Cells in tissues can adhere directly to one another (cell-cell adhesion) through specialized integral membrane protein called cell adhesion molecules (CAMs).

Cells in animal tissues also adhere indirectly (cell-matrix adhesion) through the binding of adhesion receptors in the plasma membrane to components of the surrounding extracellular matrix (ECM); A complex interdigitating meshwork of proteins and polysaccharides secreted by cells into the spaces between them.

CAMs and ECM can bind cells together, and transfer of information between the exterior and interior cells.

Cell Junctions are relatively stable, ultrastructurally (i.e., in EM) distinct sites where cells are joined to each other or the extracellular matrix.

- Adhesion molecules are one component of adhering junctions
- Adhesion Molecules are cell surface molecules that stick to each other to allow cell-cell or cell-ECM adhesion
  - Usually less stable

Inflammatory bowel disease:

**Blue:** nuclei
**Green:** secrete hyaluronan

**Connective tissue,** connecting fibers, basal lamina

**Indirect adhesion**
Cell-adhesion molecules bind to one another and to intracellular protein

**Cell Adhesion Molecules (CAMs)**

CAMs fall into four major families: 1. Cadherins; 2. immunoglobulin (Ig) superfamily; 3. integrins and 4. selectins.

- **Homophilic adhesion**: the same cell type adhesion.
- **Heterophilic adhesion**: different cell type adhesion.
- **Homotypic adhesion**: the same adhesive molecule interaction.
- **Heterotypic adhesion**: different adhesive molecule interaction.

Three abundant ECM

1. Proteoglycans, a glycoprotein
2. Collagens, protein that form fibers
3. Fibronectin, soluble multiadhesive matrix protein

Diversity of animal tissues depends on evolution of adhesion molecules with various properties.

Cell-cell adhesion of two types of molecular interaction

Cis (lateral) interaction: on one cell associated laterally through their extracellular domain or cytosolic domain or both into homodimers or higher-order oligomers in the plane of the cell’s plasma membrane.

Trans interaction: on one cell bind to the same or different CAMs on an adjacent cell.

**Types of epithelia**

Different types had different function.

Epithelial tissues provide cellular coats that protect exposed internal & external surfaces from water loss and wear & tear.

Seal surfaces

Regulate flow of materials across surface via secretion and transcytosis.

Cell junctions key to formation and maintenance of epithelial sheets.
Classification of cell junctions

Occluding (封閉) junctions
- Tight junctions and septate (分開) junctions

Anchoring junctions
- Actin filament attachment sites
  1. Cell-cell (adherens junctions)
  2. Cell-matrix (focal adhesions)

Intermediate filament attachment sites
- 1. Cell-cell (desmosomes)
- 2. Cell-matrix (hemi desmosomes)

Communicating junctions
- Gap junctions

Specialized junctions help define the structure and functions of epithelial cells

Cell Junctions
Occur at many points of cell-cell and cell-matrix contact in all tissues and can be classified according to their function.

Cell membrane did not directly contact (fusion), need protein molecule

Classified into 3 functional groups:
- Occluding/Tight Junctions: seal cells together in an epithelial sheet stops molecules from leaking from one side of the sheet to the other
- Anchoring Junctions: mechanically attach cells (and their cytoskeleton) to their neighbors, or to ECM
- Communicating Junctions: mediate passage of chemical, or electrical signals from one cell to its adjacent neighbor

Ca²⁺ dependent homophilic cell-cell adhesion in adherens junctions and desmosomes is mediated by cadherins

The cadherin family of Ca²⁺ dependent cell-cell adhesion molecules comprises ~80 members.
Most cadherins are integral membrane proteins that contain a specific number of extracellular cadherin (EC) domains.
**Cadherins mediate Ca\(^{2+}\)-dependent homophilic cell-cell adhesion**

**E-cadherin:** expressed on early embryonic cells in mammals. Later becomes restricted to embryonic and adult epithelial tissue

**P-cadherin:** Trophoblast cells (placental)

**N-cadherin:** First mesodermal, later CNS

**EP-cadherin:** frog blastomere adhesion

**Protocadherins:** not connected to catenin

- The C-terminal cytoplasmic domain associates with the cytoskeleton
- N-terminal extracellular domain forms dimers and, through homophilic interactions, forms tetramers
- Each cadherin has a characteristic tissue distribution

---

**How to proof the Ca\(^{2+}\) dependent adhesion**

When Ca\(^{2+}\) → generate cell-cell adhesion (anchoring junctions and tight junctions)

Block by add cadherin antibody

Cadherin induced cell adhesion is Ca\(^{2+}\) dependent

---

**Protein constituents (組成) of typical adherens junctions**

**Binding partners:**
- catenins, and via catenins to cytoskeleton (actin)

**Cytosolic domains of the E-cadherin bind direct or indirectly to multiple adapter protein that connect the junctions to cytoskeleton and participate in intracellular signaling pathways (catenin)**

---

**Desmosomal cadherins (desmosomes)**

**Pemphigus Vulgaris, PV**

Auto-antibody attack desmosome → skin disease

---
Tight junctions seal off body cavities and restrict diffusion of membrane components

Freeze-fracture preparation of tight junction zone between two intestinal epithelial cells.

Major protein tight protein, JAM the junction adhesion molecule

Occludin comes in two splice variants; TJs may form without occludin in certain cell types

Claudin (-1 and -2) appears to form the ridge backbone; claudin-transfection into fibroblasts establishes TJs. Claudin expression is cell- and tissue specific

JAMs associate laterally with TJs (→ Adhesion Molecules)
Adherens junctions (adhesion belt); attach to actin
Desmosomes; attach to intermediate filaments
Focal adhesions
Hemidesmosomes


Exp: demonstrates the impermeability of certain tight junctions to many water-soluble substance

Differences in permeability of tight junctions can control passage of small molecules across

The freeze fracture, freeze etch method

Figure 5–38

Figure 5–39

Figure 5–39

Paracellular pathway
Transcellular pathway
Basolateral membrane

Lanthanum hydroxide (between cells)
Many cell-matrix and some cell-cell interactions are mediated by integrins

