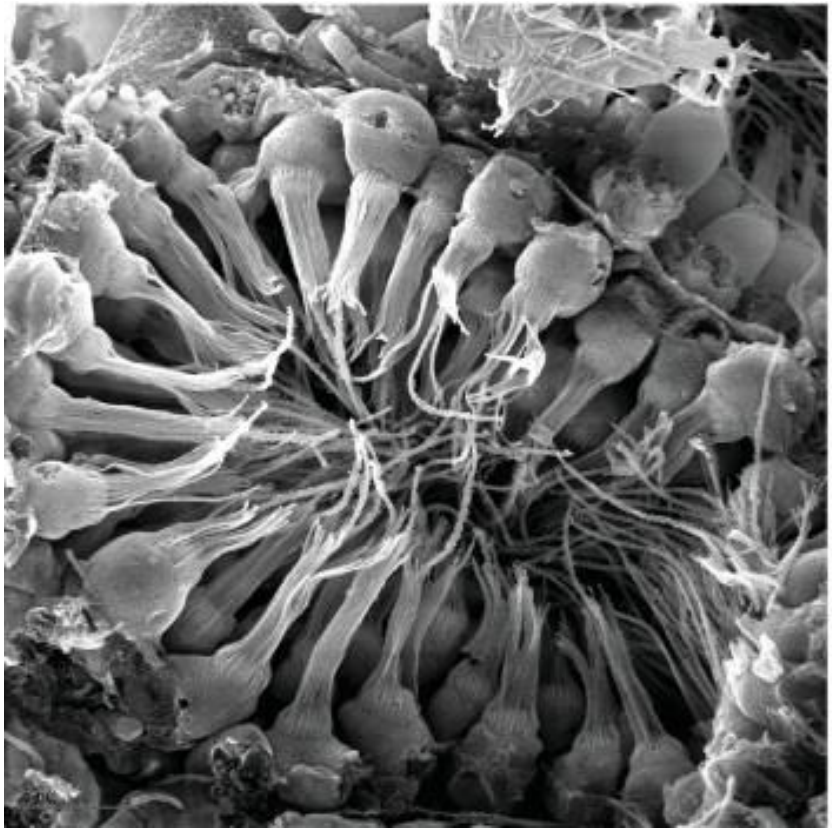
CHAPTER 11

The Extracellular Matrix and Cell Interactions



Courtesy of Scott Nichols

11.1 | Overview of Extracellular Interactions

Materials present outside the plasma membrane play an important role in the life of a cell.

Most cells in a multicellular plant or animal are organized into clearly defined tissues in which the component cells maintain a defined relationship with one another and with the extracellular materials that lie between the cells.

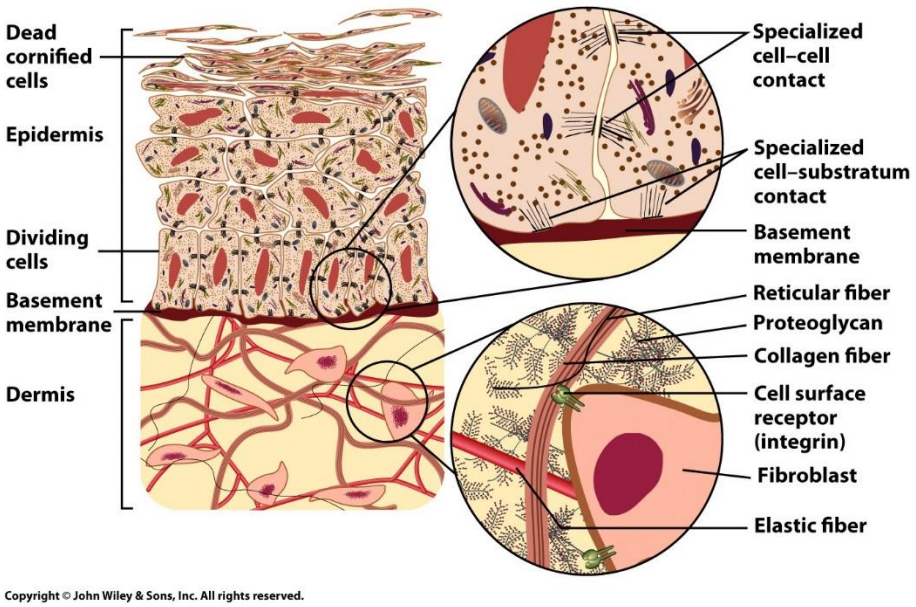
These interactions regulate such diverse activities as cell migration, cell growth, and cell differentiation, and they determine the three- dimensional organization of tissues and organs that emerges during embryonic development.

11.1 | Overview of Extracellular Interactions

The epidermis has closely packed cells attached to one another and to an underlying non-cellular layer by specialized contacts.

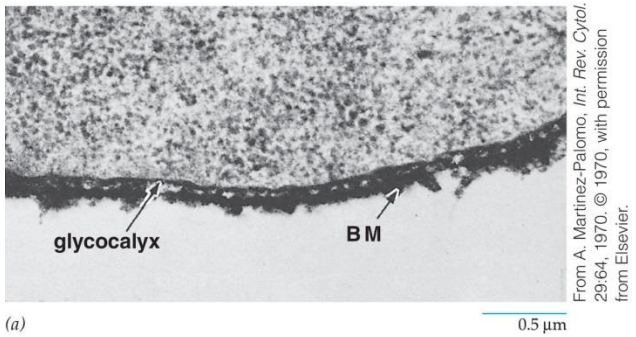
The dermis is a type of connective tissue made up of extracellular material.

Fibroblasts of the dermis have receptors that mediate interactions and transmit messages between cytoplasmic proteins in the cell and the environment.



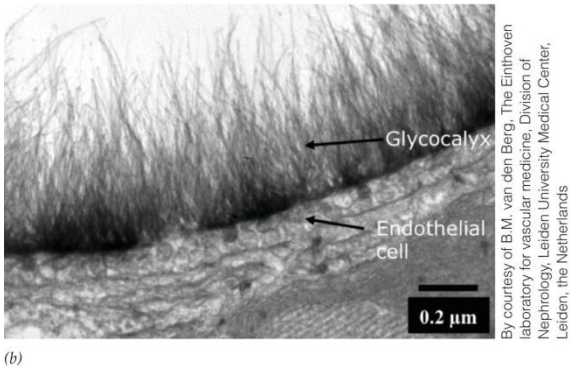
An overview of how cells are organized into tissues and how they interact with one another and with their extracellular environment.

11.2 | The Extracellular Matrix



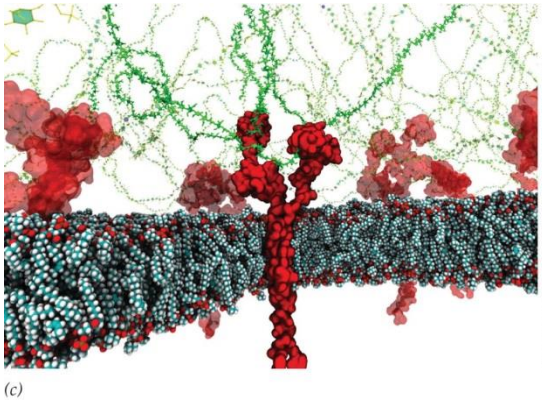
From A. Martinez-Palomo, *Int. Rev. Cytol.* 29:64, 1970. © 1970, with permission from Elsevier.

Basal surface of ectodermal cell, early chick embryo



By courtesy of B.M. van den Berg, The Einthoven laboratory for vascular medicine, Division of Nephrology, Leiden University Medical Center, Leiden, the Netherlands

EM: endothelial glycocalyx in a coronary capillary



Courtesy of Igor Pivkin et al., Università della Svizzera italiana. *Biophys. Journal* 106(1):232-234, 2014, with permission from Elsevier.

Molecular model of the glycocalyx

Carbohydrate projections form part of the glycocalyx (or *cell coat*) on the outer surface of the plasma membrane.

The glycocalyx mediates cell–cell and cell–substratum interactions, provides mechanical protection to cells, serves as a barrier to particles moving toward the plasma membrane, and bind important regulatory factors that act on the cell surface.

