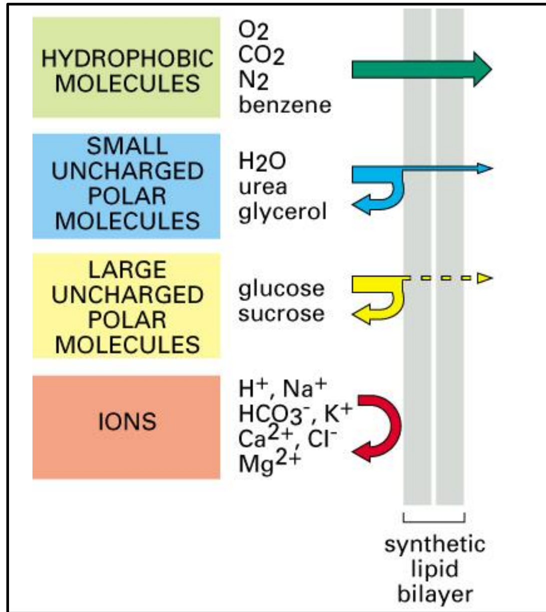
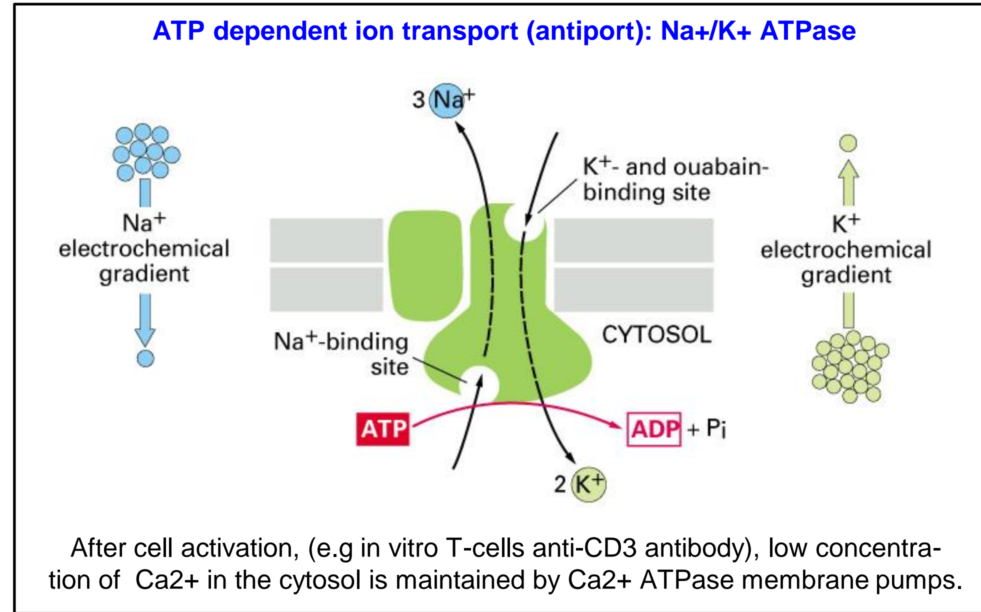
Cellular structures
(special focus on in vitro)

Manfred Kubbies, Dept. Human Genetics, Univ. Würzburg, 2014

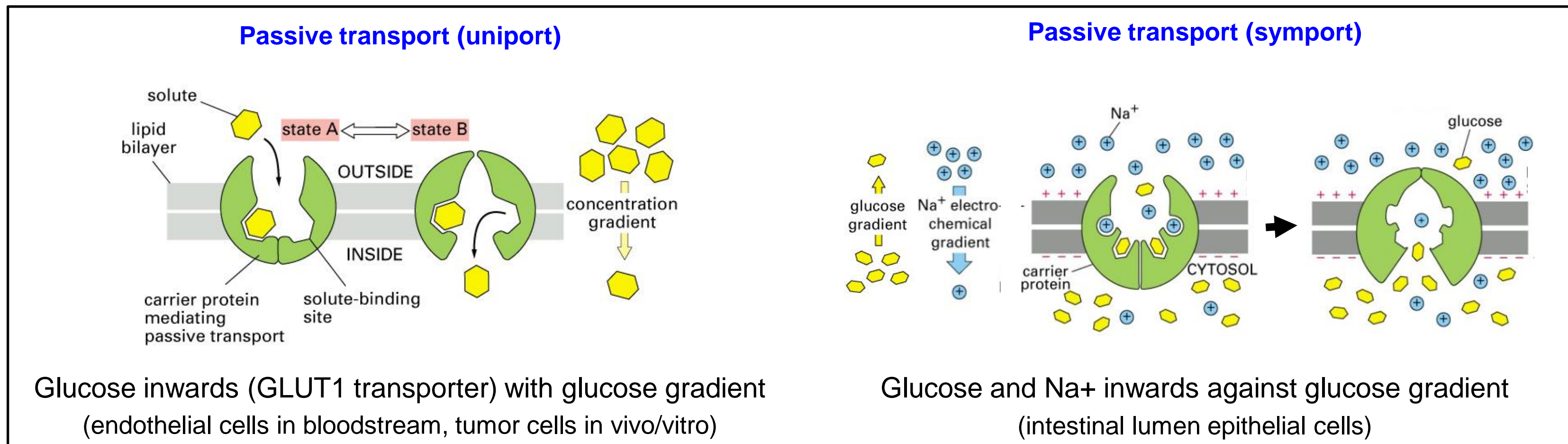
Permeability and functions of cell membranes

Charged molecules do not penetrate cell membranes. Charged or hydrophilic biomolecules may pass membranes via specific transporters.





After cell activation, (e.g in vitro T-cells anti-CD3 antibody), low concentration of Ca2+ in the cytosol is maintained by Ca2+ ATPase membrane pumps.