Integrins mediate cell-matrix and cell-cell interactions

Part of family cell adhesion receptors; receptor proteins
Roles in binding ligand for cell signaling and in adhesion esp to matrix
Two transmembrane glycoprotein subunits, non-covalently bound, alpha and beta
Now, 18 alpha and 8 beta subunits to 24 integrins
Not all permutations viable, eg, \( \beta_4 \) can form only with \( \alpha_6 \),
but \( \beta_1 \) can form partners with ten different \( \alpha \)
•“Combinatorial Diversity” = small number of components to a large number of functions

P223
Adhesive interactions and nonepithelial cells

Gap junction composed of connexins allow small molecules to pass between adjacent cells

Gap junctions

Connections at the lateral surfaces of cells that allow transport of ions and small molecules (as large as 12 nm)
Channels directly link the cytosol of adjacent cells
The extent to which channels are open is highly regulated (ex. very high calcium ion concentration closes the channels)
In neurons, the passage of ions can lead to propagation of action potentials
In smooth muscle, calcium transfer can induce contraction
Passage of cyclic AMP can lead to signal transduction
A hormonal stimulation of one cell can be passed to neighboring cells
6 connexin subunits on each cell form a connexon. Each connexin crosses the membrane four times. Different connexins form junctions that differ in channel size and regulation. Hetero-oligomeric connexons can form heterotypic gap junction channels.

Summary: Cell junctions

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tight junction</td>
<td>seals neighboring cells together in an epithelial sheet to prevent leakage of molecules between them</td>
</tr>
<tr>
<td>Adherens junction</td>
<td>joins actin bundles in one cell to a similar bundle in a neighboring cell</td>
</tr>
<tr>
<td>Desmosome</td>
<td>joins intermediate filaments in one cell to those in a neighboring cell</td>
</tr>
<tr>
<td>Gap junction</td>
<td>allows the passage of small water-soluble ions and molecules</td>
</tr>
<tr>
<td>Hemidesmosome</td>
<td>anchors intermediate filaments in a cell to the basal lamina</td>
</tr>
</tbody>
</table>

Cell adhesion molecules

- **Cadherins**: mainly Ca²⁺-dependent cell-cell adhesion
- **Immunoglobulin superfamily**: Ca²⁺-independent cell-cell adhesion in neuronal and other tissues
- **Integrins**: mainly cell-ECM interaction
- **Selectins**: movement of leucocytes into tissue

The extracellular matrix (ECM)

Three types of molecules are abundant in the extracellular matrix of all tissues:

1. **Proteoglycan**: a glycoprotein, high viscosity, it can bound variety of ECMs
2. **Collagen fibers**: provide mechanical strength and resilience.
3. **Soluble multiadhesive matrix proteins**: bind to and cross-link cell-surface adhesion receptors and other ECM components
The basal lamina provides a foundation for epithelial sheets

- Separates endothelial and muscle cells from connective tissue
- In the kidney, it filters capillary blood that eventually becomes the urine

Columnar and epithelia is a foundation on one surface of the cells rests

Muscle or fat the basal lamina surrounds each cell

**Basal Lamina - A Loose Connective Tissue**
- A thin ECM linked to cells by membrane receptors
- Loose connective tissue, gel-like, containing proteins, cells and capillaries - used for nutrient transfer
- Components serve to guide migrating cells during development

**Interstitial Connective Tissues**

Interstitial ECM’s have the same pattern of organization as basement membrane ECMS

- Fibrillar proteins
- Glycoproteins
- Proteoglycans

Some examples of Interstitial Connective Tissues:
- Bone, cartilage, tendons, ligaments, fascia, lamina propria, submucosa, vitreous humor
The basal lamina provides a foundation for epithelial sheets

Basal lamina has other functions:
1. Helps four and eight-celled embryos adhere together
2. Development of neurons migrate
3. Tissue repair

Most of ECM components in the basal lamina are synthesized by the cells that rest. About four types:
1. Type IV collagen: trimeric molecules (rodlike & globular), form 2D network
2. Laminins: form 2D network with collagen, also can bind to integrins
3. Entactin: cross-link collagen IV and laminin, and helps incorporate other components into the ECM; a proteoglycan
4. Perlecan: a proteoglycan, can binds to and ECM and cell surface molecules

Sheet-forming type IV collagen is a major structural component in basal laminae (基底層)

20 types of collagen participate in the formation of ECM
All collagen are trimeric protein made from three polypeptide called collagen a chain; May homotrimeric or heterotrimeric

Has triple helical structure, because of an unusual abundance of three amino acids: glycine, proline, and hydroxyproline (modified from proline)

The unique properties of each type of collagen by difference:
1. The number and lengths of the triple-helical segment
2. The segment effect 3-D structure
3. Covalent modification

Motif: Gly-X-Y, X and Y are any, but often are pro and (OH)-pro

The triple helix is interrupted by non-helical segments

A lateral association of triple helices combined with C-terminal associations results in sheet formation

Type IV collagen assembly

- Triple helix associations (small arrow)
- C-Terminal associations (large arrow)

EM of in vitro formed network
- Thin arrows: side-to-side binding
- Thick arrows: C-term domain binding

Goodpasture’s syndrome (dysfunction of basal lamina)

Autoimmune disease

Abs against α3 chains of type IV collagen of kidney and lungs

Cellular damage, progressive renal failure and pulmonary hemorrhage
Laminins provide an adhesive substrate for cells

Laminin, a multiahesive matrix protein helps cross-link components of the basal lamina

LAMININ: a heterotrimeric protein found in all basal lamina. It binds to cell surface receptors as well as various matrix components. Multiahesive matrix proteins are long and flexible with multiple domains. They bind collagen, other matrix proteins, polysaccharides, cell-surface adhesion receptors, and extra-cellular ligands. Function in organization of extracellular matrix, regulating cell-matrix adhesion, cell migration, and cell shape. Laminin, principal multiahesive matrix protein in basal. Heterotrimeric 820,000 daltons.

Secreted and cell surface proteoglycan are expressed by many cell types.

