Aquaporin, the water channel, consists of four identical transmembrane polypeptides

The phospholipid bilayer is a barrier that controls the transport of molecules in and out of the cell.

- **Gases**: CO₂, N₂, O₂
- **Small uncharged polar molecules**: Ethanol, NH₃, Urea, Water
- **Large uncharged polar molecules**: Glucose, fructose
- **Ions**: K⁺, Mg²⁺, Ca²⁺, Cl⁻, HCO₃⁻, HPO₄²⁻
- **Charged polar molecules**: Amino acids, ATP, glucose 6-phosphate, proteins, nucleic acids

- **Electrochemical gradient**: The differences in ion concentrations across the membrane establishes a membrane electrochemical gradient
- **Membrane potential**: Ion concentration gradients across the membranes establishes the membrane electric potential
- **Concentration gradient**

Studies of synthetic lipid bilayers help define which types of transport will require the activity of a protein. Hence, transport of an ion should require a protein.
An electrochemical gradient combines the membrane potential and the concentration gradient.

Cell membrane
- Barrier to the passage of most polar molecule
- Maintain concentration of solute

Diffusion rate depends on:
1. Concentration gradient or electrochemical gradient
2. Hydrophobicity (i.e., higher partition coefficient)
3. Particle size

The rate at which a molecule diffuses across a synthetic lipid bilayer depends on its size and solubility. The smaller the molecule and the less polar it is, the more rapidly it diffuses across the bilayer.

Membrane proteins mediated transport of most molecules and all ions across biomembrane.

Three main classes of membrane protein
1. ATP-power pump (carrier, permease)
   - couple with energy source for active transport
   - binding of specific solute to transporter which undergo conformation change
2. Channel protein (ion channel)
   - formation of hydrophilic pore
   - allow passive movement of small inorganic molecule
3. Transporters
   - uniport
   - symport
   - antiport
Overview of membrane transport proteins

1. All transmembrane proteins
2. Some transport has ATP binding sites
3. Move molecules uphill (upward) against its gradient

The four mechanisms of small molecules and ions are transported across cellular membranes

**Free Diffusion**
- A. Non-channel mediated
  - lipids, gasses (O2, CO2), water
- B. Channel mediated
  - ions, charged molecules

**Facilitated diffusion**
- Carrier mediated
  - glucose, amino acids

**Facilitated Diffusion**
- Rate of diffusion is determined by:
  - concentration gradient
  - amount of carrier protein
  - rate of association/dissociation
Several features distinguish uniport transport from passive diffusion:

1. **Higher diffusion rate for uniport than passive diffusion.**
2. Transported molecules never enter the membrane and are irrelevant to the partition coefficient (do not cross the membrane).
3. Transport rate reaches $V_{max}$ when each uniport works at its maximal rate.
4. Transport is specific. Each uniport transports only a single species of molecules or single or closely related molecules.

**Mammalian glucose transporters**

<table>
<thead>
<tr>
<th>Name</th>
<th>Tissue distribution</th>
<th>Proposed function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glut1</td>
<td>all fetal and adult tissues</td>
<td>basal glucose transport</td>
</tr>
<tr>
<td>Glut2</td>
<td>hepatocytes, pancreatic β-cells, intestine, kidney</td>
<td>transepithelial transport from and to the blood</td>
</tr>
<tr>
<td>Glut3</td>
<td>widely distributed</td>
<td>basal glucose transport mostly in brain</td>
</tr>
<tr>
<td>Glut4</td>
<td>skeletal muscle, heart, adipocytes</td>
<td>insulin-dependent transport</td>
</tr>
<tr>
<td>Glut5</td>
<td>intestine, lesser amounts in others</td>
<td>fructose transport</td>
</tr>
<tr>
<td>Glut7</td>
<td>gluconeogenic tissues</td>
<td>mediates flux across endoplasmic reticulum</td>
</tr>
<tr>
<td>Glut8</td>
<td>preimplantation blastocyst</td>
<td>embryonic insulin-dependent transport</td>
</tr>
</tbody>
</table>

**Glucose transporter (GLUT): Facilitated Diffusion**

Families of GLUT proteins (1-12):
- Highly homologous in sequence, containing 12 membrane-spanning α-helices.
- Different isoforms → different cell type expression, and different function:
  - GLUT2: expressed in liver cells (glucose storage) and β cell glucose uptake in pancreas.
  - GLUT4: found in intracellular membrane, increased expression by insulin to remove glucose from blood to cell.
  - GLUT5: transports fructose.
- Other isoforms: ???

