprotein was called LEU-CAM1 & later L-selectin)
   C. Selectins possess a small cytoplasmic domain, a single membrane-spanning domain, & a large extracellular segment consisting of a number of separate modules (outermost domain acts as the lectin)
   D. 3 known types - E-selectin (endothelial cells); P-selectin (platelets, endothelial cells); L-selectin (present on all types of leukocytes or white blood cells)
      1. All 3 recognize a particular grouping of sugars found on ends of carbohydrate chains of certain complex glycoproteins; binding of selectins to their carbohydrate ligands requires calcium
      2. As a group, selectins mediate transient interactions between circulating leukocytes & vessel walls at inflammation & clotting sites

II. Immunoglobulins (Igs) & Integrins - blood-borne antibody (immunoglobulin) structure was elucidated in the 1960s; this was a milestone in the understanding of the immune response
   A. Igs are a large family of proteins; they consist of polypeptide chains composed of a number of similar domains; most are present on lymphocyte surfaces as integral proteins
   1. Each Ig domain is composed of 70 – 110 amino acids organized into tightly folded structure
      2. Ig-type domains were seen in wide variety of proteins, which together constitute the immunoglobulin superfamily (IgSF)
   B. Most IgSF members are involved in various aspects of immune function, but some of them mediate calcium-independent cell-cell adhesion
      1. Ig-like domains were discovered in cell-adhesion receptors in invertebrates, animals that lack a classic immune system
      2. This suggests that Ig-like proteins originally evolved as cell-adhesion mediators & only secondarily took on their functions as effectors of the vertebrate immune system
   C. Most IgSFs mediate specific interactions of lymphocytes with cells needed for immune response (other lymphocytes, macrophages, target cells) but some mediate adhesion between nonimmune cells
      1. VCAM (vascular cell-adhesion molecules)
      2. NCAMs (neural cell-adhesion molecules)
      3. L1 – NCAMs & L1 play roles in nerve outgrowth, synapse formation & other events during nervous system development
   D. Like fibronectin & many other cell-adhesion proteins, IgSF cell-adhesion molecules exhibit modular construction; composed of individual domains similar in structure to domains in other proteins
   E. L1 importance in neural development has been revealed in several ways - human *L1* gene mutations can have devastating consequences

1. In extreme cases, babies are born with a fatal condition of hydrocephalus (water on the brain)
2. Children with less severe forms of the mutation typically exhibit mental retardation & difficulty in controlling limb movements (spasticity)
3. Autopsies of those who died of L1-deficiency disease are often missing 2 large nerve tracts: one that runs between the 2 halves of brain & the other running between the brain & spinal cord
4. Such missing nerve tracts suggest L1 is involved in axon growth within embryonic nervous system

F. Growing axon tip is a highly motile portion (growth cone) that is responsible for guiding axon to its correct target in the embryo
   1. Growth cone is an exploratory structure that creeps along substratum, sensing & responding to substances in its environment that influence axonal growth in one direction or another
   2. *In vitro* studies have shown that:
   a. Growth cones of certain nerves contain the L1 protein (& other IgSF molecules)
   b. These growth cones will grow outward over an L1-containing substratum
   c. Nerve outgrowth in these experiments is blocked by addition of antibodies directed against L1
   3. Studies suggest that interaction between L1 extracellular domains present on the cell surface & in the substratum generates signals within the growth cone that promote its extension

G. Various types of proteins serve as ligands for IgSF cell-surface molecules - may bind to same or different IgSFs on other cells or a few integrins
   1. Most integrins facilitate adhesion of cells to their substratum, but a few integrins mediate cell-cell interactions by binding to proteins on other cells
   2. Leukocyte surface integrin ($\alpha_4\beta_1$) binds VCAM (an IgSF protein on the endothelial lining of certain blood vessels)

III. Cadherins – large family of glycoproteins mediating Ca^{2+}-dependent cell-cell adhesion; also transmit signals from ECM to cytoplasm; found in many different cell types with specific body distribution

A. Join cells of similar type to one another mostly by binding to the same cadherin present on neighboring cell surface; this was demonstrated by the following experiment:
   1. Genetically engineer nonadhesive cells to express one of a variety of cadherins —> then mix them in various combinations & their interactions were monitored
   2. Cells expressing one species of cadherin preferentially adhered to other cells expressing the same cadherin (may mold cohesive tissues in embryo & hold them together in adult)

B. Cadherins are found on the surfaces of many different animal cell types with each particular family member having a specific distribution in body & distinguished by cell type on which they are found
   1. E-cadherins (epithelial), N-cadherins (neural), P-cadherins (placental)
   2. Cadherins appear to be important in holding cells together in tightly cohesive tissues

C. These "classical" cadherins have a relatively large extracellular segment consisting of 5 tandem domains of similar size & structure, 1 transmembrane segment & a small cytoplasmic domain
   1. Cytoplasmic domain is often associated with members of catenin family of cytosolic proteins
   2. Catenins (like integrins) have 2 roles: tie cadherins to cytoskeleton & transmit signals to cytoplasm

D. X-ray crystallography has been carried out on the extracellular portions of cadherins
   1. Cadherins from the same cell surface associate laterally to form parallel dimers; studies also shed light on role of calcium, which for decades has been known to be essential for cell-cell adhesion
   2. Ca^{2+} ions form bridges between successive domains of a given molecule, not between molecules from different cells, as had long been presumed
   3. Ca^{2+} ions apparently maintain rigid conformation of extracellular portion of each cadherin needed for cell adhesion

E. Cell-cell adhesion results from interaction between extracellular domains of cadherins from opposing cells to form a "cell adhesion zipper", which, if extensive, would hold cells together with great strength

1. Controversy has arisen over the degree to which cadherins from opposing cells overlap with one another; different cell types might engage in different types of interactions
2. Thus, more than one configuration may occur within an organism
3. Cadherin interdigitation can be compared to a zipper; cadherin clusters can be compared to Velcro.
4. The greater the number of interacting cadherins in a cluster, the greater the strength of adhesion between apposing cells

F. Cadherin-mediated adhesion may be responsible for ability of like cells to sort out of mixed aggregates
1. Cadherins may be the single most important factor in molding cells into cohesive embryonic tissues & holding them together in the adult
2. The loss of cadherin function may play a key role in the spread of malignant tumors

G. Embryonic development is characterized by changes in gene expression, cell shape, cell motility, cell adhesion, etc.
1. Some embryonic developmental (morphogenetic) events involve cells changing from mesenchyme (loose, mostly nonadhesive cells) to epithelium (tightly adherent, organized cell layer) or vice versa
2. Mesodermal cell movement in gastrulation exemplifies mesenchymal-epithelial transition; usually, cells leave cohesive layer at early gastrula surface, wander into interior regions as mesenchymal cells
3. Later, some of them get adhesive again & form epithelium (somites along embryo dorsal midline)
4. Even later, some somite cells lose adhesiveness & wander again as mesenchyme into developing limb (become cartilage or muscle) or beneath developing epidermis (become dermal tissues)

H. Cadherins & other cell-adhesion molecules play a key role in such activities by changing cell adhesive properties
1. Aggregation of cells into an epithelium (as in somite) correlates with N-cadherin appearance on cell surfaces; this event would be expected to promote cell adhesion
2. In contrast, cell dispersion from epithelium is correlated with disappearance of N-cadherin from cell surface
3. Cadherins are typically distributed diffusely over cell surfaces of 2 adherent cells but they also participate in formation of specialized intercellular junctions

Linking Cells to Other Cells: Synaptic Junctions

I. Mechanism by which growing axon tip hooks up with particular target cell is a great biological mystery
A. During human eye development, each axon grows out of a specific site in the retina & eventually forms a synaptic junction with a specific cell or group of cells in the optic tectum of the brain
1. This relationship between retina neurons & optic tectum neurons is necessary for us to understand spatial relationships in our visual field – how does tip of retina axon select the right brain cell?