11.2 | The Extracellular Matrix

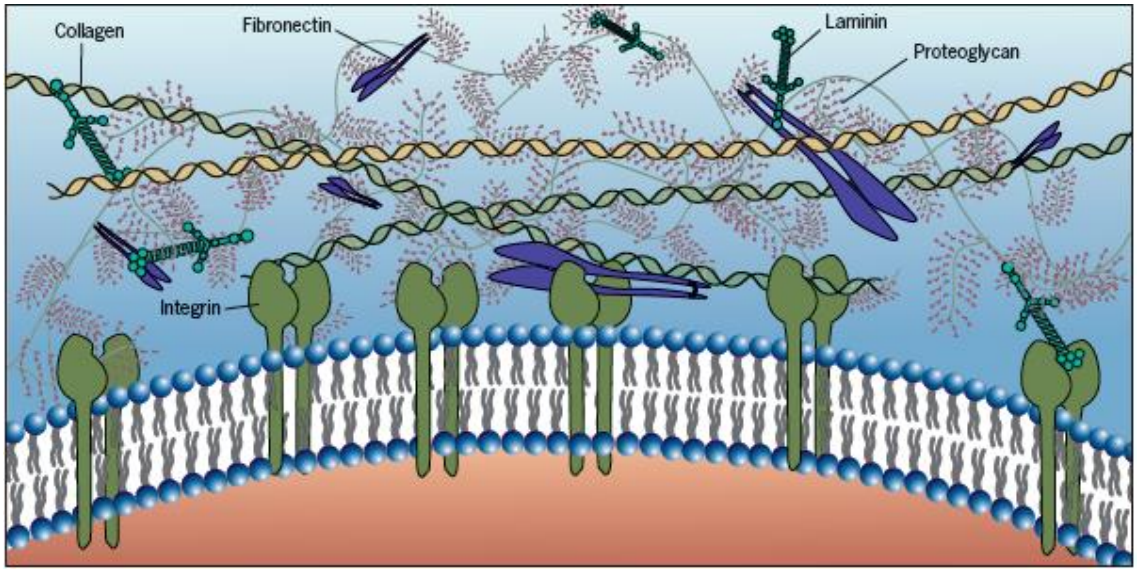
Basement membranes provide mechanical support for the attached cells, generate signals that maintain cell survival, serve as a substratum for cell migration, separate adjacent tissues within an organ, and act as a barrier to the passage of macromolecules.

Basement membranes of capillaries prevent the passage of proteins out of the blood and into the tissues, particularly important in kidney function.

Kidney failure in long-term diabetics may result from an abnormal thickening of the basement membranes surrounding the glomeruli.

Basement membranes also serve as a barrier to invasion of tissues by cancer cells.

11.2 | The Extracellular Matrix



Organized network of extracellular materials outside plasma membrane, provides support and determines shape and activity of cell.

The ECM may take diverse forms in different tissues and organisms, yet is composed of similar macromolecules.

Most proteins inside cells are globular, while those in the ECM are fibrous.

These proteins are secreted into the extracellular space where they are capable of self-assembling into an interconnected 3D network.

11.3 | Components of the Extracellular Matrix

Collagen

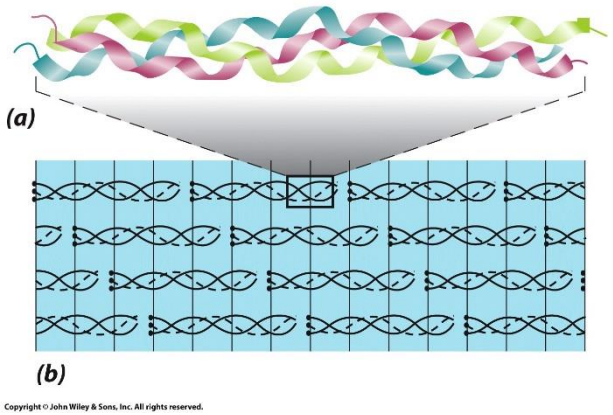
Collagens comprise a family of fibrous glycoproteins present only in the ECM, with 28 types found in humans.

Collagen is the single most abundant protein in the human body constituting more than 25 percent of all protein.

Collagen is produced by fibroblasts, smooth muscle and epithelial cells.

Collagen types are restricted to particular locations, but two or more different types are often present together, and mixing of different types can occur in the same fiber.

Collagen molecule: triple helix of three helical alpha chains



Collagen I molecules become aligned in staggered rows

11.3 | Components of the Extracellular Matrix

Collagen

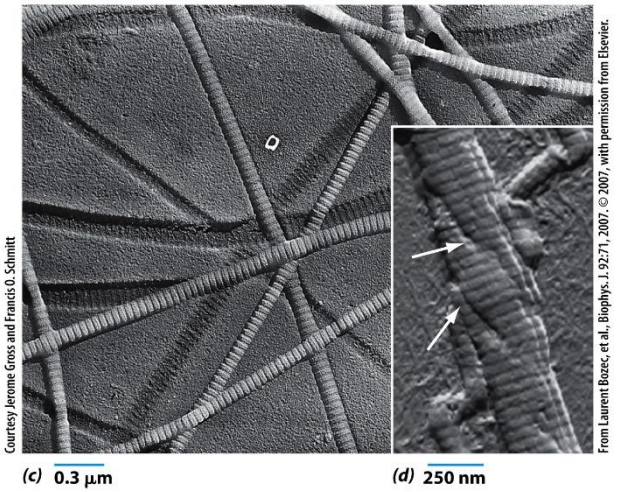
All collagen molecules are **trimers** consisting of three polypeptide chains, called α chains.

Along part of their length, the three polypeptide chains are wound around each other to form a rod-like triple helix.

Collagen molecules contain large amounts of proline, and many of the proline/lysine residues are hydroxylated to form hydrogen bonds between chains.

Ascorbic acid is required as a coenzyme by the enzymes that add -OH groups to the lysine and proline residues.

EM of human collagen fibrils after metal shadowing



Atomic force micrograph of a collagen fibril surface

11.3 | Components of the Extracellular Matrix

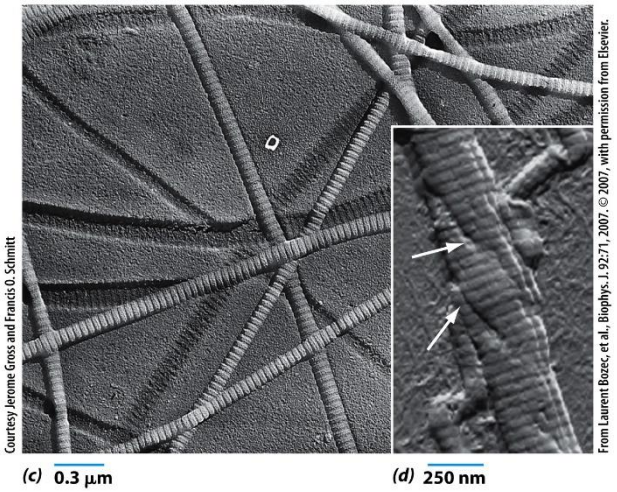
Collagen

Type I, II, and III collagens are fibrillar because they assemble into cable-like fibrils and then are packaged into thicker fibers.

The fibrils are strengthened by covalent cross-links between lysine and hydroxylysine residues on adjacent molecules.

This cross-linking process continues through life and may contribute to the decreased skin elasticity and increased bone brittleness among the elderly.

EM of human collagen fibrils after metal shadowing



Atomic force micrograph of a collagen fibril surface

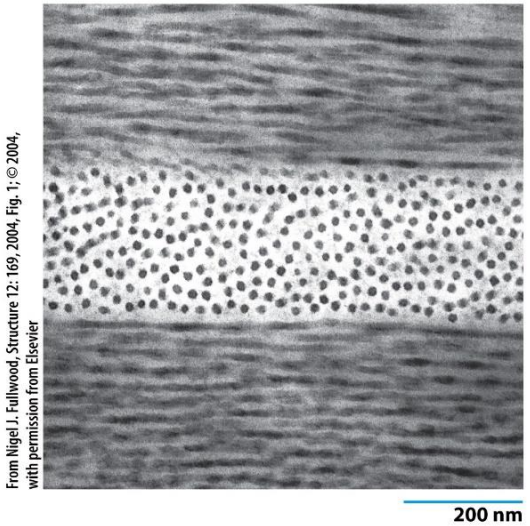
11.3 | Components of the Extracellular Matrix

Collagen

Collagen provides the insoluble framework that determines many of the mechanical properties of the matrix.

Tissue properties can often be correlated with the 3D organization of its collagen (e.g. tendons, cornea).

In the cornea, the fibrils of each layer are parallel to other fibrils in the layer but perpendicular to the fibrils in the layer on either side. This structure provides strength and promotes the tissue's transparency.



Corneal stroma: layers of collagen fibrils of uniform diameter and spacing arranged at right angles

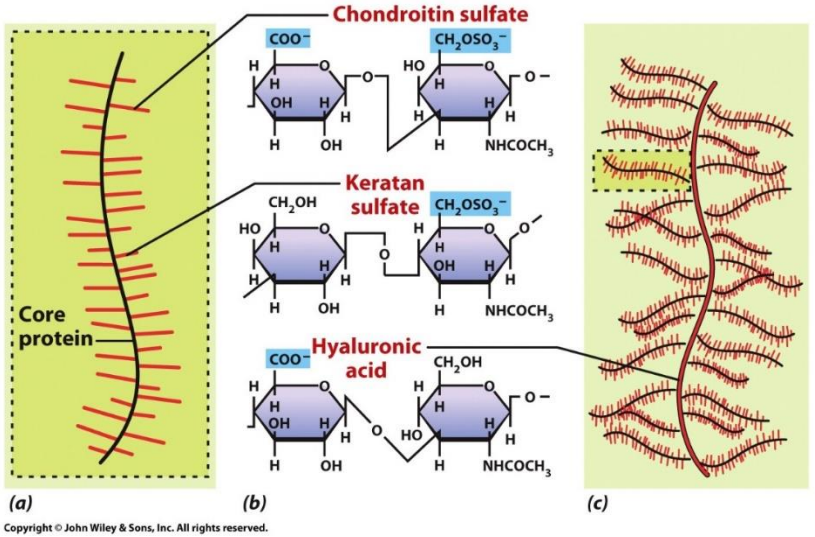
11.3 | Components of the Extracellular Matrix

Proteoglycans

Proteoglycans – protein-polysaccharide complex, with a core protein attached to *glycosaminoglycans (GAGs).*

Have a repeating disaccharide structure, -A-B-A-B-, where A and B represent two different sugars.

