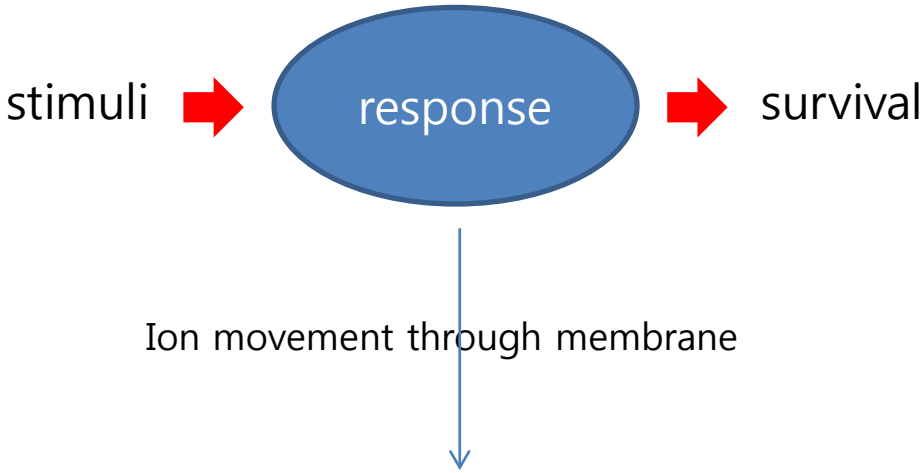
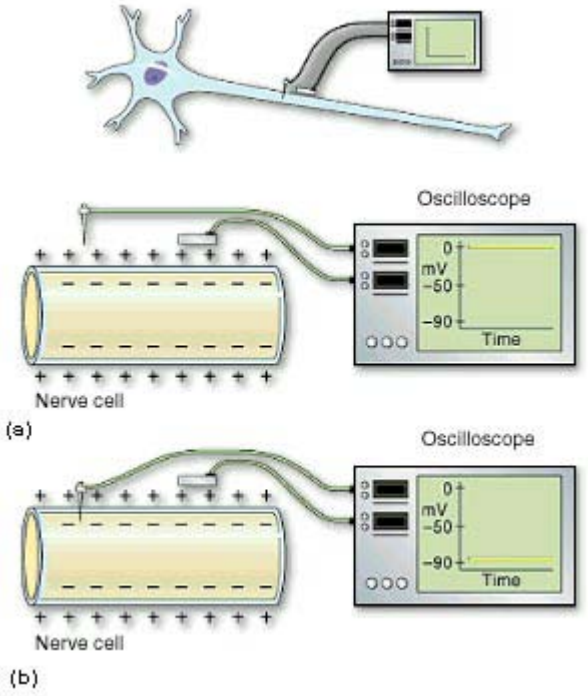
Chap 3. Electrical excitability of neurons
(Ion channel structure & function)

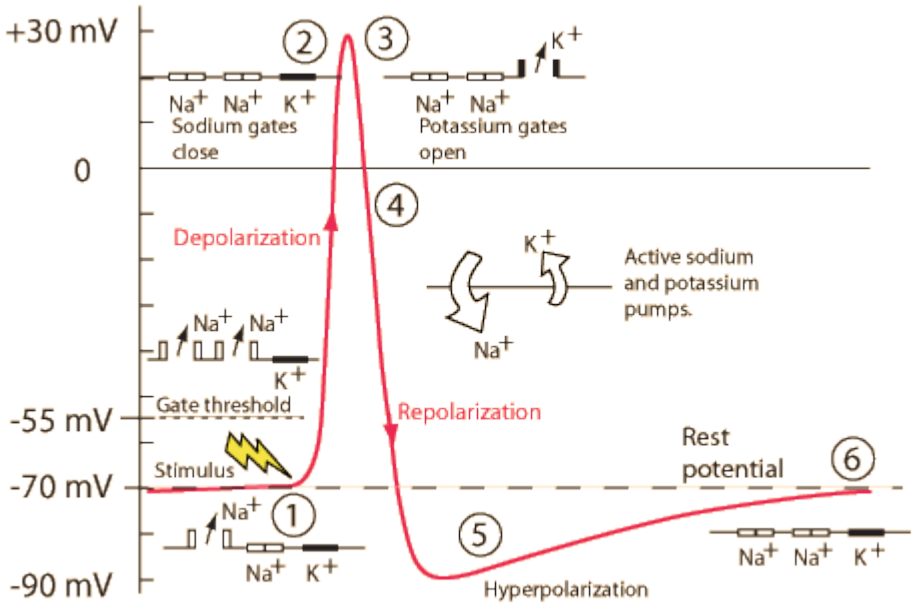


From resting potential to action potential

http://www.columbia.edu/cu/psychology/courses/1010/mangels/neuro/neurosignaling/neurosignaling.html

Action potential





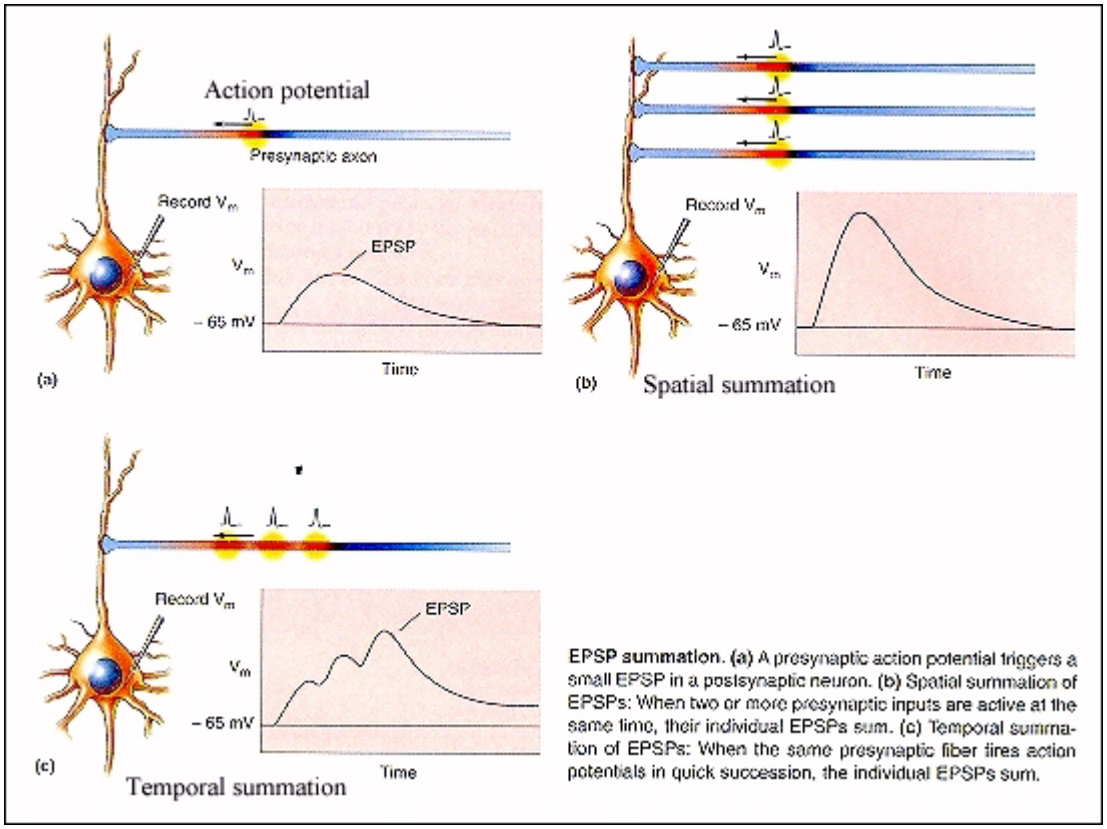
Animation:
http://bcs.whfreeman.com/thelifewire/content/chp44/4402s.swf

Synaptic summation

Graded potential
(local potential)

Summation
(in exon hillock)
Temporal summation
Spatial summation (excitatory & inhibitory)

Action potential

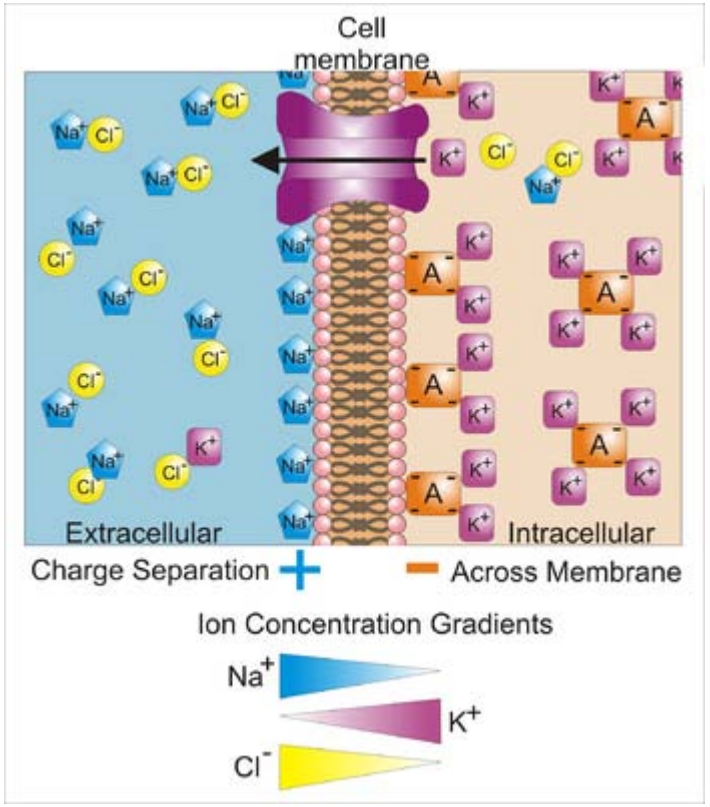


EPSP summation. (a) A presynaptic action potential triggers a small EPSP in a postsynaptic neuron. (b) Spatial summation of EPSPs: When two or more presynaptic inputs are active at the same time, their individual EPSPs sum. (c) Temporal summation of EPSPs: When the same presynaptic fiber fires action potentials in quick succession, the individual EPSPs sum.

Electrical potential across membrane (membrane potential): ion gradient

Contributed by two characteristics of neurons

- Unequal permeability of membranes to ions
  - Potassium > chloride > sodium
  - Potassium-selective leak channels
- Unequal distribution of ions
  - Higher extracellular sodium, chloride, and calcium ions
  - Higher intracellular potassium and proteins (-)

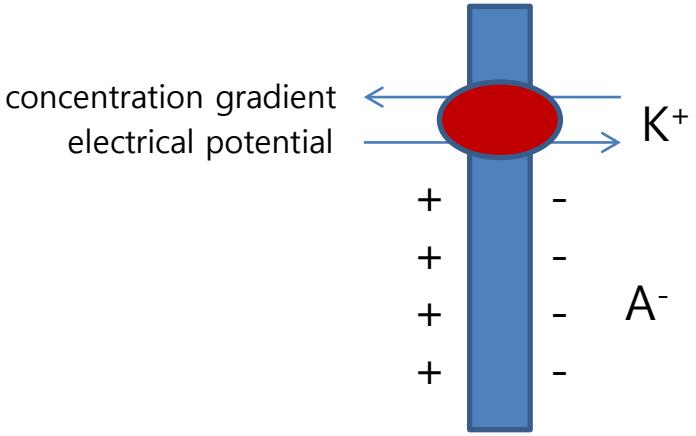


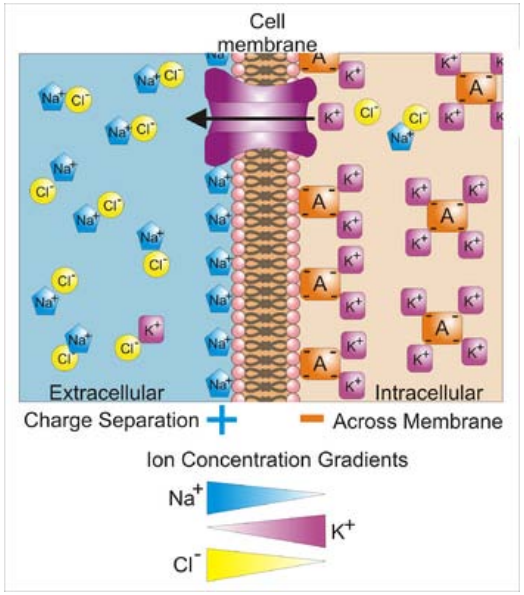
Prerequisites

- lipid bilayer is impermeable
- Ion movement through protein
  - Passive: simple, mediated
  - Active: primary, secondary
- Modulation of the proteins

Ion channels function
(needs structural insight)

