K^+ channels

Cheng Long, chenglong_scnu@qq.com
School of Life Sciences, South China Normal University
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The outline…

Required Readings:

Yellen G. (2002) The voltage-gated potassium channels and their relatives. Nature 419(6902): 35-42.

Shieh CC, Coghlan M, Sullivan JP, Gopalakrishnan M. (2000) Potassium channels: molecular defects, diseases, and therapeutic opportunities. Pharmacol Rev. 52: 557-594.

International Union of Pharmacology (2002). Potassium channels, 59-63.

Further Readings:

Bichet D, Haass FA, Jan LY. (2003) Merging functional studies with structures of inward-rectifier K^+ channels. Nature Reviews Neuroscience 4: 957-967.

Niwa N, Nerbonne JM. (2010) Molecular determinants of cardiac transient outward potassium current (I(to)) expression and regulation. Mol Cell Cardiol. 48(1): 12-25.

The outline…

This class will cover:

- **Types & structure of K^+ channels**
- **Function & blockers of K^+ channels**
- **Diseases related to K^+ channels**

Introduction

- The potassium channels are large proteins with a central pore that pierces the cell membrane and allows only potassium ions to pass through.
- In the field of cell biology, potassium channels are the most widely distributed type of ion channel and are found in virtually all living organisms. They are found in most cell types and control a wide variety of cell functions. They are the largest and most diverse group of ion channels, represented by some 70 known loci in the mammalian genome.
- The first cloned potassium channel gene was the Drosophila voltage-gated shaker channel, and this was rapidly followed by the identification of other voltage- and ligand-gated potassium channel genes in flies, mammals, and many other organisms.
- The K^+ channels are precise molecular machines that propagate electrical impulses in the brain, heart and other cell types.

Introduction

- Potassium channels present in both excitable and nonexcitable cells.
- Members of this channel family play critical roles in cellular signaling processes regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume regulation.
- Over 50 human genes encoding various K^+ channels have been cloned during the past decade, and precise biophysical properties, subunit stoichiometry, channel assembly and modulation by second messenger and ligands have been addressed to a large extent.
- The crystal structure of a K^+ channel from Streptomyces lividans has become available.

Abbreviations

- K_v, voltage-gated K^+ channel;
- BK_{Ca}, large conductance Ca^{2+}-activated K^+ channel; SK_{Ca}, small conductance Ca^{2+}-activated K^+ channel;
- EAG, *ether-a-go-go* K^+ channel;
- hERG, human *ether-a-go-go*-related K^+ channel;
- IK_{Ca}, intermediate conductance Ca^{2+}-activated K^+ channel;
- IK_r, cardiac rapid delayed rectifier; IKs, cardiac slow delayed rectifier;
- IK_{ur}, ultrarapid delayed rectifier; IK_{TO}, transient outward delayed rectifier;
- K_{ATP}, ATP-sensitive K^+; KCsA, K^+ channel from *Streptomyces lividans*;
- K_{ir}, inward rectifier K^+ channel; K_{CO}, K^+ channel opener;
- M-channel, muscarine-sensitive K^+ channel;
- TWIK, two-pore weak inward rectifier; TREK, two-pore weak inward rectifier-related K^+ channel;
- TEA, tetraethylammonium; 4-AP, 4-aminopyridine;
- MiRP, minK related peptide; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; PS, presenilin;
- SUR, sulfonylurea receptor ……

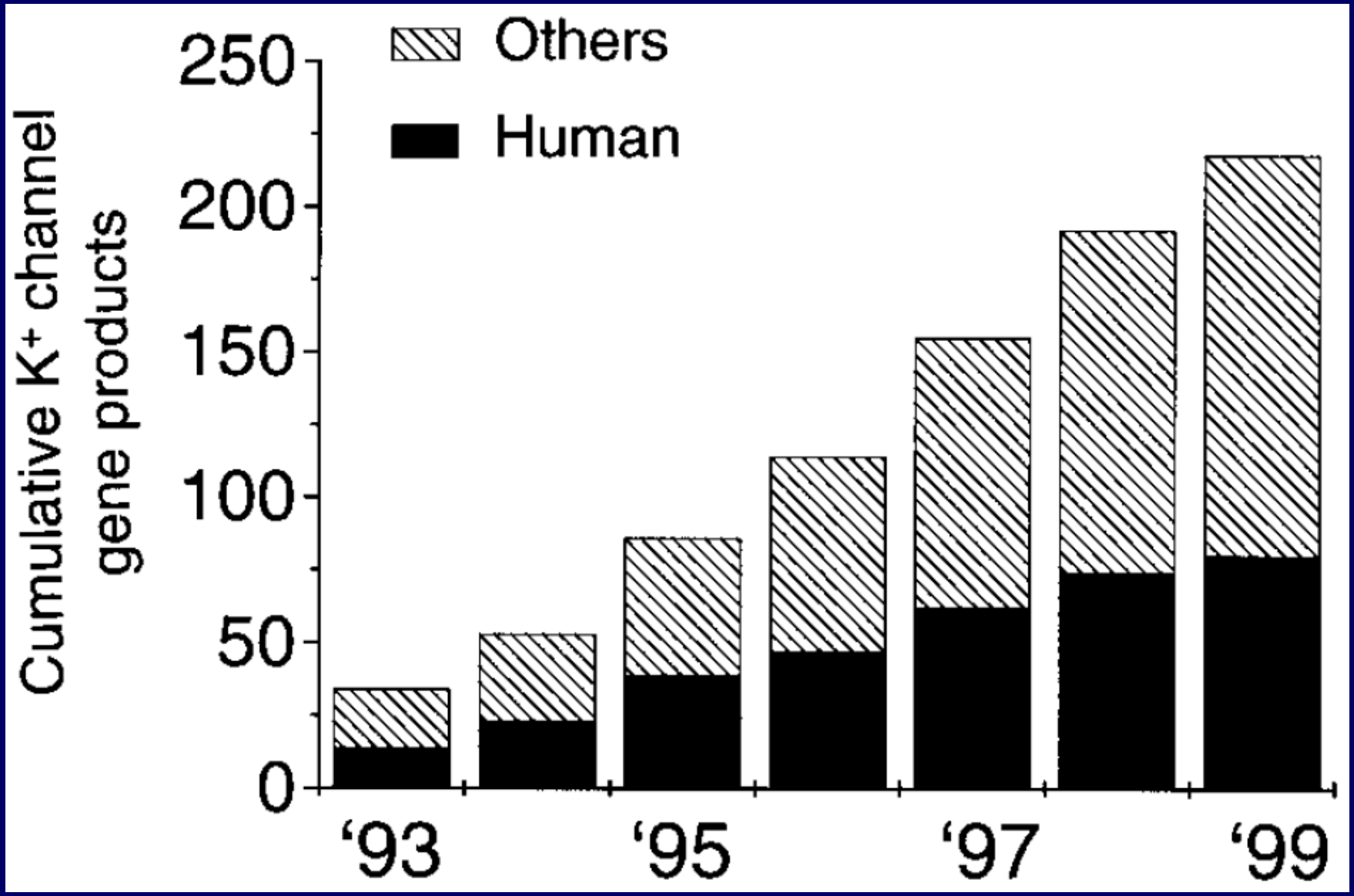
Nomenclature

- A standardised nomenclature for the six-transmembrane domain (TM), voltage-gated K^+ channel genes – the K_V naming system – was widely adopted. This nomenclature was based on deduced phylogenetic relationships; channels that shared 65% sequence identity being assigned to one subfamily.
- A parallel nomenclature – KCN – was developed by the Human Genome Organisation (HUGO). Since then, the K^+ channel superfamily of genes has greatly expanded, requiring an update of the naming system.