Proteoglycans of the ECM may be assembled into gigantic complexes by linkage of their core proteins to a molecule of *hyaluronic acid*, a non-sulfated GAG.



Schematic representations of a single proteoglycan, repeating disaccharide structure of GAGs, and linkage to hyaluronic acid to form a giant complex

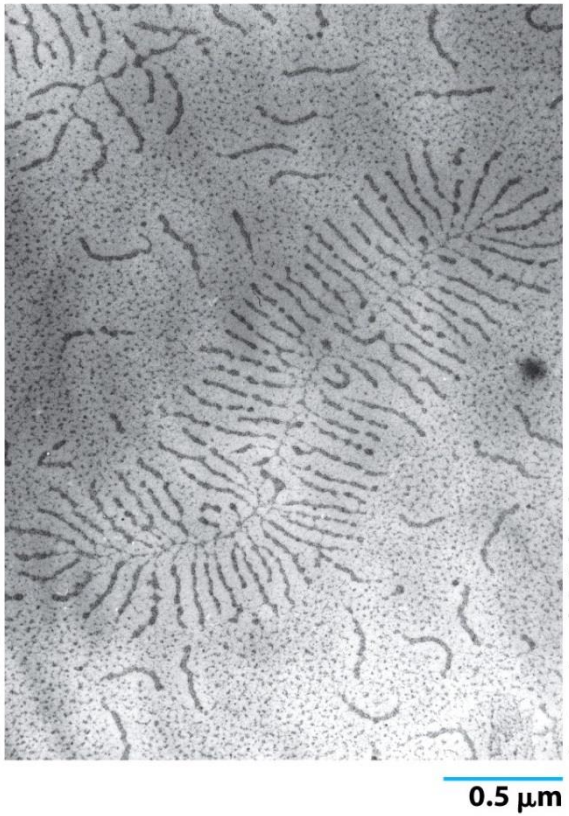
11.3 | Components of the Extracellular Matrix

Proteoglycans

Negative charges on the sulfated GAGs, attract cations, which in turn bind large numbers of water molecules.

As a result, proteoglycans form a porous, hydrated gel that fills the ECM to resist crushing (compression) forces.

Collagens and proteoglycans give cartilage and other ECM strength and resistance to deformation.



Electron micrograph of a proteoglycan complex isolated from cartilage matrix.

11.3 | Components of the Extracellular Matrix

Fibronectin

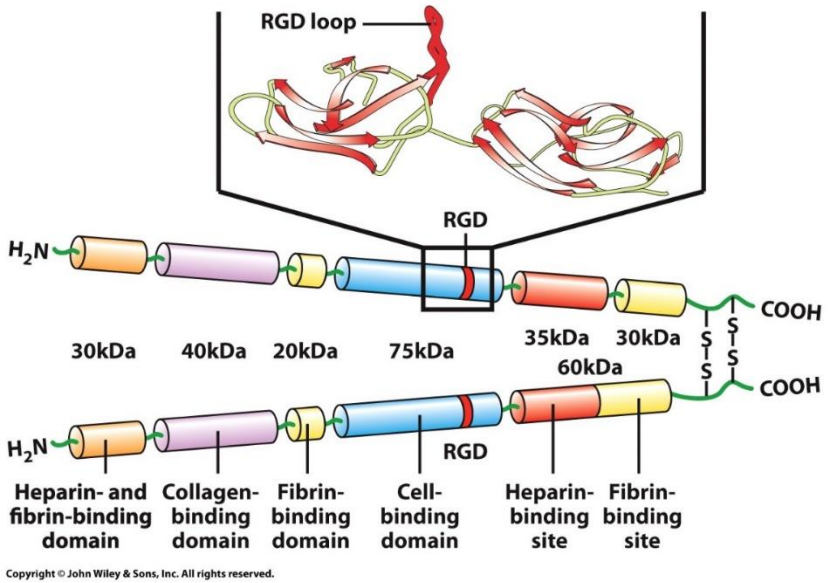
Fibronectin consists of a linear array of 30 Fn domains to give a modular construction, which combine to form five or six larger functional units.

Fn-type domains are found in blood clotting factors and receptors.

Each of the two polypeptide chains that make up fibronectin contain:

1) Binding sites for ECM molecules such as collagens, proteoglycans, and other fibronectin molecules.

2) Binding sites for receptors on the cell surface.



Human fibronectin molecule consists of two similar polypeptides joined by disulfide bonds.

11.3 | Components of the Extracellular Matrix

Laminin

Laminins comprise a family of at least 15 extracellular glycoproteins.

Laminin has three polypeptide chains linked by disulfide bonds and organized into a molecule resembling a cross with three short arms and one long arm.

They can greatly influence a cell's potential for migration, growth, and differentiation.

Primordial germ cells possess a cell surface protein that adheres strongly to a subunit of laminin.

Primordial germ cells (green) migrating along a tract of laminin (red) from the dorsal mesentery to the developing gonad.

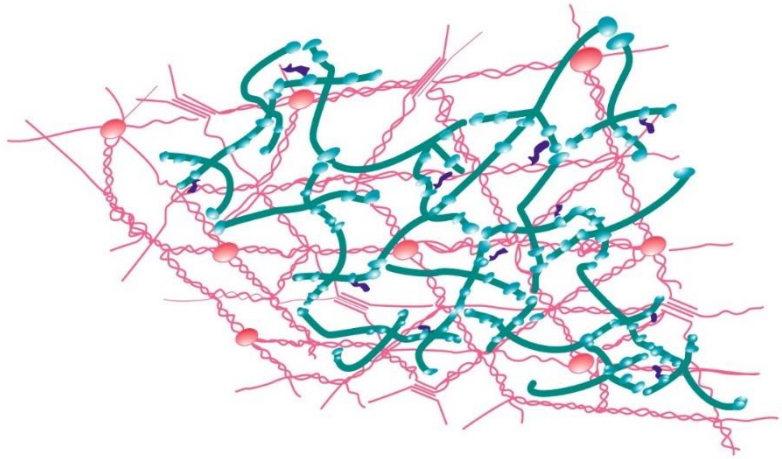
11.3 | Components of the Extracellular Matrix

Laminin

Laminins can bind to other laminin molecules, proteoglycans, and other components of basement membranes.

Laminin and type IV collagen molecules of basement membranes may form separate, interconnected networks.

These interwoven networks give basement membranes both strength and flexibility.



A model of the basement membrane scaffold. Basement membranes contain two network-forming molecules, collagen IV (pink), and laminin (green) that are connected by entactin molecules (purple).

11.4 | Dynamic Properties of the Extracellular Matrix

Although images can portray the ECM as a static structure, in actuality it can exhibit dynamic properties both in space and time.

Spatially, ECM fibrils can stretch several times their normal length as they are pulled on by cells and contract when tension is relieved.

Temporally, ECM components are subject to continual degradation and reconstruction, even the calcified matrix of bones.

These processes serve to renew the matrix and to allow it to be remodeled during embryonic development or following tissue injury.

11.4 | Dynamic Properties of the Extracellular Matrix

ECM degradation is accomplished by a family of zinc-containing enzymes called matrix metalloproteinases (MMPs) secreted into the extracellular space or anchored to the plasma membrane.

As a group, MMPs can digest nearly all of the diverse ECM components, although individual family members are limited as to the types of extracellular molecules they can attack.

The physiological roles of MMPs are thought to be involved in tissue remodeling, embryonic cell migration, wound healing, and the formation of blood vessels.

Inappropriate MMP activity has been implicated in arthritis, tooth and gum disease, formation of blood clots and heart attack, and tumor progression.

11.5 | Integrins

Integrins are a family of membrane proteins found only in animals that play a key role in integrating the extracellular and intracellular environments, and are important for the attachment of cells to their extracellular microenvironment.

On the outer side of the plasma membrane, integrins bind to a diverse array of ligands present in the extracellular environment.

On the intracellular side of the membrane, integrins interact either directly or indirectly with dozens of different proteins to influence the course of events within the cell.

