Exam 2 Review: Nichols & Mercer Lectures

Brittany Brumback

11/4/2017
Membrane Potential and Ion Channels

Background readings:


Hille Ionic channels of excitable membranes 3rd ed.
The Lipid Bilayer is a Selective Barrier

- **inside**
  - hydrophobic molecules (anesthetics)
  - small uncharged polar molecules
  - large uncharged polar molecules
  - charged polar molecules (amino acids)
  - water

- **outside**
  - gases ($O_2, CO_2$)
  - ions
Ion gradients and membrane potential

[Diagram showing ion concentrations and membrane potential]

Na: 117
K: 3
Cl: 120
Anions: 0
Total: 240

Na: 30
K: 90
Cl: 4
Anions: 116
Total: 240

[+ charge] = [- charge]

0 mV

-89 mV

How does this membrane potential come about?
Why does it stop?
- The Nernst Equation

Calculates the membrane potential at which an ion will be in electrochemical equilibrium.
At this potential: total energy inside = total energy outside

Electrical Energy Term: \( zFV \)
Chemical Energy Term: \( RT \ln[\text{Ion}] \)

\( Z \) is the charge, 1 for \( \text{Na}^+ \) and \( \text{K}^+ \), 2 for \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \), -1 for \( \text{Cl}^- \)
\( F \) is Faraday’s Constant = 9.648 \( \times \) 10\(^4\) Coulombs / mole
\( R \) is the gas constant = 8.315 Joules / °Kelvin * mole
\( T \) is the temperature in °Kelvin
Nernst Potential Calculations

• **First K and Cl**
  \[ E_K = 60 \text{ mV} \log \left( \frac{3}{90} \right) = 60 \times -1.477 = -89 \text{ mV} \]
  \[ E_{Cl} = (60 \text{ mV} / -1) \log \left( \frac{120}{4} \right) = -60 \times 1.477 = -89 \text{ mV} \]

  Both Cl and K are at electrochemical equilibrium at -89 mV

• **Now for Sodium**
  \[ E_{Na} = 60 \text{ mV} \log \left( \frac{117}{30} \right) = 60 \times 0.591 = +36 \text{ mV} \]

  When \( Vm = -89 \text{ mV} \), both the concentration gradient and electrical gradient for Na are from outside to inside
At Electrochemical Equilibrium:

The concentration gradient for the ion is exactly balanced by the electrical gradient.

There is no net flux of the ion.

There is no requirement for any energy-driven pump to maintain the concentration gradient.
Deviation from the Nernst Equation

Resting membrane potentials in real cells deviate from the Nernst equation, particularly at low external potassium concentrations.

The Goldman, Hodgkin, Katz equation provides a better description of membrane potential as a function of the concentration of all the ions contributing to it in cells.
The Goldman Hodgkin Katz Equation

\[ V_m = 60 \text{mV} \times \log \left( \frac{P_{K} * [K]_{\text{out}} + P_{Na} * [Na]_{\text{out}} + P_{Cl} * [Cl]_{\text{in}}}{P_{K} * [K]_{\text{in}} + P_{Na} * [Na]_{\text{in}} + P_{Cl} * [Cl]_{\text{out}}} \right) \]

- Resting \( V_m \) depends on the concentration gradients and on the relative permeabilities to Na, K and Cl. The Nernst Potential for an ion does not depend on membrane permeability to that ion.
- The GHK equation describes a steady-state condition, not electrochemical equilibrium.
- There is net flux of individual ions, but no net charge movement.
- The cell must supply energy to maintain its ionic gradients.
Channels Exist in at least 2 States

![Diagram showing current (pA) with ACh and end-plate channel models showing closed and open states](image)
Current $\sim 3.2$ pA  $= 3.2 \times 10^{-12}$ Coulombs/sec
Charge on 1 ion  $\sim 1.6 \times 10^{-19}$ Coulombs
Ions per second  $= \frac{3.2}{1.6} \times 10^7$

$\sim 20$ million
Summary:

I. Cell membranes form an insulating barrier that acts like a parallel plate capacitor (1 µF/cm²)

II. Ion channels allow cells to regulate their volume and to generate membrane potentials

III. Only a small number of ions must cross the membrane to create a significant voltage difference
   ~ bulk neutrality of internal and external solution