If K^+ is permeable

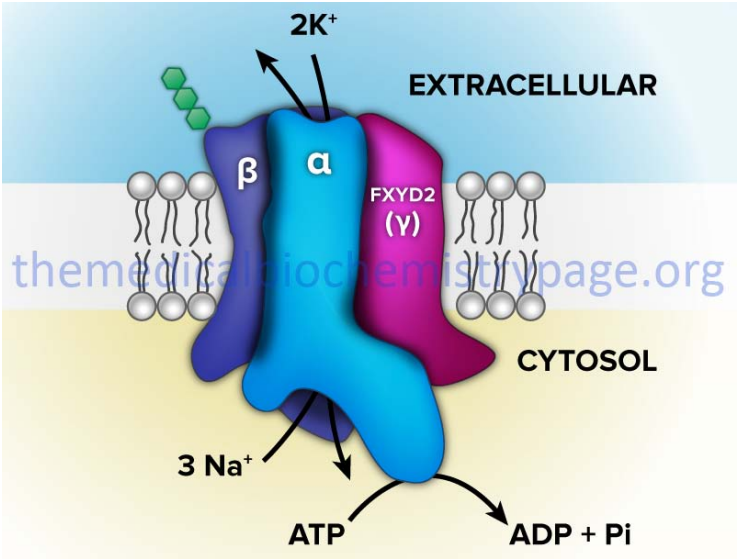




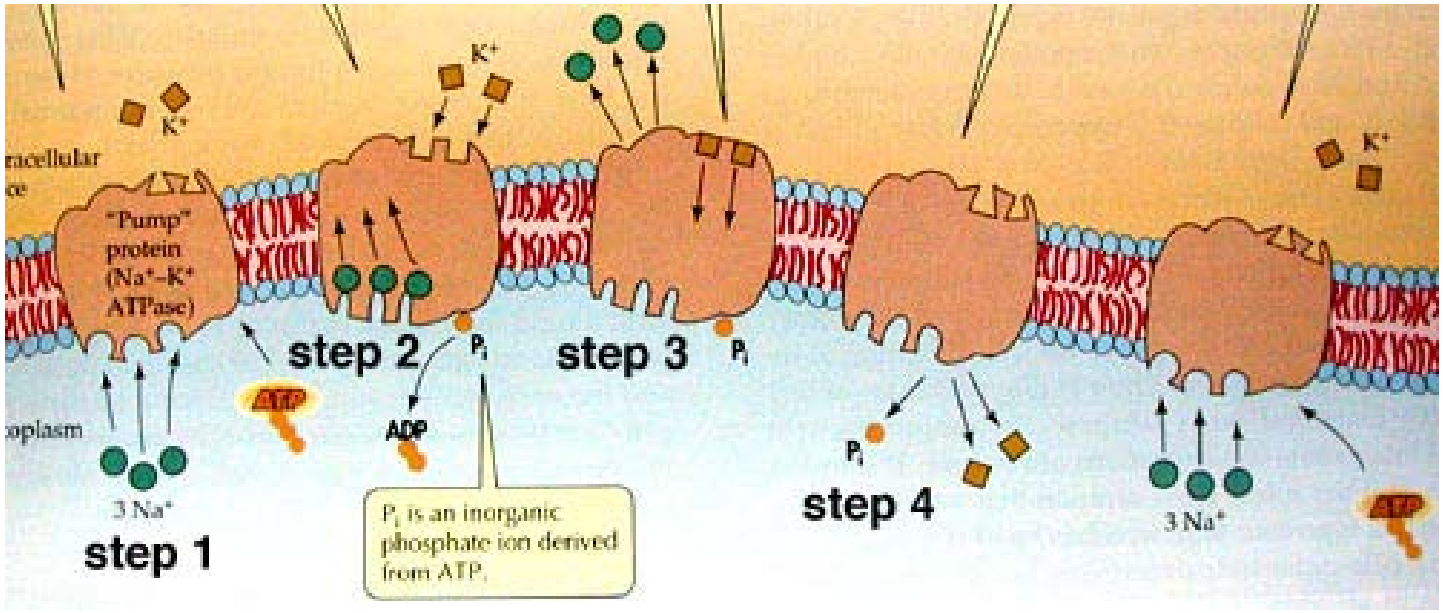
concentration gradient counterbalanced by electrical potential
Nernst equation: $E = (RT/zF)\ln([I_o]/[I_i]) = 58\log(([I_o]/[I_i])$

Thermodynamics of transport across membrane: $\Delta G = zF\Delta\Psi + RT\ln([I_i]/[I_o])$

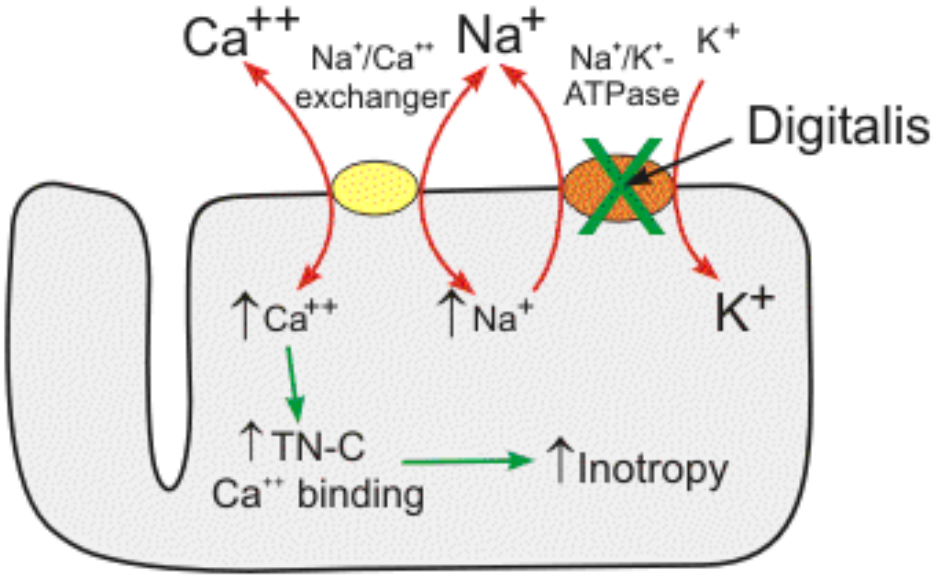
Maintenance of resting potential by Na $^{+}$-K^{+} ATPase (pump)



https://www.youtube.com/watch?v=yz7EHJFDEJs



Na^{+}-Ca^{2+} pump



Cardiac glycosides (ouabain and digoxin) cause
increased contractile force of cardiac muscle due to high intracellular Ca^{2+}

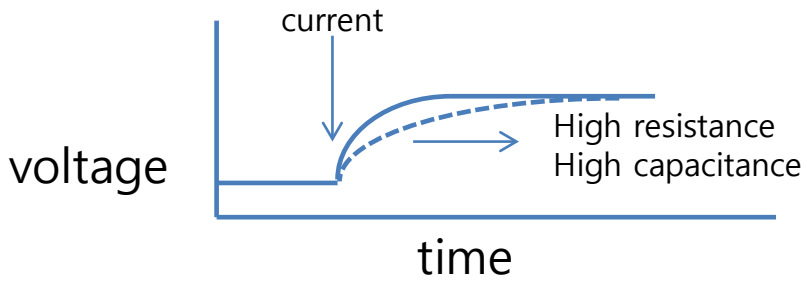
Two biophysical properties of the cell membrane

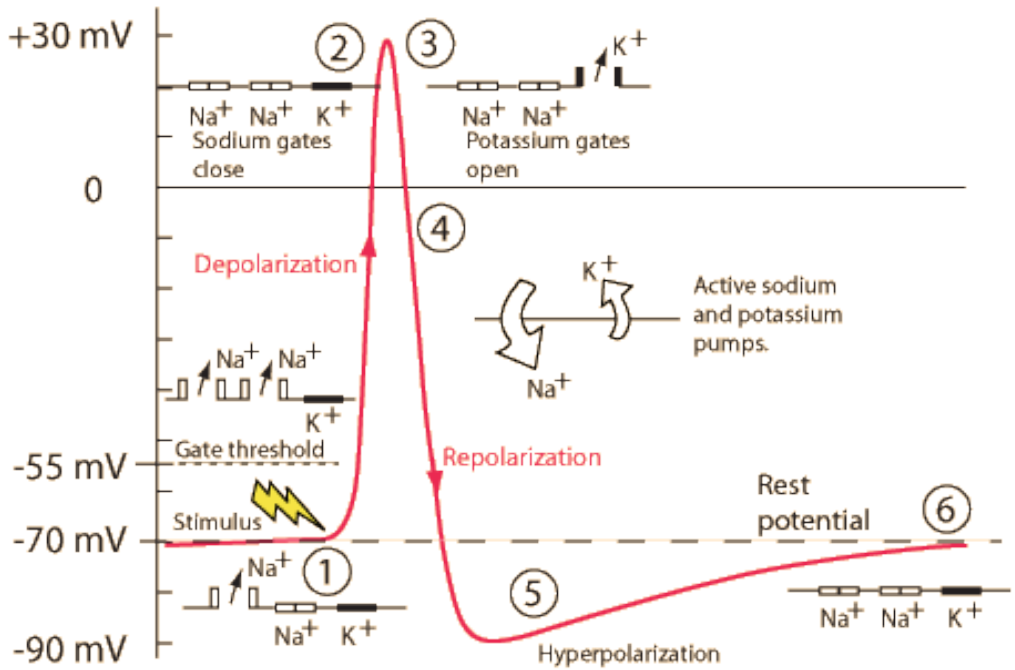
V (potential) = Q (transferred charge)/C (capacitance)

Membrane can be thought of as a resistor: ions can pass but with some difficulty
determined by the number of ion channels
the more open channels the lower resistance

Membrane acts as a capacitor: charge store
a membrane's ability to store charge is the capacitance
it decreases with thickness and increases with size

Myelin increases the resistance and decreases the capacitance of the axonal membrane,
allowing past movement of current to the next node (saltatory conductance)





Voltage-dependent Na+ channels

Transient opening
Continued opening

Volate-dependent K+ channels:

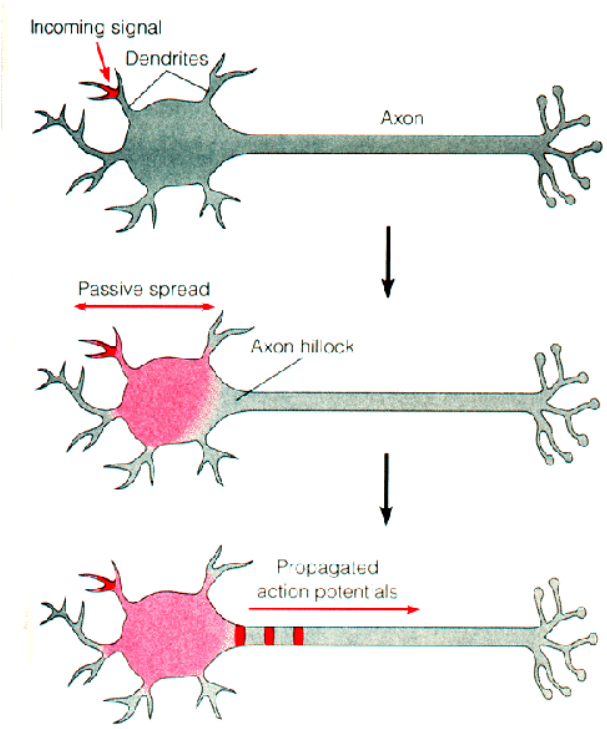
The amount and properties determine the duration of action potential & the rate of firing

Dendritic action potential

Presence of voltage-dependent Na+, K+, Ca2+ channels
Amplify incoming signal

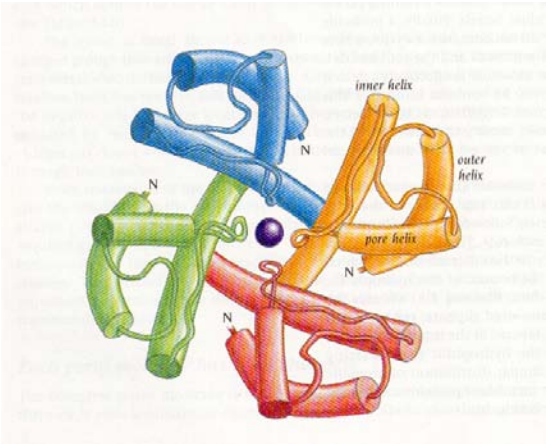
Dendrites were once believed to merely convey stimulation passively. Although passive cable theory offers insights regarding input propagation along dendrite segments, it is important to remember that dendrite membranes are host to an abundance of proteins some of which may help amplify or attenuate synaptic input. Sodium, calcium, and potassium channels are all implicated in contributing to input modulation. It is possible that each of these ion species has a family of channel types each with its own biophysical characteristics relevant to synaptic input modulation. Such characteristics include the latency of channel opening, the electrical conductance of the ion pore, the activation voltage, and the activation duration. In this way, a weak input from a distal synapse can be amplified by sodium and calcium currents en route to the soma so that the effects of distal synapse are no less robust than those of a proximal synapse.