Features of K^+ channel

- K^+ channels selectively conduct K^+ ions across the cell membrane along its electrochemical gradient at a rate of 10^6 to 10^8 ions/s.
- A set of salient features:
  - a water-filled permeation pathway (pore) that allows K^+ ions to flow across the cell membrane;
  - a selectivity filter that specifies K^+ as permeant ion species;
  - a gating mechanism that serves to switch between open and closed channel conformations.
- Since the first gene encoding a K^+ channel was cloned from *Drosophila Shaker* mutant, more than 200 genes encoding a variety of K^+ channels have been identified, all containing a homologous pore segment (S5-S6 linker) selective for K^+ ions.

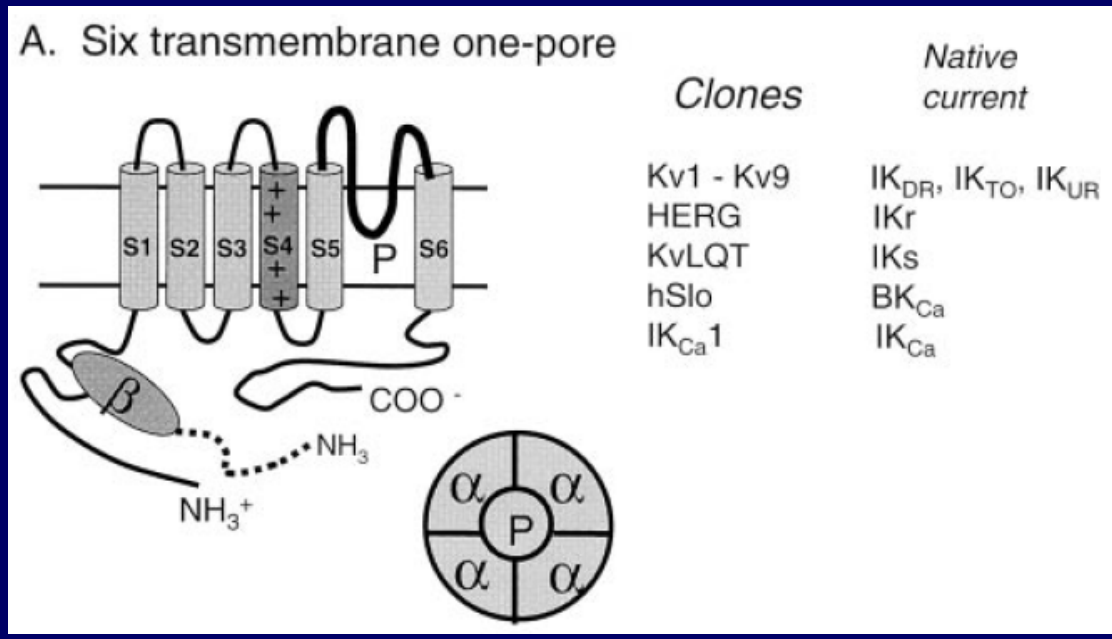
Growth of genes encoding diverse K^+ channels