IV. Permeable ions move toward electrochemical equilibrium
   • \( E_{\text{ion}} = (60 \text{ mV / z}) \times \log \left( \frac{[\text{Ion}]_{\text{out}}}{[\text{Ion}]_{\text{in}}} \right) @ 30^\circ\text{C} \)
   • Electrochemical equilibrium does not depend on permeability, only on the concentration gradient
Summary (continued):

V. The Goldman, Hodgkin, Katz equation gives the steady-state membrane potential when Na, K and Cl are permeable

\[ V_m = 60 \text{mV} \times \log \left( \frac{P_K \times [K]_{out} + P_{Na} \times [Na]_{out} + P_{Cl} \times [Cl]_{in}}{P_K \times [K]_{in} + P_{Na} \times [Na]_{in} + P_{Cl} \times [Cl]_{out}} \right) \]

- In this case, \( V_m \) does depend on the relative permeability to each ion and there is steady flux of Na and K

The cell must supply energy to maintain its ionic gradients
Summary (continued):
Voltage-Gated Channels and Action Potentials

- Colin Nichols
cnichols@wustl.edu
The Variety of Action Potentials

Skeletal muscle

Cardiac muscle
Excitation-Calcium-Function coupling
Membrane Potential as a Function of Time

What would cause this?
AChR - non-selective cation channel
Membrane Potential as a Function of Time

$E_{Na} = +36 \text{ mV}$

$E_{K} = -89 \text{ mV}$

$P_{Na} = 0$

$P_{Na} = 0.1$

$P_{Na} = 0$
Changing Membrane Potential

Physical Model

Electrical Model
Membrane Potential as a Function of Time

\[ I_{\text{Tot}} = I_R + I_C \quad \text{where} \quad I_R = \frac{V_m}{R} \quad \text{and} \quad I_C = C \times \frac{\partial V_m}{\partial t} \]

\[ \Rightarrow \frac{\partial V_m}{\partial t} = \frac{I_{\text{Tot}}}{C} - \left( \frac{V_m}{(R \times C)} \right) \]

The solution of this differential equation is:

\[ V_m = I_{\text{Tot}} \times R \times (1 - \exp(-t / \tau)), \quad \text{where} \quad \tau = R \times C \]
Passive vs. Active

- All cells exhibit passive changes in membrane potential when stimulated
- Only excitable cells fire action potentials
- Excitability depends on specialized channels
The action potential - Physics in cell biology
Currents During an Action Potential

Time Course of Currents
Sodium Channel Gating States
Voltage sensing – structural basis?
Voltage sensing – more than just channels!

Ciona intestinalis voltage-sensor containing phosphatase, Ci-VSP, which couples a VSD to a phosphatase and tensin homolog (PTEN)-like domain. Murata et al. Nature 435, 1239-1243 (2005).
Voltage dependent inactivation - structural basis?
Cardiac Bioelectricity
Burst frequency modulation - the role of Ca activated $K$ channels

Simultaneous recordings of membrane potential and absorbance changes from an ameba-bilinjected *Aplysia* neuron. (A) During each spontaneous burst of action potentials, $[Ca^{2+}]_i$ rises. In the quiet intervals, it falls. (B) At higher resolution in another cell, the Ca$^{2+}$ buildup is seen to occur during individual action potentials, $7^\circ$ - $16^\circ$ C. [From Soriano and Thomas, 1979.]
Summary:

I. Action potentials require voltage-gated channels
   • They open with depolarization and carry a net inward current
   • Inactivation and / or voltage-gated outward current underlie repolarization
   • Inactivation imposes a refractory period
   • The final effect of an action potential is to elevate calcium

II. Channel structure gives insight into gating and permeation
Central role of $K_{ATP}$ channel in control of insulin secretion

Normal β-cell response to a rise in blood glucose

$K_{ATP}$ channel is inhibited, turning on insulin secretion in pancreatic β-cells...?
Hyperinsulinemia in absence of $K_{ATP}$ channel activity?