**Three carrier proteins, appropriately positioned in the plasma membrane, function to transport glucose across the intestinal epithelium.**

---

*Oded Meyuhas*
There are 2 main classes of membrane transport proteins

Common: specific or selective

(A) CARRIER PROTEIN
1. Energy or ion force depend
2. Or without

(B) CHANNEL PROTEIN
1. Normal is open
2. Need ligand

Two types of transport are defined by whether metabolic energy is expended to move a solute across the membrane.

Passive transport: no metabolic energy is needed because the solute is moving down its concentration gradient.
- In the case of an uncharged solute, the concentration of the solute on each side of the membrane dictates the direction of passive transport.

Active transport: metabolic energy is used to transport a solute from the side of low concentration to the side of high concentration.
Liposome containing a single type of transport protein are very useful in studying functional properties of transport proteins

- It is a major experimental tool to study the biochemistry of transport protein function in vitro
- Widely used as a drug delivery system and for gene transfection

**ATP powered pump**

1. **P-class**
   - P$_2$ and P$_2$β subunit; can phosphorylation
   - i.e. Na$^+$K$^+$ ATPase, Ca$^+$ ATPase, H$^+$ pump

2. **F-class**
   - Locate on bacterial membrane, chloroplast, and mitochondria
   - Pump proton from exoplasmic space to cytosolic for ATP synthesis

3. **V-class**
   - Maintain low pH in plant vacuole
   - Similar to F-class

4. **ABC (ATP-binding cassette) superfamily**
   - Several hundred different transport proteins

**P-class pumps**
- Plasma membrane of plants, fungi, bacteria (H$^+$ pump)
- Plasma membrane of higher eukaryotes (Na$^+$K$^+$ pump)
- Apical plasma membrane of mammalian stomach (H$^+$K$^+$ pump)

**F-class pumps**
- Plasma membrane of all eukaryotic cells (Ca$^{2+}$ pump)
- Sarcolemmal reticulum membrane in muscle cells (Ca$^{2+}$ pump)

**Exoplasmic face**
- P$_{α}$ and P$_{β}$ subunit
- Specific ion binding site
- ATP
- ADP

**Cytosolic face**
- P$_{α}$ and P$_{β}$ subunit
- Specific ion binding site
- ATP
- ADP
**P class : Ca\(^{2+}\) ATPase:**

(a) Consists of 10 transmembrane alpha helices that form a channel for Ca\(^{2+}\) ions movement. By site directed mutagenesis four residues on four different helices (4, 5, 6, 8) are involved in calcium binding.

(b) Found on cell surface membrane and in sarcoplasm reticulum of the muscle cells involved in muscle contraction.

(c) Relaxed muscle Ca\(^{2+}\) is low (0.1 \(\mu\)m) and during contraction it is higher (>1.0 \(\mu\)m).

(d) Transport process requires ATP hydrolysis in which the free energy is liberated by breakdown of ATP into ADP and phosphate. This is stored in an acyl phosphate bond on the aspartate residue of the alpha subunit of the ATPase protein.

---

**F-class proton pumps**

- Bacterial plasma membrane
- Inner mitochondrial membrane
- Thylakoid membrane of chloroplast

Inside: 4H\(^+\)  
Outside: 4H\(^+\)

Inside: ADP + P\(_i\)  
Outside: ATP

**V-class proton pumps**

Vacuolar membranes in plants, yeast, other fungi

Endosomal and lysosomal membranes in animal cells

Plasma membrane of osteoclasts and some kidney tubule cells

**ATP powered pump**

1. **P-class**
   - 2\(\alpha\), 2\(\beta\) subunit
   - i.e. Na\(^{+}\)-K\(^{+}\) ATPase, Ca\(^{2+}\) ATPase, H\(^{+}\) pump

2. **F-class**
   - locate on bacterial membrane, chloroplast and mitochondria
   - pump proton from exoplasmic space to cytosolic for ATP synthesis

3. **V-class**
   - maintain low pH in plant vacuole
ATP-powered ion pumps generate and maintain ionic gradients across cellular membranes