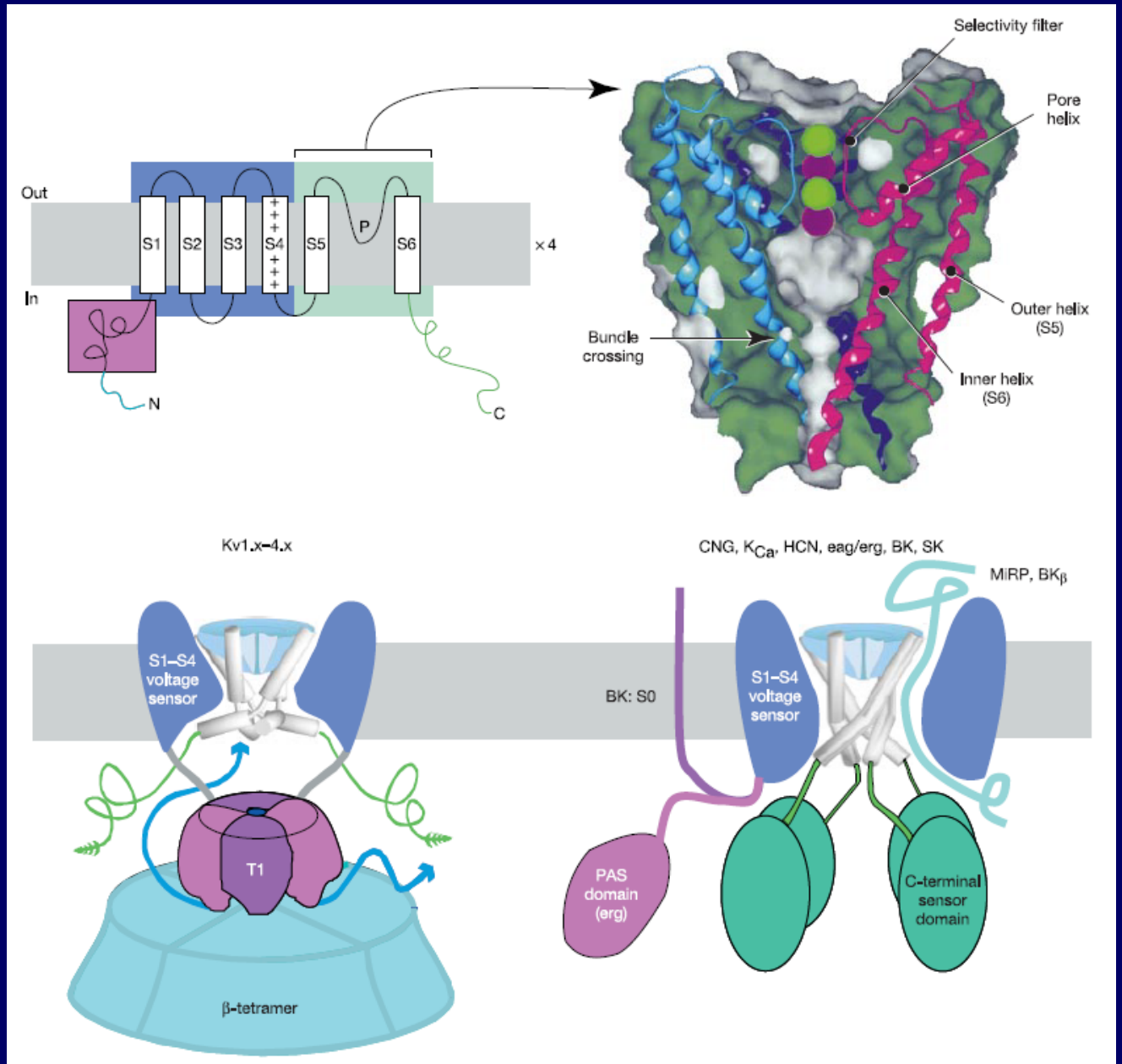
Channel diversity and classification

Structural classification of K^+ channel subunits

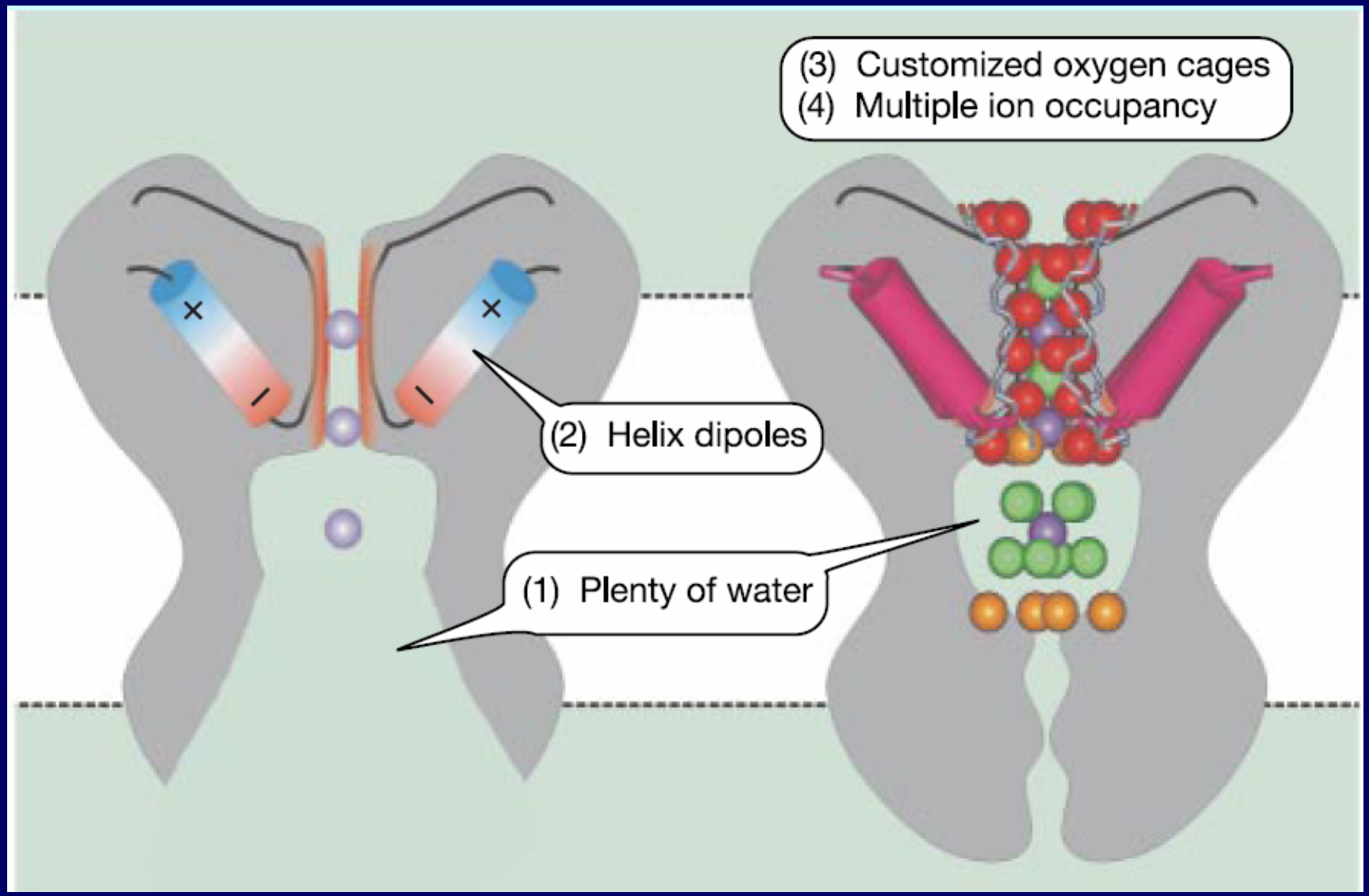
- A general classification of K^+ channels into families is based upon the primary amino acid sequence of the pore-containing subunit.
- Three groups with six, four, or two putative transmembrane segments are recognized.
  - voltage-gated K^+ channels (*Shaker*-like) containing six transmembrane regions (S1-S6) with a single pore;