Viscous proteins and glycoprotein, covalently linked to charged polysaccharides, also called GAG (specialized polysaccharide chains). GAGs are a polymeric repeating disaccharide, sulfate residues. Four classes: hyaluronan, chondroitin sulfate, heparan sulfate, keratan sulfate. Proteoglycans are very diverse. Modifications in GAC chains can determine proteoglycan functions (Fig 6-19)

Gels of Polysaccharide and Protein Fill Spaces and Resist Compression

GAGs in general; strongly hydrophilic. Adopt highly extended conformations, having volume relative to their mass. Form gels at very low concentrations, multiple negative charges attract cations, leading to osmotically active large amounts of water adsorbed into the matrix.

Create swelling pressure that is counterbalanced by tension in the collagen fibres and interwoven with the PGs.
The repeating disaccharides of glycosaminoglycans (GAGs), the polysaccharide components of proteoglycans:

**Localization**
1. Cell surface receptors
2. Extracellular

**Function**
1. Bind & present growth factors
2. Extracellular matrix

---

Glycosaminoglycan (GAG)

(a) Hyaluronan (n = 5,000)

(b) Chondroitin (or dermatan) sulfate (n = 250)

(c) Heparin/Heparan sulfate (n = 200)

(d) Keratan sulfate (n = 20–40)

---

Biosynthesis of heparan and chondroitin sulfate chains in **proteoglycans**

- **GAG + protein = proteoglycan**
- **Core protein**
- **Linking sugars**
- **Chondroitin sulfate repeats**
- **Gal = galactose**
- **GalNAc = N-acetylgalactosamine**
- **Xyl = xylose**

Glycosaminoglycans (heparan or chondroitin sulfate) are covalently linked to serine residues in the core protein via linking sugars (three); keratan sulfate attached to asparagine residues, N-linked oligosaccharides.

Core protein synthesis at ER; GAG chains assembled in Golgi complex.
Addition of keratan sulfate chains are oligosaccharide chains attached to asparagine residues: N-linked oligosaccharides.

---

**N-linked oligosaccharides**

(a) Man = N-acetylglucosamine

(b) Gal = galactose

---

Single proteoglycan poly-proteoglycan GAG
Red (sulfate group) are essential for heparin function
Blue may be present but are not essential.

Pentasaccharide GAG sequence that regulates
the activity of antithrombin III; after
modification, heparin bind to ATIII and activated
for inhibited blood clotting

ECM can regulated many functions

Modifications in GAG chains can determine proteoglycan functions

Syndecans

proteoglycan regulators of
cell-surface microdomains

Four members:
- Syndecan-1/syndecan-1
- Syndecan-2/fibroglycan
- Syndecan-3/N-syndecan
- Syndecan-4/ryudocan

Syndecans are proteoglycans that has an inherent transmembrane and
cyttoplasmic domain
- Syndecan-1 interacts with intracellular microfilaments
- Syndecan-1 has both HS and CS GAG chains
- Syndecan-4 connects to focal adhesion molecules

Heparan sulfate proteoglycans are cell surface coreceptors

Heparan sulfate proteoglycans are a subset of proteoglycans.
- They contain chains of the glycosaminoglycan heparan sulfate.

Most heparan sulfate is found on two families of membrane-bound proteoglycans:
- the syndecans

Heparan sulfates are composed of distinct combinations of more than 30 different
sugar subunits.
- This allows for great variety in heparan sulfate proteoglycan structure and
function.

Cell surface heparan sulfate proteoglycans:
- are expressed on many types of cells
- bind to over 70 different proteins
Heparan sulfate proteoglycans are cell surface coreceptors

Cell surface heparan sulfate proteoglycans
- assist in the internalization of some proteins
- act as coreceptors for:
  • soluble proteins such as growth factors
  • insoluble proteins such as extracellular matrix proteins

Glycoproteins are vast in number & structurally very diverse

Proteoglycans are few and share a simple structure

Glycoproteins
- Proteoglycans = protein + GAG

Two main types of linkage: O & N

Core protein

Repeating sugar pair

Conserved attachment

Core protein

S - Sugar in chain

GLYCOPROTEINS VERSUS PROTEOGLYCANS

Asparagine - S - S
Serine - S - S
Threonine - S
Asparagine - N
Serine - O

CORE PROTEIN

Two main types of linkage: O & N & several core attachment structures

PGs - Only O linkage

Repeating sugar pair

 сахарозамена  

Conserved attachment

CORE PROTEIN

GLYCOPROTEINS VERSUS PROTEOGLYCANS

Sugar varied, not all hexose

Sugar chains short (sometimes very short, or a single sugar)

Less negative charge

Sugar chains can branch

Characteristic core proteins

Sugar chains are all glucoseaminoglycans (GAGs)

Sugar chains are long

GAGs often sulfated

Large negative charge

Sugar chains do not branch

Sugars - small repertoire

Own core proteins

GAG can be independent of protein or have PGs attached, eg., hyaluronan
The ECM of nonepithelial tissue

Fibrillar collagen is the major protein in the ECM of connective tissue

Most volume is made up of ECM rather than cell, and packed with insoluble protein fiber and contain proteoglycan, various multiadhesive proteins, and hyaluronan (non sulfated GAG.)

Collagen found in all multicellular animals, mammals; approx 25 different genes
Are main proteins in bone, tendon and skin. approx. 25% of total protein
Connective Tissue = mainly types I, II, III, V and XI, type-I by far most common; 80-90% of the collagen in the body consists of types I, II and III.

Rope-like super-helix with 3 collagen polypeptide chains wound around each other
Packed together in ordered fashion → collagen fibrils = thin cables, 10-300 nm diameter → these pack together → thicker collagen fibers

Characterizations of COLLAGEN

The various isoforms are the most abundant proteins in the animal kingdom
There are at least 16 types (or 24 types)
Types I, II and III are the most abundant and form fibrils
Type IV forms sheets (found in the basal lamina)
They form triple helices
They have unique segments that interrupt the triple helix and are responsible for the unique properties of individual collagen
They contain a three residue repeat of: glycine, proline, X
They are rich in hydroxyproline
There are three amino acids per turn of the helix, with pyrrolidine rings on the outside of the helix
The helix is stabilized by hydrogen bonds
The fibrous backbone of the extracellular matrix