B. Roger Sperry, Caltech (1950s) – suggested that neural specificity was governed by a large family of cell surface molecules; over the years, supporting evidence has accumulated in favor of his proposal
1. A given axon & its target cell would have a specific pair of complementary surface molecules that would allow the growing axon to find its target among the many potential candidates
2. Recent evidence points to proteins of the cadherin superfamily as playing a key role in mediating synaptic connection

II. Presynaptic & postsynaptic synapse membranes are separated from each other by narrow synaptic cleft
A. The spacing between membranes is maintained by cadherins projecting from the 2 parallel plasma membranes & adhere to one another within the center of the synapse

B. Classic cadherins have been known to localize in synapses for years; there is evidence that cells in different parts of brain/spinal cord contain different combinations of classic cadherins on surface

C. These proteins do not appear to have sufficient diversity to account for the high degree of specificity needed to mediate synapse formation throughout the central nervous system

D. In the past few years, a unique cadherin subfamily (**protocadherins**) has been suggested to carry the molecular code for mediating highly specific synaptic connections

E. Protocadherins have been localized to synapses within brain & different members of the family are expressed by different neurons in a given region of the brain
   1. 60 or more genes encode the protocadherin subfamily; their structure & organization has made them attractive as mediators of synaptic specificity
   2. They have a unique genomic architecture that enables an organism to generate a significant amount of protein diversity from a relatively small cluster of protocadherin genes
   3. Neurons expressing the same set of protocadherins might be able to form synapses, whereas neurons expressing different sets might be incapable of interacting with one another

Linking Cells to Other Cells: Adherens Junctions and Desmosomes

I. Cells of certain tissues (epithelia, cardiac muscle) are notoriously difficult to separate from one another, because they are held together tightly by specialized Ca^{2+}-dependent adhesive junctions
   A. Two main types of adhesive junctions: adherens junctions & desmosomes
   B. Other types of epithelial cell junctions (gap junctions & tight junctions) are also located along lateral cell surfaces near apical lumen
   C. When these junctions are in specific array, the assortment of surface specializations called **junctional complex**

II. Adherens junctions – found in a variety of body sites; particularly common in epithelia (like the lining of the intestine)
   A. Occur as belt encircling each cell at apical end; binds cell to its surrounding neighbors; may transmit signals between them, too; called **zonulae adherens**
      1. Cells of adherens junction are tightly held together by Ca^{2+}-dependent linkages formed between extracellular cadherin domains bridging 30 nm gap between neighboring cells
      2. Cytoplasmic cadherin domains in junctions are linked by α- & β-catenins to a variety of cytoplasmic proteins, like actin filaments of cytoskeleton
   B. Thus, junction cadherins, like focal adhesion integrins, connect external environment to actin cytoskeleton & provide potential pathway for signal transmission from cell exterior to cytoplasm
      1. Adherens junctions between endothelial cells lining blood vessel walls transmit signals that ensure cell survival
      2. Mice lacking an endothelial cell cadherin are unable to transmit these survival signals —> these animals die during embryonic development as result of the death of cells lining vessel walls

III. Desmosomes (**maculae adherens**) - disk-shaped adhesive junctions (~1 μm in diameter) found in a variety of tissues (most notably in epithelia where they are basal to the zonulae adherens)
   A. Particularly numerous in tissues subjected to mechanical stress (skin epithelial layers, cardiac muscle, gingiva [gums], uterine cervix epithelial layers)
   B. Like adherens junctions, they contain cadherins that link the 2 cells across a narrow (30 nm) extracellular gap
      1. They have a different domain structure from that of adherens junction classical cadherins; they are called **desmogleins** & **desmocollins**
   C. Dense cytoplasmic plaques on inner membrane surfaces serve as sites of anchorage for looping intermediate filaments (like those in hemidesmosomes)
      1. These filaments extend into cytoplasm & connect with other desmosomes
      2. The 3D network of ropelike intermediate filaments gives structural continuity & tensile strength to the entire cell sheet
      3. Intermediate filaments also link to desmosomal cadherin cytoplasmic domains via additional proteins

D. Region between cells (desmoglea) filled with lightly staining material, which may act as glue
E. Autoimmune disease pemphigus vulgaris illustrates importance of cadherins in maintaining epithelium structural integrity
   1. Antibodies made against a desmoglein causing loss of epidermal cell-cell adhesion & severe blistering of skin

The Role of Cell Adhesion Receptors in Transmembrane Signaling

I. All 4 types of cell-adhesion molecules have the potential to transfer information across plasma membrane
   A. Integrins & cadherins can transmit signals from extracellular environment to cytoplasm via links with cytoskeleton & with cytosolic regulatory molecules, like protein kinases (**transmembrane signaling**)
   B. Binding of integrin with its ligand can induce variety of cell responses via transmembrane signaling
      1. Changes in cytoplasmic pH
      2. Changes in Ca^{2+} ion concentration
      3. Changes in protein phosphorylation
      4. Changes in gene expression
   C. Such changes can alter subsequent cell behavior - growth potential, migratory activity, differentiation state, survival; an example is mammary gland epithelial cells

II. Remove cells from mammary gland & grow them on bare culture dish, in absence of extracellular glycoproteins (laminin & fibronectin)
   A. They appear as flattened, undifferentiated cells & lose their ability to produce milk proteins
   B. Add laminin or other extracellular molecules to culture —> it binds cell surface integrins —> cells regain their differentiated appearance
      1. They also become organized into milk-producing, glandlike structures (milk protein production rises 50 X)
   C. Laminin may work by binding cell surface integrins & activating kinases at inner membrane surface

Tight Junctions: Sealing the Extracellular Space

I. A simple epithelium (lining of intestine or lungs) is comprised of a layer of cells that adhere tightly to one another to form a thin cellular sheet
   A. Certain types of epithelia (frog skin, urinary bladder wall) can be mounted between 2 compartments containing different solute concentrations
   B. When this is done, the membranes allow very little diffusion of ions or solutes across the wall of the epithelium from one compartment to the other
   C. Plasma membranes are impermeable so solutes cannot diffuse freely through cells of the epithelial layer but why can't solutes pass between cells by a paracellular pathway?