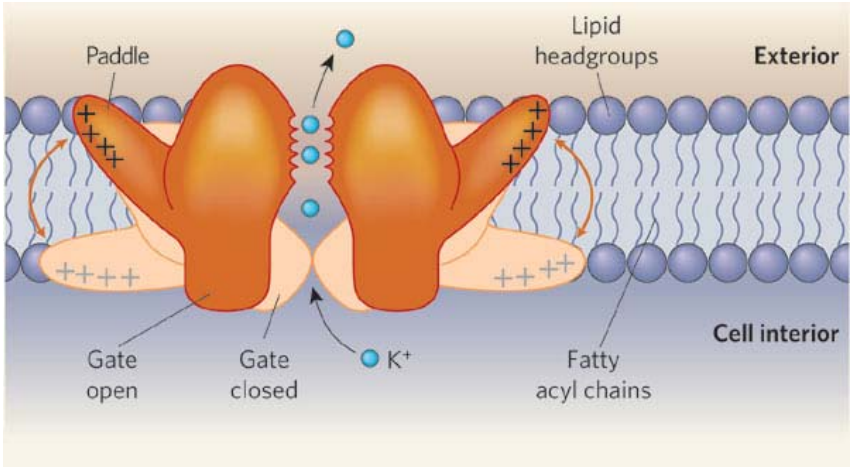
One important feature of dendrites, endowed by their active voltage gated conductances, is their ability to send action potentials back into the dendritic arbor. Known as backpropagating action potentials, these signals depolarize the dendritic arbor and provide a crucial component toward synapse modulation and long-term potentiation. Furthermore, a train of backpropagating action potentials artificially generated at the soma can induce a calcium action potential (a dendritic spike) at the dendritic initiation zone in certain types of neurons. Whether or not this mechanism is of physiological importance remains an open question.

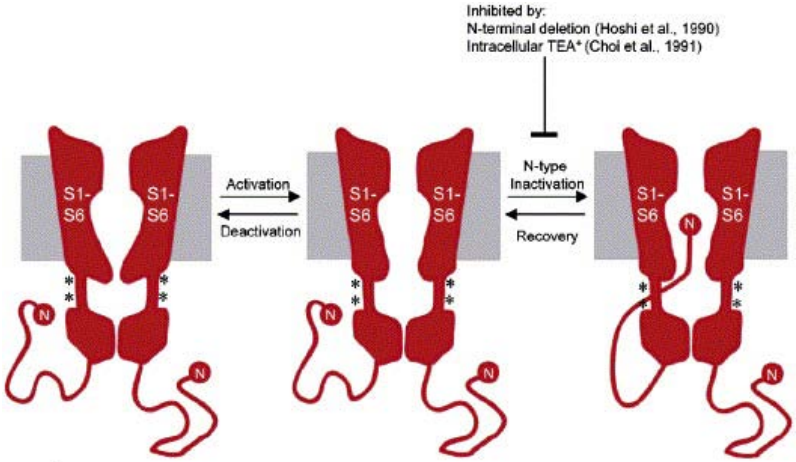


Molecular properties of ion channels

Na+, K+, Ca2+ channels share common structural properties







Voltage-gated potassium channels contain positively charged 'paddles', loosely associated with the surface of the channel, that move within the lipid bilayer to open the channel. After ruling out some explanations for what bilayer composition is required for correct paddle operation, MacKinnon and colleagues[1] conclude that negatively charged phosphate groups are an essential component. Nature (2006) 444:697

https://www.youtube.com/watch?v=hSvHSNs1f1o

Ion channels

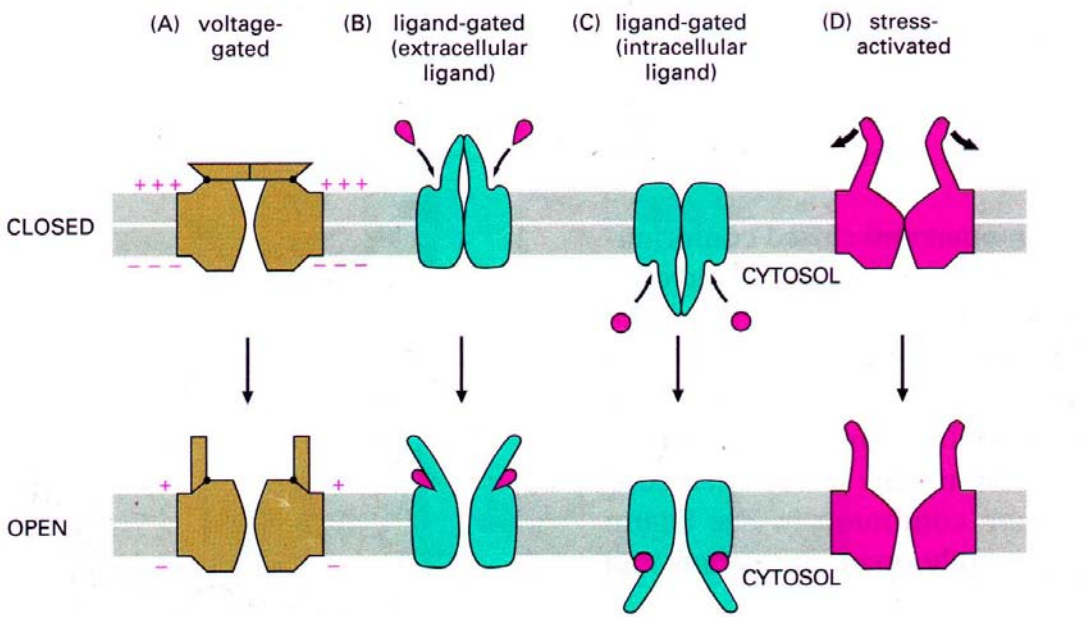
Outward rectifier

Inward rectifier

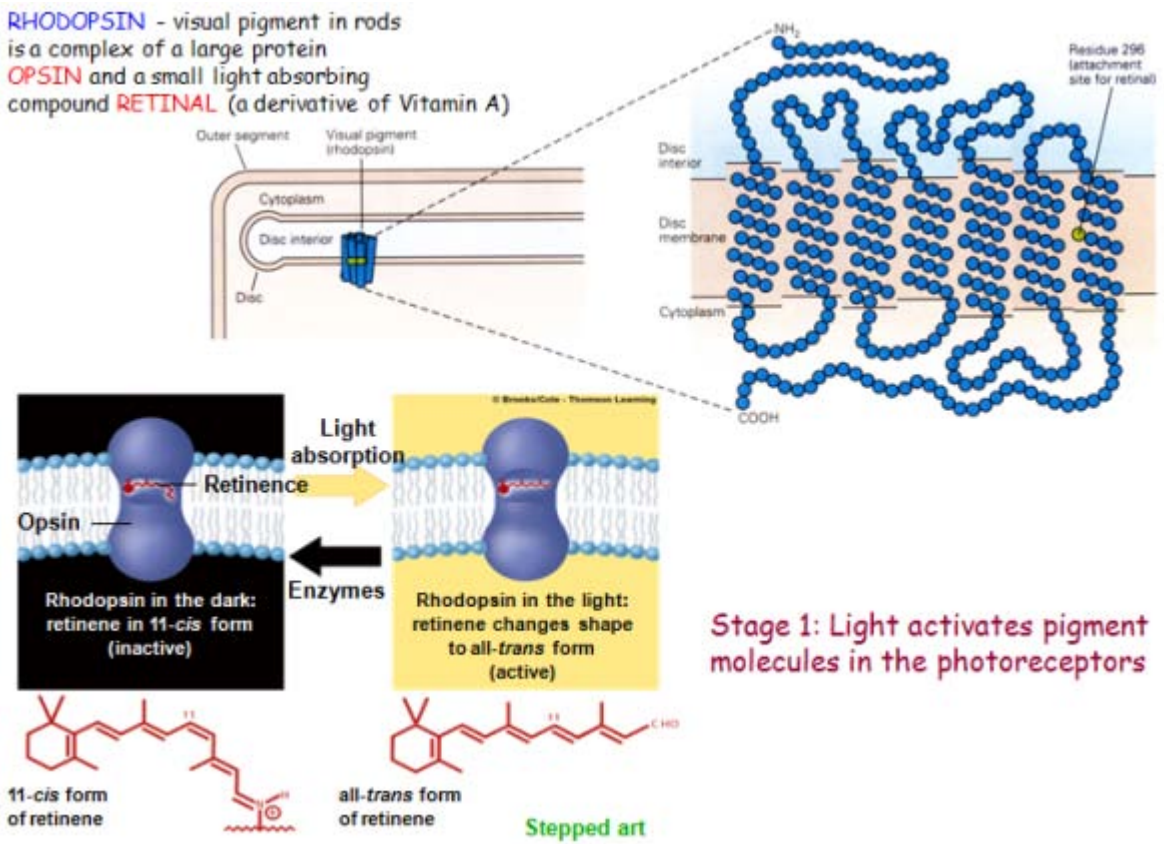
A rectifier is an electrical device that converts alternating current (AC)

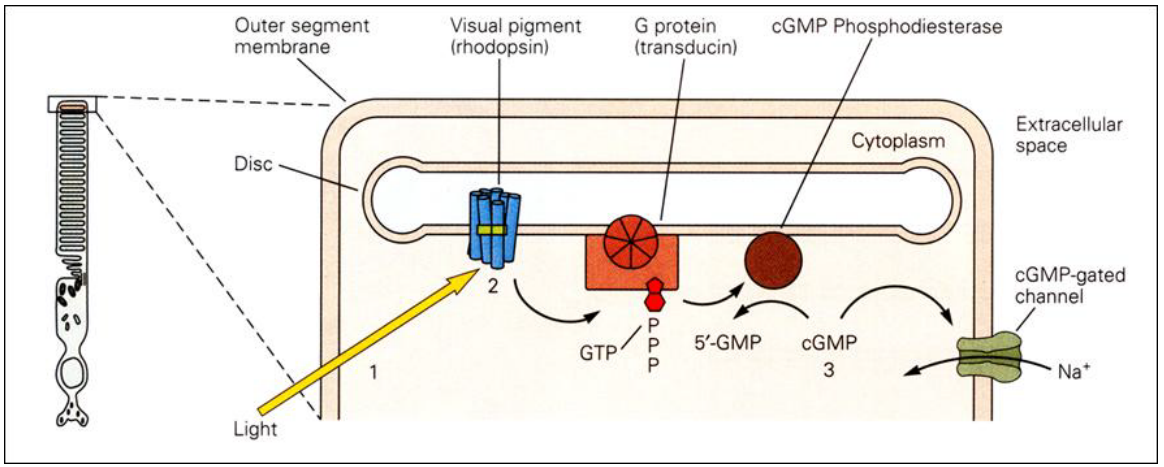
Non-gated: leak channels (always open)