Integrins are composed of two membrane-spanning polypeptide chains, an α chain and a β chain, that are non-covalently linked. Eighteen different α subunits and eight different β subunits have been identified.

11.5 | Integrins

Only about two dozen different integrins have been identified on the surfaces of cells, each with a specific distribution within the body.

Whereas many subunits occur in only a single integrin heterodimer, the β1 subunit is found in 12 different integrins.

Most cells possess a variety of different integrins, and conversely, most integrins are present on a variety of different cell types.

Many ECM proteins through an arginine-glycine-aspartic (RGD) motif.

TABLE 7.1 Classification of Integrin Receptors Based on Recognition of RGD Sequences

RGD Recognition		Non-RGD Recognition	
Integrin receptor	Key ligands	Integrin receptor	Key ligands
$\alpha_3\beta_1$	Fibronectin	$\alpha_1\beta_1$	Collagen
$\alpha_5\beta_1$	Fibronectin	$\alpha_2\beta_1$	Collagen
$\alpha_v\beta_1$	Fibronectin		Laminin
		$\alpha_3\beta_1$	Collagen
$\alpha_{IIb}\beta_3$	Fibronectin		Laminin
	von Willebrand factor	$\alpha_4\beta_1$	Fibronectin
	Vitronectin		VCAM
	Fibrinogen	$\alpha_6\beta_1$	Laminin
$\alpha_v\beta_3$	Fibronectin	$\alpha_L\beta_2$	ICAM-1
	von Willebrand factor		ICAM-2
	Vitronectin	$\alpha_M\beta_2$	Fibrinogen
$\alpha_v\beta_5$	Vitronectin		ICAM-1
$\alpha_v\beta_6$	Fibronectin		

Source: S. E. D'Souza, M. H. Ginsberg, E. F. Plow, *Trends Biochemical Sciences* 16:249, 1991.

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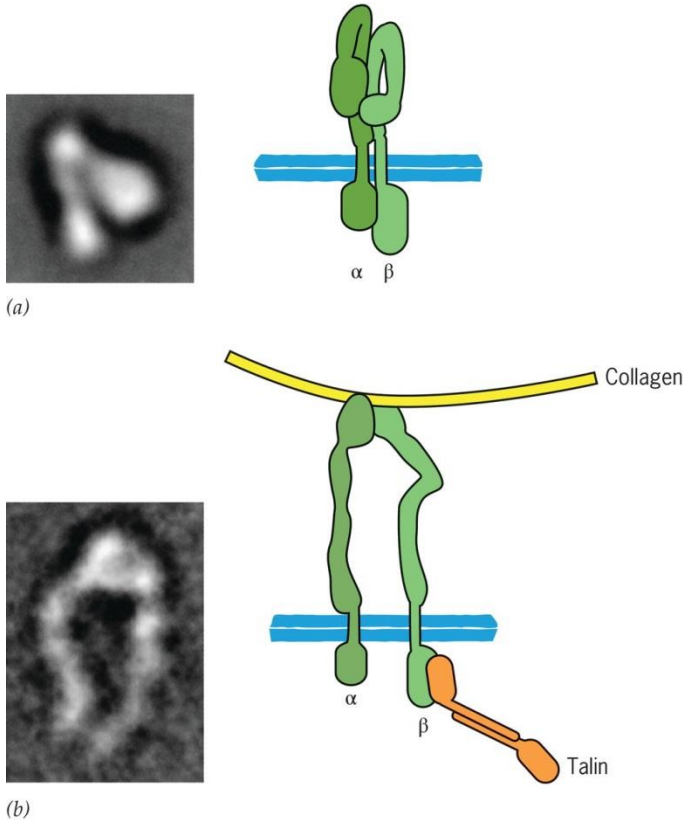
11.5 | Integrins

The bent conformation of integrin corresponds to its inactive state, incapable of binding a ligand.

Integrins with a bound ligand are found in an upright conformation.

The transmembrane domains of the two subunits are in close proximity, held together by noncovalent interactions between residues of the two helices.

The cytoplasmic domains bind a wide array of proteins including talin, which causes separation of the α and β subunits via “inside-out” signaling.



EMs and ribbon drawings of the extracellular domains of an integrin ($\alpha_v\beta_3$) in the “bent”/inactive and “upright”/active conformation. Changes driven by divalent metal ions.

11.5 | Integrins

Integrins have two major activities: adhesion of cells to their substratum (or to other cells) and transmission of signals between the external environment and the cell interior.

Integrin-ligand binding can induce a conformational change at the cytoplasmic end of the integrin, especially its β subunit.

Outside-in signals can induce a conformational change in talin, initiating a cascade of events leading to the polymerization of actin filaments.

Cytoplasmic protein kinases (e.g. FAK and Src) can be activated to phosphorylate other proteins; this chain reaction could lead activation of a specific group of genes.

11.5 | Integrins

Outside-in signals transmitted by integrins can influence differentiation, motility, growth, and cell survival.

Most malignant cells are capable of growing in liquid suspension, their survival no longer depends on integrin binding.

Normal cells can only grow and divide if they are cultured on a solid substratum to transmit life-saving signals to the interior of the cell; if they are placed in suspension cultures, they die.

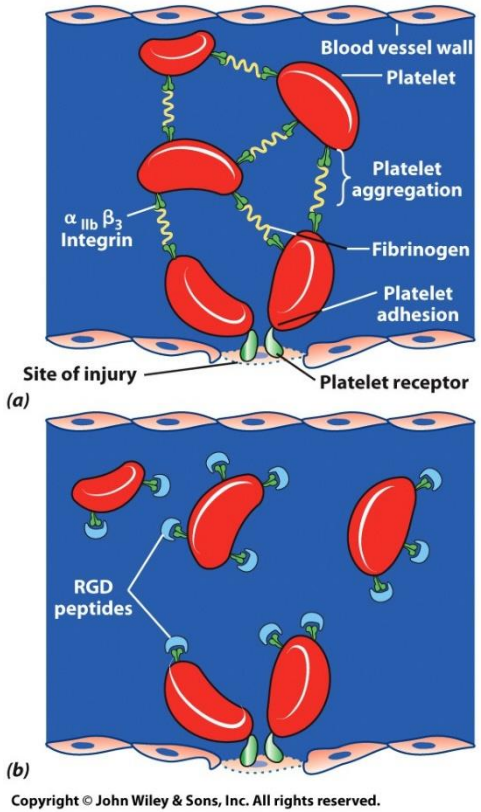
11.5 | Integrins

The RGD sequence is the basis for treatments of conditions that involve receptor-ligand interactions.

Platelets aggregation involves platelet-specific integrins and RGD-containing blood proteins (e.g. fibrinogen), acting as linkers that hold platelets together.

New class of antithrombotic agents (Aggrastat and Integrelin) resemble the RGD structure but bind selectively to the platelet integrin.

ReoPro: antibody directed against the RGD binding site of αIIβ3 integrins to prevent blood clots.



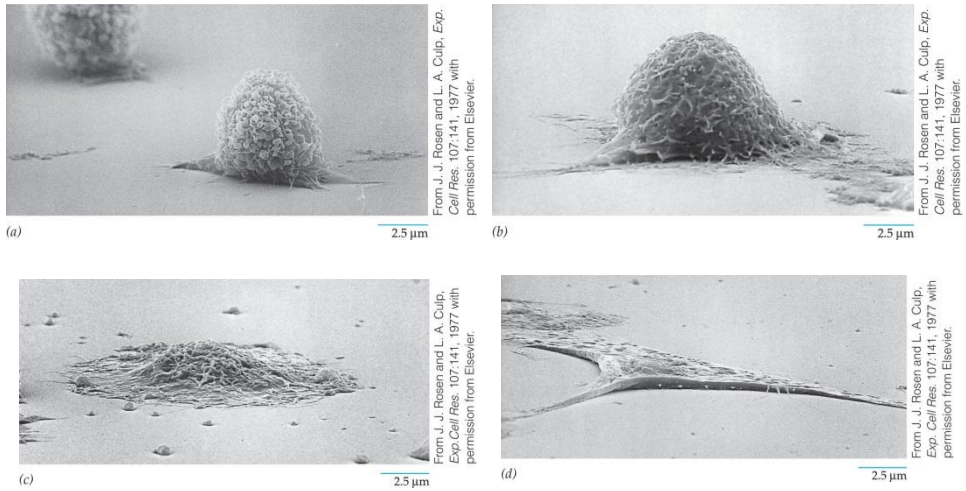
Blood clots form when platelets adhere to one another through fibrinogen bridges that bind to the platelet-integrins

11.6 | Anchoring Cells to Their Substratum

Since it is easier to study the cell interactions with a culture dish than with an ECM inside an animal, in vitro experiments have been instrumental in understanding cell-matrix interactions.

At first, the cell has a rounded morphology, but once the cell makes contact with the substratum, it sends out projections that form increasingly stable attachments.

Over time, the cell flattens and spreads itself out on the substratum.



Steps in the process of cell spreading.