$\beta$-cell response to a fall in blood glucose in absence of $K_{ATP}$

$K_{ATP}$ channel is not activated, insulin secretion is maintained, and blood glucose drops further...?
The “ChLoride Channel” Family

<table>
<thead>
<tr>
<th>CLC-1</th>
<th>skeletal muscle</th>
<th>myotonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLC-2</td>
<td>epithelia, neurons</td>
<td>epilepsy</td>
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<tr>
<td>CLC-Ka</td>
<td>kidney/inner ear</td>
<td>deafness</td>
</tr>
<tr>
<td></td>
<td>kidney/inner ear</td>
<td>Bartter’s Syndrome</td>
</tr>
<tr>
<td>CLC-Kb</td>
<td>synaptic vesicles, intracellular</td>
<td>missing hippocampus disorder</td>
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<tr>
<td>CLC-3</td>
<td>endosomes</td>
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<tr>
<td>CLC-4</td>
<td>endosomes</td>
<td>Dent’s Disease</td>
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<tr>
<td>CLC-5</td>
<td>osteoclasts, lysosomes</td>
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<tr>
<td>CLC-6</td>
<td></td>
<td>osteopetrosis</td>
</tr>
<tr>
<td>CLC-7</td>
<td></td>
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</tr>
</tbody>
</table>
MEMBRANE TRANSPORT

Bob Mercer
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Membrane Transport Defects

Cardiac arrhythmias
Renal transport defects
Cystic Fibrosis
Epilepsy
Diabetes
Deafness
Parkinson disease
Autism
Ataxia
Hypertension
Mental Retardation
THE RELATIVE PERMEABILITY OF A SYNTHETIC LIPID BILAYER TO DIFFERENT MOLECULES

HYDROPHOBIC MOLECULES
- O₂
- CO₂
- N₂
- benzene

SMALL UNCHARGED POLAR MOLECULES
- H₂O
- urea
- glycerol

LARGE UNCHARGED POLAR MOLECULES
- glucose
- sucrose

IONS
- H⁺, Na⁺, K⁺, HCO₃⁻, Ca²⁺, Cl⁻, Mg²⁺

synthetic lipid bilayer
Simple Diffusion

- Flux is proportional to external concentration
- Flux never saturates
Membrane Flux (moles of solute/sec)

- Simple Diffusion
- Carrier Mediated Transport
  - Facilitated Diffusion
  - Primary Active Transport
  - Secondary Active Transport
- Ion Channels
TRANSPORT OF MOLECULES THROUGH MEMBRANES

PASSIVE TRANSPORT (FACILITATED DIFFUSION)

ACTIVE TRANSPORT

transported molecule

lipid bilayer
simple diffusion
channel-mediated diffusion
carrier-mediated diffusion

ENERGY

electrochemical gradient
CARRIER MEDIATED TRANSPORT

- **COUPLED TRANSPORT**
  - **UNIPORT**
  - **SYMPORT OR COTRANSPORT**
  - **ANTIPORT OR COUNTERTRANSPORT**

- **lipid bilayer**
Membrane Potential Review

- The lipid bilayer is impermeable to ions and acts like an electrical capacitor.
- Cells express ion channels, as well as pumps and exchangers, to equalize internal and external osmolarity.
- Cells are permeable to K and Cl but nearly impermeable to Na.
- Ions that are permeable will flow toward electrochemical equilibrium as given by the Nernst Equation.
  \[ E_{\text{ion}} = \left( \frac{60 \text{ mV}}{z} \right) \log \left( \frac{[\text{ion}]_{\text{out}}}{[\text{ion}]_{\text{in}}} \right) \quad @ \quad 30^\circ \text{C} \]
- The Goldman-Hodgkin-Katz equation is used to calculate the steady-state resting potential in cells with significant relative permeability to sodium.

\[
V_m = 60 \text{mV} \times \log \left( \frac{P_K [K]_{\text{out}} + P_{Na} [Na]_{\text{out}} + P_{Cl} [Cl]_{\text{in}}}{P_K [K]_{\text{in}} + P_{Na} [Na]_{\text{in}} + P_{Cl} [Cl]_{\text{out}}} \right)
\]
Carrier-Mediated Transport