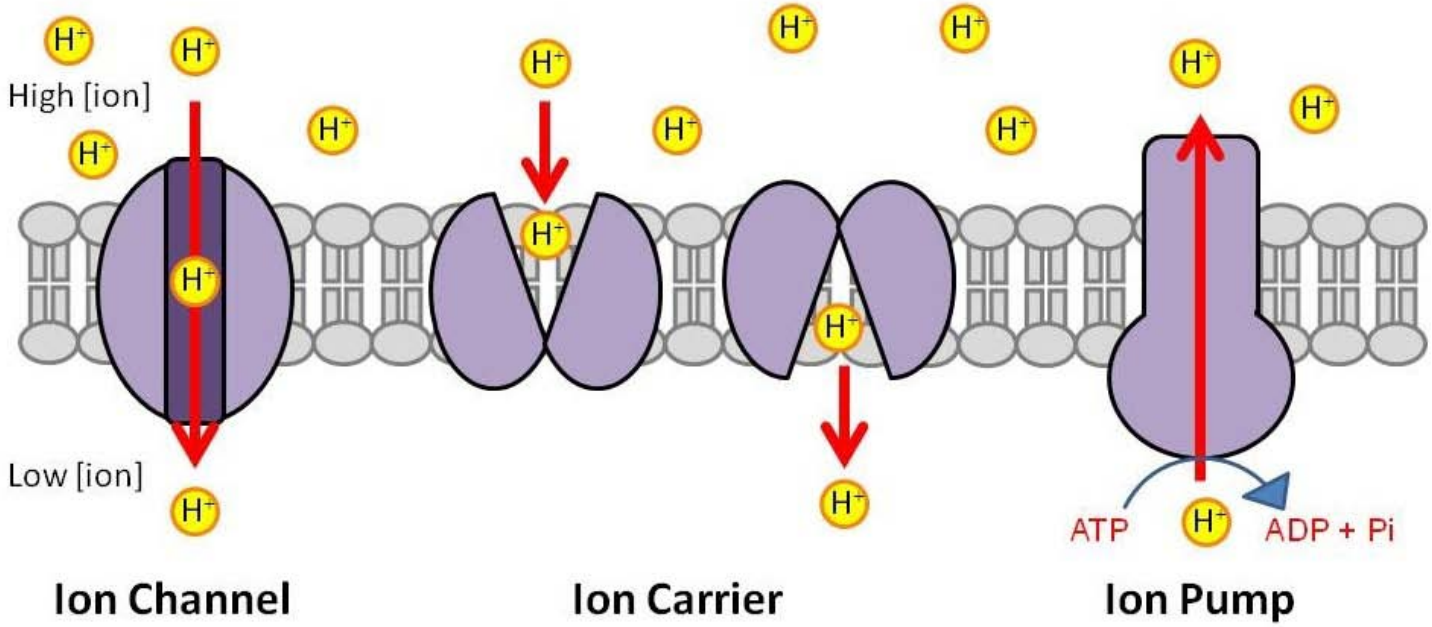
Gated: voltage-gated, ligand-gated (extra- or intra-cellular), mechano-sensitive



cGMP-gated channel in photosignalling





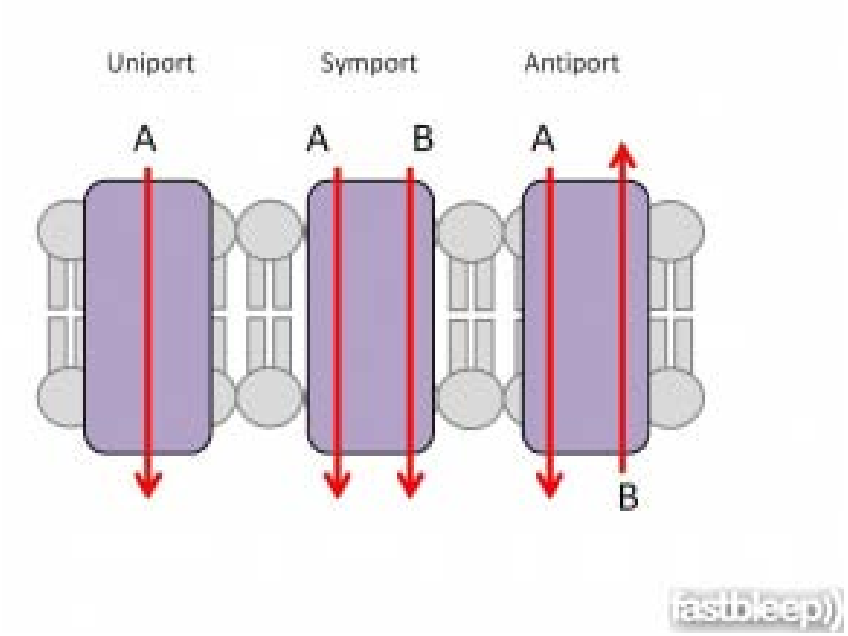


Carrier proteins

These can transport many different types of small molecules, not just ions and they are much slower than channels as they undergo a conformational change every time they move a molecule across the lipid bilayer. (Carrier proteins transport molecules/ion at a rate of 1-1000 per second whereas ion channels transport ions at up to 10^8 per second) There are three main types of carriers:

- **Uniporters**- move one molecule at a time, using facilitated diffusion
- **Symporters**- move two types of molecule in the same direction simultaneously, e.g. the Na+/glucose symporter.
- **Antiporters**- transport one type of molecule in one direction while moving another in the opposite direction

Symporters and antiporters use **secondary active transport**: the movement of one type of molecule down its concentration gradient is used to power the transport of another molecule against its concentration gradient.



Ion pumps: These use primary active transport to move ions against their concentration gradient. The energy required to do this is gained by the hydrolysis of ATP, which is used to drive a conformational change in the pump and to push the ion/molecule to the other side of the membrane. Pumps can be divided into four categories, three of which are used exclusively for ion transport.

P-class pumps- these transport all types of ions, although each type of pump is specific to only one or two kinds of ions. They are composed of two α subunits and sometimes two regulatory β subunits. P-class pumps use the hydrolysis of ATP to pump ions by forming a phosphorylated intermediate which results in a conformational change. Some P-class pumps can transport more than one type of ion at a time, e.g. the Na+/K+ pump.

V-class pumps- These only transport protons and do not form a phosphoprotein intermediate like P-class proteins. They have a very different structure to P-class pumps, with at least 13 subunits, all of which are unconnected to the subunits in P-class pumps. V-class pumps are normally used to maintain a low pH in vacuoles and lysosomes.

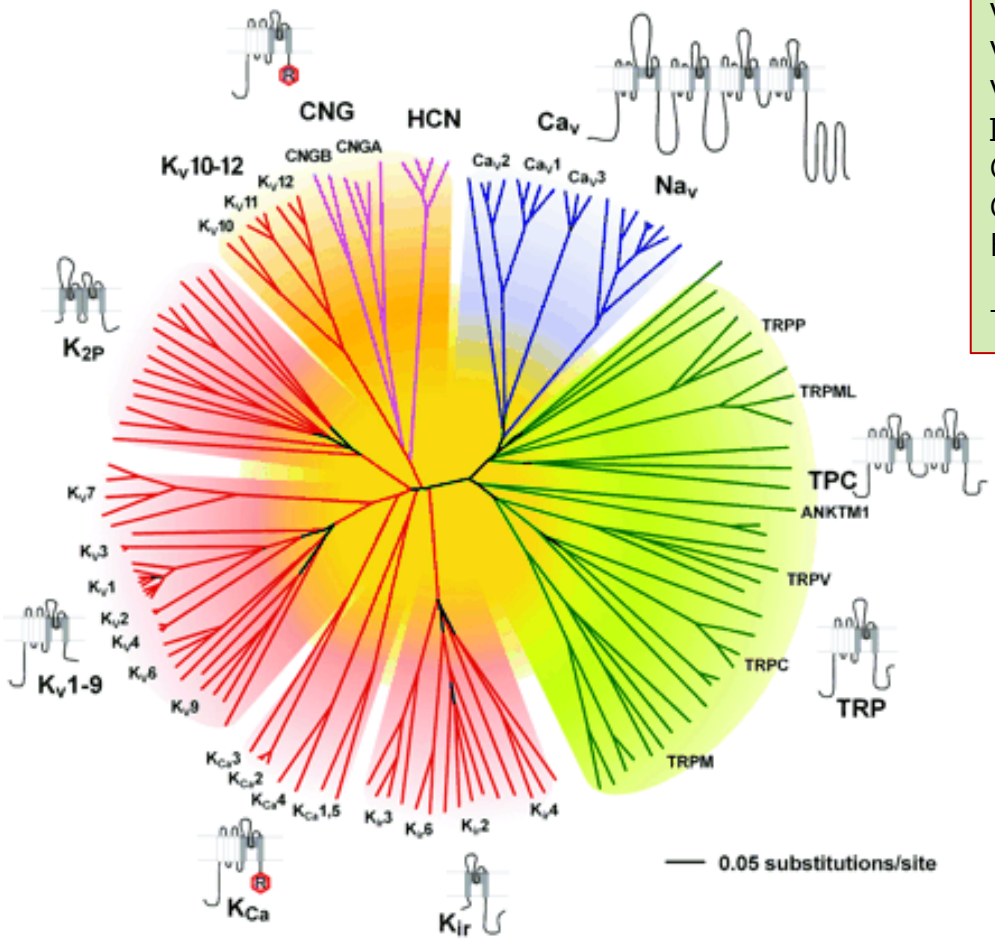
F-class pumps- these are very similar to V-class pumps in that they only transport protons and do not form a phosphoprotein intermediate. They also have similar structures. However, F-class pumps transport protons down their electrochemical gradient, using the movement of the protons to power ATP synthesis, such as the ATP synthase pump found in mitochondria.

ABC (ATP Binding Cassette) superfamily pumps- this is a very diverse class of pumps which can transport all kinds of molecules, including polysaccharides and proteins. All pumps in the ABC superfamily contain two transmembrane domains and two cytosolic domains which bind ATP. ABC pumps are found in both prokaryotic and eukaryotic organisms and are needed for many essential functions in the human body, e.g. the CFTR pump in the membrane of epithelial cells, which can cause cystic fibrosis if mutated.

P-class pumps	V-class pumps	F-class pumps	ABC Superfamily
•Ca^{2+} pump in plasma membrane and sarcoplasmic reticulum •H^+/K^+ pump in the stomach •Na^+/K^{2+} pump in plasma membrane	•Only pumps protons (H^+) •Maintains difference in pH between organelles and cytoplasm •Uses ATP to generate a proton gradient	•Only pumps protons (H^+) •Uses proton gradient to generate ATP	•ATP-Binding Cassette transporters •Small molecule transporters in plasma membrane e.g. cholesterol

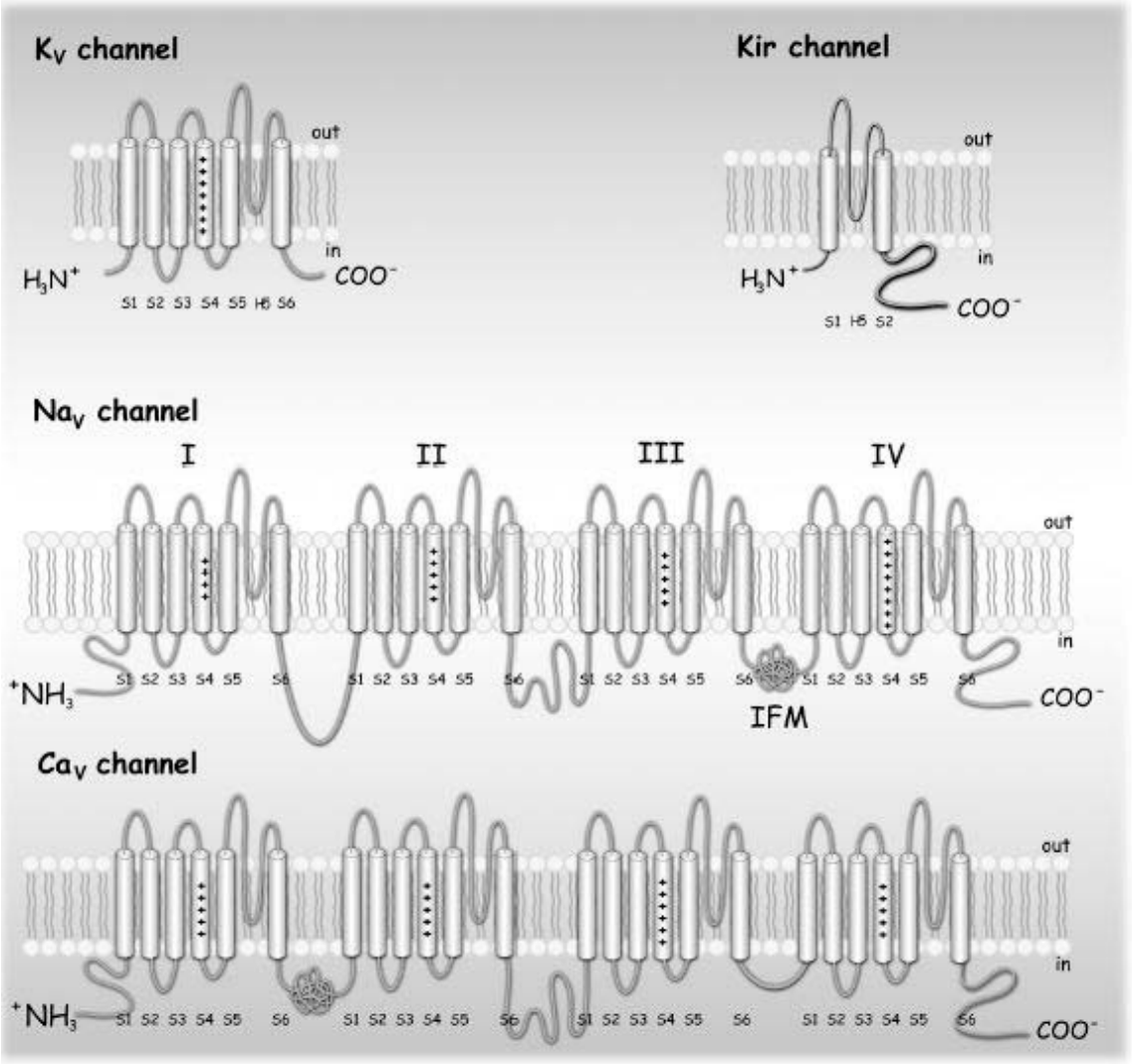
Representation of the amino acid relationships of the minimal pore regions of the voltage-gated ion channel superfamily.