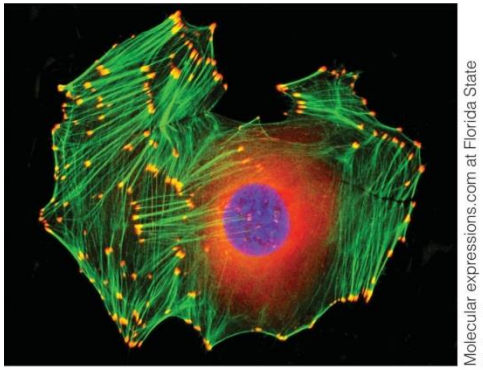
11.6 | Anchoring Cells to Their Substratum

Focal Adhesions

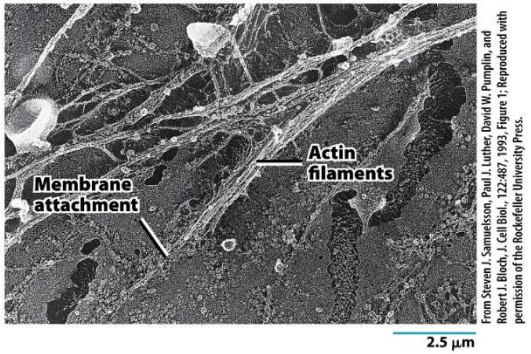
Cultured cells are anchored to the surface of the dish only at scattered, discrete sites, called focal adhesions.

Focal adhesions play a key role in cell locomotion, during which the integrins develop transient interactions with extracellular materials.

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



Cultured cell: actin filaments (gray-green), integrins (red); sites of focal adhesions



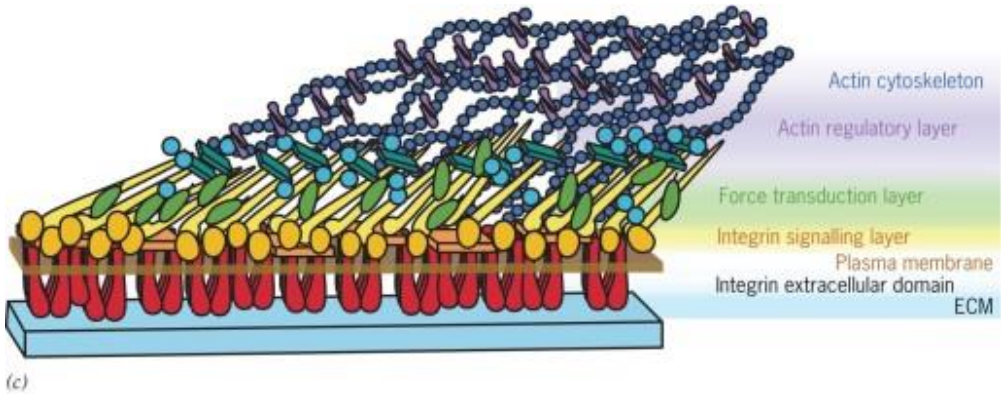
Amphibian cell processed for quick-freeze, deep-etch analysis

11.6 | Anchoring Cells to Their Substratum

Focal Adhesions

Integrin cytoplasmic domains are connected to cytoskeletal actin filaments through stratified layers of adaptor proteins (e.g., talin, α-actinin and vinculin).

Pulling of the ECM can lead to activation of protein kinases, such as FAK or Src, to dramatically alter cell behavior.



Focal adhesions are sites where cells adhere to their substratum and send signals to the cell interior

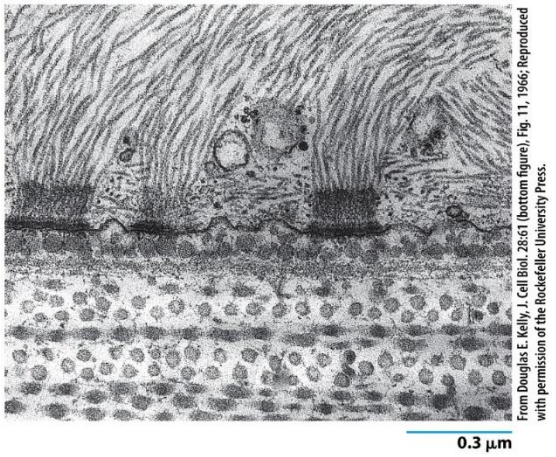
11.6 | Anchoring Cells to Their Substratum

Hemidesmosomes

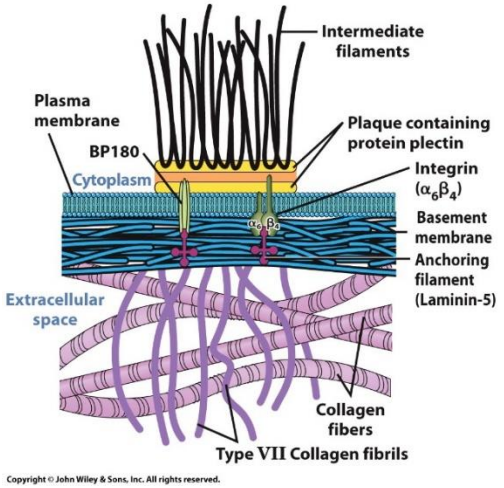
Cell-matrix attachment in vivo is seen at the basal surface of epithelial cells, anchored to the underlying basement membrane by a specialized adhesive structure called a hemidesmosome.

Hemidesmosomes contain a dense cytoplasmic plaque with keratin filaments coursing outward into the cytoplasm.

Keratin filaments are linked to the ECM by integrins, which can transmit signals to alter cell shape and activities.



Hemidesmosomes: EM and schematic diagram.



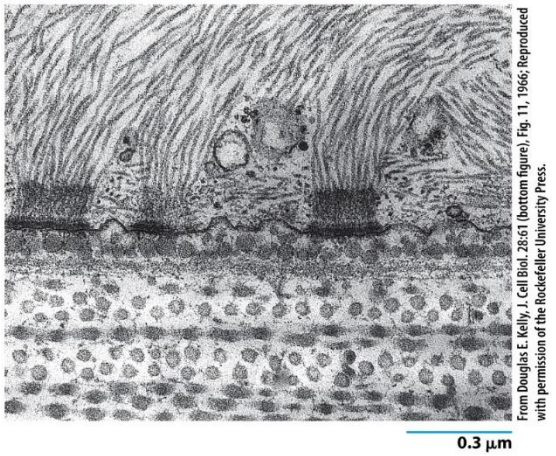
11.6 | Anchoring Cells to Their Substratum

Hemidesmosomes

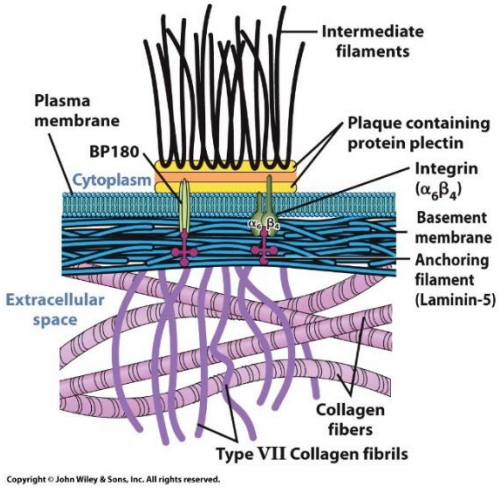
In the autoimmune disease *bullous pemphigoid*, antibodies are made towards proteins present in these adhesive structures.

These auto-antibodies cause the epidermal layer to lose attachment to the underlying basement membrane and result in severe skin blistering.

Epidermolysis bullosa results from genetic alterations in hemidesmosomal proteins, including the α6 or β4 integrin subunit, collagen VII, or laminin-5.



Hemidesmosomes: EM and schematic diagram.

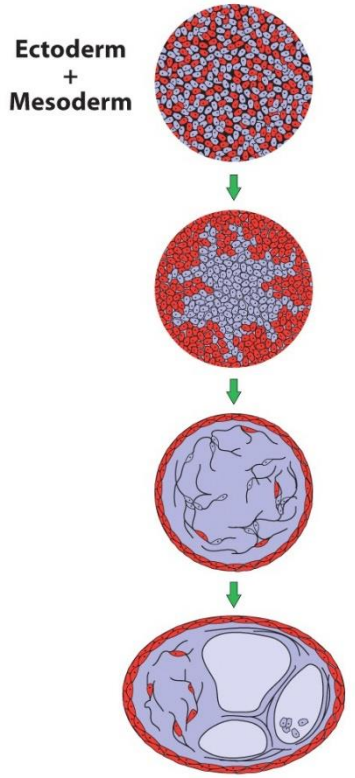


11.7 | Interactions of Cells with Other Cells

Organs have a complex architecture involving a variety of different cell types, although the mechanisms behind this organization are not fully understood.

This process may depend on selective interactions between cells of the same type, as well as between cells of a different type.