II. Tight junctions (TJs; **zonula occludens**) - discovered in 1960s; found between adjacent epithelial cells at the very apical end of the junctional complex between those cells (frog skin, urinary bladder wall)
   A. In tight junctions, the membranes make contact at intermittent points; not fused over large surface area
   B. The points of cell-cell contact are sites where integral proteins of the 2 adjacent membranes meet within the extracellular space
      1. Freeze fracture (allows observation of internal membrane faces) shows that plasma membranes of a TJ contain interconnected strands (or grooves in opposite face of fractured membrane)
      2. The interconnected strands run mostly parallel to one another & to the epithelium's apical surface; they wrap around the cell like a belt around waist
      3. These strands or grooves in the opposite face of the fractured membrane correspond to paired rows of aligned integral membrane proteins

4. The integral TJ proteins form continuous fibrils that completely encircle the cell like a gasket & contact neighboring cells on all sides

C. Thus, TJs serve as a barrier (a seal) to free diffusion of water & solutes from extracellular compartment on one side of epithelial sheet to that on the other side
   1. Place piece of tissue in lanthanum (electron-dense heavy metal) & take EM pictures —> lanthanum penetrates between cells as far as upper & lower edge of tight junctions

D. TJs also serve as fences that help maintain the polarized character of epithelial cells; they block diffusion of integral proteins between the apical domain of membrane & its lateral & basal domains

III. TJs are occluding junctions & form a continuous permeability barrier, but not all of them exhibit the same permeability properties

A. TJs with several parallel strands form better seals than those with one or a couple strands
   1. Leakiest junctions (proximal renal tubule) - a single strand & little resistance
   2. Tightest junctions (bladder wall, brain capillaries) - a number of parallel, interconnected strands; high resistance

B. Some are permeable to specific ions or solutes to which others are impermeable

1. All cells of human kidney tubule are connected to neighbors by TJs
2. Only one small region of tubule has TJs permeable to Mg^{2+} ions (thick ascending limb; TAL)
3. TAL is site along tubule where Mg^{2+} ions are reabsorbed from tubular fluid back into blood

C. Until 1998, TJ strands were thought to be composed of single protein, **occludin**
   1. But it was found that cultured cells that lacked occludin gene (& could not make the protein), but were still able to form TJ strands of normal structure & function
   2. S. Tsukita et al., Univ. of Kyoto – discovered family of proteins (**claudins**) that form major structural TJ strand component; occludin & claudin are found together in linear TJ fibers
   3. At least 24 different claudins have been identified; differences in their distribution may explain differences in TJ permeability

D. Example of permeability differences is seen in one small region of a human kidney tubule, the thick ascending limb (TAL); the TAL has TJs that are permeable to magnesium (Mg^{2+}) ions
   1. It is thought claudin-containing TAL strands have pores that are selectively permeable to Mg^{2+} ions
   2. Support for this idea comes from the finding that one specific member of the claudin family (claudin-16) is expressed primarily in TAL
   3. 1999 - patients were found to have rare disease characterized by abnormally low Mg^{2+} levels in their blood; they had mutations in both copies of their claudin-16 gene
   4. Their blood levels of Mg^{2+} were low since TJs with the abnormal claudin were impermeable to Mg^{2+}, thus Mg^{2+} fails to be reabsorbed from the tubule & is simply excreted in the urine

IV. Another important TJ function has recently come to light – we rely on our skin to protect us from what would otherwise be a continual loss of water

A. For decades, the impermeability of mammalian skin to water was thought to be a property of the outer, cornified layer of the skin, which contains tightly packed protein filaments & associated lipids

B. Tsukita et al. (2002) – reported that mice lacking a gene for claudin-1 died shortly after birth as a result of dehydration
   1. The epidermis of these animals appeared normal, but it lacked the watertight character exhibited by the epidermis of normal littermates
   2. Later, it was shown that cells in one of the outer layers of normal epidermis are connected to one another by TJs (not previously fully described in one of the best studied mammalian tissues)
   3. Animals lacking the gene for claudin-1 were unable to assemble watertight epidermal TJs & thus suffered from uncontrolled water loss

V. TJs also present between endothelial cells lining capillary walls; they are particularly evident in brain

A. They help form blood-brain barrier (prevents substances from passing from bloodstream into brain)

B. Although small ions & even water molecules may not be able to penetrate the blood-brain barrier, immune system cells are able to pass across endothelium through these junctions
   1. These cells are thought to send a signal that opens the junction, allowing the cells to pass
C. While protecting the brain from unwanted solutes, the blood-brain barrier also prevents access of many drugs to the central nervous system (CNS)
   1. Major pharmaceutical industry goal is to develop drugs that open brain TJs allowing passage of therapeutic compounds

Mediating Intercellular Communication: Gap Junctions

I. Gap junctions - plasma membranes of adjacent cells come very close together (~3 nm) but they make no direct contact; they are sites between animal cells that are specialized for intercellular communication
   A. Very fine strands span gap between cells, form molecular pipeline connecting adjacent cell cytoplasms
   B. Link cells of most mammalian tissues, except skeletal muscle & most nerve tissue

II. Gap junctions have simple molecular composition made entirely of an integral membrane protein connexin (multigene family; ~12 reported)
   A. Connexins clustered in membrane to form multisubunit complex (connexon); totally spans membrane
      1. Each connexon is composed of 6 connexin subunits arranged around a central opening (**annulus**; ~16 Å in diameter at its extracellular surface)
      2. Connexons assembled between connexin production in RER & arrival at membrane
      3. Some cells make >1 connexin; don't know if single connexon contains >1 connexin
   B. During gap junction formation, connexons in membranes of apposing cells become tightly linked to one another through extensive interactions of connexin subunit extracellular domains
      1. Once aligned, connexons in apposing membranes form complete intercellular channels connecting neighboring cell cytoplasms
      2. The channels become clustered in specific membrane regions; form gap-junction plaques that can be visualized when the membrane is split down the middle by freeze-fracture

III. Gap junctions mediate **g**ap **j**unction **i**ntercellular **c**ommunication (GJIC) – communication sites between adjacent cell cytoplasms
   A. GJIC is revealed through passage of ionic currents or low MW dyes (fluorescein) from one cell to its neighbors
      1. Mammalian gap junctions allow diffusion of molecules with molecular mass < ~1000 daltons like ions & dyes like fluorescein
      2. Unlike highly selective ion channels connecting a cell to the external medium, gap junction channels are relatively nonselective
   B. However, recent studies suggest that some metabolites can be discriminated against even if they are smaller than 1000 daltons; molecular basis of such selectivity is unclear
      1. Gap junctions are also thought to be gated; channel closure is probably triggered primarily by phosphorylation of connexin subunits by protein kinase
      2. Several treatments can cause closure of gap junctions, including elevated intracellular [Ca^{2+}]; inject Ca^{2+} ions —> gap junctions close