This global view of the 143 members of the structurally related ion channel genes highlights seven groups of ion channel families and their membrane topologies. Pharmacol Rev (2005) 57: 387-395



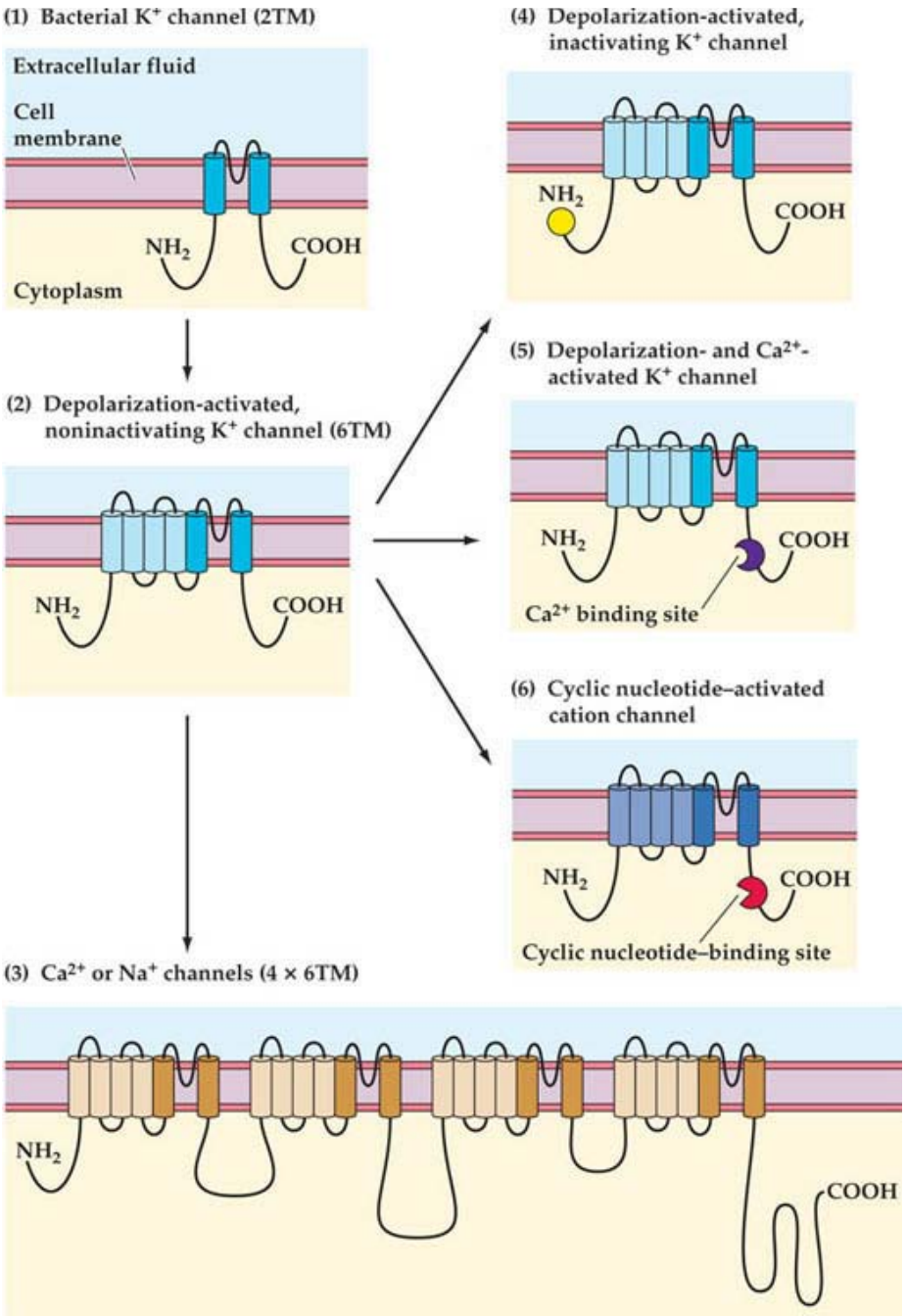
Voltage-gated sodium (Na_V) channels
Voltage-gated calcium (Ca_V) channels
Voltage-gated potassium (K_V) channels
Inwardly-rectifying potassium (K_{ir}) channels
Calcium-activated potassium (K_{Ca}) channels
Cyclic nucleotide-gated (CNG)
Hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels
Transient receptor potential (TRP) channels

Structural similarities among voltage-gated channels



Hypothetical sequence in the evolution of voltage-gated channels

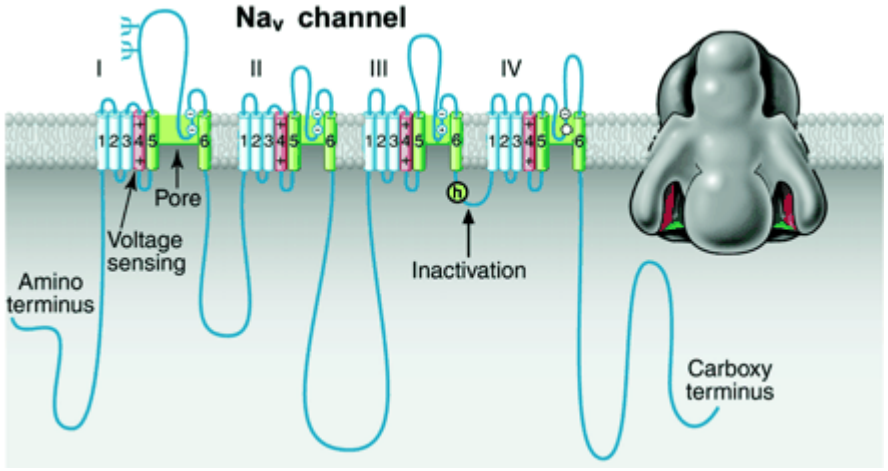
http://sites.sinauer.com/animalphys3e/boxex12.01.html

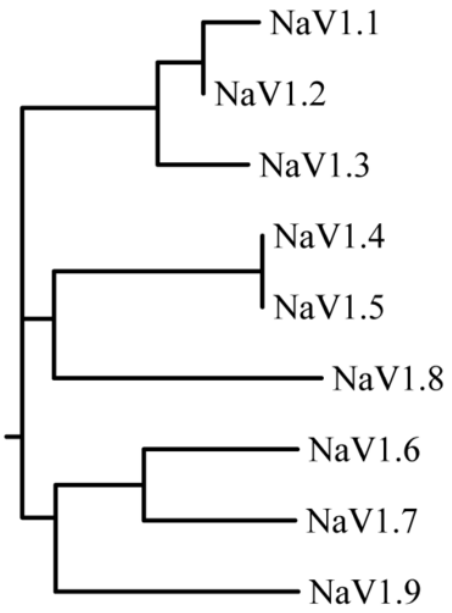


Sodium channels

Voltage-gated: consist of alpha and beta subunits
- alpha (>9 types)
- beta (4 types)

Ligand-gated: nicotinic receptors (acetylcholine) in neuromuscular junction





http://en.wikipedia.org/wiki/Sodium_channel

Pharmacology and toxicology of sodium channels

Toxins

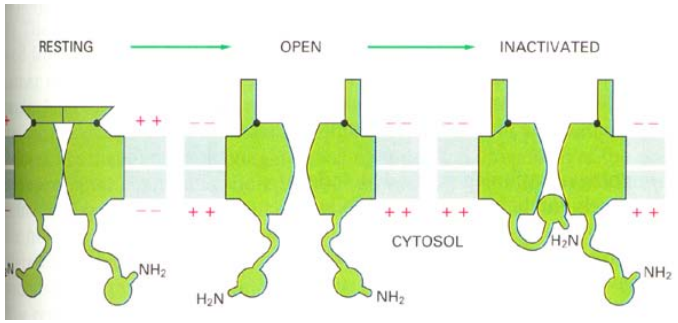
Blocker: Tetrodotoxin (TTX), saxitoxin (STX), SS1-SS2 segment
Agonists: Scorpion and sea anemone toxins, S3-S4 loop

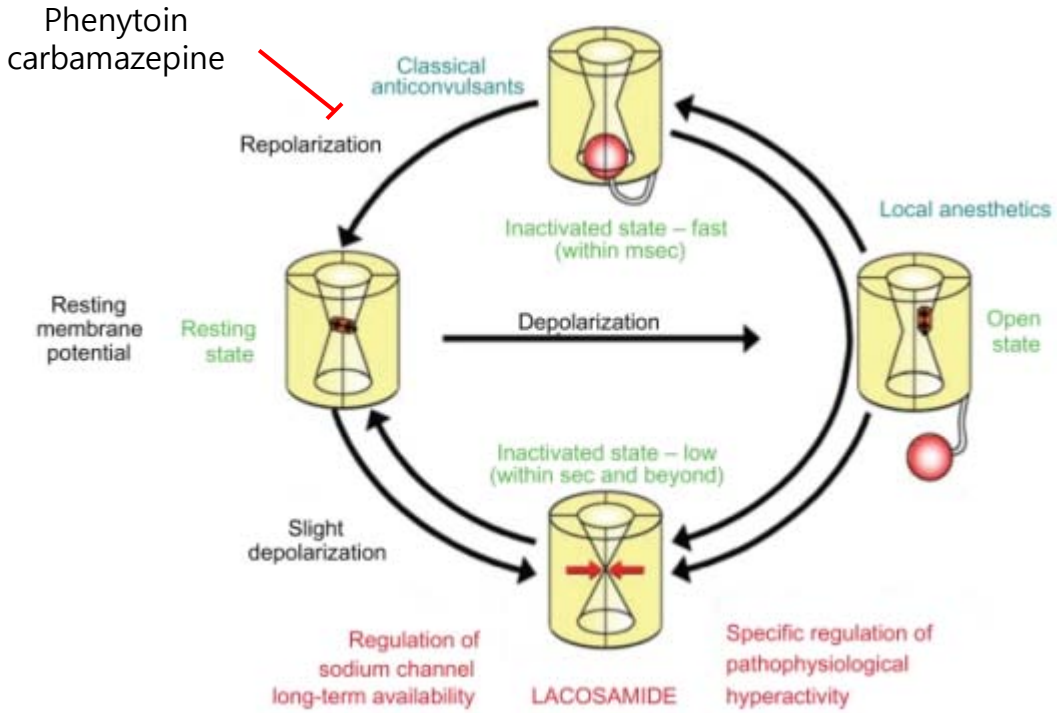
Drugs

For epilepsy: Phenytoin, carbamazepine
Local anesthetics (blocker): cocaine, lidocaine, procaine
membrane permeable acting in cytoplasm

Acquired and inherited disorders

Myotonias (http://en.wikipedia.org/wiki/Myotonia)
Periodic paralysis

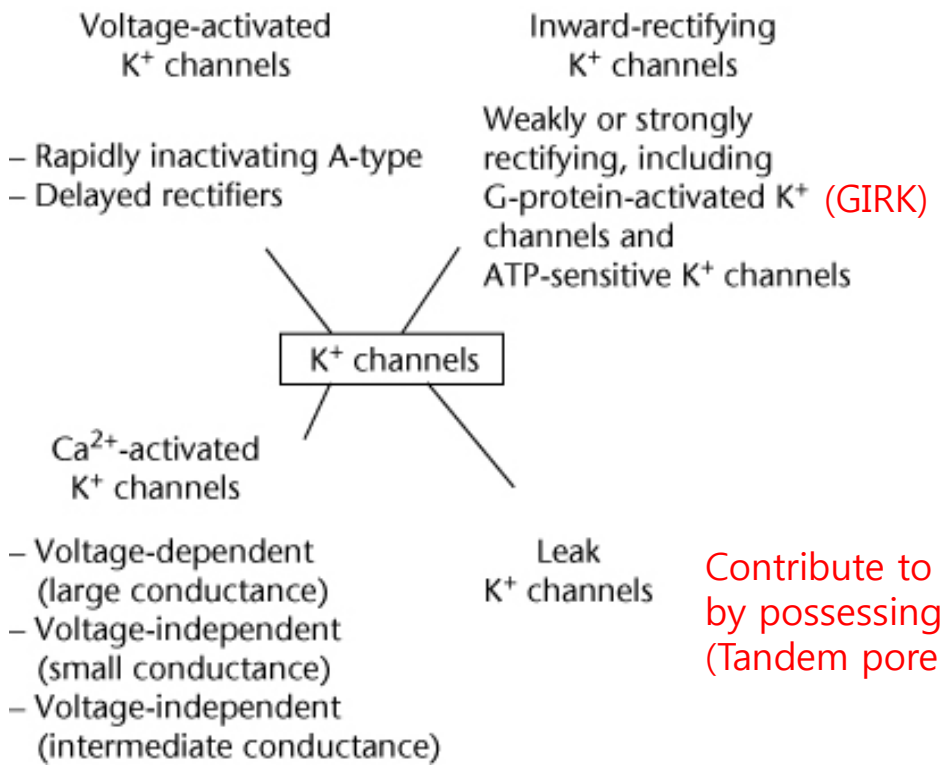




Working modes of voltage-gated sodium channels. At the resting potential, sodium channels are closed and can be opened by depolarization of the membrane potential which allow the flux of sodium ions into the cell. The channels close within a few milliseconds from inside of the neuron and go into a fast inactivated state from which they cannot be reactivated. W hen the membrane potential returns to baseline, the sodium channel goes back to its resting state.
Under conditions of slight prolonged depolarization and repetitive neuronal activity, the sodium channel can go into a slow inactivated state by closing the pore from inside. This process happens on a second-to-minute time scale. Drugs can either block the open channel (eg, local anesthetics), or enhance fast inactivation (classical anticonvulsants), or enhance slow inactivation (lacosamide). Lacosamide: a review of preclinical properties. CNS Drug Rev. 2007;13:21–42. http://openi.nlm.nih.gov/detailedresult.php?img=2938295_ndt-6-465f1&req=4