Dissociation-reaggregation experiments showed that a suspension of single cells would initially aggregate to form a mixed clump.



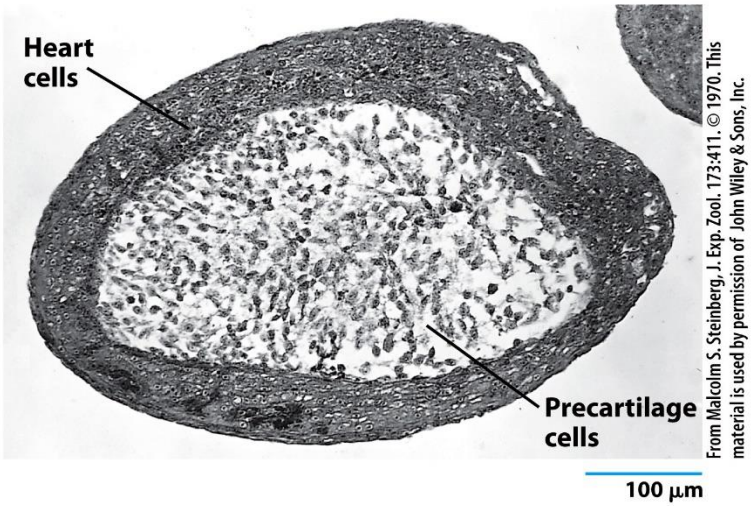
Ectoderm and mesoderm from an early amphibian embryo re-associate after initial random dissociation

11.7 | Interactions of Cells with Other Cells

Over time, cells would redistribute themselves so that each cell adhered only to cells of the same type.

Once segregated, cells would differentiate into many of the structures they would have formed within an intact embryo.

Four distinct families of integral membrane proteins play a major role in mediating cell–cell adhesion: (1) selectins (2) certain members of the immunoglobulin superfamily (IgSF), (3) certain members of the integrin family, and (4) cadherins.



Light micrograph: mixed pre-cartilage cells from a chick limb and chick heart ventricle cells re-sort based on cell type

11.7 | Interactions of Cells with Other Cells

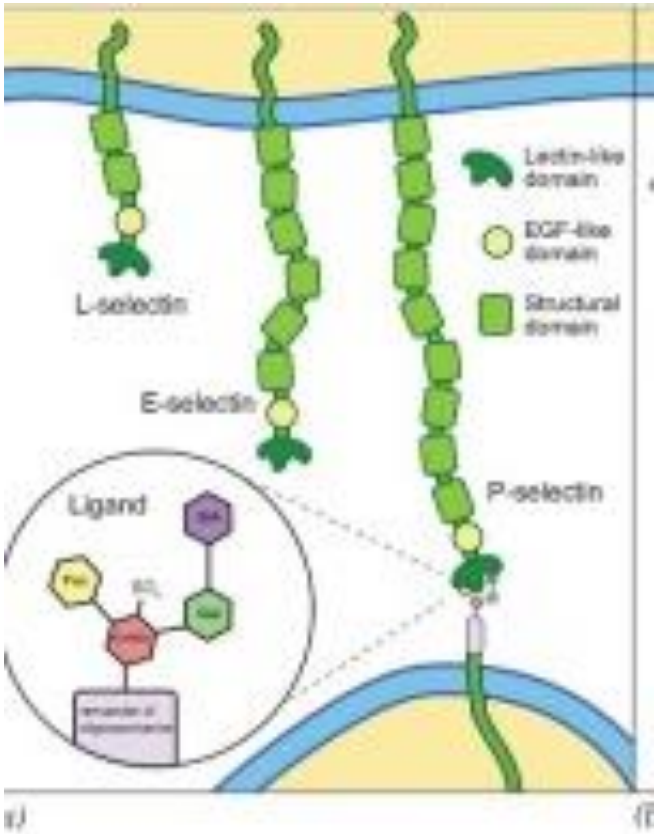
Selectins

Selectins are a family of membrane glycoproteins that bind to specific oligosaccharides on the surfaces of neighboring cells.

"Lectin," is a term for a compound that binds to specific carbohydrate groups.

Selectins have a small cytoplasmic segment, a single membrane-spanning domain, and a large extracellular portion.

Three cell-specific selectin types:
E-selectin, endothelial cells;
P-selectin, platelets and endothelial cells;
L-selectin, leukocytes (white blood cells).



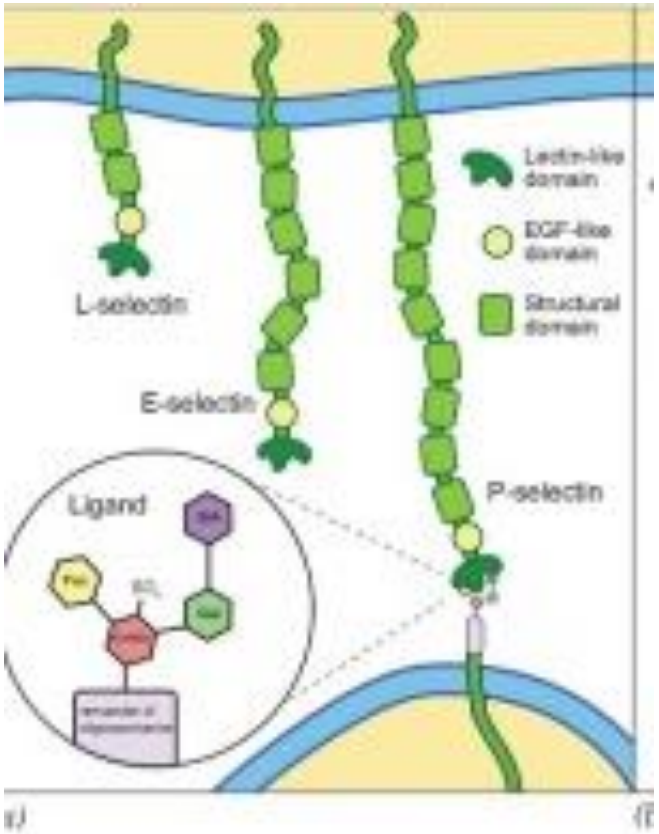
Schematic of the three selectins and their ligand

11.7 | Interactions of Cells with Other Cells

Selectins

Binding of selectins to their carbohydrate ligands requires calcium.

As a group, selectins mediate transient interactions between circulating leukocytes and vessel walls at sites of inflammation and clotting.



Schematic of the three selectins and their ligand

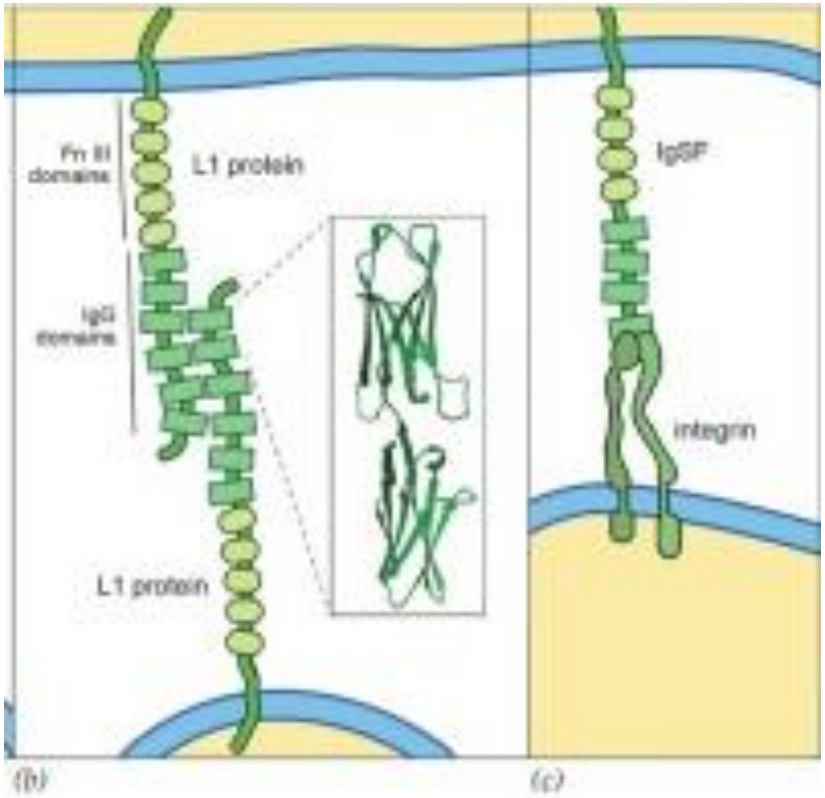
11.7 | Interactions of Cells with Other Cells

The Immunoglobulin Superfamily

Antibodies are immunoglobulin proteins with Ig domains, 70-110 amino acids organized into a tightly folded structure.

The human genome encodes 765 distinct Ig domains, making it the most abundant domain in human proteins.

These proteins are members of the immunoglobulin superfamily, or IgSF, and most are involved with mediating interactions of lymphocytes with cells required for an immune response (e.g., macrophages, lymphocytes, and target cells).