IV. While skeletal muscle cells are stimulated by chemicals released from the tips of nearby nerve cells, cardiac or smooth muscle stimulation occurs by a different process involving gap junctions
   A. Contraction of mammalian heart is stimulated by an electrical impulse generated in small region of specialized heart muscle (**sinoatrial node**); acts as the heart's pacemaker
      1. Impulse spreads rapidly as ion current flows from 1 cardiac cell to its neighbors via gap junctions, causing the cells to contract in synchrony
      2. Intestinal/esophageal wall smooth muscle coordinated peristaltic waves that move down the length of wall are due to current (ion) flow through gap junctions interconnecting smooth muscle cells

B. Gap junctions also occur between the presynaptic & postsynaptic membranes of adjacent nerve cells in certain parts of brain
   1. They allow nerve impulses to be transmitted directly from one neuron to another without requiring the release of chemical neurotransmitters

V. Gap junctions can put many cells of tissue into intimate cytoplasmic contact; has important physiological consequences; with respect to molecules small enough to pass, connected cells form 1 giant compartment
   A. Highly active regulatory molecules (cAMP, inositol phosphates) pass through gap-junction channels
      1. Thus, individual connected cells' activities can be integrated so that they act as a functional unit
   B. Connected cells respond together even if only a small portion of the cells are stimulated by hormone
      1. If only a few cells near a particular blood vessel happen to be stimulated by a hormone, the stimulus can be rapidly transmitted to all cells of the tissue
   C. Gap junctions also allow cells to cooperate metabolically by sharing key metabolites (ATP, sugar phosphates, amino acids, many coenzymes), small enough to pass through these intercellular channels

VI. Connexins (Cx), the proteins of which gap junctions are made, are members of a multigene family
   A. ~20 different connexins with distinct tissue-specific distributions have been identified
      1. Connexons made of different connexins —> variations in conductance, permeability, regulation
      2. Sometimes, connexons in neighboring cells made of different connexins can dock & form functional channels, other times they cannot
   B. These compatibility differences may play important roles in either promoting or preventing communication between different types of cells in an organ
      1. Connexons joining cardiac muscle cells are made of connexin Cx43; those joining cells of heart's electrical conduction system made of Cx40 - they are incompatible, can't form working channels
      2. These cells are electrically insulated from each other, even though they are in physical contact
   C. Some inherited disorders have been associated with mutations in genes encoding connexins; consequences of these disorders include deafness, blindness, skin abnormalities or nerve degeneration

Mediating Intercellular Communication: Plasmodesmata

I. What are plasmodesmata? - a little like gap junctions of animal cells; cylindrical cytoplasmic channels (30 - 60 nm diameter) that pass through the cell walls of adjacent cells; plasmodesma is singular
   A. Unlike animals, whose cells are in intimate contact with each other, plant cells are separated by cell wall
   B. Plants lack the specialized junctions of animal tissues; but most plant cells connected by plasmodesmata; thus, plants lack the cell adhesion molecules typical of animals

II. They are lined by plasma membrane & usually contain a dense central structure (**desmotubule**)
   A. Serve as sites of intercellular communication allowing plant tissue to function as a metabolic unit
   B. Desmotubule is derived from smooth endoplasmic reticulum of the two cells - passage usually limited to space between desmotubule & inner membrane surface
   C. Until recently, it was thought that they were impermeable to molecules > ~1 kDa
      1. Based on studies in which different-sized fluorescent dyes were injected into cells
      2. More recent studies suggest that some plasmodesmata allow much larger molecules (up to 50 kDa) to pass between cells
   D. Unlike gap junctions, which have fixed openings, plasmodesmatal pore is capable of dilation so larger molecules (RNA, protein) can pass
      1. Insight into this dynamic property was gained from studies on plant viruses in the 1980s
      2. These viruses spread from one cell to another through plasmodesmata
      3. Viral infections increase plasmodesmata permeability so virus particles/nucleic acids can pass
      4. Virus encodes a movement protein that interacts with plasmodesma wall increasing pore diameter
      5. It was found later that plant cells make their own movement proteins that mediate cell-cell macromolecule transport of RNA & protein

Cell Walls

I. Naked cells covered by just a 10 nm lipid-protein membrane are extremely fragile structures (offers minimal protection for cells; cells of nearly all organisms but animals are enclosed in a protective outer envelope
   A. Protozoa have thickened outer coat
   B. Bacteria, fungi & plants have distinct cell walls (plant cell walls were first cell structures observed with light microscope)

II. Vital functions of plant cell walls:
   A. Plant cells develop osmotic turgor pressure that pushes against their surrounding wall —> the wall gives the enclosed cell its characteristic polyhedral shape
   B. They provide mechanical support for individual cells &, taken collectively, serve as a type of "skeleton" for the entire plant
   C. They protect cells against damage from mechanical abrasion, osmotic influx of water & pathogens
   D. They mediate cell-cell interactions
   E. Like ECM at animal cell surface, plant cell wall can be a source of signals that alter activities of cells that it contacts; example: fate of epidermal root cell
      1. Epidermal root cell fate may be governed by the composition of the underlying cell wall; 2 types of epidermal root cells have been distinguished: hair (H) cells & nonhair cells (N)
      2. H cells develop over cell-wall junctions; N cells develop between cell wall junctions
      3. If a cell positioned to become N cell is moved to a site overlying a cell-wall junction —> the fate of the cell is altered & it develops into an H cell
   F. Primary barrier to large molecular substance penetration, while ions/small molecules pass freely

III. Cell wall structure – often likened to fabricated materials like reinforced concrete or fiberglass; contain fibrous element embedded in nonfibrous, gel-like matrix
   A. Cellulose is fibrous component of cell wall; organized into microfibrils that confer rigidity on cell wall & provide resistance to tensile or pulling forces
      1. Microfibrils - ~5 - 10 nm in diameter; typically composed of bundles of 36 cellulose molecules oriented parallel to one another & held together by H bonds
      2. Walls of many plant cells are made of layers in which the microfibrils of one layer are oriented at ~90° to those of adjacent layers (similar to collagen fiber layers in corneal stroma)
   B. Proteins & pectin provide the matrix

IV. Cellulose molecules are polymerized at cell surface - glucose subunits are added to end of growing cellulose molecule by multisubunit enzyme complex embedded in membrane (**cellulose synthase**)
   A. Subunits of enzyme are organized into 6-membered ring (rosette), which is embedded in membrane
   B. Contrasts with matrix synthesis - made in cytoplasm, carried to surface in secretory vesicles
   C. Cortical microtubules just under the membrane set microfibril orientation at outer membrane surface