Potassium channels

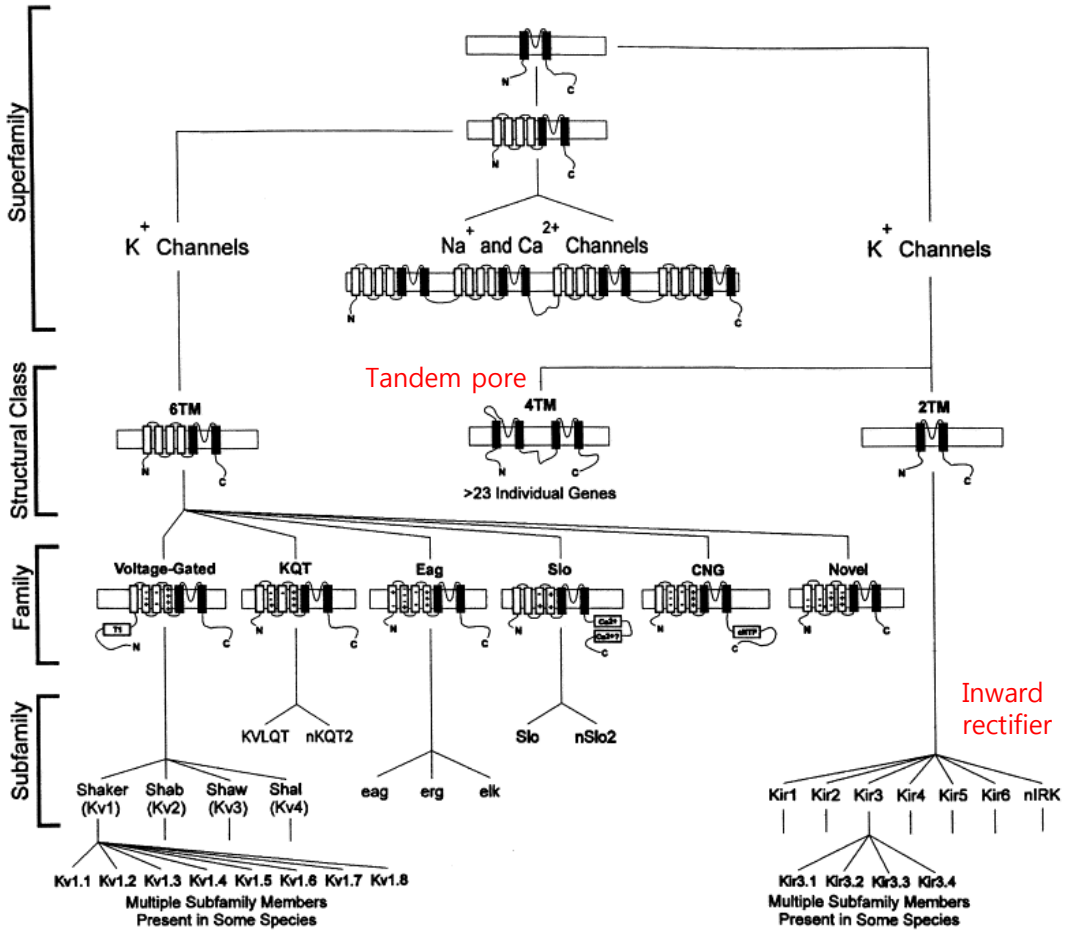
Ubiquitous in most cell types: A variety of cellular functions



Contribute to resting potential
by possessing high basal activation
(Tandem pore domain channel)

http://www.columbia.edu/cu/psychology/courses/1010/mangels/neuro/neurosignaling/neurosignaling.html

3 structural classes



A malfunctioning potassium channel
generally causes some form of hyperexcitability

Long QT syndrome: delayed ventricular repolarization in heart muscle

Benign familial neonatal convulsions: KCNQ2/3 channel

Episodic ataxia: Human Shaker-like channel in the cerebellum
abnormal firing of cerebellar cells

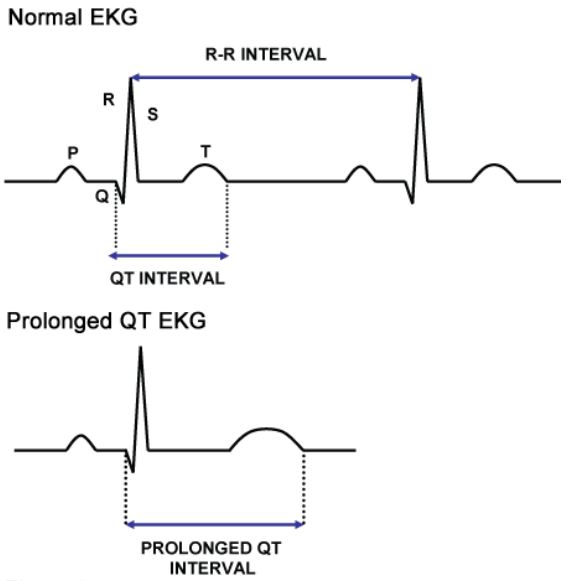
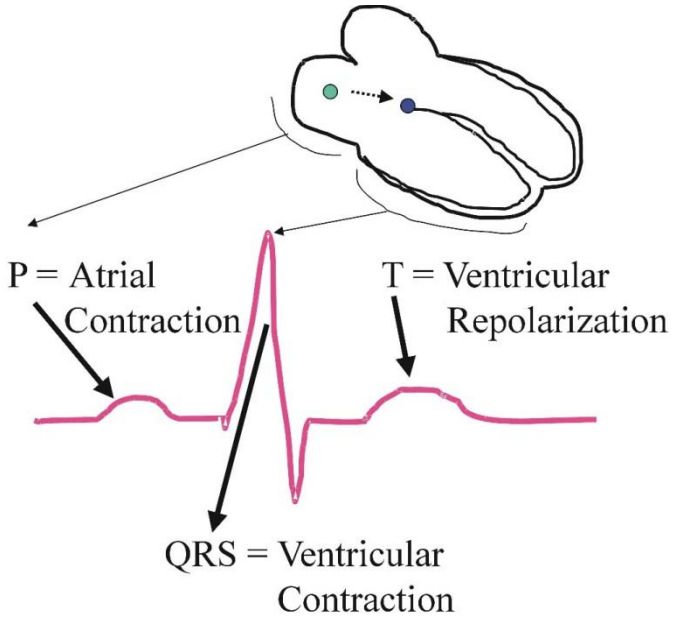


Figure 1



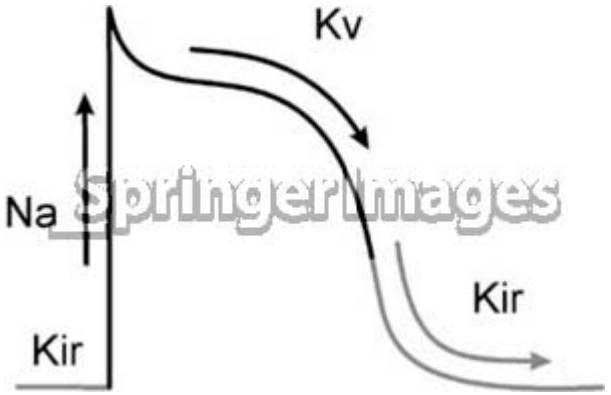
http://www.meditech.cn/meditech-edu/ecg-1.asp

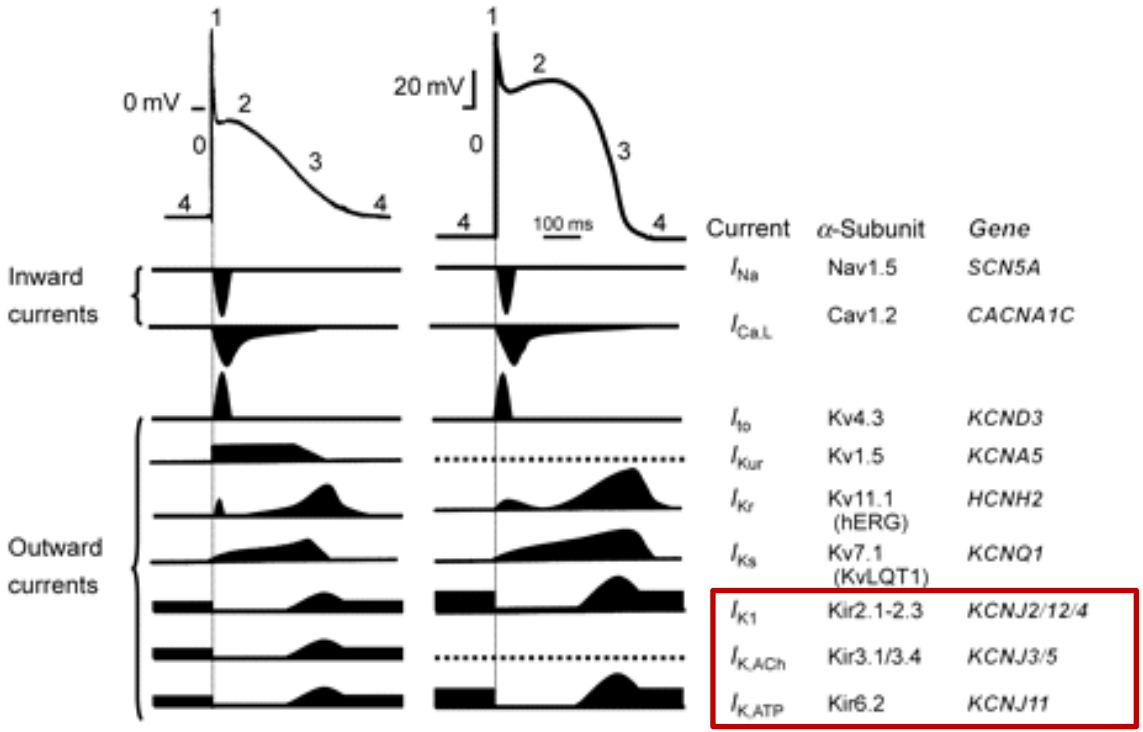
Inward rectifiers K^+ channel: anomalous rectifier

Helping maintain a more prolonged action potential
(Close when depolarized but open when hyperpolarized)

Stabilize near rest membrane potential without depolarization by conducting outward current
Shut off when the cell is sufficiently depolarized for further depolarization

Prevent outflow of potassium ions by shutting down at more positive potentials

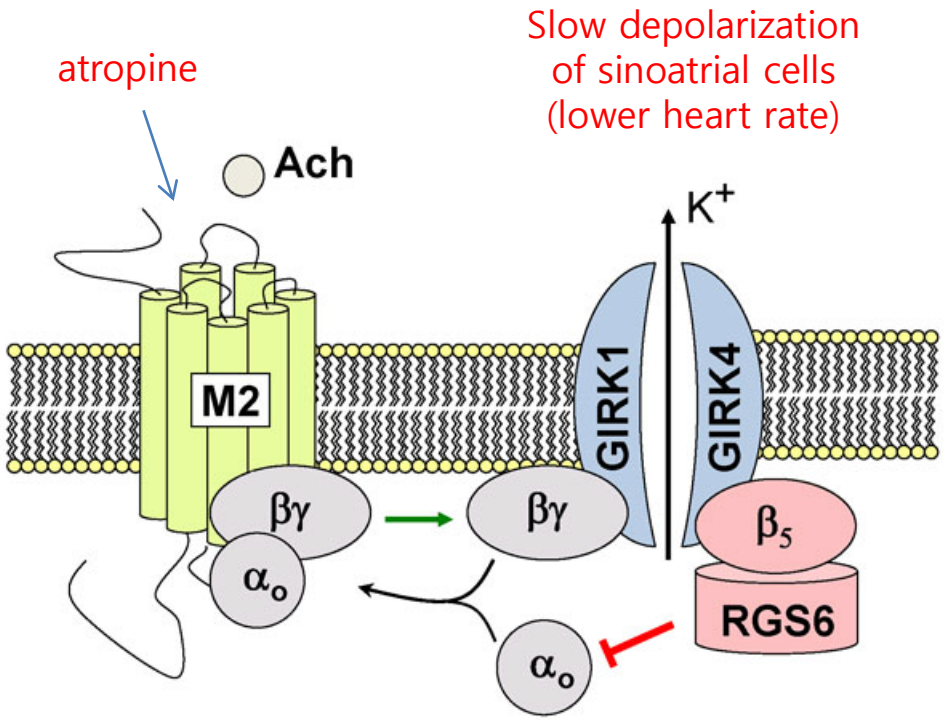




Role of potassium currents in cardiac arrhythmias

Inward, depolarizing and outward, repolarizing currents that underlie the atrial and ventricular action potential. Inward currents: I_{Na} sodium current; $I_{Ca,L}$ L-type calcium current; I_{to} transient outward curren t; I_{Kur} ultra rapidly activating delayed rectifier current; I_{Kr} and I_{Ks} rapidly and slowly activating delayed rectifier current; I_{K1} inward rectifier current; $I_{K,ACh}$ acetylcholine-activated potassium current. Note that I_{Kur} is present in atria only. Phase 0, rapid depolarization; phase 1, rapid early repolarization phase; p hase 2, slow repolarization phase ('plateau' phase); phase 3, rapid late repolarization phase; phase 4, resting membrane potential.