TABLE 6.1 Selected Collagens

<table>
<thead>
<tr>
<th>Type</th>
<th>Molecular Composition</th>
<th>Structural Features</th>
<th>Representative Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FIBRILLAR COLLAGENS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>[α1(II)2α2(I)]</td>
<td>300 nm-long fibrils</td>
<td>Syn: tendon, bones, ligaments, skin, interstitial tissues</td>
</tr>
<tr>
<td>II</td>
<td>[α1(III)]</td>
<td>300 nm-long fibrils</td>
<td>Cartilage, vitreous humor</td>
</tr>
<tr>
<td>III</td>
<td>[α1(III)]</td>
<td>300 nm-long fibrils</td>
<td>Skin, muscle, blood vessels</td>
</tr>
<tr>
<td>IV</td>
<td>[α1(IV), α2(IV)]</td>
<td>390 nm-long fibrils with globular N-terminal extensions</td>
<td>Cornea, teeth, bone, plasma, skin, smooth muscle</td>
</tr>
<tr>
<td>V</td>
<td>[α1(V), α2(V)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>[α1(VI)]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIBRIL-ASSOCIATED COLLAGENS**

| VI       | [α1(VI)]             | Lateral association with type I; periadnexal fiber, collagenous bundles | Multicellular cushions |
| IX       | [α1(IX),α2(IX)]      | Lateral association with type II; A-terminal globular domain; bound GAG | Cartilage, vitreous humor |

All collagens are organized into triple helical, coiled-coil "collagen subunits."
- They are composed of three separate collagen polypeptides.

Collagen subunits are:
- secreted from cells
- then assembled into larger fibrils and fibers in the extracellular space
Formation of collagen fibrils begins in the endoplasmic reticulum and is completed outside the cell

1. Synthesis of procollagen a on ribosomes (ER)
2. Formed trimers and glycosylation (modification)
3. Facilitate zipperlike formation and stabilization of triple helices, and binding by chaperone Hsp47. it procollagen
4. Transport to golgi complex
5. folded precollagens
6. Secretion
7. N- and C- terminal propeptides removed
8. Trimers assemble into fibrils and are covalently the corss-link

**PROCcollagen:**

Transfers to the Golgi
- There is a further addition of oligo-saccharides
- There is further processing to remove disulfide-containing regions and insertion into transport vesicles
- Exocytosis results in the removal of termini by extracellular enzymes and assembly of cross-linked fibers

**Synthesized by fibroblasts in connective tissue**
- Made by osteoblasts in bone
- Secreted by cells as “procollagen” → collagenase cuts off terminal domains at each end → assembly only after molecules emerge into extracellular space
- Propeptides function to:
  - guide intracellular formation of triple-strand structure
  - prevent intracellular formation of large collagen fibrils
Hydroxylysine and hydroxyproline residues. These modified amino acids are common in collagen; they are formed by enzymes that act after the lysine and proline are incorporated into procollagen molecules.

The covalent intramolecular and intermolecular cross-links formed between modified lysine side chains within a collagen fibril. The cross-links are formed in several steps. First, certain lysine and hydroxylysine residues are deaminated by the extracellular enzyme lysyl oxidase to yield highly reactive aldehyde groups. The aldehydes then react spontaneously to form covalent bonds with each other or with other lysine or hydroxylysine residues. Most of the cross-links form between the short nonhelical segments at each end of the collagen molecules.

Type I and II collagens from diverse structure and associate with different non-fibrillar (非纖維) collagens.

Includes Types VI and IX
Type IX cannot form fibrils due to interruptions in the helical structure, but it can associate with fibrils of other collagen types.
Type VI is bound to the sides of Type I fibrils, linking them together. Non-helical regions anchor Types VI and IX to proteoglycans/other ECM components.

Interaction of fibrous collagens with nonfibrous associated collagens.

* Ascorbic acid is required to prevent scurvy, characterized by fragile tendons, skin and blood vessels.
Collagen found in all multicellular animals, mammals; approx 25 different genes
Are main proteins in bone, tendon and skin → approx. 25% of total protein
Connective Tissue = mainly types I, II, III, V and XI, type-I by far most common
Rope-like super-helix with 3 collagen polypeptide chains wound around each another
Packed together in ordered fashion → collagen fibrils = thin cables, 10-300 nm
diameter → these pack together → thicker collagen fibres
Synthesized by fibroblasts in connective tissue
Made by osteoblasts in bone
Secreted by cells as “procollagen” → collagenase cuts off terminal domains at each
end → assembly only after molecules emerge into extracellular space
Propeptides function to:
guide intracellular formation of triple-strand structure
prevent intracellular formation of large collagen fibrils

Hyaluronan resists compression and facilitates cell migration
Also called hyaluronic acid (HA), is a nonsulfated GAG.
A long, negatively charged polysaccharide that forms hydrated gels. It synthesis by a
plasma membrane bound enzyme (HA synthase) and is directly secreted into
extracellular space.
It is not covalently linked to a protein
It imparts stiffness, resilience and lubricating qualities to connective tissues
Behaves as a random coil in solution
Takes up water (1000-fold its own weight) in the ECM
Binds via the CD44 receptor to the surface of migrating cells – keeping them apart
Degraded by the action of hyaluronidase, an extracellular enzyme

ECM Proteoglycans: Aggrecan
In cartilage the key proteoglycan is aggrecan (MW: 2 x 10^6)
• The central component of aggrecan is hyaluronan
• At 40 nm intervals aggrecan core proteins are attached (assisted
  by a linker protein) to a decasaccharide sequence in hyaluronan
• Attached to the aggrecan core protein are multiple GAGs
• The major GAGs in aggrecan are chondroitin sulphate and
  keratin sulphate
• 1-10% of cartilage glycosaminoglycans is hyaluronan
• Form aggregates- important for cartilage function

Summary - Collagen
All 16 collagen types contain a repeating gly-pro-X sequence and
form triple helices
Collagens vary in their associations to form sheets, fibrils and cross-
linkages
Most collagen is fibrillar - made of Type I molecules
The basal lamina contains Type IV collagen
Fibrous collagen molecules (I,II & III) form fibrils stabilized by
aldol cross-links
Procollagen chains are assembled into triple helices in the RER,
aligned by disulfide bonds among propeptides (which are
subsequently removed)
Fibrous collagen is subject to mutations which exhibit a dominant
phenotype
Proteoglycans provide hydration to tissues

Proteoglycans consist of a central protein "core" to which long, linear chains of disaccharides, called glycosaminoglycans (GAGs), are attached.