V. Cell wall matrix is made of 3 types of macromolecules - hemicelluloses, pectins, structural proteins
   A. Hemicelluloses - branched polysaccharides whose backbone consists of 1 sugar like glucose & side chains of other sugars like xylose
      1. Hemicellulose molecules bind to cellulose microfibril surfaces, cross-linking them into a complex structural network
   B. Pectins - heterogeneous class of negatively charged polysaccharides, containing galacturonic acid
      1. Form extensive, hydrated gel filling space between fibrous elements (attract H_2O like animal GAGs)
      2. When plant is attacked by pathogens, pectin fragments released from wall trigger defensive plant cell response
      3. When purified, pectin is used commercially to provide gel-like consistency of jams & jellies
   C. Proteins – functions not well understood, but they mediate dynamic activities

1. One class, the expansins, facilitate cell growth; they cause localized relaxation of cell wall, which allows the cell to elongate at that site in response to turgor pressure generated within cell
2. Cell wall-associated protein kinases span the plasma membrane & are thought to transmit signals from the cell wall to the cytoplasm

VI. Percentages of these various materials in cell walls are highly variable
   A. Depends on type of plant, type of cell & stage of wall
   B. Cell walls are dynamic structures modified in response to changing environmental conditions, like animal connective tissue ECMs

VII. Cell walls arise as a thin cell plate formed between membranes of newly formed daughter cells after cell division
   A. Cell wall matures by incorporating additional materials assembled inside cell & then secreted into the extracellular space
   B. Walls of young, undifferentiated cells must be able to grow along with the enormous growth of cell it surrounds along with providing mechanical support & protection from foreign agents
      1. Such walls of growing cells are called **primary walls** & they possess extensibility that is lacking in the thicker **secondary walls** present around many mature plant cells
      2. As cell enlarges, primary cell wall grows by insertion of materials into existing wall structure
   C. Transformation from primary to secondary wall occurs as wall increases in cellulose content &, in most cases, incorporates lignin (a phenol-containing polymer) that provides structural support
      1. Lignin is also the major component of wood & thus the most abundant organic molecule on Earth
      2. Lignin in water-conducting xylem cell walls gives support needed to move H_2O through the plant

The Role of Cell Adhesion in Inflammation and Metastasis

I. Inflammation is one of the primary responses to infection – although it is a protective response, it also produces negative side effects (fever, swelling due to fluid accumulation, redness, pain)
   A. If a part of the body were to become contaminated by bacteria (as with a puncture wound in skin), the site of injury would become a magnet for a variety of white blood cells
   B. White blood cells (leukocytes) that would normally stay in bloodstream instead are stimulated to traverse the endothelial layer lining the smallest veins (venules) in the region & enter the tissue
   C. Once in tissue, the leukocytes move in response to chemical signals toward the invading microorganisms, which they ingest

II. Inflammation can also be triggered inappropriately
   A. Damage to tissues of the heart or brain can occur when blood flow to these organs is blocked during a heart attack or stroke
      1. When blood flow to organ is restored, circulating leukocytes may attack the damaged tissue, causing a condition known as **reperfusion damage**
   B. An overzealous inflammatory response can also lead to asthma, toxic shock syndrome & respiratory distress syndrome

III. Research has focused on questions related to the above conditions – answers to such questions have focused on 3 types of cell-adhesion molecules: selectins, integrins & IgSF proteins
   A. How are leukocytes recruited to sites of inflammation?
   B. Why do they stop flowing through the bloodstream and adhere to vessel walls?
   C. How do they penetrate the walls of the vessels?
   D. How can some of the negative side effects of inflammation be blocked without interfering with the beneficial aspects of the response?

IV. Chain of events proposed to occur during acute inflammation – this cascade of events involves several different types of cell-adhesion molecules

A. Walls of venules become activated in response to chemical "signals" from nearby damaged tissue
   1. Endothelial cells lining these venules become more adhesive to circulating neutrophils, a type of phagocytic leukocyte that carries out a rapid, nonspecific attack on invading pathogens
   2. This change in adhesion is mediated by a temporary display of P- & E-selectins on the surfaces of the activated endothelial cells in the damaged area
B. When neutrophils encounter the selectins, they form transient adhesions that dramatically slow their movement through the vessel
   1. Neutrophils can be seen to roll slowly along the wall of the vessel
   2. Companies are trying to develop anti-inflammatory drugs that act by interfering with binding of ligands to selectins, especially P-selectin
   3. Anti-selectin antibodies block neutrophil rolling on selectin-coated surfaces *in vitro* & suppress inflammation & reperfusion damage in animals
   4. A similar type of blocking effect has been attained using synthetic carbohydrates that bind to P-selectin, thereby competing with carbohydrate ligands on the surfaces of the neutrophil
C. As neutrophils interact with the inflamed venule endothelium, integrins present on the neutrophil surface become activated, causing a marked increase in their binding activity
D. The activated integrins bind with high affinity to IgSF molecules (ICAMs) on the surface of the endothelial cells, causing the neutrophils to stop their rolling & adhere firmly to the vessel wall
E. The bound neutrophils then change their shape & squeeze between adjacent endothelial cells into the damaged tissue
   1. Invading neutrophils appear capable of disassembling the adherens junctions that form the major barrier between cells of the vessel wall
F. The above events ensure attachment of blood cells to blood vessel walls & their subsequent penetration occurs only at sites where leukocyte invasion is required

V. The importance of integrins in the inflammatory response is demonstrated by a rare disease called leukocyte adhesion deficiency (LAD)
   A. People with this disease cannot produce the β_2 subunit as part of a number of leukocyte integrins
   B. The leukocytes of these individuals lack the ability to adhere to the endothelial layer of venules, a step required for their exit from the bloodstream
   C. Patients suffer from repeated, life-threatening bacterial infections; the disease is best treated by bone marrow transplantation, which provides patient with stem cells that can form normal leukocytes
   D. Administration of antibodies against the b_2 subunits can mimic the effects of LAD, blocking the movement of neutrophils & other leukocytes out of blood vessels
      1. Such antibodies might prove useful in preventing inflammatory responses associated with diseases like asthma & rheumatoid arthritis or with reperfusion

VI. Cancer is a disease in which cells escape from the body's normal growth control mechanisms & proliferate in an unregulated manner
   A. If malignant cells remained in a single mass (as often occurs in some types of skin cancer or thyroid cancer), most cancers would be readily cured by surgical removal of the diseased tissue
   B. Most malignant tumors, however, spawn cells that are capable of leaving the primary tumor mass & entering the bloodstream or lymphatic channels
      1. This thereby initiates the growth of secondary tumors in other parts of the body
      2. The spread of a tumor within the body (**metastasis)** is the reason why cancer is so devastating
   C. Metastatic cells (cancer cells that can initiate the secondary tumors) are thought to have special cell-surface properties that are not shared by most other cells in the tumor
      1. Metastatic cells must be less adhesive than other cells to break free of the tumor mass
      2. They must be able to penetrate numerous barriers, (ECMs of surrounding connective tissues, basement membranes that line the blood vessels that carry them to distant sites
      3. They must be able to invade normal tissues if they are to form secondary colonies