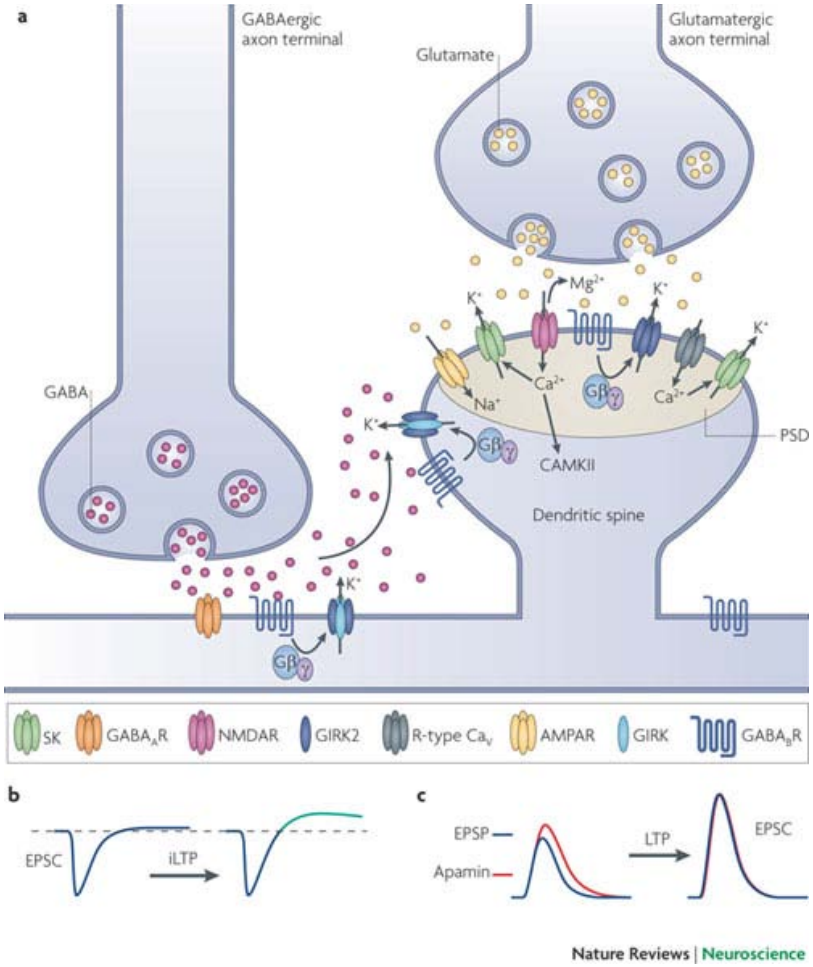
G-protein coupled Kir (GIRKs)



Atropine, a competitive antagonist, increases heart rate

Postsynaptic GIRK and SK

Nature Reviews Neuroscience (2009) 10, 475-480

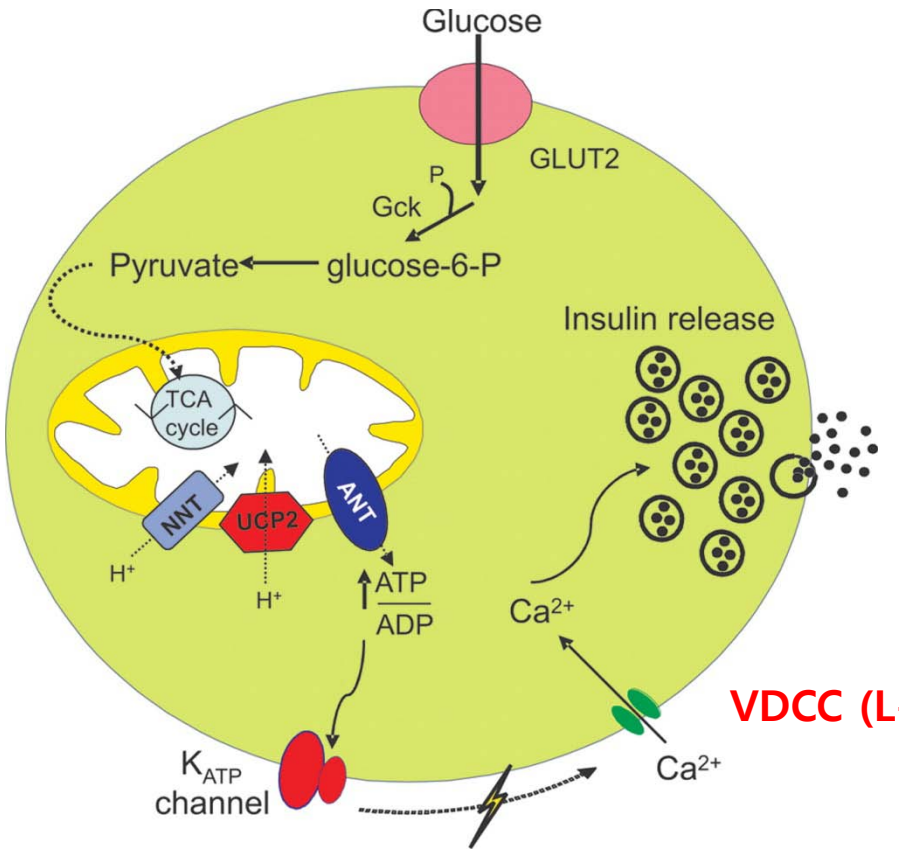


A schematic of glutamatergic excitatory and GABA-ergic inhibitory synapses established on a dendritic spine and shaft, respectively, of a hippocampal pyramidal neuron.
small-conductance Ca2+-activated K+ (SK) channels

K(ATP) channels: ATP-sensitive K channels

Closed at high ATP (0.1-1 mM)

Function in pancreatic beta cells: insulin release
Function in neuronal cells: protective function when energy level is low

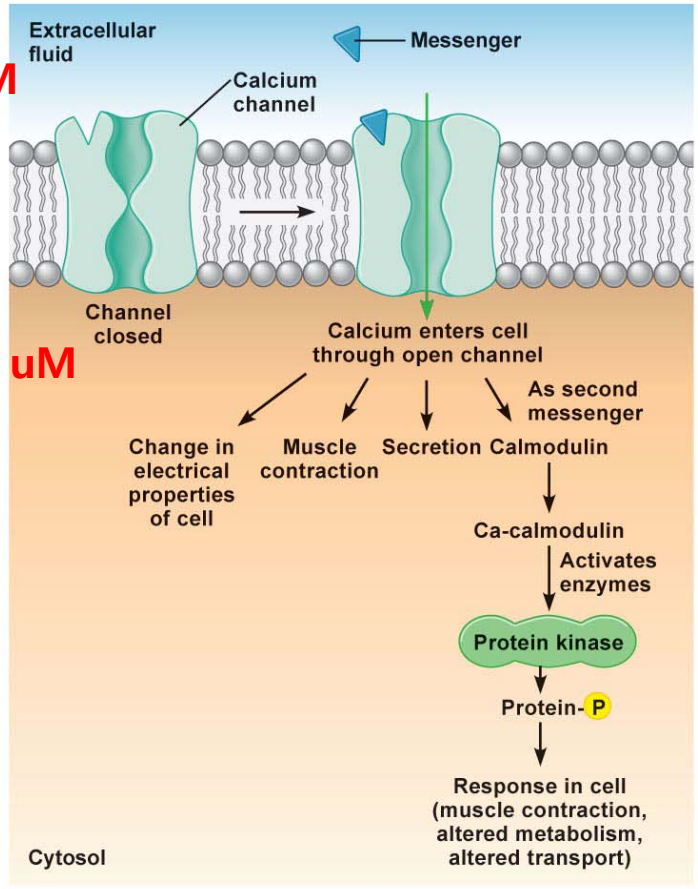


VDCC (L- and T-type)

Calcium channels

1-5 mM

0.1-0.2 uM



Categorized in terms of their
- Voltage dependence
- Kinetics
- pharmacology

Type	Named for	Electrophysiology	Location
L	Long-lasting	HVA (–20 mV)	Pyramidal cells
		Slowly inactivating	Skeletal, cardiac, and smooth muscle
			Endocrine cells
T	Transient	LVA (–65 mV)	Cardiac muscle
		Rapidly inactivating	Neurons (e.g., thalamic)
			Endocrine cells
N	Neuronal	HVA (–20 mV)	Neurons
		Moderate inactivation	
P	Purkinje cell	HVA (–50 mV)	Cerebellar Purkinje cells
		Noninactivating	Mammalian neuromuscular junction
Q	Q after P	HVA	Cerebellar granule cells
R	Remaining	HVA and LVA	

HVA, high voltage-activated; LVA, low voltage-activated.

Chloride channels

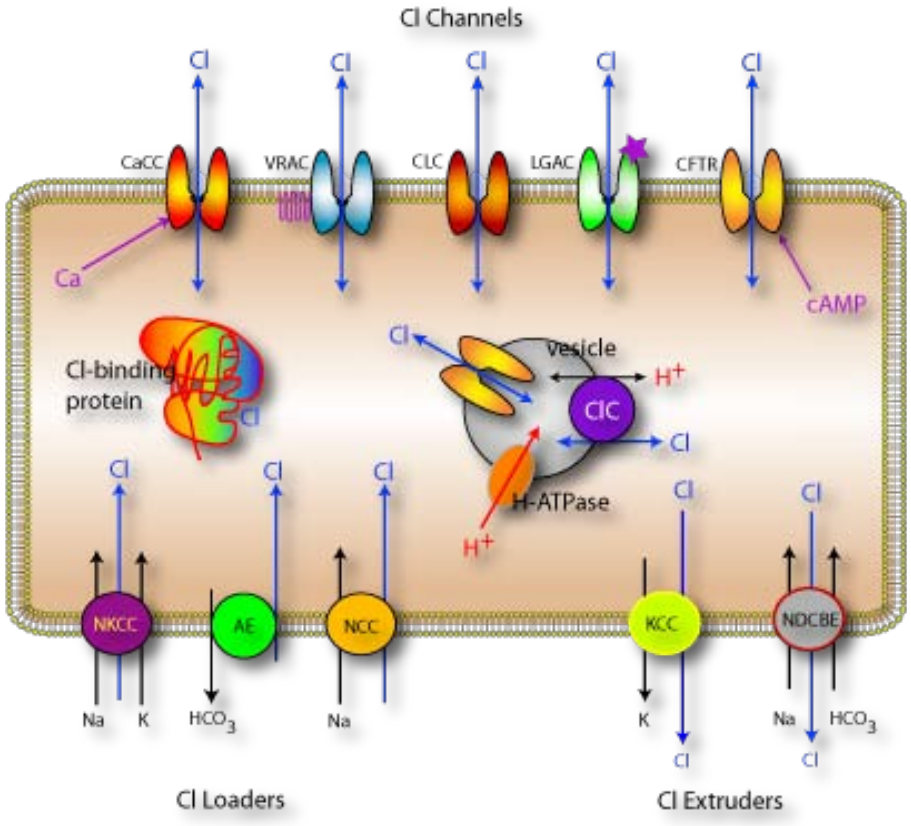
Structurally unrelated to cation channels