GAG chains on proteoglycans are negatively charged.

- This gives the proteoglycans rodlike, bristly shape due to charge repulsion.

Structure of proteoglycan aggregate from cartilage

Association of hyaluronan and proteoglycans forms large, complex aggregates

Proteoglycans form large aggregates

- proteoglycans attached to a hyaluronate backbone
- can be as long as 4000 nm and a diameter of 500 nm

Function of aggregation:
- increased water retention
- increased stiffness
- regulate collagen fibril deposition

Aggregated proteoglycans

Hyaluronan is a glycosaminoglycan enriched in connective tissues

Hyaluronan is a glycosaminoglycan.

- It forms enormous complexes with proteoglycans in the extracellular matrix.

These complexes are especially abundant in cartilage.

- There, hyaluronan is associated with the proteoglycan aggrecan, via a linker protein.

Hyaluronan is highly negatively charged.

- It binds to cations and water in the extracellular space.
  - This increases the stiffness of the extracellular matrix.
  - This provides a water cushion (墊子) between cells that absorbs compressive forces.

Unlike other glycosaminoglycans, hyaluronan chains are:

- synthesized on the cytosolic surface of the plasma membrane
- translated out of the cell

Cells bind to hyaluronan via a family of receptors known as hyladherins.

- Hyladherins initiate signaling pathways that control:
  - cell migration
  - assembly of the cytoskeleton
Proteoglycan synthesis begins with core protein in ER: secreted, GPI-linked, or transmembrane forms of core protein. Sugars added in ER. More sugars added in golgi. HA synthesis in membrane.

Glycosaminoglycans

<table>
<thead>
<tr>
<th>GAG</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronate</td>
<td>synovial fluid, vitreous humor, ECM of loose connective tissue</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>cartilage, bone, heart valves</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>basement membranes, components of cell surfaces</td>
</tr>
<tr>
<td>Heparin</td>
<td>mast cells lining the arteries of the lungs, liver and skin</td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>skin, blood vessels, heart valves</td>
</tr>
<tr>
<td>Keratan sulfate</td>
<td>cornea, bone, cartilage aggregated with chondroitin sulfates</td>
</tr>
</tbody>
</table>

Dermatan Sulphate: absent in cartilage identified in meniscus, tendon, skin and joint capsule.

The extracellular matrix (ECM)

Three types of molecules are abundant in the extracellular matrix of all tissues:

1. Proteoglycan: a glycoprotein, high viscosity, it can bound variety of ECMs.
2. Collagen fibers: provide mechanical strength and resilience.
3. Soluble multiadhesive matrix proteins: bind to and cross-link cell-surface adhesion receptors and other ECM components.
**Fibronectins** connect many cells to fibrous collagens and other matrix components

Fibronectin = example of “adhesive” ECM protein
Is a protein with multiple domains with number of specific binding sites for macromolecules and for receptors on cell surface.

**Plasma Fibronectin**: soluble, circulates in the blood and other body fluids → enhances blood clotting, wound healing and phagocytosis.
**Fibronectin Fibrils**: insoluble → assemble on surface of cells & deposited in ECM
Fibrils assemble on surface of cells via fibronectin-binding integrins.
Usually aligned with adjacent intracellular actin fibres.

Fibronectin is a dimer, two chains linked via disulfide bonding at the carboxyl terminus.

- **Fibrin, heparan sulfate proteoglycan, and collagen**:
  - bind to distinct regions in fibronectin
  - integrate fibronectin fibers into the extracellular matrix network

Some cells express integrin receptors that bind to the Arg-Gly-Asp (RGD) sequence of fibronectin.

At least 20 different forms of fibronectin have been identified.
- All of them arise from alternative splicing of a single fibronectin gene.

The soluble forms of fibronectin are found in tissue fluids.
The insoluble forms are organized into fibers in the extracellular matrix.
Fibronectin fibers consist of crosslinked polymers of fibronectin homodimers.
Fibronectin proteins contain six structural regions.
- Each has a series of repeating units.

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The insoluble forms are organized into fibers in the extracellular matrix.
Fibronectin fibers consist of crosslinked polymers of fibronectin homodimers.
Fibronectin proteins contain six structural regions.
- Each has a series of repeating units.

FIBRONECTINS: attach cells to collagen matrices
Regulates cell shape/cytoskeleton
Dimers of two similar polypeptides linked by disulfide bonds
Forms fibrils when exposed to cells expressing integrins
Circulating fibronectin can bind to fibrin, with the result that platelets associate at the site of blood clots via integrin binding

- **窄域蛋白（220-250KD）**
- **窄域蛋白模型**
- **窄域蛋白的主要功能：**
  - 介導細胞黏著，進而調節細胞的形狀和細胞骨架的組織，促進細胞移動；
  - 在胚胎發生過程中，纖連蛋白對於許多類型細胞的遷移和分化是必要的；
  - 在創傷修復中，纖連蛋白促進巨細胞和其他免疫細胞移動到受損部位；
  - 在血管新生中，纖連蛋白促進血小板附著於血管受損部位。
Specific tripeptide sequence RGD is important role of the cell-bind region of fibronectin is require adhesion of cells

- Arg-Gly-Asp
- Cell binding region
- Type III repeat/binds integrins

Elastic fibers impart flexibility to tissues

The principal function of elastin is to impart elasticity to tissues. Elastic monomers (known as tropoelastin subunits) are organized into fibers. The fibers are so strong and stable they can last a lifetime.

- The strength of elastic fibers arises from covalent crosslinks formed between lysine side chains in adjacent elastin monomers.
- The elasticity of elastic fibers arises from the hydrophobic regions, which:
  - are stretched out by tensile forces
  - spontaneously reaggregate when the force is released.