The tetrameric 6TM architecture of the K^+ channel family

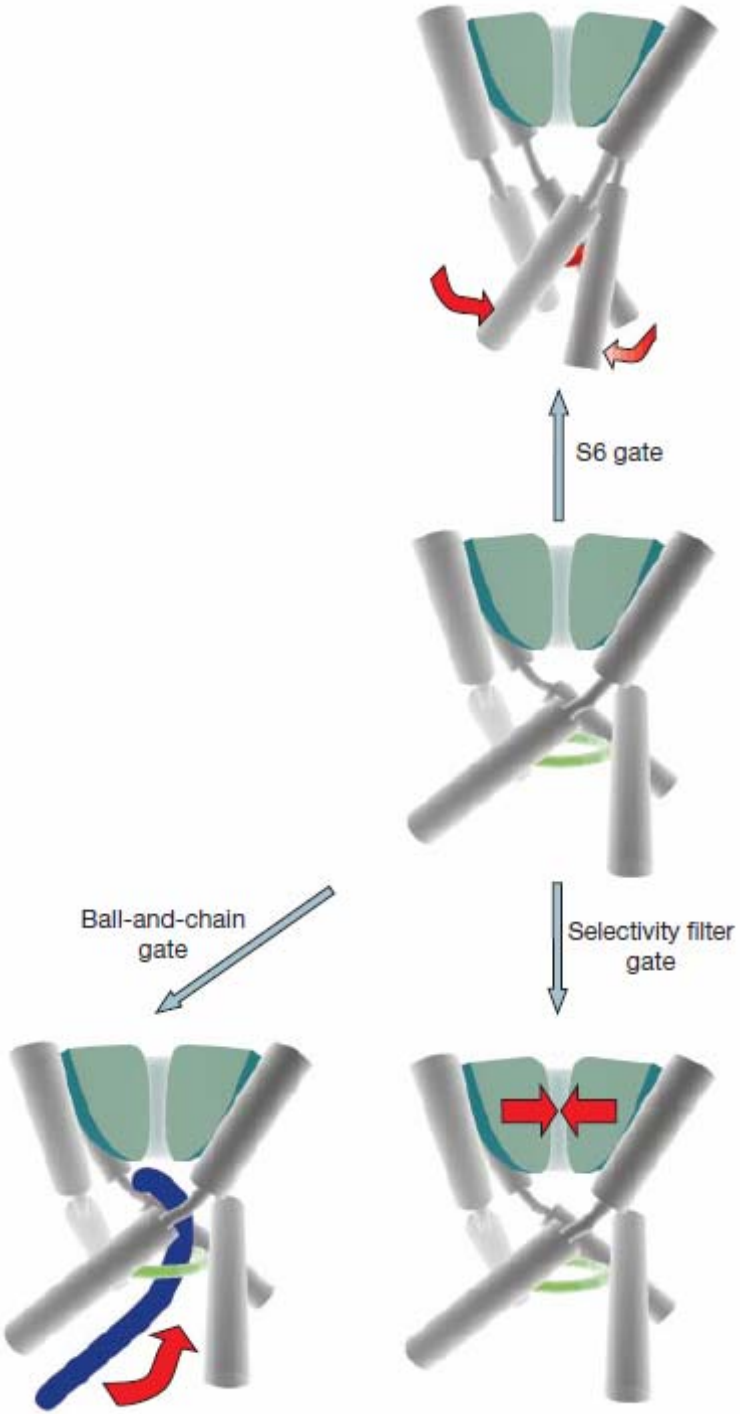


Architectural features of K^+ channels important for ion permeation

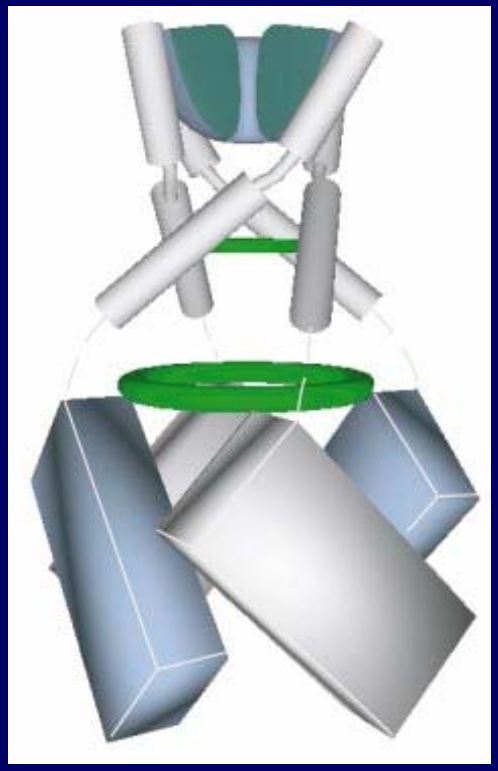


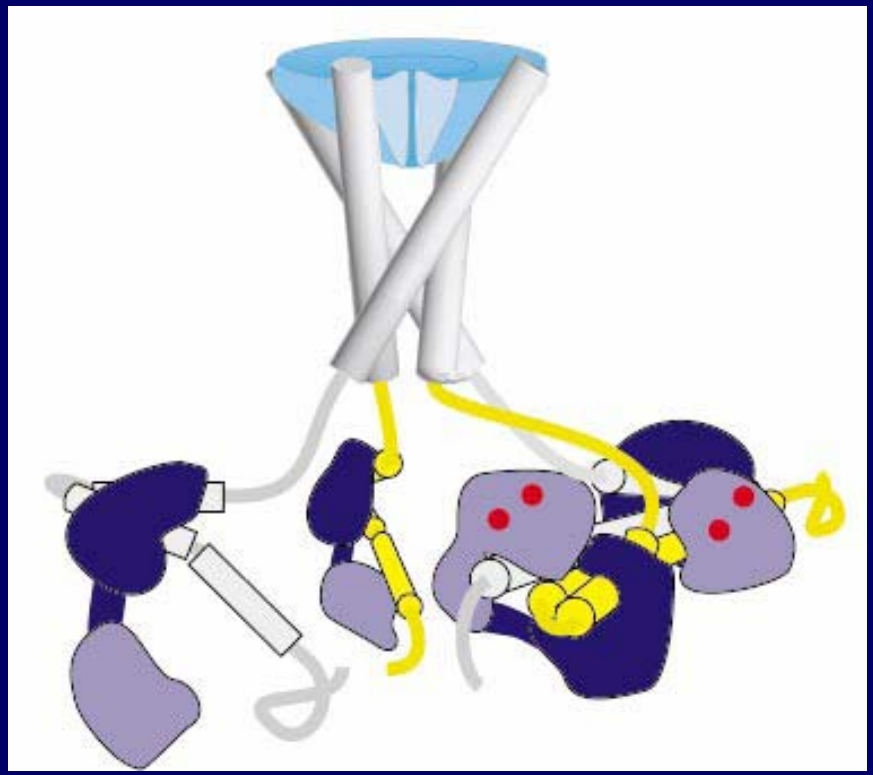
The conformational changes that gate the K^+ channel pore

- The three best understood conformational changes for closing potassium channels
- Green: the selectivity filter
- Blue: an auto-inhibitory peptide



Two sensor domains that govern gating in the K^+ channel family





Six transmembrane one-pore channels

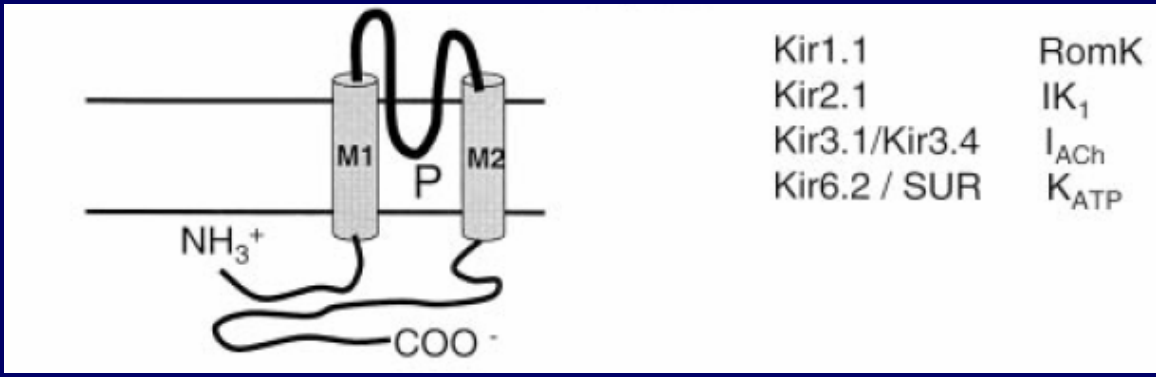
- **Pore and selectivity filter**
  - **The tripeptide sequence motif G(Y/F)G located in the S5-S6 linker is common to the pore or P-loop of these and other K^+ channels and hence is considered as the K^+-selectivity signature motif.**
  - **The external entry to the channel pore consisting of portions of the P-loop and adjacent residues in both S5 and S6 segments constitutes binding sites for toxins and K^+ channel blockers.**
  - **The internal vestibule of pore composed of residues from S5 and S6 segments facing the intracellular side contributes to binding sites for compounds such as 4-aminopyridine, tetraethylammonium, and quinidine. The S4-S5 linker lies close to the permeation pathway and forms part of the receptor for the inactivation ball.**

Six transmembrane one-pore channels

- **Voltage sensor and channel activation**
  - **In voltage-dependent ion channels, membrane depolarization is required to cause conformational changes leading to channel opening, which allows permeant ions to flow.**
  - **The transmembrane S4 segment represents the major component of the voltage sensor. The S4 segment that contains positively charged residues (lysine or arginine) at approximately every third position resulting in a regularly spaced array of 5 to 7 positive charges is conserved within the voltage-gated K^+ channel family.**
- **Inactivation**
  - **Many voltage-dependent K^+ channels activate and inactivate rapidly when membrane potential becomes more positive. Inactivation is a nonconducting state during maintained depolarization. Three types of inactivation, i.e., N-, P-, and C-type, have been characterized and associated with distinct molecular domains of the channel.**
- **Subunit interaction and assembly domains**