Glucose inwards (GLUT1 transporter) with glucose gradient
(endothelial cells in bloodstream, tumor cells in vivo/vitro)

Glucose and Na+ inwards against glucose gradient
(intestinal lumen epithelial cells)

Alberts, Molecular Biology of the Cell ,2002

Eukaryotic membrane transport mechanisms

Passive diffusion*
CO_2, N_2, O_2, steroids, fatty acids

Passive transport
Uniport*: glucose (GLUT1), amino acids, nucleosides
Symport#: Na^+/glucose (2 in/1 in), Na^+/amino acid, Na^+/nucleoside
Antiport#: Na^+/Ca^{2+} (3 in/1 out), Na^+/H^+ (1 in/1 out)

Active transport# (ATP-dependent)
Na^+/K^+ ATPase (3 out/2 in), Ca^{2+} ATPase (1 out), P-glycoprotein

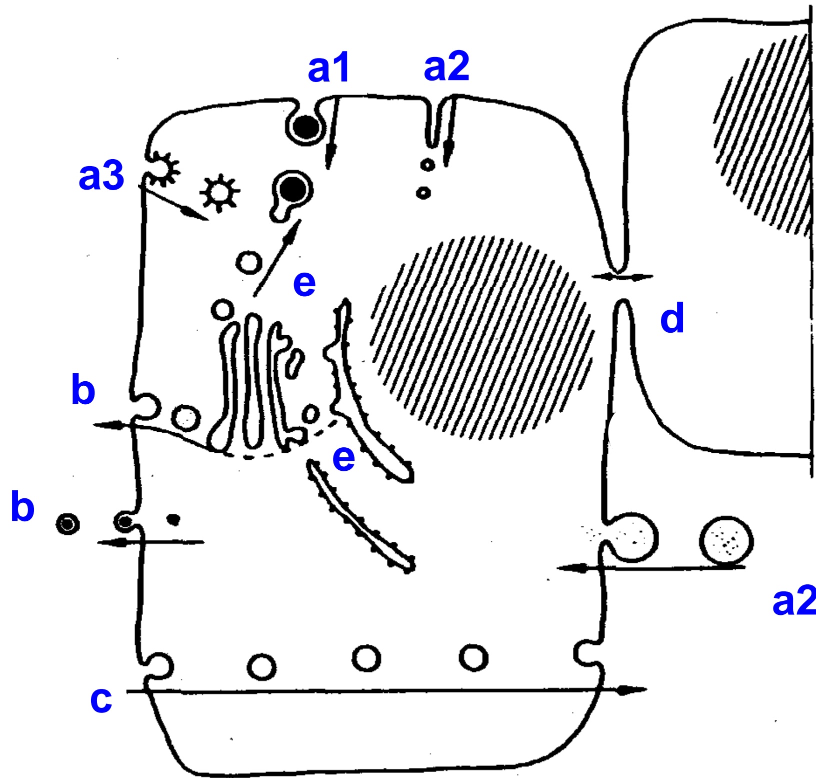
Ion channels
Potential sensitive and/or receptor activated: K^+, Na^+ Ca^{2+}, Cl^-

Endocytosis, pinocytosis
Uptake of small or high molecular weight compounds from the extracellular milieu.

Gap junctions*
Intercellular exchange of compound up to ~1000 D molecular weight
(e. g. inorganic ions, sugars, amino acids, nucleotides, vitamins, analytes like fluorochromes)

* Diffusion towards concentration gradient
\# Diffusion against concentration gradient

Cellular vesicle transport: cytoses



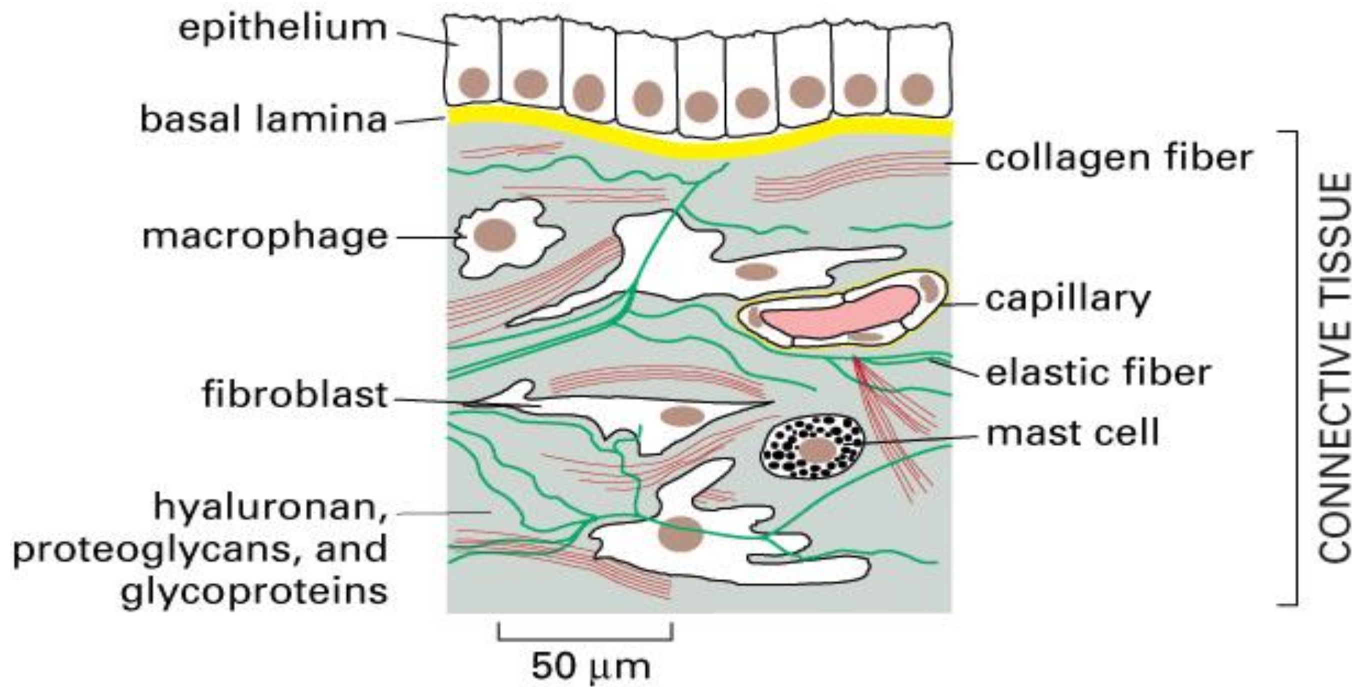
(a) Endocytosis (uptake of solid or liquid material)
a1: phagocytosis (vesicle uptake)
a2: pinocytosis (vesicle uptake and fusion)
a3: coated vesicle (vesicle uptake)
(b) Exocytosis (release of cellular material)
(c) Transcytosis (material passage through cells)
(d) Syncytosis (fusion of cells)
(e) Intracytosis (intracellular vesiculation/fusion of vesicles)