Cell–cell adhesion from homotypic interactions of two L1 molecules through Ig domains at the N-termini.

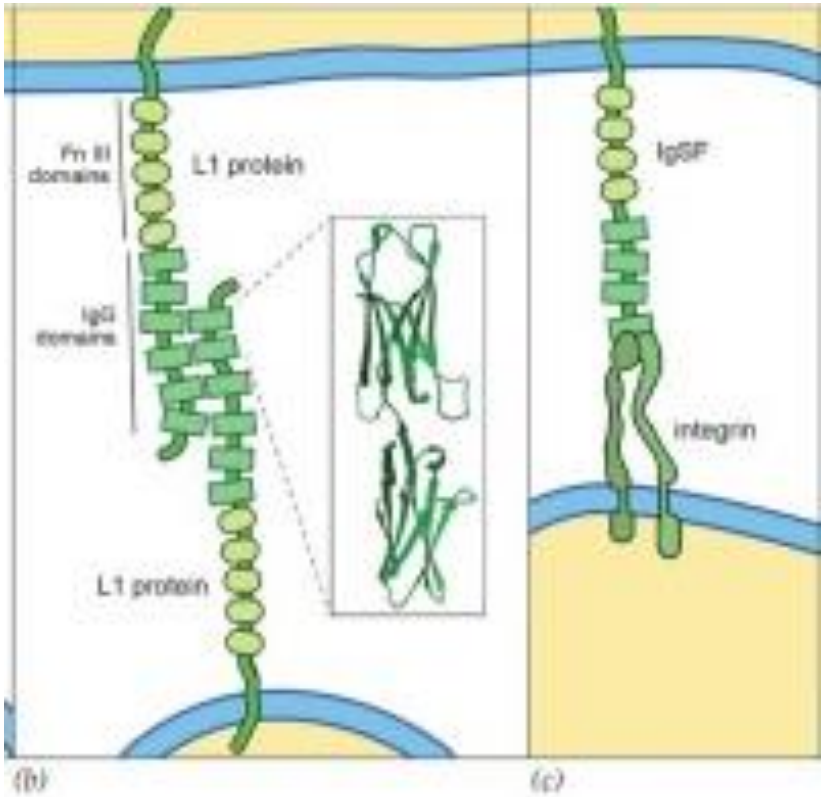
11.7 | Interactions of Cells with Other Cells

The Immunoglobulin Superfamily

VCAM (vascular cell-adhesion molecule), NCAM (neural cell-adhesion molecule), and L1 (also called L1CAM), mediate adhesion between nonimmune cells.

NCAM and L1 have roles in nervous system development.

Various types of proteins serve as ligands for IgSF cell surface molecules, such as integrins.



Cell–cell adhesion from homotypic interactions of two L1 molecules through Ig domains at the N-termini.

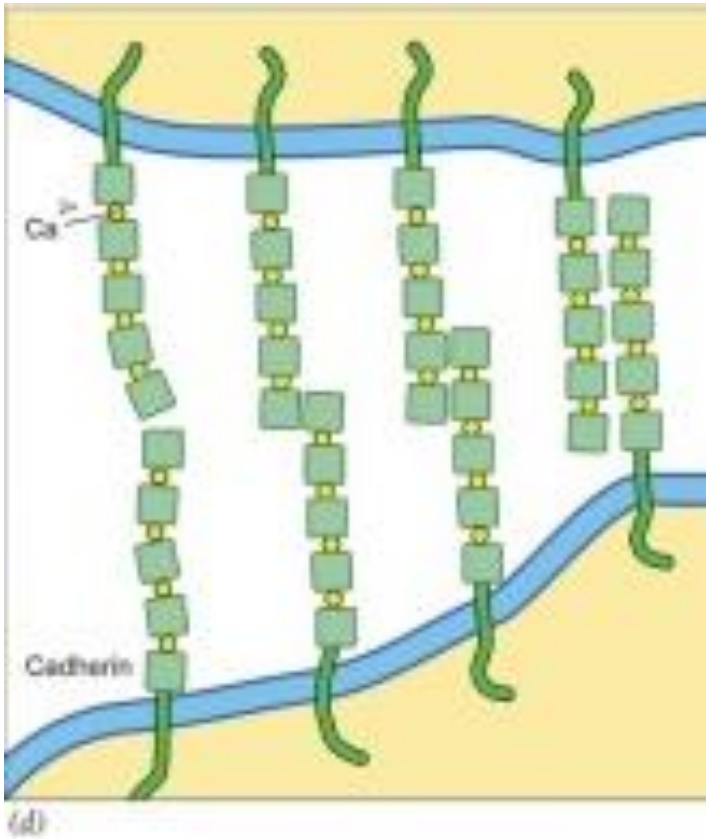
11.7 | Interactions of Cells with Other Cells

Cadherins

Cadherins are family of glycoproteins that mediate Ca^{2+}-dependent cell–cell adhesion and transmit signals from the ECM to the cytoplasm.

Cadherins typically join cells of similar type to one another and do so predominantly by binding to the same cadherin present on the surface of the neighboring cell.

Cadherins may be the single most important factor in molding cells into cohesive tissues in the embryo and holding them together in the adult.



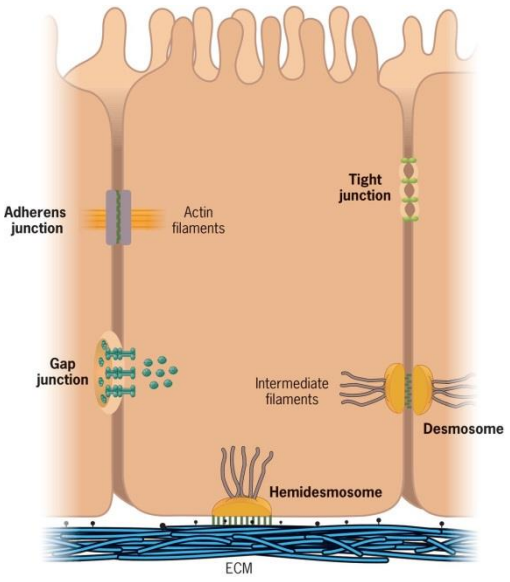
Schematic of two adhering cells due to interactions between cadherins projecting from the plasma membrane of each cell

11.8 | Adherens Junctions and Desmosomes

Cadherins are found in specialized intercellular junctions: adherens junctions (AJs) and desmosomes.

AJs are commonly found in epithelia as "belts" (*zonulae adherens*) that encircle cells near their apical surface, binding a cell to its surrounding neighbors.

These calcium-dependent linkages formed between the extracellular domains of cadherin molecules that bridge the 30–nm gap between neighboring cells.



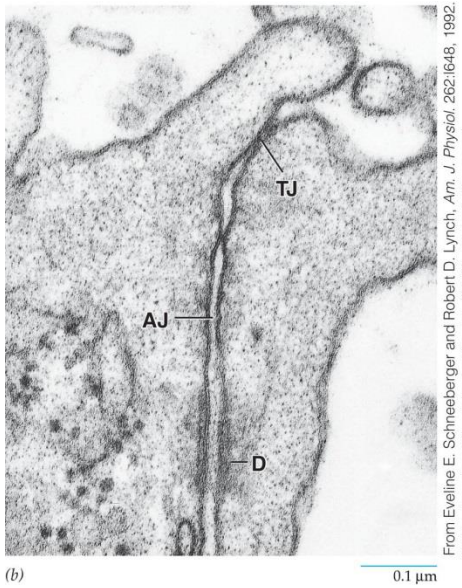


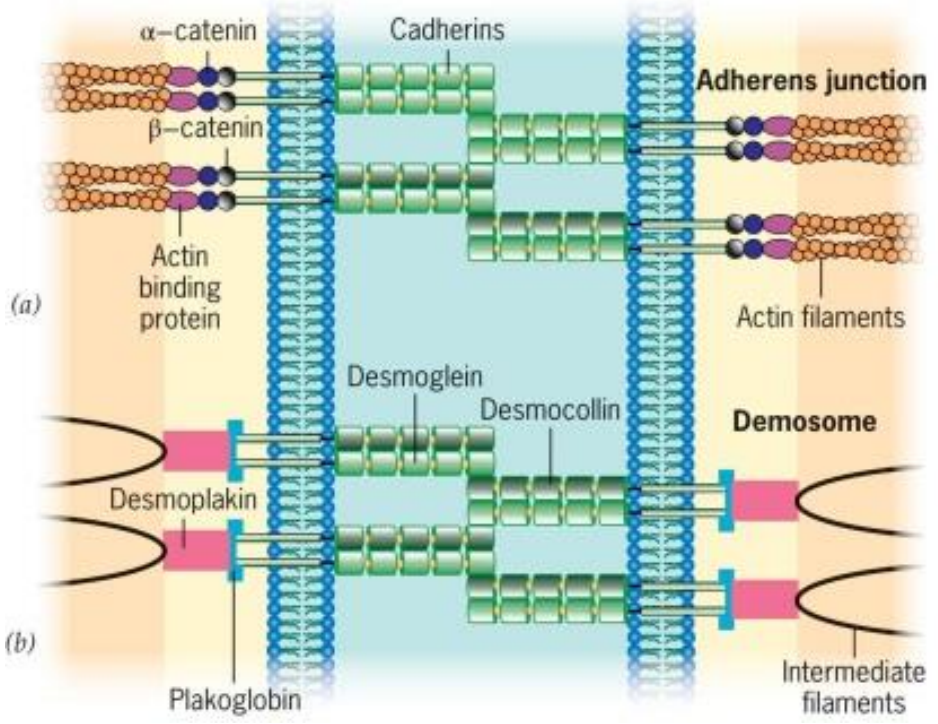
Diagram and EM showing the junctional complexes on the lateral surfaces of a simple columnar epithelial cell

11.8 | Adherens Junctions and Desmosomes

The cytoplasmic domain of cadherins is linked by α- and β-catenins to cytoplasmic proteins including actin filaments.