**Operational model of the Ca^{2+}-ATPase in the SR membrane of skeletal muscle cells**

Plays a major role in muscle relaxation by transporting released Ca^{2+} back into SR

- A single subunit protein with 10 transmembrane fragments
- Is highly homologous to Na,K-ATPase

**Structure of the catalytic a subunit of the muscles Ca^{2+} ATPase**

**Table 7.1**: Typical Intracellular and Extracellular Ion Concentrations

<table>
<thead>
<tr>
<th>Ion</th>
<th>Intracellular (mM)</th>
<th>Extracellular (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K^{+}</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Na^{+}</td>
<td>150</td>
<td>145</td>
</tr>
<tr>
<td>Cl^{-}</td>
<td>40 to 130</td>
<td>560</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>0.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Mg^{2+}</td>
<td>0.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Need large energy: RBC need 50% ATP for Na/K pump; nerve and kidney need 25% for ion transport.
Four major domains:

M - Membrane-bound domain, which is composed of 10 transmembrane segments

N - Nucleotide-binding domain, where adenine moiety of ATP and ADP binds

P - Phosphatase domain, which contains invariant Asp residue, which became phosphorylated during the ATP hydrolysis

A domain – essential for conformational transitions between E1 and E2 states

Calmodulin-mediated activation of plasma membrane Ca2+ ATPase leads to rapid Ca2+ export → keep cytosolic Ca2+ very low

**Na+/K+ ATPase**

(maintain the intracellular Na and K concentration in animal cell)

Greatest consumer cellular energy

Sets up concentration & electrical gradients

Hydrolysis of 1 ATP moves 2K+ in and 3Na+ out against their concentration gradients
**Na⁺,K⁺-ATPase**

maintains uneven distribution of Na⁺ and K⁺ ions across cell membrane by transporting 3Na⁺ and 2K⁺ per each ATP hydrolyzed during the transport cycle. The ions become "occluded" cycles between two major conformational states E₁ which has high affinity for Na⁺ and ATP and E₂, which has high affinity for K⁺-ions. Can be specifically inactivated by ouabain.

**Effect of proton pumping by V-class ion pumps on H⁺ concentration gradients and electric potential gradients across cellular membrane**

V-class H⁺ ATPase pump protons across lysosomal and vacuolar membranes. Only transport H⁺. Effects of proton pumping by V-class ion pumps on H⁺ concentration gradients and electric potential gradients across cellular membranes.

**Generation of electrochemical gradient**

Electrochemical gradient combines the membrane potential and concentration gradient which work additively to increase the driving force.

**H⁺,K⁺-ATPase**

Mediates acid secretion in gastric mucosa by exporting protons in exchange for extracellular potassium ions. Structurally, it is very similar to Na⁺,K⁺-ATPase. Gastric and duodenal ulcer depend on acid secretion, therefore H⁺,K⁺-ATPase is an important pharmacological target.

**The Gastric H⁺,K⁺-ATPase**

This is the largest concentration gradient across a membrane in eukaryotic organisms! H⁺,K⁺-ATPase is similar in many respects to Na⁺,K⁺-ATPase and Ca⁺-ATPase.
Osteoclast Proton Pumps

How your body takes your bones apart!

Bone material undergoes ongoing remodeling
- osteoclasts tear down bone tissue
- osteoblasts build it back up

Osteoclasts function by secreting acid into the space between the osteoclast membrane and the bone surface – acid dissolves the Ca-phosphate matrix of the bone.

An ATP-driven proton pump in the membrane does this!

F-class Ion Pump – the mitochondrial “ATPase” - SYNTHASE

The structure of F and V class pumps are similar

Find in
- bacterial plasma membranes
- mitochondria
- chloroplasts

The movement of electrons through the electron transport chain is coupled to proton ejection from the matrix.

The movement of protons back via the F-ATPase is coupled to the synthesis of ATP.

\[ F_0: \text{located in membrane, multimeric - containing 3 types of subunit, incorporation into vesicles leads to } H^+ \text{ permeability.} \]

\[ F_1: \text{water soluble complex, 5 polypeptides.} \]

F and V pumps - only transport protons (H\(^+\))
No phosphoprotein intermediate (Unlike P pumps)

F pumps: function to power ATP synthesis using proton gradient.

V pumps: use ATP to pump protons into vacuoles, lysosomes.
Bacterial permeases are ABC proteins that import a variety of nutrients from the environment. About 50 ABC small-molecule pumps are known in mammals.