Cell culture relevant examples:

(a) Endocytosis (uptake of solid or liquid material)
a1: granulocyte/macrophage phagocytosis studies
a3: clathrin coated vesicle receptor uptake
(b) Antibody secretion
(c) Epithelial cell barrier assays (BBB, intestine)
(d) Cell fusion myoblasts
(e) Autophagy, lysosomal degradation

Lysosomes contain enzymes for biostructure degradation:
Proteases (e.g. collagenase, cathepsin)
Nucleases (e.g. DNAse, RNAse, phosphatase)
Lipases (e.g. phospholipase A and C, esterase)
Glycosidases (e.g. galactosidase, hyaluronidase)

Structure of the extracellular matrix



Alberts, Molecular Biology of the Cell, 2002

The extracellular matrix consists of fibrous protein embedded in a hydrated polysaccharide gel.

The basal membrane is a specialized extracellular matrix that consists primarily of collagen type IV, proteoglycan and laminin.

Collagen is the major protein of the extracellular matrix. Elastin is a protein network of crosslinked elastin units (gives elasticity).

Organs consist of connective tissue (extracellular matrix and fibroblasts) with embedded, specialized cells.

Tissue type collagen

Collagen	Polymere form	Tissue distribution	
type I	fibril	skin, tendon, cornea, inner organs	
type II	fibril	cartilage, intervertebral discs, vitreous body	
type III	fibril	skin, blood vessel, inner organs	
type IV	basal membrane	epithelial basal membrane	→ most frequently used for cell culture plate coating

Collagen producing cells

Collagen	Anchor protein associated	Proteoglycan associated	Cellular receptor	Producer cells
Type I	Fibronectin	Dermatan- and chondroitin sulfate	Integrins	Fibroblasts
Type II	Fibronectin	Chondroitin sulfate	Integrins	Chondrocytes
Type III	Fibronectin	Heparan sulfate, heparin	Integrins	Hepatocytes; Hepatocyte associated fibroblasts
Type IV	Laminin	Heparan sulfate, heparin	Laminin receptor	Epithel-/endothelial cells, regenerating hepatocytes
Type V	Fibronectin	Heparan sulfate	Integrins	Resting hepatocytes
Type VI	Fibronectin	Heparan sulfate, heparin	Integrins	Resting hepatocytes

Secreted matrix fibers adhere to cell culture flasks thereby facilitating adhesion of cells in culture.

Glycosaminoglycans

Polysaccharide gels are glycosaminoglycan chains that generate hydrated gels.

Glycosaminoglycan	Molecular weight	Disaccharide repeat $(A\text{-}B)_n$	Bound to protein	Tissue distribution
Hyaluronic acid	$4 \times 10^3 – 8 \times 10^6$	D-glucuronic acid/N-acetyl-D-glucosamine	-	Skin, vitreous body, cartilage
Chondroitin sulfate	5000-50000	D-glucuronic acid/N-acetyl-D-galactosamine	+	Cartilage, cornea, bone, skin, arteries
Dermatan sulfate	15000-40000	D-glucuronic acid/N-acetyl-D-galactosamine	+	Skin, arteries, heart
Heparan sulfate	5000-12000	D-glucuronic acid/N-acetyl-D-glucosamine	+	Lung, arteries, cell surfaces, basal membranes
Heparin	6000-25000	D-glucuronic acid/N-acetyl-D-glucosamine	+	Lung, liver, skin, mast cells
Keratan sulfate	4000-19000	D-galactose/N-acetyl-D-glucosamine	+	Cartilage, cornea, intervertebral disc

Dissociation of tissues for cell isolation may require enzymes that degrade not only matrix fibers and adhesion molecules but also glycosaminoglycans !

Cell adhesion molecules

	SOME FAMILY MEMBERS	Ca^{2+} OR Mg^{2+} DEPENDENCE	HOMOPHILIC OR HETEROPHILIC	CYTOSKELETON ASSOCIATIONS
Cell–Cell Adhesion				
Classical cadherins	E, N, P, VE	yes	homophilic	actin filaments (via catenins)
Desmosomal cadherins	desmoglein	yes	homophilic	intermediate filaments (via desmoplakin, plakoglobin, and other proteins)
Ig family members	N-CAM	no	both	unknown
Selectins (blood cells and endothelial cells only)	L-, E-, and P-selectins	yes	heterophilic	actin filaments
Integrins on blood cells	$\alpha_L\beta_2$ (LFA-1)	yes	heterophilic	actin filaments
Cell–Matrix Adhesion				
Integrins	many types	yes	heterophilic	actin filaments (via talin, filamin, α-actinin, and vinculi
	$\alpha_6\beta_4$	yes	heterophilic	intermediate filaments (via plectin)
Transmembrane proteoglycans	syndecans	no	heterophilic	actin filaments