Two transmembrane one-pore channels

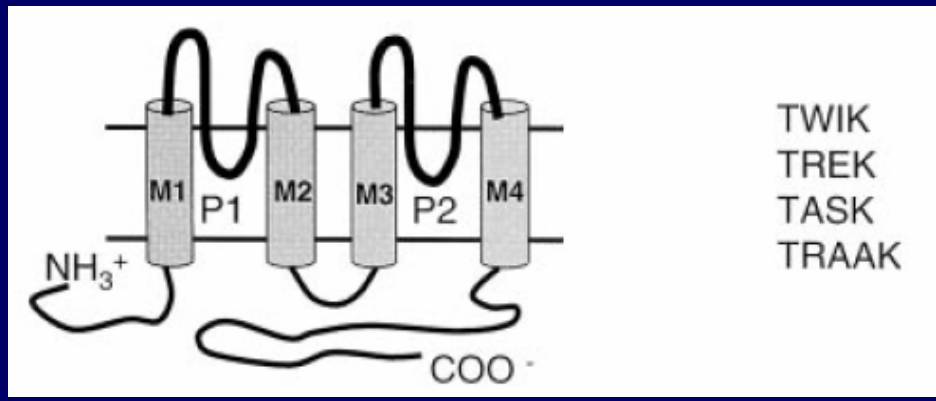
- **Inward rectifier K^+ channels containing only two transmembrane regions and a single pore loop in between.**



- **This inward rectification is attributed to gating mechanisms by internal Mg^{2+} and polyamines (spermine, spermidine, etc.) that occlude access of K^+ to the internal vestibule of a conducting pore.**
- **They are important in setting the resting membrane potential.**

Four transmembrane two-pore channels

- **Two-pore K^+ channels containing four transmembranes with two pore regions.**



- **They represent the most abundant class of K^+ channels (at least in *C. elegans*), with >50 distinct members. The G(Y/F)G residues of K^+-selective motif is preserved in the first pore loop of the two-pore K^+ channel, but it is replaced by GFG or GLG in the second pore loop.**
- **All the two-pore channels have a conserved core region between transmembrane segments M1 and M4. The amino- and carboxyl-terminal domains are quite diverse. Two-pore domain subunits would presumably form a channel to retain the tetrameric arrangement.**

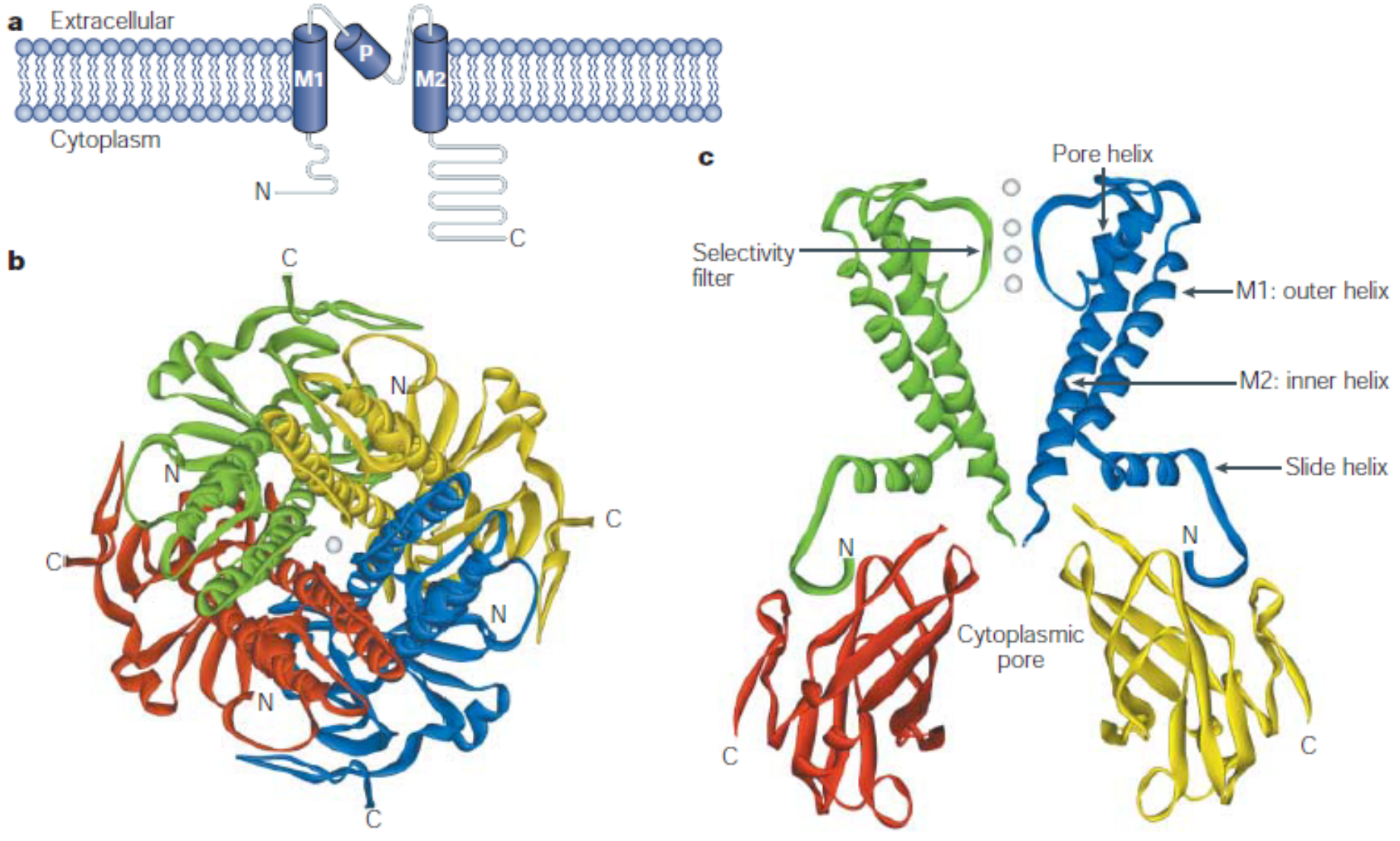
Auxiliary subunits

- The Kv1 channels associate with cytoplasmic β-subunits to alter channel kinetics.
- Chaperone proteins, such as KChAP, regulating the function and expression of some of the Kv channels, such as Kv2.1, Kv1.3, and Kv4.3, have been reported.
- Certain other Kv channels, such as Kv5, Kv6, Kv8, and Kv9, do not form functional channels themselves but associate with Kv2.1 channels to alter the biophysical properties.
- Distinct β-subunits that associate with the calcium-activated K^+ channels, sulfonylurea receptors for the inward rectifiers Kir6.1 or Kir6.2, and minK and minK-related peptides (MiRPs) for the cardiac delayed rectifier channels. These subunits play roles as diverse as modulation of gating properties such as inactivation, cell surface expression, and/or trafficking of the ion channel complex, to serving as binding sites for both endogenous and exogenous ligands.
- Given the diversity of K^+ channel subunits and the potential to vary the constituents to form diverse α-α or α-β heteromeric channel complexes to alter expression, cellular targeting, and biophysical and pharmacological properties in native cell types.
- Understanding the precise composition of channel complexes in vivo remains a challenge.