- Higher flux than predicted by solute permeability
- Flux saturates
- Binding is selective (D- versus L-forms)
- Competition
- Kinetics: ⇒

\[
[S]_0 < < K_m \quad M \propto [S]
\]

\[
[S]_0 = K_m \Rightarrow M = M_{\text{max}} / 2
\]

\[
[S]_0 \gg K_m \quad M = M_{\text{max}}
\]
Facilitated Diffusion

• Uses bidirectional, symmetric carrier proteins

• Flux is always in the directions you expect for simple diffusion

• Binding is equivalent on each side of the membrane

Examples include: Glucose Transporters (GLUT); Anion Exchanger; Organic Anion Transporters; Urea Transporters; Monocarboxylate (lactate) Transporters (MCTs); Amino Acid Transporters; Zn Transporters (ZIP)
Primary Active Transport: **Driven by ATP**

- **Class P** – all have a phosphorylated intermediate
  - Na,K-ATPase  
  - H,K-ATPase  
  - Ca-ATPase  
  - Cu-ATPase  
  - H-ATPase  
  - bacterial K-ATPase  
  - Phospholipid Flippase

- **Class V**
  - $H^+$ transport for intracellular organelles

- **Class F**
  - Synthesize ATP in mitochondria

- **ABC ATPases**
  - ATP Binding Cassette  
  - 48 known members- Multiple Drug Resistance; Sulfonylurea receptor; CFTR
Primary Active Transport: Na,K-ATPase

- 3 Na outward / 2 K inward / 1 ATP
- $K_m$ values: $[Na]_{in} \approx 20$ mM, $[K]_{out} \approx 2$ mM
- Inhibited by digitalis and ouabain
- Palytoxin “opens” ion channel
- 2 subunits, beta and alpha (the pump)
- Two major conformations E1 & E2
- Turnover = 300 Na$^+$/sec/pump site @ 37°C
Membrane Transport and Cellular Functions that Depend on the Na,K-ATPase
Secondary Active Transport

• Energy stored in the Na\(^+\) (H\(^+\) or K\(^+\)) gradient is used to power the transport of a variety of solutes

  glucose, amino acids, ions and other molecules are pumped in (cotransport)

Ca\(^{2+}\) or H\(^+\) are pumped out 2 or 3 Na\(^+\) / 1 Ca\(^{2+}\); 1 Na\(^+\) / 1 H\(^+\)
(countertransport)

• These transport proteins do not hydrolyze ATP directly; but they work at the expense of the ion gradient which must be maintained by an ATPase
Cotransport Proteins

Extracellular space

- Na+/glucose cotransporter
  - SGLT1
  - 2 Na⁺, 1 Glucose

- Na+/phosphate cotransporter
  - NaPi IIa/b
  - 3 Na⁺, 1 Pᵢ

- Na+/iodide symporter
  - NIS
  - 2 Na⁺, 1 I⁻

- Na⁺/K⁺/Cl⁻ cotransporter
  - NKCC
  - 1 Na⁺, 1 K⁺, 2 Cl⁻

- Na⁺/Cl⁻ cotransporter
  - NCC
  - 1 Na⁺, 1 Cl⁻

- K⁺/Cl⁻ cotransporter
  - KCC
  - 1 K⁺, 1 Cl⁻

Cytoplasmic space

- Phlorizin
- Bumetanide
- Furosemide
- Thiazide

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Countertransport / Exchanger Proteins

**Extracellular space**

- Na⁺/Ca²⁺ exchanger: NCX
  - 3 Na⁺
  - 1 Ca²⁺

- Na⁺/H⁺ exchanger: NHE
  - 1 Na⁺
  - 1 H⁺

- Cl⁻/HCO₃⁻ exchanger: AE
  - 1 Cl⁻
  - 1 HCO₃⁻

**Cytoplasmic space**

Amiloride

SITS  DIDS
Summary: Membrane Flux (moles of solute/sec)

Simple Diffusion
- Flux is directly proportional to external concentration
- Flux never saturates

Carrier-Mediated Transport
- Higher flux than predicted by solute permeability
- Flux saturates
- Binding is selective D- versus L-forms
- Competition
- Kinetics

Facilitated Diffusion
- Uses bidirectional, symmetric carrier proteins
- Flux is in the direction expected for simple diffusion
- Binding is equivalent on each side of the membrane

Primary Active Transport – driven by ATP hydrolysis
Secondary Active Transport – driven by ion gradients

Ion Channels
EPITHELIAL CELLS

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EPITHELIAL CELL

- Zonula occludens
- Zonula adherens
- Macula adherens
- Gap junction
LIMITING JUNCTION / TIGHT JUNCTION
TIGHT JUNCTION