Two primary functions

- Hyperpolarization: skeletal muscle, motor neurons
- Osmotic flow of water: secretory cells (mucosal epithelium, kidney)

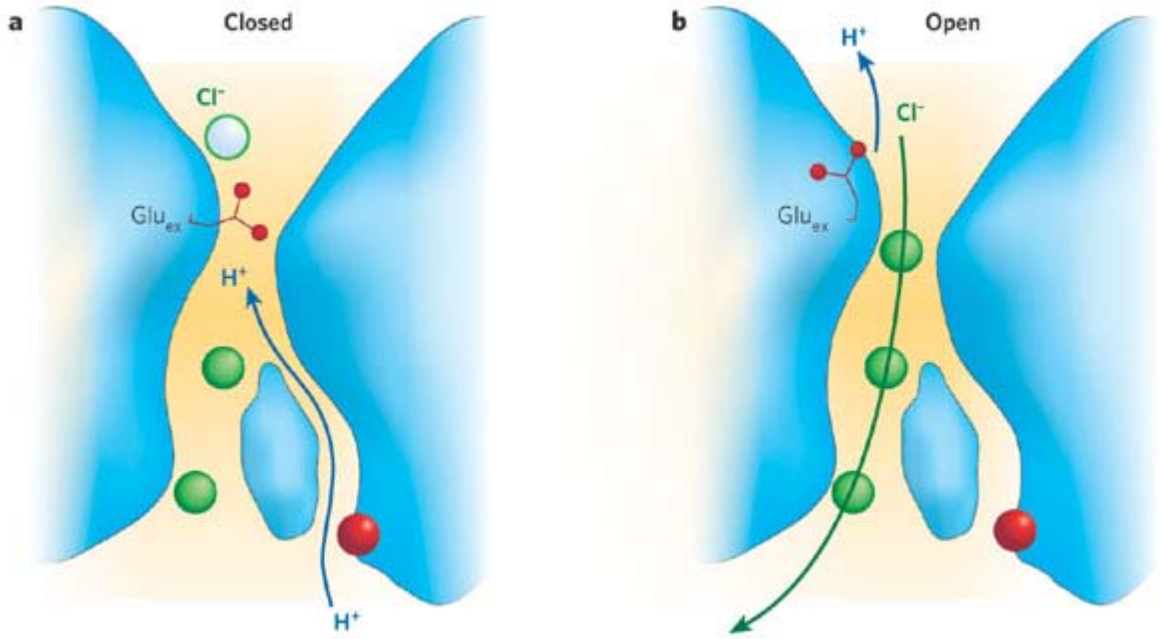
Three families

Ligand-gated channels: important in brain
CLC channels: CLC-5 is high in kidney
CFTR channels: important in epithelial cells



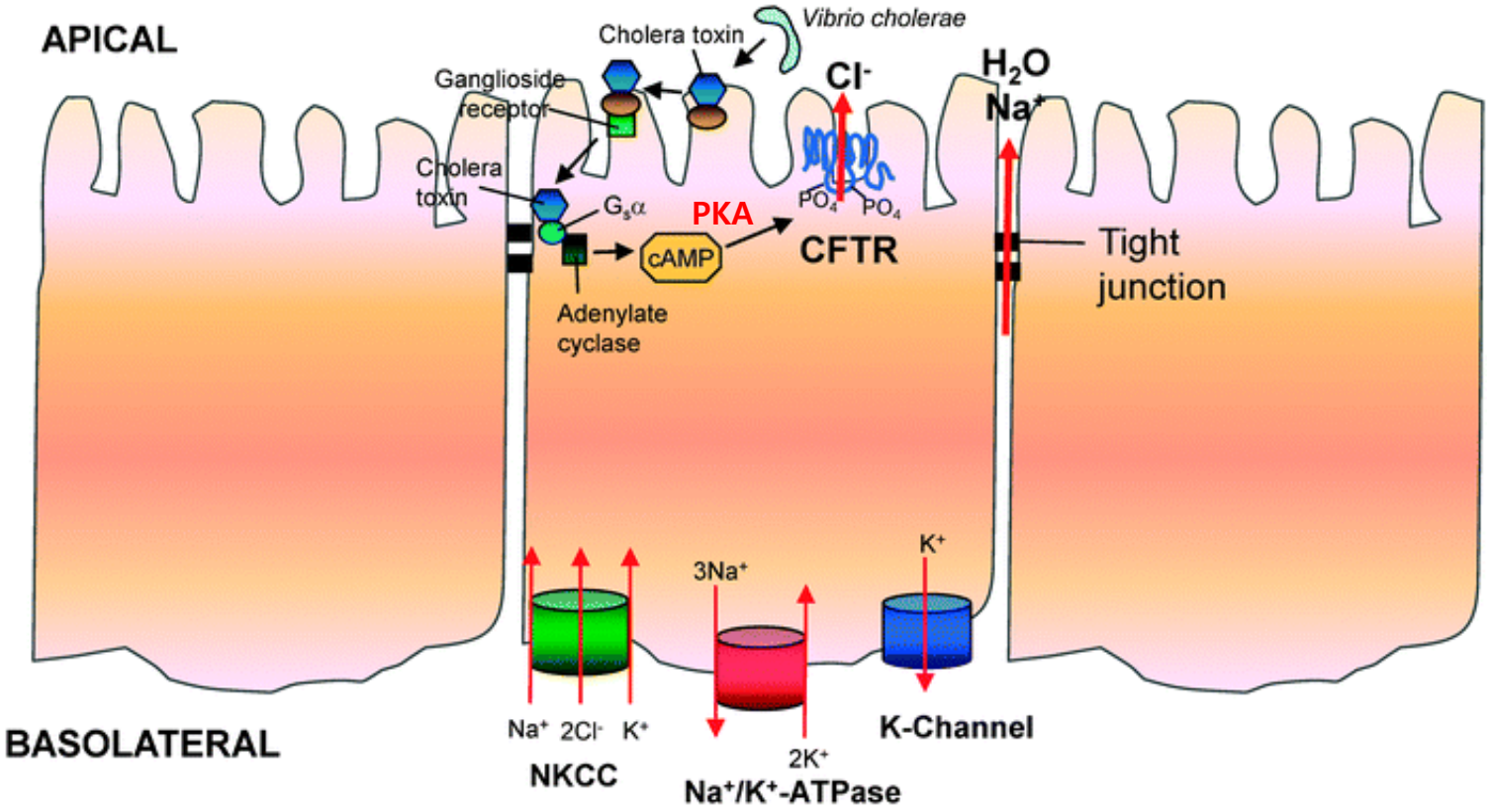
Cellular Cl signaling. Cells actively transport Cl across the plasma membrane by transporters that accumulate Cl intracellularly or extrude it from the cell. Cl flows passively across a variety of Cl channels in the plasma membrane, including Ca-activated Cl channels (CaCC), cAMP-activated Cl channels (CFTR), cell volume-regulated anion channels (VRAC), CLC voltage-gated Cl channels, and ligand-gated anion channels. In addition, Cl channels and transporters are found in intracellular membranes, such as the endosomal-lysosomal pathway, and play a role in regulating intravesicular pH and Cl concentration. Many proteins are regulated by Cl, as depicted by the Cl-binding protein. http://www.emory.edu/HEARTCELL/

CLC chloride channels



a, Closed state, with Glu_{ex} side chain deprotonated and blocking the pore. Allosteric Cl^- ion occupies hypothetical site external to Glu_{ex} and thereby allows intracellular proton transfer. **b**, Open state, with protonated Glu_{ex} side chain in externally exposed position. The red circles mark the position occupied by Glu_{in} in the transporters, but which is always valine in the channels. Nature (2006) 440:484-489

CFTR channel: a member of ABC protein

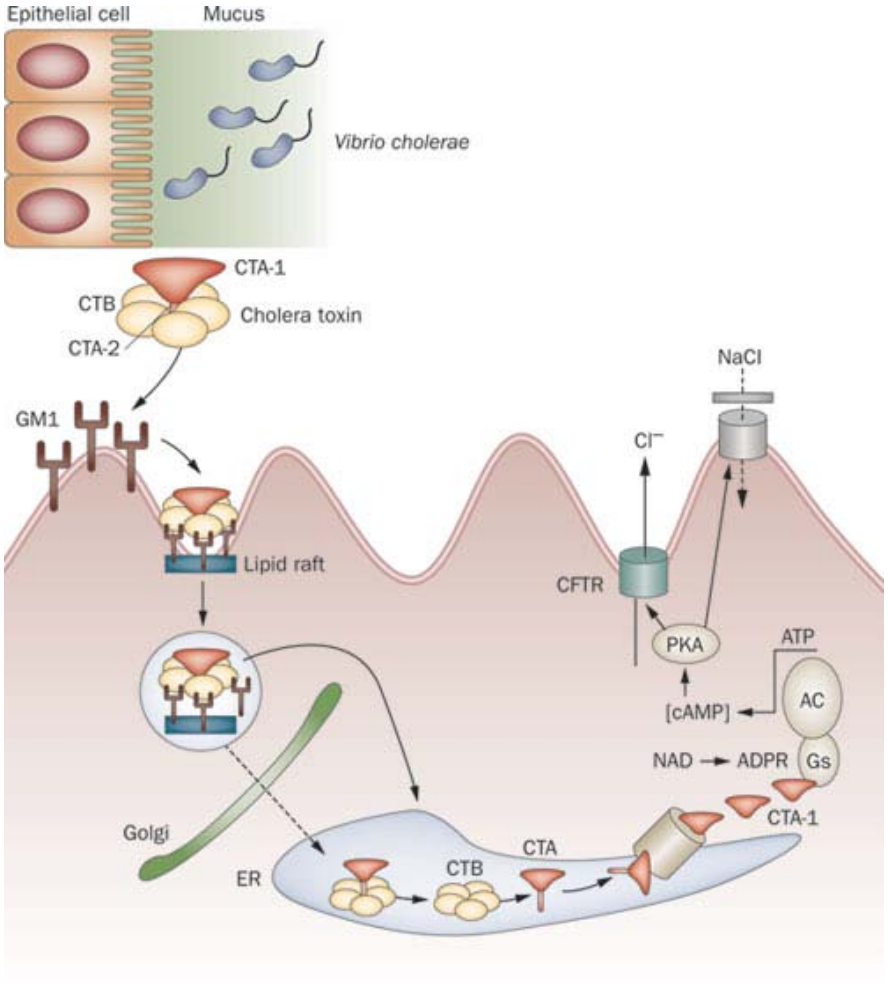


CFTR chloride channel in the apical compartments: spatiotemporal coupling to its interacting partners
Integr. Biol., 2010,**2**, 161-177

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride channel located primarily at the apical or luminal surfaces of epithelial cells in the airway, intestine, pancreas, kidney, sweat gland, as well as male reproductive tract, where it plays a crucial role in transepithelial fluid homeostasis.

CFTR hypofunctioning because of genetic defects leads to cystic fibrosis, the most common lethal genetic disease in Caucasians, whereas **CFTR hyperfunctioning** resulting from various infections evokes secretory diarrhea, the leading cause of mortality in early childhood.

Therefore, maintaining a dynamic balance between CFTR up-regulating processes and CFTR down-regulating processes is essential for maintaining fluid and body homeostasis.



After ingestion, V. cholerae colonizes the small intestine and secretes cholera toxin, which has a doughnut-like structure with a central enzymatic toxic-active A (A_1+A_2) subunit associated with pentameric B subunits (B5). After binding to GM1 ganglioside receptors, mainly localized in lipid rafts on the cell surface, the toxin is endocytosed and travels to the ER via a retrograde pathway which—dependent on cell type—may or may not involve passage through the Golgi. In the ER, the A subunit dissociates from the B subunits and through translocation via the ER degradasome pathway, A1 can reach the cytosol where it can rapidly refold. It binds to and ADP-ribosylates Gs, stimulating the AC complex to produce increased cellular levels of cAMP, leading to activation of PKA, phosphorylation of the major chloride channel, CFTR, and secretion of chloride (Cl^-) and water. Cholera toxin-induced chloride (and bicarbonate) secretion is especially pronounced from intestinal crypt cells, whilst in villus cells the increased cAMP levels instead mainly inhibits the normal uptake of NaCl and water.[14] Abbreviations: AC, adenylate cyclase; ADPR, ADP ribose; cAMP, cyclic AMP; CTA, cholera toxin A; CTB, cholera toxin B; CTFR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; Gs, GTP- binding protein, Gs; PKA, protein kinase A. Nature Reviews Gastroenterology & Hepatology 8, 701-710 (December 2011)