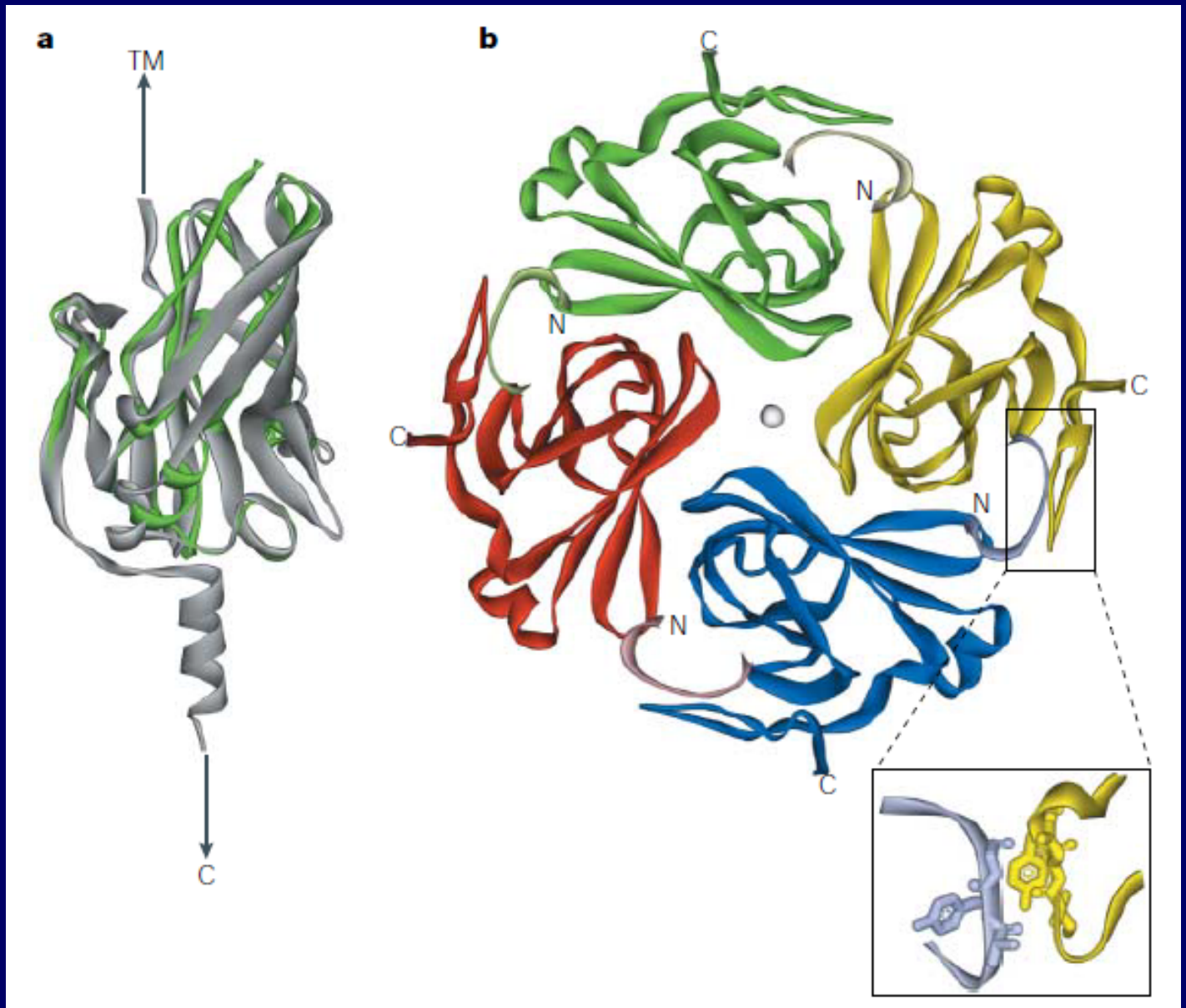
Crystal structure of K^+ channels

- Mutagenesis approache
- Biophysical approache
- X-ray analysis
  - The crystal structure of KCsA channel provides the first three-dimensional structure of the conduction pore that fits consistently with current understanding of the core functionality of K^+ channels.
  - The understanding of structural information can be applied to design selective compounds targeting K^+ channels and would revolutionize and refine approaches targeting K^+ channels for therapeutic purposes.