D. The penetration of ECMs is accomplished largely by ECM-digesting enzymes, most notably the matrix metalloproteinases (MMPs)
   1. In some cases, cancer cells secrete their own MMPs, but usually growing tumors induce the synthesis & secretion of these enzymes by the surrounding "host" cells
   2. Either way, these enzymes degrade the proteins & proteoglycans that stand in the way of the cancer cell migration
   3. MMPs also seem to stimulate cancer cell growth & promote the development of the blood vessels that nourish growing tumors

E. Because of their prominent role in malignant tumor development, MMPs became a major target of the pharmaceutical industry
   1. Synthetic MMP inhibitors were found that could reduce metastasis in mice; clinical trials ensued on patients with a variety of advanced, inoperable cancers were done
   2. So far, they have shown little promise in stopping late-stage tumor progression & have sometimes led to joint damage
   3. An MMP inhibitor has shown promise in treating patients with early-stage (non-metastatic) stomach cancer
   4. Several large-scale trials are still in progress; it is too early to tell if any of these drugs will have therapeutic value
   5. Thus far, the only FDA-approved MMP inhibitor (Periostat) is us3ed to treat periodontal disease
   6. The failure of these studies have been evaluated in light of potential flaws in the ways that anti-cancer drugs are selected for testing

F. Changes in the numbers & types of various cell-adhesion molecules (& thus the ability of cells to adhere to other cells or to ECMs) have also been implicated in the promotion of metastasis
   1. Major studies have focused on E-cadherin, the predominant epithelial cell-cell adhesion molecule
   2. In one survey of epithelial cell tumors (breast, prostate & colon cancers), it was found that the greater the level of E-cadherin expression, the less the cell's metastatic potential
   3. Other studies suggest that the progressive loss of E-cadherin from cancer cell surfaces as they grow in a tumor increases the level of malignancy of the cells & worsens the patient's prognosis
   4. Thus, the presence of E-cadherin favors the adhesion of cells to one another & suppresses the dispersal of tumor cells to distant sites
   5. In fact, when malignant cells are forced to express extra copies of the E-cadherin gene, they become much less capable of causing tumors when injected into host animals
   6. E-cadherin importance - study of native New Zealander family (lost 25 members to stomach cancer over 30-years); DNA analysis shows that susceptible individuals have E-cadherin gene mutations

LECTURE HINTS

The Extracellular Space

Start your lecture on cell interactions by talking about the extracellular space and its importance. Emphasize that the extracellular space and the molecules contained within it determine how cells will interact with one another and/or any adjacent noncellular surfaces. For historical perspective, introduce the terms cell coat (carbohydrate chains attached to proteins of the membrane outer leaflet) and fuzzy layer (carbohydrates attached to the carbohydrate chains of the cell coat by weak interactions). Point out that they are now known collectively as the glycocalyx since they are so difficult to distinguish from each other. Mention that these sugars can be removed from the cell surface apparently without permanent cell damage. They are much like fur on the pelt of an animal. If the fur is removed, the animal might be uncomfortable for a while, but the hair will come back as will the carbohydrate chains. Stress the presumed functions of the glycocalyx: mediation of cell - cell and cell -

substratum interactions, mechanical protection for the cell, and serving as a barrier to particles reaching the membrane. Also, give examples of some extracellular structures typically found in organisms as part of their extracellular matrix: jelly coats and thick capsules around eggs, the basement membrane, extracellular components of bone and cartilage. Emphasize perhaps the major common trait of these materials - that they self-assemble. Also, stress that these extracellular materials have a major influence on the activities of the cells they surround.

Components of the Extracellular Matrix

Once you have defined the extracellular matrix and outlined its functions, it is useful to describe the molecules that make up these extracellular materials, stressing the similarities and differences between their structures. Since fibronectin is so well studied, it serves well as an example of glycoproteins in the extracellular matrix and the roles they play. Fibronectin has high affinity binding sites for other extracellular molecules (collagens and proteoglycans) and the cell surfaces of the cells producing it. Its function may be to link the cell surface and the collagen- and GAG-containing substrate. Do not forget to describe the sugar groups that form a significant part of most of these proteins. Their sequences are highly variable and they are branched oligosaccharides. Also, mention the non-covalent association of fibronectin and its relatives (laminin, chondronectin, etc.) with the cell membrane via glycolipid receptors. You may also wish to mention other examples of these proteins (laminin, tenascin, entactin, thrombospondin) and briefly describe them, their locations and their functions.

Next, move onto glycosaminoglycans (GAGs) that were previously known as mucopolysaccharides. Unlike the sugars of the glycoproteins, the GAGs are composed of repeating disaccharides with one sugar always an amino sugar; they also contain no branching and are extremely long molecules. All of the GAGs except hyaluronic acid contain sulfated sugars. Since the molecules also contain carboxyl groups, GAGs are highly acidic and thus highly hydrophilic. GAGs usually exist in combination with proteins (proteoglycan or mucoprotein) in a structure called a proteoglycan aggregate. Running down the center of this aggregate is the GAG, hyaluronic acid, which forms the core of the structure. Projecting periodically from the hyaluronic acid core are individual proteoglycan monomers. Each of these consists of a central core protein with sulfated GAGs extending from it at regular intervals. Such a structure is both enormous and extremely hydrophilic. Consequently, proteoglycan aggregates form porous, amorphous, highly hydrated gels; they occupy huge volumes for their weight and are capable of resisting fairly high compression (crushing) forces. They interact with glycoproteins and collagen to form extracellular structures. While most proteoglycans are completely extracellular, the heperan sulfate proteoglycans (HSPGs) function at the cell surface. In fact, the core protein of the HSPGs spans the membrane.

Finally, I suggest that you briefly mention collagen. Stress that there are numerous types of collagen and that particular types are restricted to specific tissues although two or more types can be found together. Emphasize the triple helical arrangement of collagen chains, the cross-links that strengthen their association and their high tensile strength. Point out that the arrangement of the fibers in a particular tissue is related to that tissue's function, the forces routinely acting upon it and its function in the tissue. If you wish, you may spend more time on the structures of the different types of collagen, although I do not recommend it since this time can be used more effectively for other things.

Illustration

THE CORNEAL STROMA

The formation of the corneal stroma in the embryonic chick serves as an excellent example of the roles collagens and GAGs play in the sculpting of tissues in living organisms. The cornea of the chick is composed of three layers: an outer and inner epithelium and a greatly thickened stroma. The stroma consists of collagen fibers embedded in a GAG matrix with flattened fibroblasts scattered throughout. Corneal development begins on day 3 when the portion of the embryonic outer ectoderm that will become the corneal epithelium begins to secrete collagen and the GAG chondroitin sulfate from its basal surface. The result is completely non-cellular (no fibroblasts present) and called the primary stroma. It contains about 20 collagen fiber layers arranged orthogonally with each layer perpendicular to those above and below it. The arrangement of these fibers arises by self-assembly of the secreted collagen fibers. On day 6, the stroma swells following the secretion of hyaluronic acid (another GAG) into the stroma. Due to its hydrophilic nature, the hyaluronic acid attracts water into the stroma thus accounting for the swelling. The swelling introduces space between the collagen fiber layers and fibroblasts wander into the stroma using the collagen fibers as a substrate. Once inside the stroma, the fibroblasts secrete more collagen and chondroitin sulfate, which then organize into the secondary (adult) stroma. The collagen fibers of this stroma are also organized in an orthogonal fashion. On day 10, the enzyme hyaluronidase is secreted into the stroma. It digests the hyaluronic acid secreted earlier and thus water exits the stroma; the collagen fiber layers collapse onto each other as the swelling decreases, trapping the fibroblasts and flattening them between the layers.