**ABC transporter**
- 2 T (transmembrane) domain, each has 6 α-helix
- Form pathways for transported substance
- 2A (ATP-binding domain)
- 30-40% homology for membranes

i.e. bacterial permease
- Use ATP hydrolysis
- Transport a.a., sugars, vitamins, or peptides
- Inducible, depend on the environmental condition

i.e. mammalian ABC transporter (Multi Drug Resistant)
- Export drug from cytosol to extracellular medium
- mdr gene amplified by drugs stimulation
- Mostly hydrophobic for MDR proteins
- Cancer cell resistant to drug mechanisms

**Examples of a few ABC proteins**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Transport substrate (description)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>Cholesterol (Gut wax/ERL deficiency)</td>
<td>Human</td>
</tr>
<tr>
<td>ABCA4</td>
<td>Neutral phospholipids</td>
<td>Human</td>
</tr>
<tr>
<td>ABCG5</td>
<td>Unclear</td>
<td>Human</td>
</tr>
<tr>
<td>ABCG8</td>
<td>Unclear</td>
<td>Human</td>
</tr>
<tr>
<td>ABCG2</td>
<td>Unclear</td>
<td>Human</td>
</tr>
<tr>
<td>ABCG1</td>
<td>Unclear</td>
<td>Human</td>
</tr>
<tr>
<td>ABCG12</td>
<td>Unclear</td>
<td>Human</td>
</tr>
<tr>
<td>ABCG13</td>
<td>Unclear</td>
<td>Human</td>
</tr>
<tr>
<td>ABCG15</td>
<td>Unclear</td>
<td>Human</td>
</tr>
</tbody>
</table>

**Auxiliary transport system associated with transport ATPases in bacteria with double membranes**

The transport ATPases belong to the ABC transporter superfamily.
The Multidrug Resistance Protein (MDR)

ABC (ATP-binding cassette)

170 Kdalton P-glycoprotein that pumps hydrophobic drugs out of cells in an ATP-dependent fashion.

Uses the energy derived from ATP hydrolysis to export a large variety of drugs from the cytosol to the extracellular medium.

It reduces the cytoplasmic concentration of drugs and hence their toxicity. It therefore reduces the effectiveness of chemotherapeutic drugs. It is overexpressed in some tumor cells. Need high concentration to killed cell.

It transports a wide range of chemically unrelated proteins including the anthracyclines, actinomycin D, valinomycin, and gramicidin.

Action of the Multi-drug Resistant Transport Protein

Mode of action of MDR1 involves flipping (flippase) or pumping of the lipid soluble drugs that typically have some positive charges across the membrane (ATPase side) to the exterior where the drug is released to the outside.

MDR1 is found in high activity in organs like liver and kidney that play a major role in the breakdown of drugs and other toxic substances but unfortunately reaches highly unregulated levels in corresponding tumor cells.

A typical ABC transporter consists of four domains – two highly hydrophobic domains and two ATP-binding catalytic domains

ATP binding leads to dimerization of the two ATP-binding domains and ATP hydrolysis leads to their dissociation.

ABC protein that transport lipid-soluble substrates may operated by a flippase mechanism

Structural model of E. coli lipid flippase, and ABC protein homologous to mammalian MDR1
Proposed mechanisms of action for the MDR1 protein

**Flippase model of transport by MDR1 and similar ABC protein.**

1. Spontaneously Diffuses laterally
2. Flips the charged substrate molecule

Lipids can be moved from one monolayer to the other by flippase proteins

Some flippases operate passively and do not require an energy source

Other flippases appear to operate actively and require the energy of hydrolysis of ATP

Active flippases can generate membrane asymmetries

**ATP driven pumps**

<table>
<thead>
<tr>
<th>Pump Type</th>
<th>Transports</th>
<th>Example</th>
<th>Phosphoprotein?</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>H⁺, Na⁺, K⁺, Ca²⁺</td>
<td>plasma membrane Na⁺/K⁺ ATPase</td>
<td>yes</td>
</tr>
<tr>
<td>F</td>
<td>H⁺</td>
<td>F-ATPase in vacuolus</td>
<td>no</td>
</tr>
<tr>
<td>V</td>
<td>H⁺</td>
<td>Na⁺/K⁺ ATPase in mitochondria</td>
<td>no</td>
</tr>
<tr>
<td>ABC (ATP binding cassette)</td>
<td>various</td>
<td>p-glycoprotein and drugs from liver cells</td>
<td>bind ATP – do not hydrolyse unless ions are simultaneously transported</td>
</tr>
</tbody>
</table>