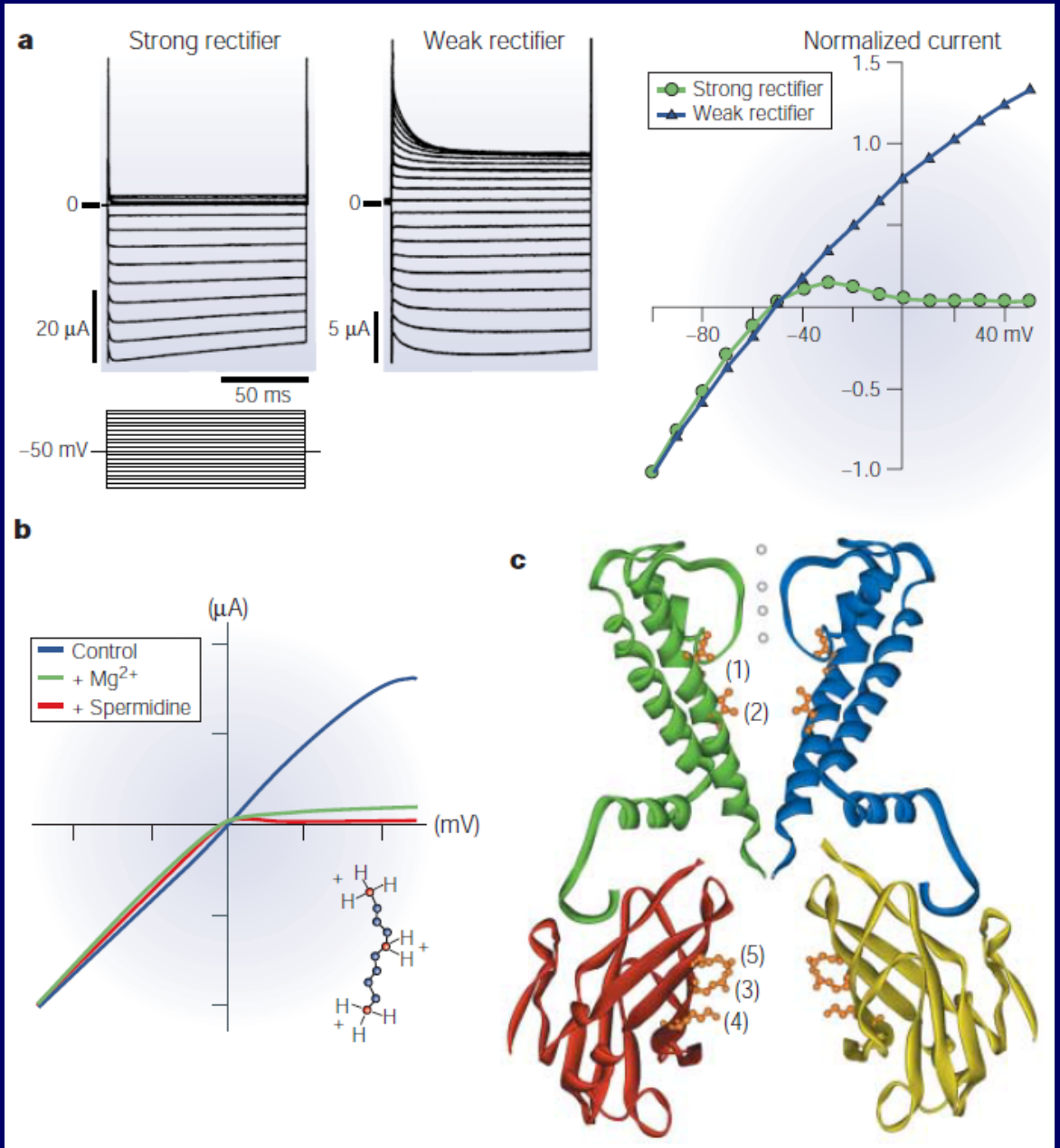
Overall architecture of K_{ir} channels



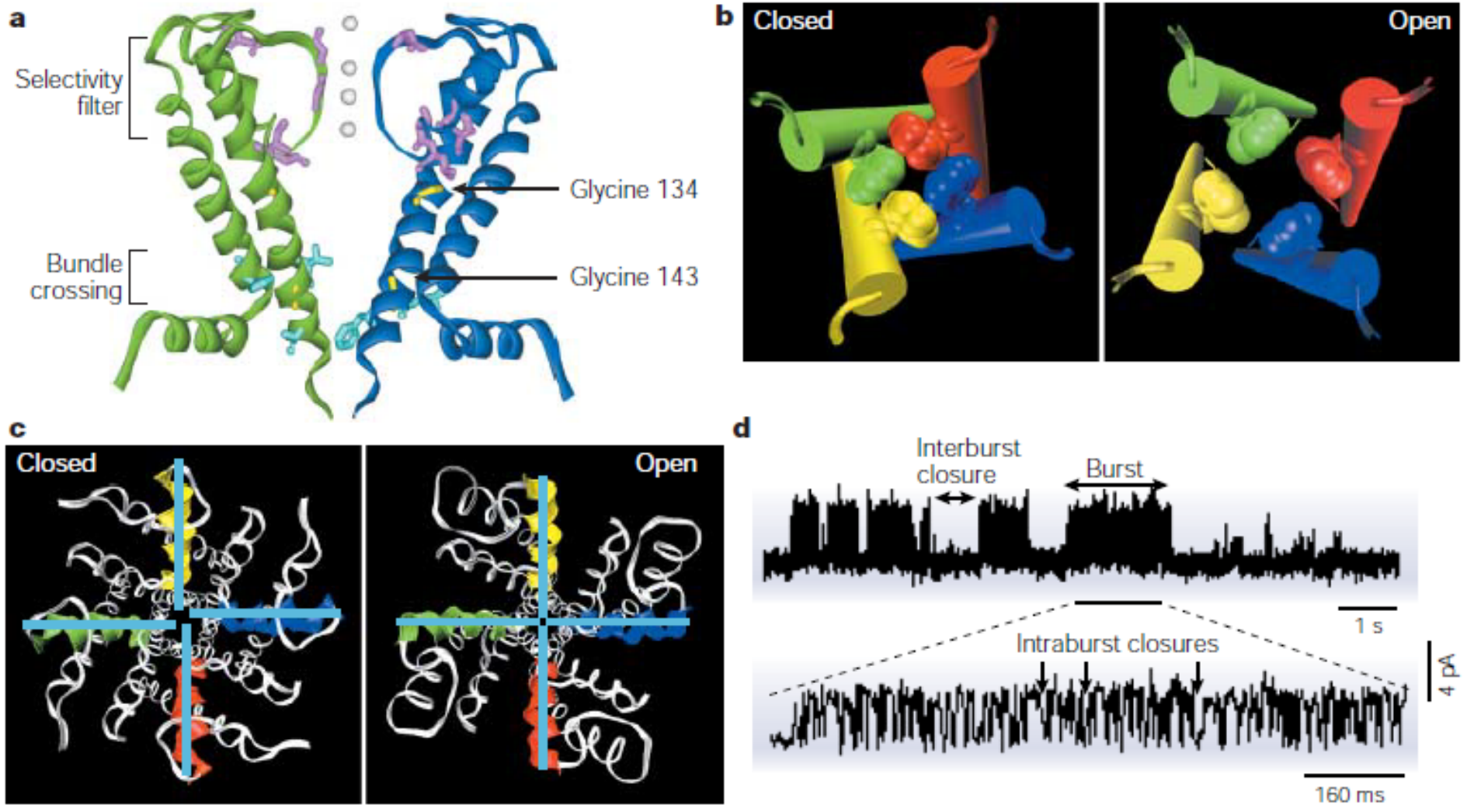
Structures of the cytoplasmic domains of KirBac1.1 and Kir3.1



Inward rectification



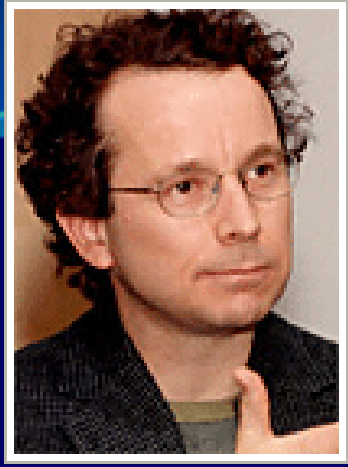
Gating of Kir channels



Structure of K^+ channels

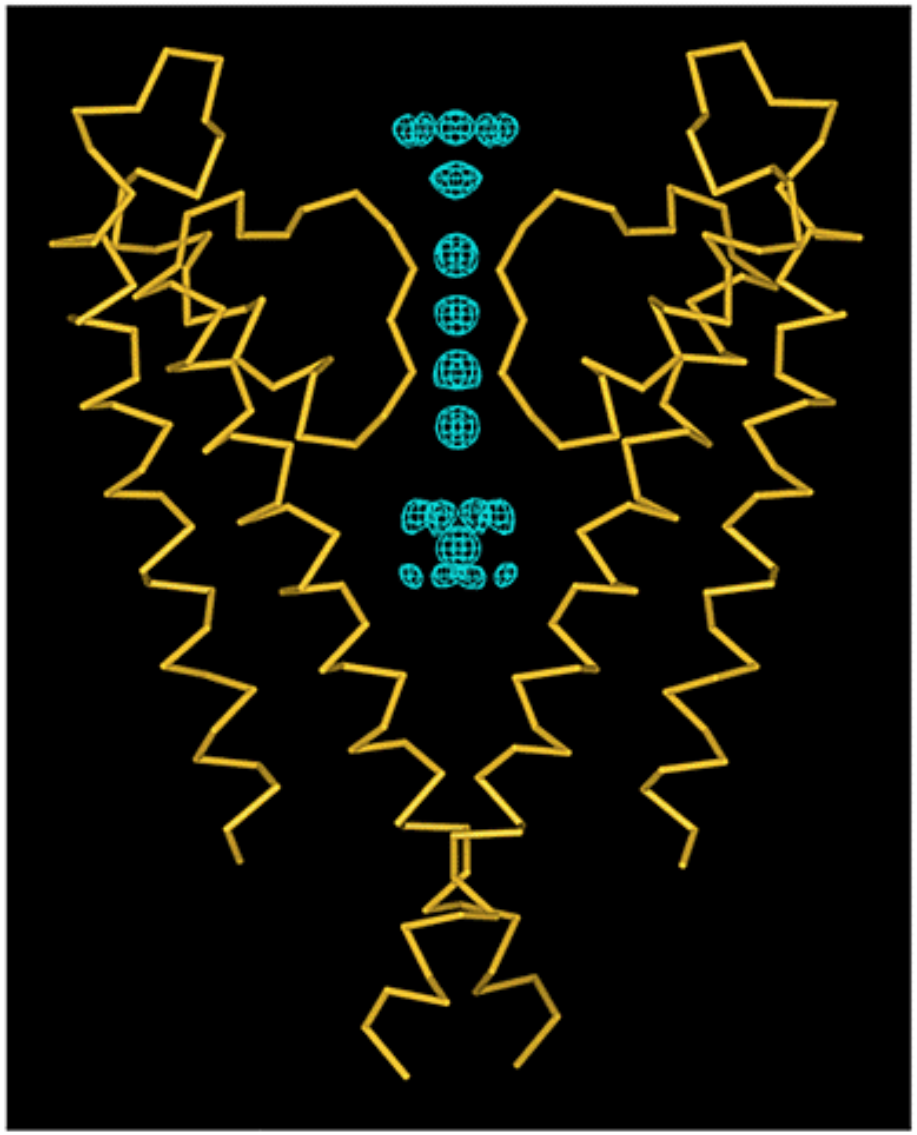
- Potassium channels have a tetrameric structure in which four identical protein subunits associate to form a four-fold symmetric (C4) complex arranged around a central ion conducting pore (i.e., a homotetramer). Alternatively four related but not identical protein subunits may associate to form heterotetrameric complexes with pseudo C4 symmetry.
- All potassium channel subunits have a distinctive pore-loop structure that lines the top of the pore and is responsible for potassium selective permeability.
- There are over 80 mammalian genes that encode potassium channel subunits.
- Using X-ray crystallography, profound insights have been gained into how potassium ions pass through these channels and why (smaller) sodium ions do not.