Cell Adhesion

As a transition to the topic of cell adhesion, I point out that the presence or absence of adhesion in certain situations is extremely important. For example, you would not want cells to stick to the surfaces of your blood vessels under normal circumstances, since this could lead to occlusions that cut off blood flow. On the other hand, most organs are composed of tissues that must be held together in particular ways. If that does not happen, the organ function is likely to be compromised. The cells of epithelia must adhere to the noncellular basement membrane or basal lamina. Without this association, the epithelia would be less able to maintain their integrity.

Adhesion to Noncellular Surfaces

Certain cells make their living by adhering to noncellular surfaces in an organism. These surfaces are a mixture of glycoproteins, GAGs and collagen. Studies of cells in *in vitro* culture systems show that naked glass or uncharged plastic do not work as well as surfaces treated with a thin layer of collagen or serum protein. With the latter surface, cells are able to attach to the surface, since it approximates the surface in an organism. This is not the case with the former surfaces.

As they sit down on a surface, cells go through stages of attachment. At first, a cell is rounded like most cells in an aqueous medium. After initial contact with the substrate, the cell sends out projections that make more stable attachments to the surface. As the number of attachments increases, the cell begins to flatten out. Initially, it looks like a fried egg. (Ask your students why.) Then as the cytoskeleton changes, probably in response to transmembrane signaling, cells adopt their final morphology. Fibroblasts spread into a long, thin bipolar shape. Hepatocytes adopt a rectangular (epithelioid) shape and chondrocytes become stellate. Emphasize that the bottom surface of the cell does not contact the surface uniformly. There are discrete sites (focal contacts) where the cell is in close proximity to the dish. These are the sites of strongest adhesion between the cell and substrate. Microfilaments are closely associated with these sites inside the cell; interference with their function results in blockage of attachment and spreading.

The attachment of these cells may be mediated by a member of the superfamily of integral membrane proteins called integrins. These proteins bind substances on both sides of the membrane and apparently can transmit signals between the inside and outside of the cell. At the focal adhesions (focal contacts) where cells in culture

contact the substrate, integrins bind to the ECM on the dish externally and to actin filaments in the cytoskeleton intracellularly. Signals generated by binding to integrins could be responsible for changes in cell behavior. Proteins that bind integrins generally have the tripeptide sequence arginine-glycine-aspartic acid (abbreviated RGD). Propose some experiments that might demonstrate the importance of the RGD sequence. For example, ask the students what would happen if the RGD tripeptide were added to a fibronectin-coated culture dish along with some cells; integrins can bind to fibronectin via the fibronectin RGD sequences.

Adhesion of Cells to Other Cells

In classic experiments, investigators disaggregated tissues and plated their cells out on culture dishes. Some of these cells were then radiolabeled and exposed to the different cell types on the plates. The radiolabeled cells would stick only to cells of their own type. Explain the experiment to students and ask them to predict the results. If they are reluctant to answer, turn it into a multiple-choice question. It is clear that cells must be able to recognize selectively the surfaces of other cells. Cells stick to each other by forming high affinity noncovalent bonds between complementary cell-surface-associated molecules. Four distinct families of membrane proteins are responsible for these interactions: selectins, certain members of the immunoglobulin superfamily, some integrins, and cadherins. Describe and define each group. Selectins bind to specific carbohydrate groups on the cell surface. Point out that different tissues use different selectins (E-, P-, and L-selectins; which are found on endothelial cells, platelets-endothelial cells and leukocytes, respectively). The integrins and immunoglobulin super family adhesion molecules have similar domains. Most of them are present as integral proteins on lymphocyte surfaces. The cadherins, which are glycoproteins that require calcium for cell adhesion, join cells of similar types. Different tissues possess different cadherins: epithelia contain E-cadherins, neural tissues contain N-cadherins and P-cadherins are found in placenta.

As an example of cell-cell adhesion, I have found it useful to tell students about sponge dissociation and reaggregation. Start by describing Wilson's experiments of the early 1900s and progress to the work of others. Ask the students why sponges rather than mammals are suited for such studies (only a few cell types, ease of dissociation, potential moral problems with mammals). Two methods of dissociation have been used to break sponges up into single cell suspensions: mechanical dissociation and chemical dissociation. Sponges can be forced through a silk mesh and since they are loosely attached, this treatment separates them into single cells or perhaps small clumps of cells. Sponges can also be dissociated chemically by exposure to EDTA, a Ca^{2+} chelator, or placement into Ca^{2+} - Mg^{+2}-free (artificial) seawater. Once dissociated, mechanically dissociated cells will reassociate. Eventually, new sponges form with the cells taking up appropriate positions in the organism. This suggests that the cells possess all the information needed for cell self-assembly. If two different species are dissociated and mixed together, they will sort themselves out, demonstrating species specificity. Chemically dissociated cells that have been washed repeatedly, when kept at 4°C, do not reassociate because the adhesion molecules that hold them together have been removed. At 4°C, these molecules cannot be resynthesized. When the temperature is raised, the adhesion molecules are produced and the cells reassociate. These cells will reassociate at 4°C if the original wash supernatant is added back to the cells. Propose a number of experiments mixing mechanically and chemically dissociated sponges. Ask your students to predict the results. The aggregation factor responsible for this behavior has been identified as a giant multimeric proteoglycan large enough to be seen in the electron microscope. It looks like a sunburst and requires Ca^{2+} to maintain its integrity, explaining chemical dissociation of sponges.

Vertebrate cell adhesion is different. It appears to be more tissue driven with tissue specificity seeming more important than species specificity. Furthermore, glycoproteins seem to be more important as adhesion molecules. Malcolm Steinberg proposed the differential adhesion theory, which stated that the number of contacts between cells determines the position cells adopt within an aggregate. Cells with a larger number of contacts will tend to move to the center of the aggregate, while cells making a smaller number of contacts will stay on the outside of the aggregate. Steinberg suggested that the configuration adopted by the cells is the one that is most thermodynamically favored. Once again, propose experiments such as the following one and ask the students to predict the results. If tissue A and tissue B are mixed, B ends up inside and A on the outside of the

aggregate. If tissues B and C are mixed, B ends up outside and C inside. What happens when tissues A and C are mixed? A ends up on the outside and C on the inside. Steinberg's work suggested that there is a hierarchy of adhesiveness.