TJ PLASMA MEMBRANE PROTEINS

Tetra span proteins:

TJ-associated marvel protein (TAMP):

**Occludin** (marvelD1; 65 kDa; 522 aa; 4 splice isoforms identified)

```
marvel:myelinandlymphocyteproteinandrelatedproteins
for vesicle trafficking and membrane link
```

**Claudins** (23-30 kDa; 211-260 aa, 27 identified)

**Tricellulin** (marvelD2; 64 kD; ≈550 aa, 4 splice isoforms identified)

**MarvelD3** (2 splice isoforms, 410, 401 aa)
TJ PLASMA MEMBRANE PROTEINS

Single span proteins:

**Junctional Adhesion Molecule (JAM)** 3 proteins identified (JAM1-3) 43kDa; Ig Superfamily cytoPDZ (PSD-95, discslarge, ZO-1) domain binds ZO-1, PAR-3/PAR-6/

aPKC, cingulin

role in endothelial leukocyte exit

**Coxsackievirus & Adenovirus Receptor (CAR)** Ig-like domain, cyto PDZ domain binds ZO-1
TIGHT JUNCTIONAL PROTEINS
OCCLUDIN AND TRICELLULIN EXPRESSION
CLAUDINS

- Claudin-1
- Claudin-2
- Claudin-4
- Claudin-5
- Claudin-7
- Claudin-8
- Claudin-10
- Claudin-11
- Claudin-14
- Claudin-15
- Claudin-16
- Claudin-18
- Claudin-19

Nonpolar Hydrophobic: R E L I P F K
Uncharged Hydrophilic: S T N V E
Basic Hydrophilic: K R H
Acidic Hydrophilic: D E

T(M): Transmembrane domain; TER: Transendothelial Resistance
PM: Plasma Membrane; Cyto: Cytoplasm; Extra: Extracellular Domain

- T(M): TFR
  D: Sclerosing cholangitis; KO: Epidermal water loss

- T(M): TER
  D: Deafness; KO: Conductance in CNS; Male infertility

- T(L), T(MLC): TER
  D: Deafness; KO: Conductance in CNS; Male infertility

- T(MLC): TER
  D: Blood-brain-barrier impairment

- T(MLC): TER
  D: Hypomagnesemia; Hypercalcinuria

- T(MLC): TER
  D: Hypomagnesemia; Hypercalcinuria; KO: Conductance in PNS

- T(MLC): TER
  D: Deafness; KO: Conductance in CNS; Male infertility
Absence of tricellulin may lead to paracellular permeability barrier defects. The intramembranous particles formed by bicellular tight junction proteins are shown between the plasma membrane lipid bilayers. These proteins form lateral associations with junctional proteins in the adjacent cell obliterating the intercellular space. (A) At tricellular junctions in Tric+/+ mice, tricellulin and other proteins from the 3 cells associate with each other to form the central sealing elements, where the bicellular junction strands unite with it. (B) In the absence of tricellulin, the tricellular junctions are no longer continuous and the disconnected particles are possibly formed by other as yet unknown proteins. These strands are no longer able to associate with the elements of the bicellular junctions, potentially resulting in “channels” or conduits for paracellular permeability and barrier defect in the TricR497X/R497X mice. The arrows depict paracellular leak of ions or small signaling molecules through the “channels” at the tricellular junctions of TricR497X/R497X mice. From: Nayak G, et al., J Clin Invest. 2013 Sep 3;123(9):4036-49
TJ CYTOPLASMIC PROTEINS

Membrane-Associated Guanylate Kinase proteins:
- **ZO-1** (210-250 kDa), **ZO-2** (160 kDa), **ZO-3** (130 kDa)
  - 3 PDZ; Src homology, SH-3;
  - guanylate kinase-like, GUK domains

- **Cingulin**- actin, myosin binding

- **ZONAB**- ZO-1 Associated Nucleic Acid Binding;
  - Y-box transcription factor
PROTEIN INTERACTIONS AT THE ZO

- 7H6
- Symplekin
- ASIP
- Sec6/8
- Myosin
- rab13
- rab3B
- PKC
- G
- AF-6
- BAP-1
- ZO-1
- ZO-2 or ZO-3
- ZAK
- ZONAB
- Claudins
- Occludin
- Actin
- Paracellular Space
BELT DESMOSOMES