In animals the ECM is composed of:

- **Structural fiber** -> Collagen and elastin
- **Matrix** -> proteoglycans
- **Adhesive** -> fibronectins & laminin
- **Receptors** -> integrin

Assembly of tropoelastin into fibers:
- occurs in the extracellular space
- is controlled by a three-step process

Mutations in elastin give rise to a variety of disorders, ranging from mild skin wrinkling to death in early childhood.
Multiadhesive Matrix Proteins - Long and flexible, they bind collagens and other proteins, polysaccharides, cell-surface proteins and signaling molecules (growth factors, hormones)

Summary - Components of the ECM
Laminin, a multiadhesive protein in basal lamina, binds heparan sulfate, type IV collagen and other cell surface proteins
Fibronectins, multiadhesive proteins linking collagen, other matrix proteins, attach cell to matrix
Glycosaminoglycans are sulfated disaccharides (like chondroitin sulfate, heparin, heparan sulfates)
Proteoglycans have a protein core and branching glycosaminoglycan chains
Aggrecan is a proteoglycan in cartilage – forms large aggregates
Smaller proteoglycans attach to cell surface and facilitate cell-matrix interactions
Hyaluronic acid is a long, negatively charged polysaccharide that forms viscous, hydrated gels that resist compression; can inhibit cell-cell adhesion, facilitate cell migration

Many cell-matrix and some cell-cell interactions are mediated by integrins

At least 12 distinct α-subunits and 9 β-subunits identified; single subunit can associate with more than one partner
Difficult to crystallize and therefore 3D structures not readily available
Cloning experiments indicate that integrins have short cytoplasmic tail (~50 amino acids) and 4 Ca\(^{2+}\) binding sites identified in the α-subunit
Numerous disulfide bonds - difficult to identify all of them
Integrin superfamily 的黏著分子主要參與細胞與細胞外基質的黏附，使細胞得以附著而形成 integration。此外，這些分子還參與白血球與血管內皮細胞的黏附。

這些分子都是由α、β兩條鏈以非共價鍵連接組成的 heterodimer。

α链的分子量為120~180kD, β链的分子量為90~110kD。不同的α链或β链氨基酸組成和序列有不同的同源性，在架構上有各自共同的特點。

α链和β链由胞載區、穿膜區、胞膜外區三部分組成。胞載區一般較短，可與細胞骨架相連。穿膜區富含疏水胺酸。

α subunits和β subunits組合構成並不是隨機的，多數α subunits只能與一種β subunits結合成heterodimer，而大部分β subunits則可以結合數種不同α subunits。目前依β subunits的不同將與動脈粥狀硬化相關的integrin superfamily分為3個不同的組。

Integrin在與ligand結合時所識別的只是ligand分子中由數個胺基酸組成的序列，例如Arg-Gly-Asp（RGD）序列。不同的integrin可以識別相同的序列或同一個ligand中不同的序列。

integrins在體內分佈很廣泛，多數integrins可以表現於多種組織、細胞，如VLA組的integrins在體內廣泛分佈於各種組織、細胞，而多數細胞可同時表達數種不同的integrins。
Adhesive interaction and non-epithelial cells
Integrin containing adhesive structures physically and functionally connect the ECM and cytoskeleton in nonepithelial cells

(B) Anchoring junctions display greater adhesion and mobility. Increased rates of cell proliferation and spindle shaped morphology

Characterization of integrin
1. Tie the matrix to the cell’s cytoskeleton
2. Principal cell receptors for binding most ECM proteins
3. Large family of homologous transmembrane linker proteins
4. Heterodimer structure
5. Non-covalently associated α and β subunits
6. Cells can regulate the activity of their integrins
7. Often need to be activated before they can mediate cell adhesion
8. Short cytoplasmic domains, no kinase activity

α and β subunits (α1, α2, ..., and β1, β2, ...) associate in pairs e.g. α1-β1 binds collagen, β2-α1 binds fibrinogen; α subunits attach to membrane, lack cytoplasmic domain; β subunit attaches to membrane-cytoskeletal domain

Integrins bind to various ECM proteins and Serum proteins

Diversity of ligand-integrin interaction contributes to number of biological processes
Cell-matrix adhesion is modulated by changes in the binding activity and number of integrins

Focal Contacts (adhesion plaques, focal adhesions)

Found in cells (i.e., fibroblasts) where actin bundles (stress fibers) are anchored to the plasma membrane
Important in cell movement and wound healing
Transmembrane linker proteins are integrins
Integrins bind to various ECM Proteins
Intracellular attachment proteins bind integrins to actin
The Binding of Cytoskeleton to the Extracellular Matrix Through the Integrin Molecule

Integrins are signaling receptors that control both:
- cell binding to extracellular matrix proteins
- intracellular responses following adhesion

Integrins have no enzymatic activity of their own.
- Instead, they interact with adaptor proteins that link them to signaling proteins.

Changes in integrin receptor conformation are central to both types of modulation.
They can result from changes:
- at the cytoplasmic tails of the receptor subunits or
- in the concentration of extracellular cations

Integrin vs Receptor

In inside-out signaling, changes in receptor conformation result from intracellular signals that originate elsewhere in the cell.
- For example, at another receptor

In outside-in signaling, signals initiated at a receptor are propagated to other parts of the cell.
- For example, upon ligand binding
Muscular dystrophy: connections between the ECM and cytoskeleton are defective

About 1/3300 boys, heat or lung failure
Mutation of dystrophin (a cytosolic protein), bind to dystroglycan
Dystroglycan: α-subunit (peripheral protein) plus β-subunit (transmembrane protein); the α-subunit also has O-linked oligosaccharides to bind various basal lamina components
DGC (dystrophin glycoprotein complex) links extracellular matrix to the cytoskeleton and singling pathways enzyme for muscle’s function
Mutations in components of this pathway (e.g. muscular dystrophy) results in mechanical instability of muscle cells.

Selectins: mediate transient cell-cell adhesion in the bloodstream

White blood cells (WBCs) utilize the adhesive properties of selectins (and integrins) in order to move: blood → tissue.