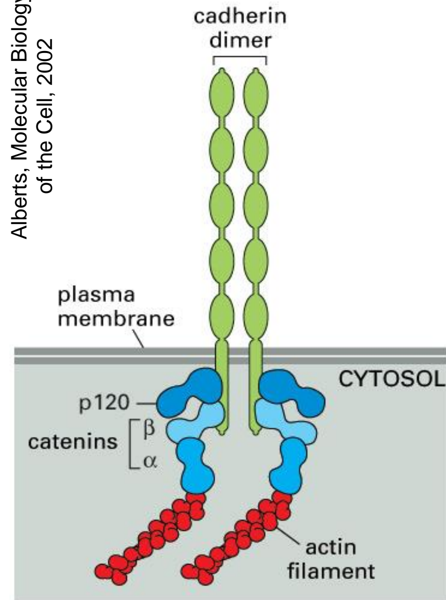
Integrins and ligands

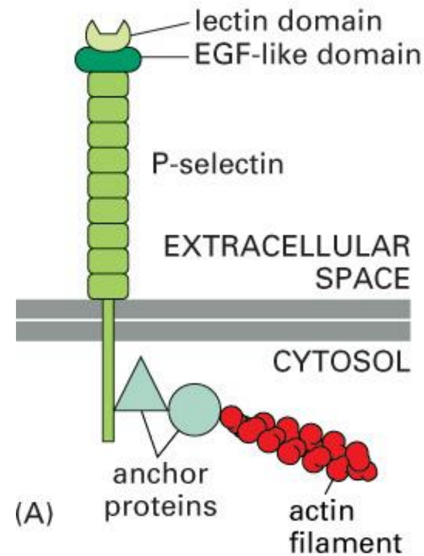
INTEGRIN	LIGAND*	DISTRIBUTION
$\alpha_5\beta_1$	fibronectin	ubiquitous
$\alpha_6\beta_1$	laminin	ubiquitous
$\alpha_7\beta_1$	laminin	muscle
$\alpha_L\beta_2$ (LFA-1, see p. 1411)	Ig superfamily counterreceptors	white blood cells
$\alpha_2\beta_3$	fibrinogen	platelets
$\alpha_6\beta_4$	laminin	epithelial hemidesmosomes

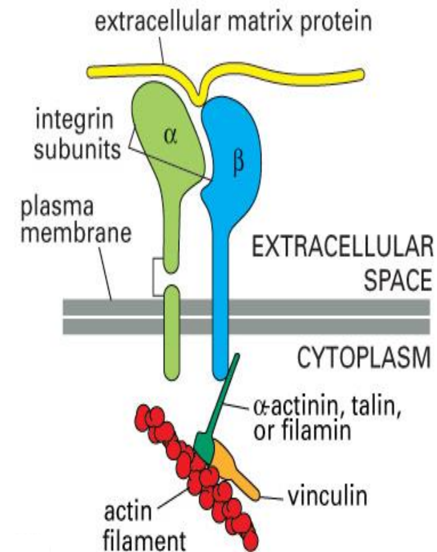
Alberts, Molecular Biology of the Cell, 2002

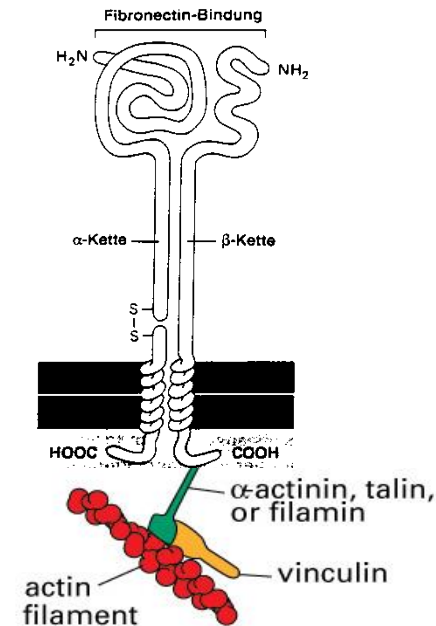
Cell adhesion molecules connect INSIDE and OUTSIDE