Flippase model of transport by MDR1 and similar ABC protein.
Diseases linked with ABC proteins

1. ALD (X-link adrenoleukodystrophy)
   - Defect in ABC transport protein (ABCD1)
   - Located on peroxisome, used for transport for very long fatty acid
   - Absence ABCD1 → fatty acid → accumulate cytosol → cell damage

2. Tangier disease
   - Deficiency in plasma ABCA1 proteins, which is used for transport of phospholipids and cholesterol
   - Ch18 p747-749

3. Cystic fibrosis
   - Mutation of CFTR (cystic fibrosis transmembrane regulator; a Cl- transporter in the apical membrane of lung, sweat gland and pancreas)
   - Lacked it → did not resorption of Cl → taste salty → This leads to abnormalities in the pancreas, skin, intestine, sweat glands and lungs

Fatty acid didn’t directly pass membrane

Incorporation of FAs into membrane lipids takes place on organelle membranes

Flippases move phospholipids from one membrane leaflet to the opposite leaflet

asymmetric distribution of phospholipids

senescence or apoptosis – disturb the asymmetric distribution

Phosphatidylserine (PS) and phosphatidylethanolamine: cytosolic leaflet

exposure of these anionic phospholipids on the exoplasmic face – signal for scavenger cells to remove and destroy

Annexin V – a protein that specifically binds to PS phospholipids

fluorescently labeled annexin V – to detect apoptotic cells

flippase: ABC superfamily of small molecule pumps

**TABLE 18.8** Selected Human ABC Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Tissue Expression</th>
<th>Function</th>
<th>Disease Caused by Defective Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>Ubiquitous</td>
<td>Exports cholesterol and phospholipid for uptake into high density lipoprotein (HDL)</td>
<td>Tangier's disease</td>
</tr>
<tr>
<td>ABCB1 (MDR1)</td>
<td>Adrenal, kidney, brain</td>
<td>Exports lipophilic drugs</td>
<td></td>
</tr>
<tr>
<td>ABCD1 (MDR2)</td>
<td>Liver</td>
<td>Exports phospholipids into bile</td>
<td></td>
</tr>
<tr>
<td>ABCD1</td>
<td>Liver</td>
<td>Exports lipids into bile</td>
<td></td>
</tr>
<tr>
<td>CFTR</td>
<td>Exocrine tissue</td>
<td>Transports Cl- ions</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>ABCD1</td>
<td>Ubiquitous in proximal renal tubule</td>
<td>Influences activity of proximal renal tubule that reabsorbs very long chain fatty acids</td>
<td>Membranous nephropathy (MN)</td>
</tr>
<tr>
<td>ABCG2</td>
<td>Liver, intestine</td>
<td>Exports cholesterol and other sterols</td>
<td>Beta-2-microglobulinosis</td>
</tr>
</tbody>
</table>
Phospholipid flipase activity of ABCB4

Yeast sec mutant – at nonpermissive temp: secretory vesicle cannot fuse with plasma membrane – purify the secretory vesicles (dithionite)

Fig 18-5 In vitro fluorescence quenching assay can detect phospholipid flipase activity of ABCB4

Nongated ion channels and the resting membrane potential
Gated: need ligand to activation; Non-gated: do not need ligand

Ion Channel (non-gate)
Generation of electrochemical gradient across plasma membrane
i.e. Ca\(^{2+}\) gradient
regulation of signal transduction, muscle contraction and triggers secretion of digestive enzyme in to exocrine pancreatic cells
i.e. Na\(^{+}\) gradient
uptake of a.a., symport, antiport; formed membrane potential
i.e. K\(^{+}\) gradient
formed membrane potential

Q: how does the electrochemical gradient formed?
Selective movement of Ions Create a transmembrane electric potential difference

**Ion gating Channel**
Depending on the type of the channel, this gating process may be driven by:
1. ligand binding (ligand-gated channels)
2. changes in electrical potential across cell membrane (voltage-gated channels)
3. mechanical forces acting on cellular components (mechanosensitive channels)