Similar to integrins of a focal adhesion, the cadherin clusters of an adherens junction:
(1)Connect the external environment to the actin cytoskeleton and
(2)Provide a pathway for signals to be transmitted from the exterior to the interior

Adherens junctions situated that line the walls of blood vessels transmit signals that ensure the survival of the cells.



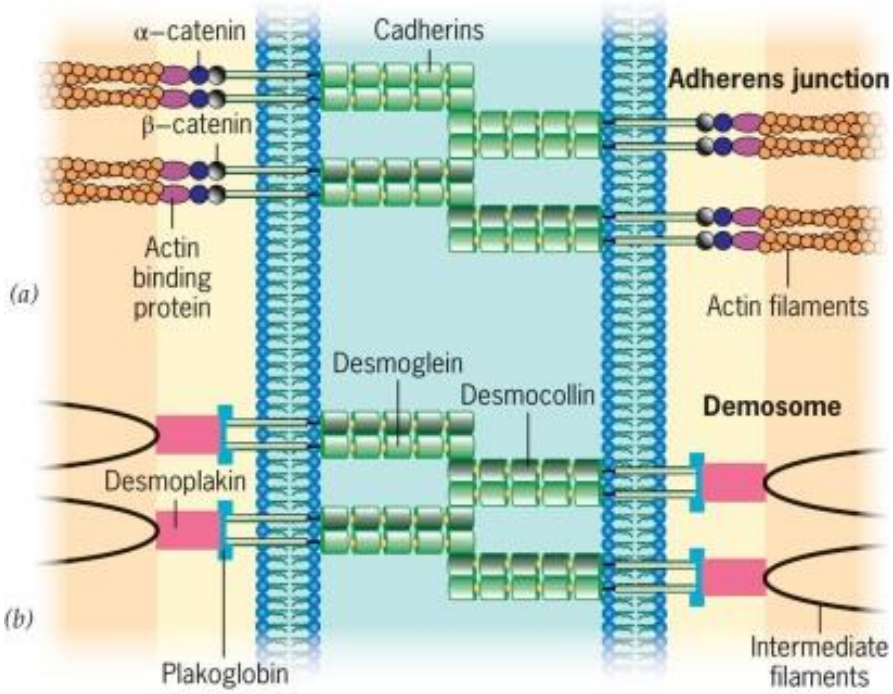
Cadherins link the two cells across a narrow extracellular gap

11.8 | Adherens Junctions and Desmosomes

Desmosomes (or *maculae adherens*) are disk-shaped adhesive junctions found in a variety of tissues subjected to mechanical stress, such as cardiac muscle, epithelial layers of the skin and uterine cervix.

Desmosomes contain cadherins with a different domain structure and are referred to as desmogleins and desmocollins.

Dense cytoplasmic plaques serve as sites of anchorage for looping intermediate filaments.



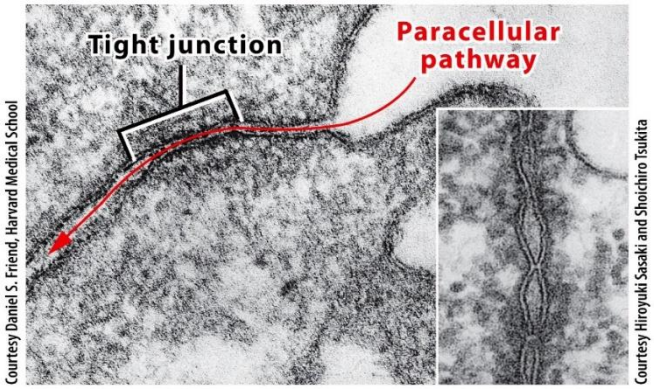
Model: molecular architecture of a desmosome

11.10 | Tight Junctions: Sealing the Extracellular Space

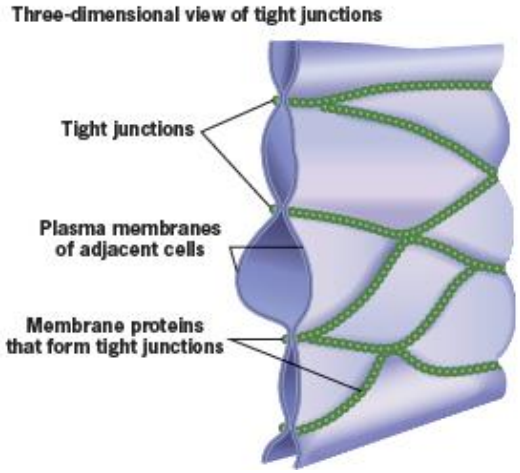
Tight junctions (*zonulae occludens*), occur between neighboring epithelial cells, and are located at the very apical end of the junctional complex between adjacent cells.

EM reveals that the adjoining membranes make contact at intermittent points, rather than being fused over a large surface area.

The points of cell–cell contact are sites where integral proteins of two adjacent membranes meet within the extracellular space.



EM: apical region of adjoining epithelial cells



Tight junction model showing intermittent contact points between proteins from two apposing membranes

11.11 | Gap Junctions and Plasmodesmata: Mediating Intercellular Communication

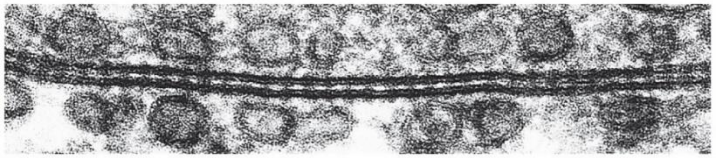
Gap Junctions

Plasma membranes of a gap junction contain channels that connect the cytoplasm of one cell with the cytoplasm of the adjoining cell.

The passage of ionic currents through gap junctions plays a pivotal role in numerous physiologic processes.

Gap junctions are sites between animal cells that are specialized for intercellular communication.

Gap junctions come very close to one another but do not make direct contact.



From Camillo Peracchia and Angela F. Duhunty, J. Cell Biol. 70:426, 1976, Fig. 5; Reproduced with permission of the Rockefeller University Press.

Electron micrograph of a section through a gap junction perpendicular to the plane of the two adjacent membranes

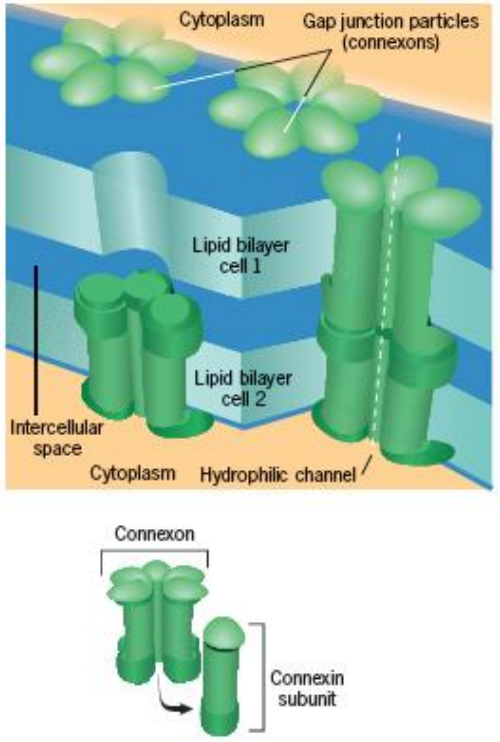
11.11 | Gap Junctions and Plasmodesmata: Mediating Intercellular Communication

Gap Junctions

Gap junctions are molecular "pipelines" that pass through the adjoining plasma membranes and open into the cytoplasm of the adjoining cells.

They are composed of an integral membrane protein *connexin*, and organized into multisubunit complexes, *connexons*, that span the membrane.

A connexon is composed of six connexin subunits arranged in a ring around a central opening, or *annulus*, around 1.5 nm in diameter at its extracellular surface.



Schematic model of a gap junction showing the arrangement of six connexin subunits to form a connexon