Alberts, Molecular Biology of the Cell, 2002









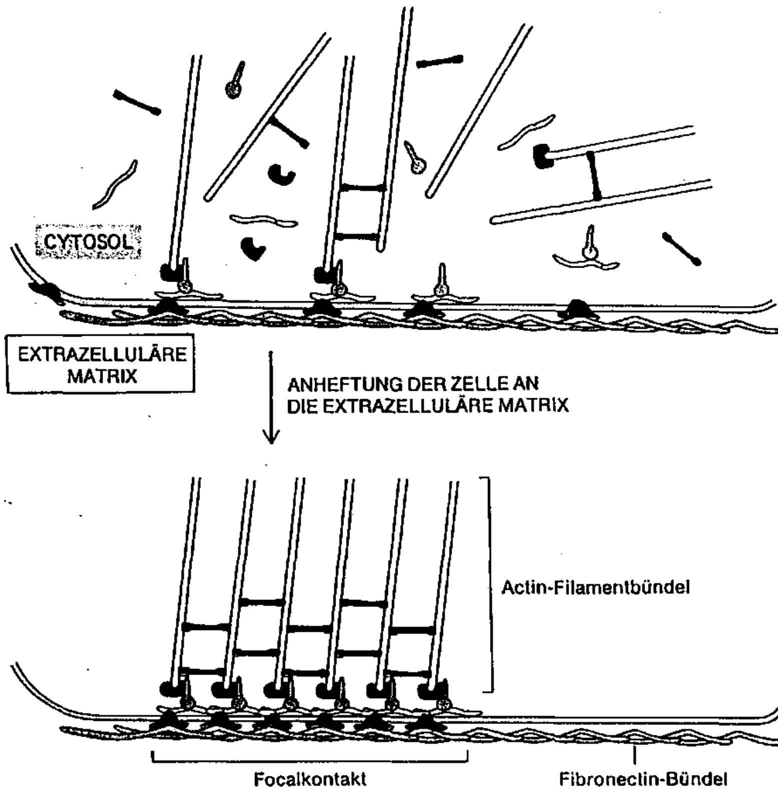
Cell binding

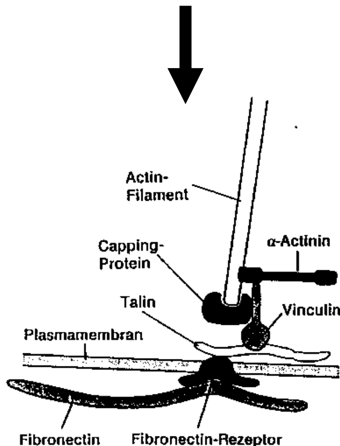
In cell culture adherent cells establish cell-cell (cadherins) and cell-matrix (e.g. collagen/integrin) adhesions.

Some cells, especially in serumfree culture, require exogenously added matrix proteins for adhesion (cell culture plate coating e.g. with collagen, fibronectin or laminin).

Proteases used for cell detachment may destroy cell adhesion receptors which are no longer detectable by immunological detection methods; use alternative techniques (e.g. EDTA release of cells).

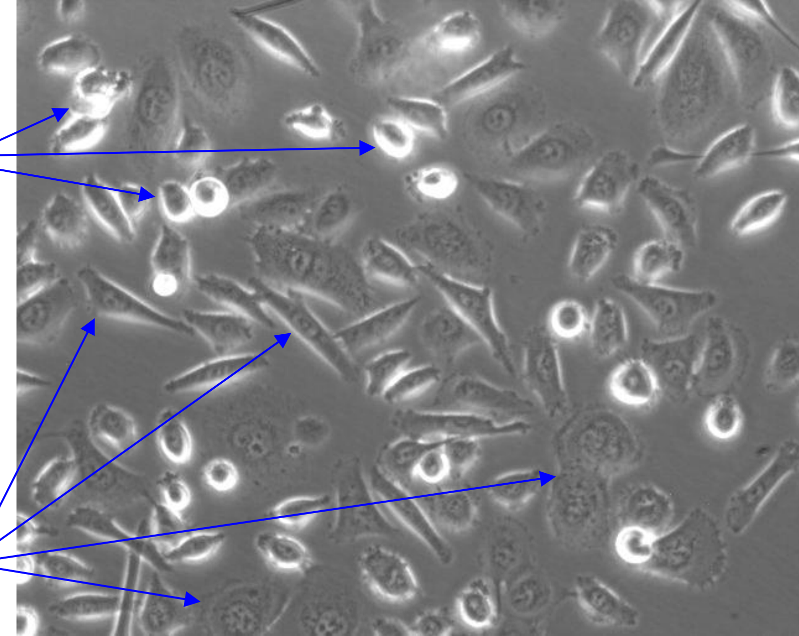
Cytoskeleton connection inside to outside





No cell adhesion or cell adhesion just started

Cells adherent to surface



Trypsin detaches fibronectin or collagen binding: cells round up !

Poor matrix protein coating or adhesion properties of cell culture plate (e.g. glas) results in insufficient adhesion and poor morphology of adherent cells.

Applications: motility assays as surrogate for metastatic capability of cells (e.g. transwell assay).

Overview of cell contact molecules

OCCLUDING JUNCTIONS

1. tight junctions (vertebrates only)
2. septate junctions (invertebrates mainly)

ANCHORING JUNCTIONS

Actin filament attachment sites

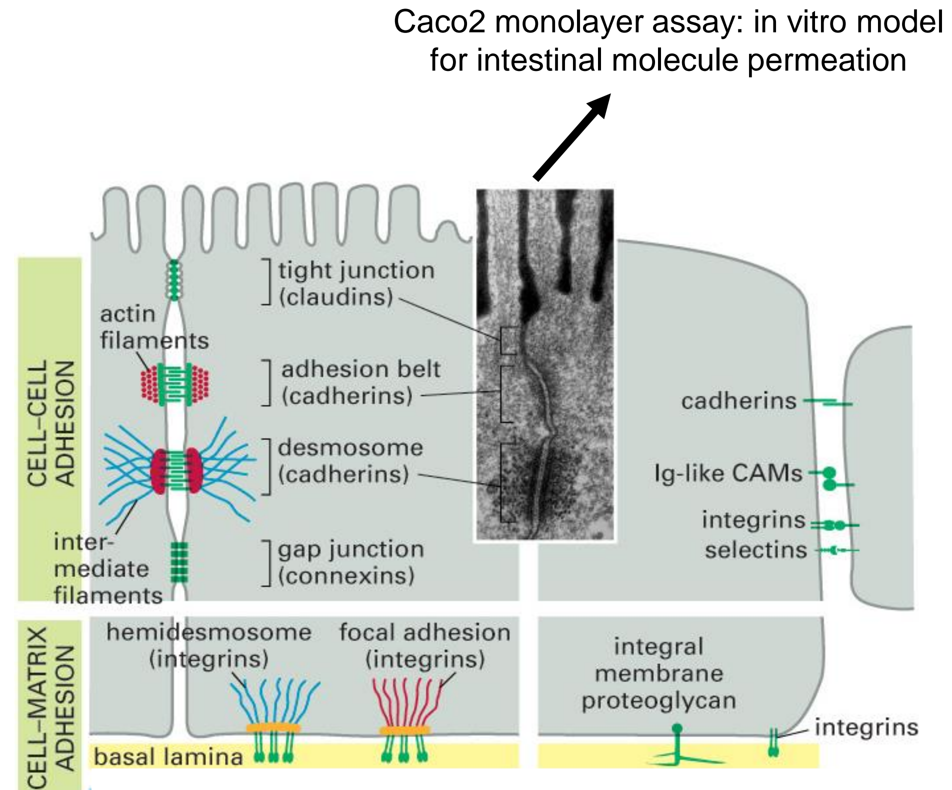
1. cell–cell junctions (adherens junctions)
2. cell–matrix junctions (focal adhesions)