- Actin filaments inside microvillus
- Microvilli extending from apical surface
- Tight junction
- Bundle of actin filaments
- Lateral plasma membranes of adjacent epithelial cells
- Basal surface
PLASMA MEMBRANE ADHEREN JUNCTION PROTEINS

E-Cadherin - Ca-dependent binding

Nectin - Ig Subfamily; Ca-Independent binding

Vezatin - Myosin binding
E-CADHERIN MEDIATES $\text{Ca}^{2+}$-DEPENDENT ADHESION OF L CELLS

Figure 20.11
*Molecular Cell Biology*, Seventh Edition
© 2013 W.H. Freeman and Company
SPOT AND HEMI-DESMOSOMES

- Desmogleins
- Cytoplasmic plaque made of desmoplakins
- Keratin filaments anchored to cytoplasmic plaque
- Interacting plasma membranes
- Intercellular space
- Basal lamina
- Hemidesmosome

0.3 µm
DESMOSOMAL PLAQUE PROTEINS

Catenin related proteins: Plakoglobin and Plakophilin

Plakin Family: Desmoplakin - most abundant

Dumbbell shaped-links plaque to intermediate filaments phosphorylated by PKA
**HUMAN DISEASES INVOLVING DESMOSOMAL MUTATIONS**

Epidermolysis bullosa (EB): blistering disorders; 1 in 50,000 live births

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dsg1</td>
<td>Amino-terminal deletion resulting in Dsg1 haploinsufficiency</td>
<td>Striate palmoplantar keratoderma. No heart defects</td>
</tr>
<tr>
<td>DP</td>
<td>Amino-terminal deletion. Autosomal dominant mutation resulting in DP haploinsufficiency</td>
<td>Striate palmoplantar keratoderma. No heart defects</td>
</tr>
<tr>
<td>DP</td>
<td>Frame shift mutation in carboxyl terminus. Autosomal recessive</td>
<td>Striate palmoplantar keratoderma, dilated left ventricular cardiomyopathy, woolly hair</td>
</tr>
<tr>
<td>DP</td>
<td>Compound heterozygosity for non-sense and missense mutations</td>
<td>Palmoplantar keratoderma more severe than with other mutations, some hair loss, nail defects. No heart defects</td>
</tr>
<tr>
<td>PG</td>
<td>Carboxy-terminal truncation, owing to frame shift. Autosomal recessive</td>
<td>Naxos disease: arrhythmic right ventricular cardiomyopathy (ARVC), striate palmoplantar keratoderma and woolly hair</td>
</tr>
<tr>
<td>PP1</td>
<td>Null mutation. Autosomal recessive.</td>
<td>Skin fragility and ectodermal dysplasia, alopecia and nail defects.</td>
</tr>
</tbody>
</table>
GAP JUNCTIONS
“GATING” OF CONNEXONS

Closed

Open

Cytoplasm (cell A)

Cytoplasm (cell B)

Plasma membrane

extracellular

Plasma membrane

Connexin
**TYPES OF EPITHELIA**

**Na⁺-Transporting Epithelia**
Examples of Na⁺-transporting epithelia include the distal segments (distal tubule and cortical collecting tubule) of the renal tubule, colon, amphibian skin, and amphibian and mammalian urinary bladder.

**Cl⁻ Transporting Epithelia**
Examples include: Regions involved in Cl⁻ absorption such as the thick segments of the loop of Henle in the mammalian kidney and the diluting segment of amphibian renal tubule and tissues involve in Cl⁻ secretion such as the trachea, corneal epithelium and the rectal gland of some fishes.

**H⁺-Transporting Epithelia**
Predominant function of this epithelia is to secrete H⁺. Transport of other ions is observed, and depending on the mechanism of H⁺ transport can be directly coupled (H,K-ATPase) or independent (H-ATPase) from H⁺ secretion.
Examples include: gastric epithelium, medullary renal collecting tubule, and reptilian urinary bladder.