Gated ion channels respond to different kinds of stimuli

(A) voltage-gated
(B) ligand-gated (extracellular ligand)
(C) ligand-gated (intracellular ligand)
(D) mechanically gated

CLOSED

OPEN
Selective movement of ions creates a transmembrane electric potential difference

Ion no move → no membrane potential
Ion move → create membrane potential

Negative charge on intracellular organic anions balanced by K⁺
High intracellular [K⁺] generated by Na⁺-K⁺ ATPase
Large K⁺ concentration gradient ([K⁺]ᵢ/[K⁺]ₒ ≈ 30)
Plasma membrane contains spontaneously active K⁺ channels ⇒ K⁺ move freely out of cell
As K⁺ moves out of cell, leaves negative charge build up ⇒ opposes further K⁺ exit
At equilibrium, electrical force balances concentration gradient and electrochemical gradient for K⁺ is zero (even though there is still a very substantial K⁺ concentration gradient)
Resting membrane potential = flow of positive/negative ions across plasma membrane precisely balanced
Membrane potential measured as voltage difference across membrane
For animal cells, resting membrane potential varies between -20 and -200 mV
Negative value due to negativity of intracellular compartment compared to extracellular fluid
Because K⁺ channels predominate in resting plasma membrane, resting membrane potential mainly due to K⁺ concentration gradient
Nernst equation permits calculation of membrane potential (V):

Potential difference exists across every cell's plasma membrane.
- cytoplasm side is negative pole, and extracellular fluid side is positive pole
Inside of cell negatively charged because:
- large, negatively charged molecules are more abundant inside the cell
- sodium potassium ATPase pump
- resting K⁺ ion channels (from in to out flow)

The membrane potential in animal cells depends largely on resting K⁺ channel

Many open K⁺ channel but few open Na⁺, Cl⁻ or Ca²⁺ channels on animal membrane
So major ionic movement across the membrane is K⁺; it form the inside out ward by the K⁺ concentration gradient → creating a positive charge on the outside; outward flow of K⁺ ions through these channels, also called resting K⁺ channels.
Ion channels contain a selectivity filter formed from conserved transmembrane α helices and P segment.

Voltage-gated K+ channels have four subunits each containing six transmembrane α helices.

Structure like but function different

Structure of resting K+ channel from the bacterium Streptomyces lividans

Transmembrane domain

Ion channels are selective pores in the membrane

Mechanism of ion selectivity and transport in resting K+ channel

Each ion contain eight water molecules

EACH OF THE binding sites closely mimics potassium ions' octahedral hydration shell, thereby minimizing the energy required to strip off their water coats. Because of their smaller size, sodium ions don't fit in these binding sites as snugly and thus find the energetic cost of trading their water coat for a spot in the selectivity filter too high.
In the vestibule, the ions are hydrated. In the selectivity filter, the carbonyl oxygens are placed precisely to accommodate a dehydrated K⁺ ion. The dehydration of the K⁺ ion requires energy, which is precisely balanced by the energy regained by the interaction of the ion with the carbonyl oxygens that serve as surrogate water molecules.

Passage of Potassium Ions Through the Channels

- 鈉離子通道（sodium channel）
  0.3×0.5大小，但更重要的是其內表面帶有極強的負電荷。這些負電荷會把鈉離子拉向通道，這是因鈉離子直徑要比其它離子小。

- 鉀離子通道
  0.3×0.3的大小，但它們不帶有負電荷，但是鈉的水合離子比鈉的水合離子要小得多，因此，體積小的水合鈉離子可以很容易地穿過這個較小的通道，而鈉離子則不行。
All pore-forming ion channels are similar in structure

Patch clamps permit measurement of ion movements through single channels
Novel ion channels can be characterized by a combination of oocyte expression and patch-clamping

1. Microinject mRNA encoding channel protein of interest
2. Incubate 24-48 h for synthesis and movement of channel protein to plasma membrane
3. Measure channel-protein activity by patch-clamping technique

Polycystic kidney disease  多囊腎病

這個病的病理是腎內生出很多個小型的囊腫，這些囊腫慢慢的長大，產生壓迫力，使周圍的腎組織功能上產生障礙，而致腎衰竭。病徵為腎小管不斷擴大，令腎臟增大，以及影響腎功能。

PDK1 or PDK2 mutation  

Regulation of ion transport