Intermediate filament attachment sites

1. cell–cell junctions (desmosomes)
2. cell–matrix junctions (hemidesmosomes)

COMMUNICATING JUNCTIONS

1. gap junctions
2. chemical synapses
3. plasmodesmata (plants only)



Alberts, Molecular Biology of the Cell, 2002

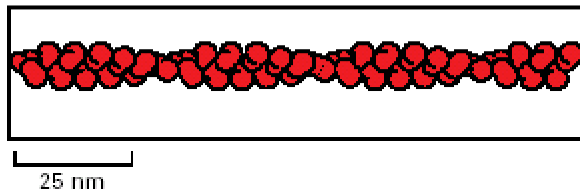
Tight junctions are present in epithelial but not in mesenchymal or hematopoietic cells.

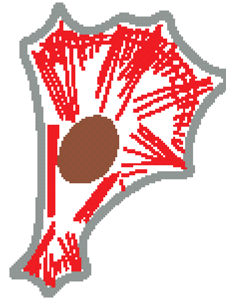
In vitro tight junctions are irrelevant for cellular vital functions of monolayer cells, but may be molecule diffusion limiting in 3D spheroids.

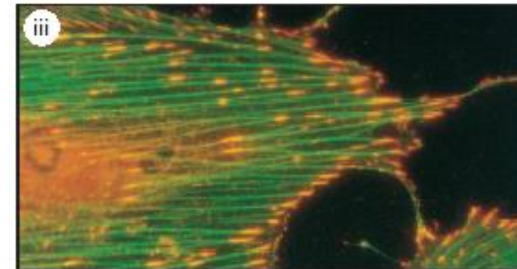
The physiological relevance of tight junctions becomes evident in monolayer transwell assays.

The three major filaments of the cytoskeleton

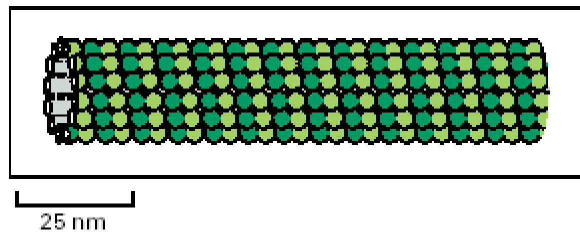
Actin filament

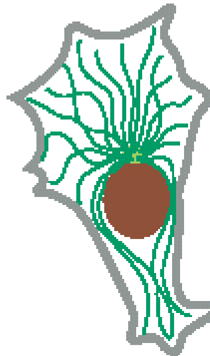


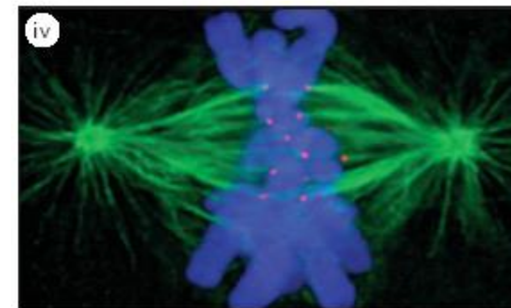




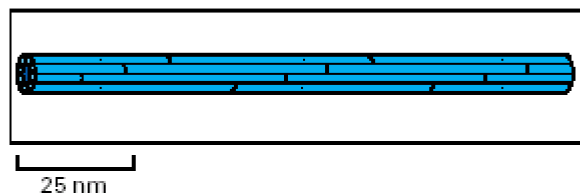
Mikrotubule

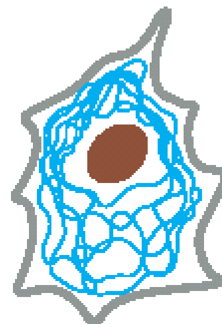


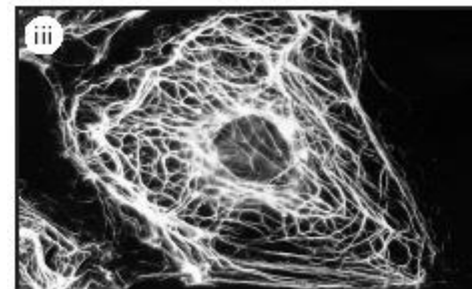




Intermediate filament







Alberts, Molecular Biology of the Cell, 2002

Protein filaments of the cytoskeleton

Filaments stabilize cells and contribute to cellular movement.

The intermediate filament is indicative of the tissue origin of cells and may be used for in vitro cell type classification.

	Diameter (nm)	Tissue distribution	Tumor type
Actin filament	6	Ubiquitous	-
Mikrotubule	25	Ubiquitous	-
Intermediate filament			
Cytokeratin	8	Epithelial cells	Carcinoma
Desmin	10	Muscle cells	Rhabdomyosarkoma
Vimentin	10	Mesenchymal cells	Sarcoma
Neurofilament	10	Neurons	Glioma
Gliafilament	10	Glia cells	Astrocytoma

Since tumors evolve in vivo, carcinoma cells may express not only cytokeratins (epithelial; more differentiated tumor cells) but also vimentin (mesenchymal; more dedifferentiated, metastasizing tumor cells). This process is called EMT transition (epithelial-mesenchymal transition).