- Selectins are “lectins” – carbohydrate binding proteins. (Ca2+ dependent)
- TM protein with a highly conserved lectin domain that binds to a specific oligosaccharide.
- Transient, calcium-dependent interactions.
- Selectin types:
  - L-selectin: WBCs
  - P-selectin: platelets and endothelial cells
  - E-selectin: activated endothelial cells
2. Non calcium dependent adhesion molecules

Evolutionarily ancient; widely expressed
Belong to the immunoglobulin (Ig) superfamily

**Structure:** single pass, transmembrane proteins which may bind to the cytoskeleton inside cells

**Type of adhesion:**
Can have both homophilic and heterophilic interactions;
home – neural specific Ig Cell Adhesion molecules (IgCAMs);
hetero systemic IgCAMs

**Functions:**
nurite outgrowth, myelination, and firm adhesion of leukocytes
免疫球蛋白超家族的成员众多，且与免疫球蛋白有一定的同源性，其中包含多个90-100个氨基酸的Ig-like domains。广泛分布于淋巴细胞、单核细胞、内皮细胞等多种细胞的表面。

重要的成员有细胞间黏附分子-1（ICAM-1）、2、3（ICAM-2、3）、血管内皮细胞黏附分子（VCAM-1）和血小板-内皮细胞黏附分子（PECAM）。

该家族的主要功能是参与不同细胞间的识别与黏附，在免疫、发炎有关的细胞黏附中发挥重要作用。

ICAM-1是最早发现的免疫球蛋白超家族黏附分子之一，之后相继发现了ICAM-2和ICAM-3。它们的氨基酸序列具有同源性，且都可以结合LFA-1分子（一种integrin）。不同的ICAM分子在体内的分布范围有较大差异，ICAM-1分子分布广泛，如白细胞、内皮细胞、某些肿瘤细胞、上皮细胞、肝细胞、平滑肌细胞等。IL-1、TNF-α、和LPS可促进ICAM-1分子的表达；ICAM-2则分布较局限，主要表现在血管内皮细胞，而ICAM-3则表现在T细胞、单核细胞。

VCAM-1为110KD跨膜糖蛋白，由6个Ig同源区组成，与ICAM-1有很高的相似性，可在内皮细胞、上皮细胞、巨噬细胞等表达，并通过配体VLA-4参与白细胞对血管内皮细胞的黏附。

PECAM-1为130KD的单链糖蛋白，由6个Ig同源区组成，PECAM-1除表达于血管内皮细胞表面外，还可表达于血小板及白细胞，细胞因子如TNF-α、IL-1和IFN可刺激其表达，其配体为β2 integrin。PECAM-1主要参与内皮细胞间的黏附，亦与单核和中性粒细胞穿越内皮进行迁移有关。此外内皮受损时PECAM-1可促使血小板黏附形成血栓。

Ig superfamily has homophilic interaction and heterophilic interaction

MACROMOLECULAR ORGANIZATION OF ECM

IgCAMs comprise a diverse group of adhesion receptors, that are defined by the presence of one or several Ig folds; classical examples are:

- Neuronal CAM (NCAM) is implicated in neuronal guidance and establishment of new synapses
- NCAM forms homotypic contacts
- Intercellular CAM (ICAM) interacts heterotypically with integrins
- CAMs differ widely in their cytoplasmic binding partners

MACROMOLECULAR ORGANIZATION OF ECM
Movement of leukocytes into tissue depends on a precise sequence of combinatorially diverse set of adhesive interaction:

- Bacterial, infection or inflammation \( \rightarrow \) tissue dysfunction \( \rightarrow \) blood (leukocyte) \( \rightarrow \) expressed special adhesion molecules at endothelial surface \( \rightarrow \) bind leukocyte \( \rightarrow \) induced adhesion molecule activation \( \rightarrow \) extravasation \( \rightarrow \) extracellular...

P-selectin, a lectin (protein that binds carbohydrates) on activated endothelial cells, binds a specific ligand (an oligosaccharide sequence) on T cells. PAF (Platelet Activating Factor - a phospholipid) activates integrin on the leukocyte surface:

1. Endothelium activation \( \rightarrow \) selectin or carbohydrate ligand \( \rightarrow \) weak, reversible binding
2. Infection or inflammation signal \( \rightarrow \) chemokines or PAF \( \rightarrow \) expressed special molecules \( \rightarrow \) attached leukocyte
3. Additional activation dependent CAM, integrins \( \rightarrow \) strong adhesion

CAM directly bind to leukocyte:
- Endothelium activation \( \rightarrow \) selectin or carbohydrate ligand \( \rightarrow \) weak, reversible binding
- Infection or inflammation signal \( \rightarrow \) chemokines or PAF \( \rightarrow \) expressed special molecules \( \rightarrow \) attached leukocyte
- Additional activation dependent CAM, integrins \( \rightarrow \) strong adhesion

Bradykinin: increases vascular permeability
Fibrin: involved in blood clot formation
Plasmin: helps to break up blood clots (tPA - tissue plasminogen activator)
Inflammation and atherosclerosis

Cell adhesion molecules:
Selectins (Select in lectins)
Integrins (Integrating proteins)
IgG adhesion molecules

• P-selectin, a lectin (protein that binds carbohydrates) on activated endothelial cells, binds a specific ligand (an oligosaccharide sequence) on T cells
• PAF (Platelet Activating Factor - a phospholipid) activates integrin on the leukocyte surface

Multi-step Model of Leukocyte Adhesion and Extravasation

When leukocyte extravasation in artery ????????????
Artery wall is so strong and thick → leukocyte did not pass through
Atheroma

Reversible interaction of leukocytes to vascular endothelium via carbohydrate ligand (x-lex) on leukocytes and E-selectin on the endothelium cannot anchor the leukocyte because of the shearing force of the blood flow and instead just slows them down and allows for the possibility of stronger binding when other adhesion molecules on the leukocyte (LFA-1) interacts with other induced cell adhesion molecules (ICAM-1)

This arrests the rolling and allows the leukocyte to extravasate (squeeze between two endothelial cells) inside the tissue the leukocyte migrates along a chemokine concentration gradient (IL-8 in this example) secreted by cells at the site of infection
This is a normal coronary artery with no atherosclerosis and a widely patent lumen that can carry as much blood as the myocardium requires.

Atherosclerosis in the coronary artery. The lumen is narrowed by half. A small area of calcification is seen in the plaque at the right.
A coronary thrombosis is occluding the lumen of this coronary artery.