Cell Junctions

In discussing cell junctions, tell your students to take note of the differences between the different junctions in terms of the distances between the membranes, the extent of cell surface involved, the appearance of materials between the adjacent membranes and the function served by the junction. It is also useful to make note of diseases that are caused by malfunctions in these structures.

Adherens Junctions and Desmosomes

Adherens junctions hold cells together tightly and form part of the intercellular junctional complex. Typically, they are found in epithelia and they make it hard to separate the cells mechanically. The cells can, however, be separated by treatment with trypsin. They are plentiful in tissues subjected to mechanical stress (skin, gingiva and cervix). If deficient, they can cause skin diseases where skin cells are readily shed.

The membrane in adherens junctions appears to be unaltered and separated by 20 - 35 nm. The space between the membranes is filled with a fine, filamentous, intercellular cement. Cadherins play a role in holding the cells together. An electron dense material underlies the membrane in this type of junction. Belt desmosomes (zonulae adherens) encircle the cell like a belt and thus help to cement the cell to all those around it. Actin microfilaments encircle the cell underneath the membrane, suggesting a contractile role for the belt desmosome as well. In cells requiring a narrowing of their apical end, the actin filaments of the belt desmosome appear to be responsible.

Spot desmosomes (maculae adherens) are disk-shaped and act like spot-welds attaching a cell to its neighbors. Tonofilaments made of keratin enter the amorphous material underlying the membrane and then loop back into the cytoplasm heading for another spot desmosome. This serves to interconnect the spot desmosomes, which spreads force experienced at any one point on the cell surface across the whole cell surface. This prevents a single spot desmosome from being pulled apart by high tension applied to the membrane.

Analogy

THE WATER BUG ANALOGY

The ability of spot desmosomes to withstand forces applied to the cell surface is similar to the ability of a water bug to walk on the surface of the water without breaking through and sinking like a stone (albeit a small one). Such bugs have long legs, which help to spread their weight over a large area. Consequently, their weight spread over such a large area is not sufficient to break through the surface tension of the water.

Hemidesmosomes, as their name suggests, look like a half of a desmosome. Their function is to attach cells to the basal lamina. Ask the students why the hemidesmosome looks like a half desmosome. They will usually be able to answer that a full desmosome requires the participation of two cells. If they have difficulty with the answer, ask leading questions.

Tight Junctions

Tight junctions (zonulae occludens) are occluding junctions. They seal off the extracellular space on one side of an epithelium from the other side. They are important in organs like the urinary bladder, which can afford no leakage. In tight junctions, the membranes of the cells involved contact each other intermittently. They run in belts around the cell circumference and there appears to be no space between the membranes at the point of contact where integral membrane proteins meet in the extracellular space. Another earmark of tight junctions is the electrical resistance they usually generate. They are also instrumental in establishing the blood-brain barrier, which prevents the passage of substances from the blood to the brain. It is also thought that they may restrict the movement of membrane proteins by preventing their movement from apical to lateral or lateral to apical surfaces.

Gap Junctions

Gap junctions are present as patches of varying size. The membranes approach each other closely (2 - 3 nm), more closely, in fact, than in desmosomes but do not touch as they do in tight junctions. There are integral membrane proteins (connexins) that assemble into a 6-protein unit called the connexon, which has a central opening (the annulus; 1.5 nm in diameter). The opening allows the passage of materials small enough to move through (~1000 daltons) and thus facilitates communication between the cytoplasms of the two connected cells. Such connections help to coordinate the contractions of cardiac (heartbeat) and smooth muscle (intestinal and esophageal peristalsis).

Analogy

THE PIPES ANALOGY

I remind my students that they may occasionally have seen two adjacent buildings with pipes running between them carrying water (or something else) between buildings. This is an effective illustration of gap junctions. The walls of the two buildings represent the cell membranes of the two cells. The pipes passing through the walls of the buildings resemble the connexons of the gap junctions and serve to connect the interiors of the two buildings. Enclosed walkways between buildings (over a street) also serve as an effective analogy. Such a walkway has just been built in the high school adjacent to our campus so it is nearby and easy for students to see. Use the architecture and natural surroundings in your local area for this and other analogies.

Point out that the effect of connecting cells with gap junctions is to connect them into one large compartment. This allows the connected cells to act in unison in response to stimuli.

Illustration

GAP JUNCTION EXPERIMENTS

Two experiments effectively illustrate the function of gap junctions. The first starts with the injection of fluorescein, a fluorescent molecule with a molecular weight of about 1000 daltons, into a cell that is connected to other cells by gap junctions. The fluorescent molecules move through the gap junctions to adjacent cells, an event, which can be seen. If gap junctions are absent, this will not happen. This experiment may also be adapted for plasmodesmata.

The second experiment involves the co-culturing of ovarian granulosa cells and cardiac cells. Ovarian granulosa cells, the follicle cells surrounding a developing oocyte, respond to follicle stimulating hormone (FSH) by producing cyclic AMP (cAMP). The production of cAMP leads to metabolic and morphological changes in these cells that are not easily observed. Cardiac cells, on the other hand, respond to norepinephrine by making cAMP, which leads directly to an increase in cellular contraction, which is easy to observe. Neither of these cell types responds to the other's hormone. When they are grown in mixed culture, the cells form gap junctions. Addition of FSH to the culture medium causes the cardiac cells to beat even though their hormone has not been added to the culture, since the cAMP is able to pass through the gap junctions from the responding ovarian granulose cells to the cardiac cells. Gap junctions are, of course, required for this to happen. Explain the parameters of the experiments and ask the students to predict the outcome.

Plasmodesmata

Plasmodesmata are cylindrical cytoplasmic channels through plant cell walls. They accomplish for plants what gap junctions accomplish for animal cells, allowing communication between the cytoplasms of adjacent plant cells. Ask your students why there are no junctions in plants analogous to the desmosomes or tight junctions of animals. The answer is that the cell wall in plants would prevent those kinds of interaction between plant cell membranes and eliminate a need for them since the cell wall performs their functions in a plant.

Cell Walls

Outline for your students the functions of cell walls. While the cell walls of plants and bacteria serve essentially the same functions, there are significant differences in their structures.

Bacterial cell walls are divided into two groups depending on their appearance after exposure to Gram's stain. Gram-positive bacteria like *Streptococcus* retain Gram stain. Such walls contain peptidoglycans and teichoic acid. It is thought that they retain the stain because of the thickness of the cell wall. Gram-negative bacteria (*E. coli*) do not retain the stain after the post-staining alcohol treatment. This is thought to be due to the thinness of the cell wall in these bacteria. This cell wall consists of a thin peptidoglycan layer sandwiched between a somewhat thicker outer layer that resembles a cell membrane and the plasma membrane itself.

Plant cell walls consist of cellulose fibrils embedded in a noncellulosic gel-like matrix composed of polysaccharides (hemicellulose, pectin, lignin), protein and water. The rigidity and strength of the wall derives from its similarity in structure to fiberglass or reinforced concrete. If time permits, you may wish to discuss the significance of the primary and secondary plant cell walls to plant cell growth.