Intermediate filaments of the cytoskeleton

The family of the intermediate filament proteins is quite diverse.
Several, different types may be expressed within one cells.

Type I intermediate filaments

Acidic keratins (epithelial cells, CK9 – CK20)

Type II intermediate filaments

Basic keratins (epithelial cells, CK1 – CK8)

Type III intermediate filaments

Desmin (muscle)
GFAP (glial fibrillary acidic protein, astrocytes)
Peripherin (peripheral neurons)
Vimentin (connective tissue, leukocytes, vascular endothelial cells)

Type IV intermediate filaments

Alpha-internexin (neurons)
Neurofilaments (neuronal axons)
Synemin (skeletal muscle cells)
Syncoilin (skeletal muscle cells)

Typ V intermediate filaments

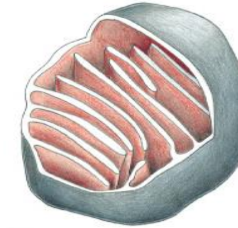
Laminin, B-Typ ubiquitous (e.g. inner, nuclear lamina; A-type gastrulation)

Typ VI intermediate filaments

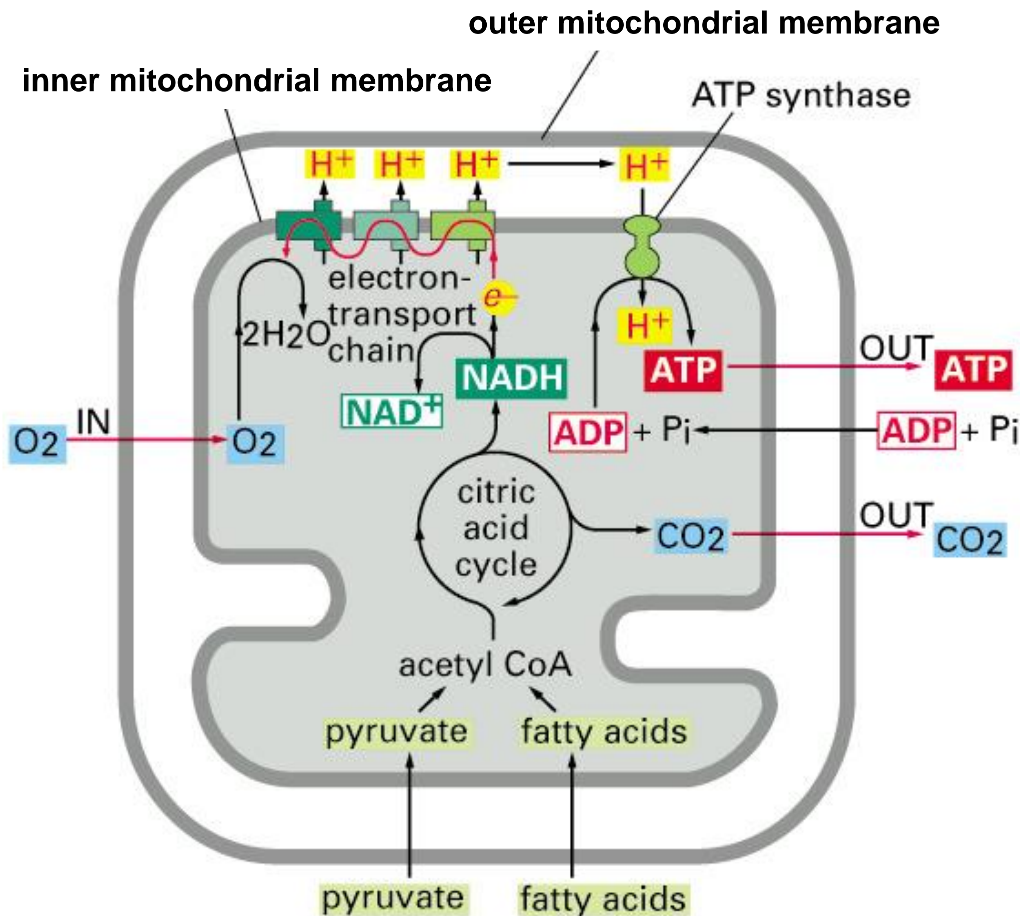
Nestin (neuronal stem cells, growing neuronal axons)

Energy metabolism of mitochondria

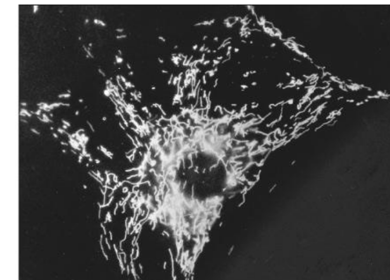
Citric acid cycle, oxydative phosphorylation, ß-oxydation of lipids.



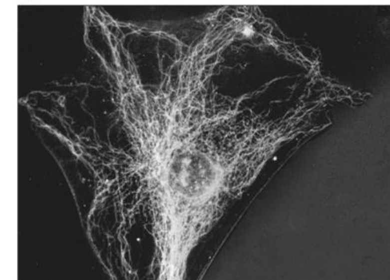
Mitochondria have a double bilayer membrane.
Mitochondria are electronegative.
Mitochondria are fixed within cells.