The Matrix Skeleton of Unstable Coronary Artery Plaque


More Detailed T-Cell Endothelial Cell Interactions

Cell-cell adhesion in leukocyte extravasation
Soluble Adhesion Molecules and Cardiovascular Risk Prediction

<table>
<thead>
<tr>
<th>Type of Population</th>
<th>sICAM-1</th>
<th>sVCAM-1</th>
<th>sE-Selectin</th>
<th>sP-Selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Individuals</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Patients at risk with established CAD</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acute Coronary Syndrome</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

+++ strong evidence;  
++ moderate evidence  
+ weak evidence  
− no evidence.

Plant tissue
Unlike animals, plants do not replace or repair old or damaged cells or tissues; only grow new organs. Plant only four broad types of cells (form four basic classes of tissue)
1. dermal tissue: interact with environment
2. vascular tissue: transport water and dissolved substances
3. ground tissue: space filling
4. sporogenous tissue: forms the reproductive organs
Contain polysaccharides: cellulose (張力 张力 张力 张力 strength), hemicellulose
Allow soluble factor to pass to cell membrane, but less permeable than animal cell matrix

Functions of cell walls:
For plants:
Mechanical strength--> plant heights
Glue cells together--> dictate the way in which plant develop
Exoskeleton--> control cell shapes
Control balance between cell turgor pressure and cell volume
Diffusion barrier for macro-molecules and pathogens
Food reserves: endosperm cell walls degrade during seed during germination
Signaling

The plant cell wall is a laminate (薄板) of cellulose fibrils in a matrix of glycoprotein
Primary wall consists of the following basic features
  – Cellulose (strength)
  – Hemicellulose
  – Pectins (flexibility)
  – Structural proteins (rigidity)
  – Non-structural proteins

CELLULOSE AND HEMICELLULOSE ARE PRESENT IN A MATRIX OFPECTIN POLYMERS
Cellulose microfibrils: Linear chains of (1→4)-linked β-D-glucose. Each microfibril may consist of 6 to 30-50 chains. Each chain has 2000 to 25,000 glucose residues. Cellulose has a high tensile strength, equivalent to steel. Insoluble, chemically stable. Excellent “bones” for building a strong cell wall.

Cellulose molecules can be arranged together to form fibrils that have great tensile strength. These fibrils are the main structural element in the cell walls of plants.

Other sugars are hydrogen bonded to cellulose:

- HEMICELLULOSE (RED BACKBONE) IS SIMILAR TO CELLULOSE BUT HAS BRANCHED SUGAR RESIDUES
- BRANCHES (BLUE) LINK MICROFIBRILS TO PECTINS (PURPLE)
- INTERLINKED NETWORK BINDS CELLS TOGETHER
Loosening of the cell wall permits elongation of plant.

Auxin → induced weakening of cell wall → water into cell → expansion of intracellular vacuole → elongation of cell

**Cell Wall Structure**

The middle lamella, primarily pectin, ‘glues’ neighbouring cells together.

**Primary cell wall:**

The first wall laid down during growth.

This is soft and flexible so that the cell can expand during growth.

It contains a mixture of biopolymers, with typically ~20-30% cellulose.

Once growth has stopped, the secondary cell wall is laid down, which provides structural support for the cells.

**Secondary Cell Wall Structure:**

Some cells have very thick secondary cell walls, to provide maximum support.

In the case of flax it can be ~μm in thickness (compared with ~100nm for the primary wall), and this was why flax was used for this study.

Typical cellulose content is ~50%, though can reach ~100% for e.g. cotton.

Structure is complex, and thought to consist of aligned layers of cellulose microfibrils in a general biopolymer matrix, with systematic misorientations between the layers.

**TABLE 15.2**

<table>
<thead>
<tr>
<th>Class of cell wall proteins</th>
<th>Percentage carbohydrate</th>
<th>Localization typically in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRGP (hydroxyproline-rich glycoprotein)</td>
<td>~55</td>
<td>Phloem, cambium, sclerenchyma</td>
</tr>
<tr>
<td>PRP (proline-rich protein)</td>
<td>~0-20</td>
<td>Xylem, fibers, cortex</td>
</tr>
<tr>
<td>GRP (glycine-rich protein)</td>
<td>0</td>
<td>Xylem</td>
</tr>
</tbody>
</table>

A repeated hydroxyproline-rich motif from a molecule of HRGP from tomato.
PLASMODESMATA INTERCONNECT CYTOPLASMS OF ADJACENT PLANT CELLS

60 nM diameter allows passage of molecules of up to 1000 MW
ER extensions (desmotubule) can pass through, allowing transit of membrane bound molecules
Elevation of cytosolic calcium inhibits transports (similar to gap junction)

Table 11.1  Extracellular Structures of Eukaryotic Cells

<table>
<thead>
<tr>
<th>Kind of Organism</th>
<th>Extracellular Structure</th>
<th>Structural Fiber</th>
<th>Components of Hydrated Matrix</th>
<th>Adhesive Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td>Extracellular matrix</td>
<td>Collagens</td>
<td>Proteoglycans and elastins</td>
<td>Fibronectins</td>
</tr>
<tr>
<td>Plants</td>
<td>Cell wall</td>
<td>Cellulose</td>
<td>Hemicelluloses and laminins</td>
<td>Pectins and extensins</td>
</tr>
</tbody>
</table>

The extracellular matrix and the cell wall are the "outside" of the cell

**Extracellular matrix (ECM)**: consists of collagen fibers and proteoglycan. Collagen are a group of insoluble glycoproteins that contain large amount of glycine and the hydroxylated forms of lysine and proline. (examples, tendons, cartilage, and bone)

**Cell wall**: consists cellulose microfibrils embedded in a matrix of other polysaccharides and small amounts of proteins (extensins)

**Primary cell wall**: cellulose fibrils and gel like polysaccharides, thus flexible and extensible

**Secondary cell wall**: additional cell wall materials deposited on the inner surface of the primary cell wall, thus thicker and rigid. Second cell wall also contains high concentration of lignin, a major component of wood

**Communication between cells**:

- **Plasmodesmata**: cytoplasmic bridges between plant cells
- **Animal cells**: gap junctions, tight junctions and adhesive junctions
- **Prokaryotes**: cell walls consist of peptidoglycans with GlcNAc-MurNAc units