**K⁺-Transporting Epithelia**
Transport of K⁺ is the predominant function. Large gradients are often established and maintained indicating a low ionic permeability. The side from which K⁺ is transported is negative. Examples include: stria vascularis epithelium of the inner ear that transports K⁺ into the endolymph and the insect midgut that secretes K⁺ into the midgut lumen.
COMMON MEMBRANE PROPERTIES OF EPITHELIA

1. Generally the Na,K-ATPase (Na,K pump) is located exclusively on the basolateral membrane.

2. $K^+$ is accumulated intracellularly by the Na,K-ATPase and the basolateral membrane is predominately $K^+$ permeable; therefore the membrane potential is typically close to the $K^+$ diffusion potential.

3. $Na^+$ activity is much lower in the cell than in the extracellular fluid. In addition to the approximate 10 fold concentration ratio, the cell negative membrane potential provides an additional driving force for $Na^+$ entry. Therefore, $Na^+$, using its electrochemical gradient can drive the accumulation of an uncharged solute, producing up to a 100 fold concentration ratio.
Aquaporins

In mammals at least 13 isoforms present
ADH Stimulated Water Permeability
CELLULAR MECHANISMS FOR INFLUENCING TRANSPORT ACTIVITY

- Expression of Specific Isoforms
- Assembly of Different Isoform Subunits
- Exocytosis / Endocytosis
- Specific Regulation by Protein Kinases
- Modification Through Inhibitors
- Assembly with Accessory / Regulatory Proteins
- Changes in Rate of Synthesis
- Changes in Rate of Degradation
Cell polarity: Asymmetry is a defining feature of eukaryotic cells.

Other examples of constitutively polarized cells: hepatocytes, neurons, osteoclasts, photoreceptor rods and cones.
BASOLATERAL AND APICAL MEMBRANES HAVE DIFFERENT PROTEIN AND LIPID COMPOSITIONS
Direct and Indirect Sorting Pathways

(A) DIRECT SORTING OF MEMBRANE PROTEINS IN THE TRANS GOLGI NETWORK

(B) INDIRECT SORTING VIA ENDOSONMES

## APICAL SORTING SIGNALS

<table>
<thead>
<tr>
<th>Signals</th>
<th>Examples</th>
<th>Elements of the sorting machinery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid rafts</td>
<td>HA, PLAP, GPI-anchored proteins</td>
<td>VIP17/MAL, galectin-4, FAPP2, annexin-13b, annexin-2, kinesin, KIFC3</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Clusterin (gp80), H,K-ATPase β-subunit, P75NTR, LPH, SI, glycoprotein g114</td>
<td>Lipid rafts, galectin-3, kinesin, KIF5B</td>
</tr>
<tr>
<td>Cytoplasmic sequences</td>
<td>Rhodopsin, megalin, receptor guanylate cyclase, M₂</td>
<td>Dynein light-chain Tctax</td>
</tr>
<tr>
<td></td>
<td>muscarinic receptors, ATP/B, copper-ATPase, NKCC2, PMCA2</td>
<td></td>
</tr>
<tr>
<td>Transmembrane sequence</td>
<td>H,K-ATPase α-subunit</td>
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</tr>
<tr>
<td>PDZ motifs</td>
<td>CFTR, Na⁺/H⁺ exchanger, NaPiII</td>
<td>NHERF, NaPi-Cap2</td>
</tr>
</tbody>
</table>

Svelto et al., *Biol. Cell* 102:75, 2010
Tyrosine-Dependent Basolateral Sorting Signals

- LDLR: 9NSINFDNPWYQKTTEDEVICHN
- LAP: 405RMQAQPGRHVDGEDHA
- HA Y543: 538NGSLQYRICI
- ASGPR H1: 1MTKEYQDLQML
- Igp120: 1RKRSHAGYQTI
- TGN38: 5VTRRPKASDYQRLNLKL

Di-Hydrophobic Basolateral Sorting Signals

- FcRII-B2: 22NTITYSLKH
- MHC II/II: 1MSSQRDLISNNEQLMGLRRPGAPESKCSR
SORTING SIGNALS

Cytoplasmic domain
- Tyrosine-based (e.g., LDL-R)
- Non-tyrosine based (e.g., IgA-R)
- Di-hydrophobic (e.g., Fc-R)

Lumenal domain
- Glycosylphosphatidylinositol (GPI) group
- Glycosylation (N-, O-glycan)

Sorting mechanisms
- Clustering by cytoplasmic coat proteins (e.g., AP1/μ1B, p200/myosin II), RhoA GTPases
- Partitioning into microdomains (e.g., lipid rafts, sorting lectins)