Alberts, Molecular Biology of the Cell, 2002



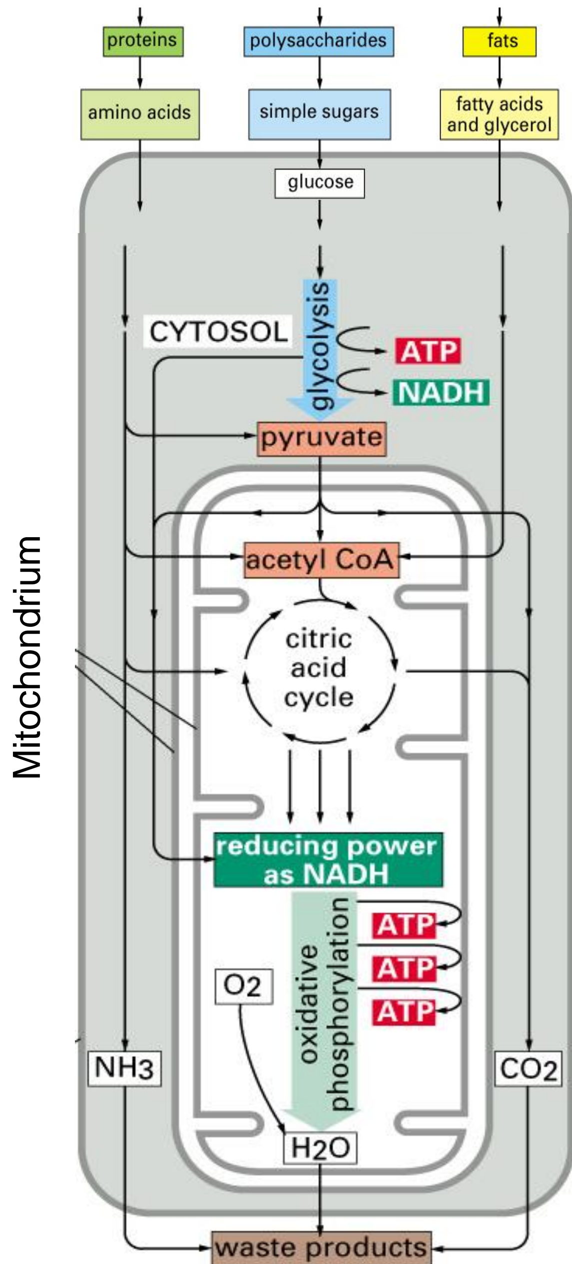
Mitochondria (R123 staining)



Microtubuli (Antibody labeling)

Pyruvate and fatty acids are not the only intermediates for ATP energy production. Glutamine is the most abundant amino acid in the plasma and an additional energy source in cells especially when glycolytic energy production is low. Glutaminolysis takes place in all proliferating cells, such as lymphocytes, thymocytes, adipocytes and especially in tumor cells. Glutamine conversion to a-ketoglutarate.

What is the dominant energy metabolism of cells in vitro ?



Alberts, Molecular Biology of the Cell, 2002

anaerob ?

aerob ?

D-Glucose

Hexokinase — ATP → ADP — - 1 ATP

Glucose-6-phosphat

Phosphohexoseisomerase

Fructose-6-phosphat

Phosphofructokinase — ATP → ADP — - 1 ATP

Fructose-1,6-bisphosphat

Aldolase — Dihydroxyaceton-phosphat

Glycerinaldehyd-3-phosphat

Phosphoglycerinaldehyd-dehydrogenase — P_i, $NADH + H^+$, NAD^+

1,3-Bisphosphoglycerat (3-Phosphoglyceroyl-phosphat)

Phosphoglyceratkinase — ADP → ATP — + 2 ATP

3-Phosphoglycerat

Phosphoglyceratmutase

2-Phosphoglycerat

Enolase — H_2O

Phosphoenolpyruvat

Pyruvatkinase — ADP → ATP — + 2 ATP

Pyruvat

Lactatdehydrogenase — $NADH + H^+$, NAD^+

Lactat

LDH analysis: dead cells

pH-indicator phenol red

anaerob: 2 ATP
aerob: 36 ATP

Löffler, Biochemie, 2004

Energy metabolism of tumor cells in vitro and in vivo

A meta-analysis of the studies done (again, mainly on cultured cells) over 40 years (Zu and Guppy, 2004) showed that the average contribution of glycolysis to ATP production in a range of 27 tumor types was 17-18%, was not significantly different from the glycolytic ATP contribution of 20-21% in 16 normal tissues. In contrast, Busk et al. (2008) found that glycolysis accounted for about 60% of ATP production in other types of cultured cells.

Remark: in vitro exposure of cells to cyanide KCN (inhibits mitochondrial cytochrome oxidase and mitochondrial oxydative phosphorylation) does not necessarily induce cell death. Especially in tumor cells there seems to be sufficient energy available for survival via glycolysis .

An analysis of the published data has shown that only ~12% of the energy requirement of these tumors came from the phosphorylation of ATP during glycolysis to lactate; the remainder came from oxidative metabolism.

SUBSTRATE uptake and utilisation data from balance studies on human tumors grown either in nude rats or studied *in situ*.

	Human tumour xenografts in nude rats[a]	Human colon carcinomas *in situ*[b]
Lactic acid output (nmol/g/min)	527	220
Glucose consumption (nmol/g/min)	401	320
O_2 consumption (nmol/g/min)	588	–
Glucose available for oxidation (nmol/g/min)	144[c]	208[d]
CO_2 output (from O_2)(nmol/g/min)	588	–
CO_2 output (from glucose)(nmol/g/min)	850	1296[e]
ATP from glycolysis (nmol/g/min)	527	220
ATP from glucose oxidation (nmol/g/min)	4402	7055
% ATP from glycolysis	**12%**	**3.1%**

These studies, both in cultured cells and tumors in vivo, suggest that cellular ATP was mainly provided by oxidative metabolism. These results do not support the assumption that tumor cells rely on aerobic glycolysis for their energy needs.

…… the apparently wasteful consumption of large quantities of glucose to generate small amounts of ATP could still give cancer cells a selective advantage either by causing hyperacidity of the surrounding host tissues ……
…….or simply by starving adjacent oxidative cells ……

Stubbs et al, Adv Enzyme Regulation, 2010