Dr. John Roderick Rockefeller

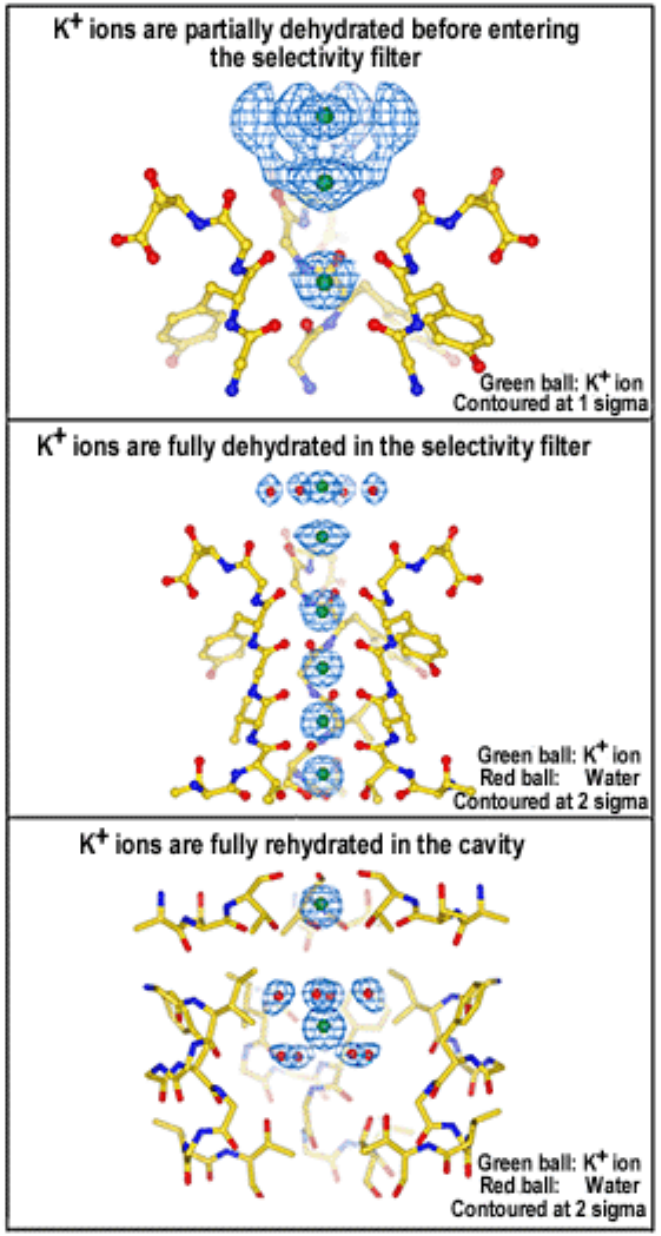


- won the 2003 Nobel Prize in Chemistry for discoveries concerning channels in cell membranes
- a Howard Hughes Medical Institute (HHMI) investigator at The Rockefeller University, a member of the National Academy of Sciences
- determined the three-dimensional structure of a pore that allows cells to control their intake of potassium ions
- solved a riddle that has perplexed biophysicists for decades: How does a potassium channel admit millions of potassium ions per second, while allowing only one smaller sodium ion to slip through for every 1,000 potassium ions?

Passage of K^+ ions through the channels



Two subunits of the KcsA tetramer are shown as an α-carbon trace in yellow color.
Electron density is contoured at 2 sigma.



Classes of K^+ channels

- **Calcium-activated potassium channel**
  - **open in response to the presence of calcium ions or other signalling molecules.**
- **Inwardly rectifying potassium channel**
  - **passes current (positive charge) more easily in the inward direction (into the cell).**
- **Tandem pore domain potassium channel**
  - **are constitutively open or possess high basal activation, such as the "resting potassium channels" or "leak channels" that set the negative membrane potential of neurons. When open, they allow potassium ions to cross the membrane at a rate which is nearly as fast as their diffusion through bulk water.**
- **Voltage-gated potassium channel**
  - **are voltage-gated ion channels that open or close in response to changes in the transmembrane voltage.**

Potassium channel classes, function, and pharmacology

Class	Subclasses	Function	Blockers	Activators
Calcium-activated 6T & 1P	• BK channel • SK channel	• inhibition following stimuli increasing intracellular calcium	• apamin • charybdotoxin	• 1-EBIO • NS309 • CyPPA
Inwardly rectifying 2T & 1P	• ROMK ($K_{ir}1.1$)	• recycling and secretion of potassium in nephrons	• Nonselective: Ba^{2+}, Cs^{+}	• none
	• GPCR regulated ($K_{ir}3.x$)	• mediate the inhibitory effect of many GPCRs	• GPCR antagonists • ifenprodil[5]	• GPCR agonists
	• ATP-sensitive ($K_{ir}6.x$)	• close when ATP is high to promote insulin secretion	• glibenclamide • tolbutamide	• diazoxide • pinacidil
Tandem pore domain 4T & 2P	• TWIK • TRAAK • TREK • TASK	• Contribute to resting potential	• none	• halothane
Voltage-gated 6T & 1P	• hERG ($K_v11.1$) • KvLQT1 ($K_v7.1$)	• action potential repolarization • limits frequency of action potentials (disturbances cause dysrhythmia)	• tetraethylammonium • 4-aminopyridine • dendrotoxins (some types)	• retigabine (K_v7) [6]

Overview of calcium-activated potassium channels

- The second major group of six/seven transmembrane potassium-selective channels consists of the K_{Ca} channels
- These channels are form two well defined but only distantly related groups

Function of K_{Ca} channels

- In excitable cells such as neurons, they shape action potentials and set the resting membrane potential.
  - By contributing to the regulation of the action potential duration in cardiac muscle, malfunction of potassium channels may cause life-threatening arrhythmias.
- They regulate cellular processes such as the secretion of hormones (e.g., insulin release from beta-cells in the pancreas)
  - so their malfunction can lead to diseases (such as diabetes)

Calcium-activated potassium channels

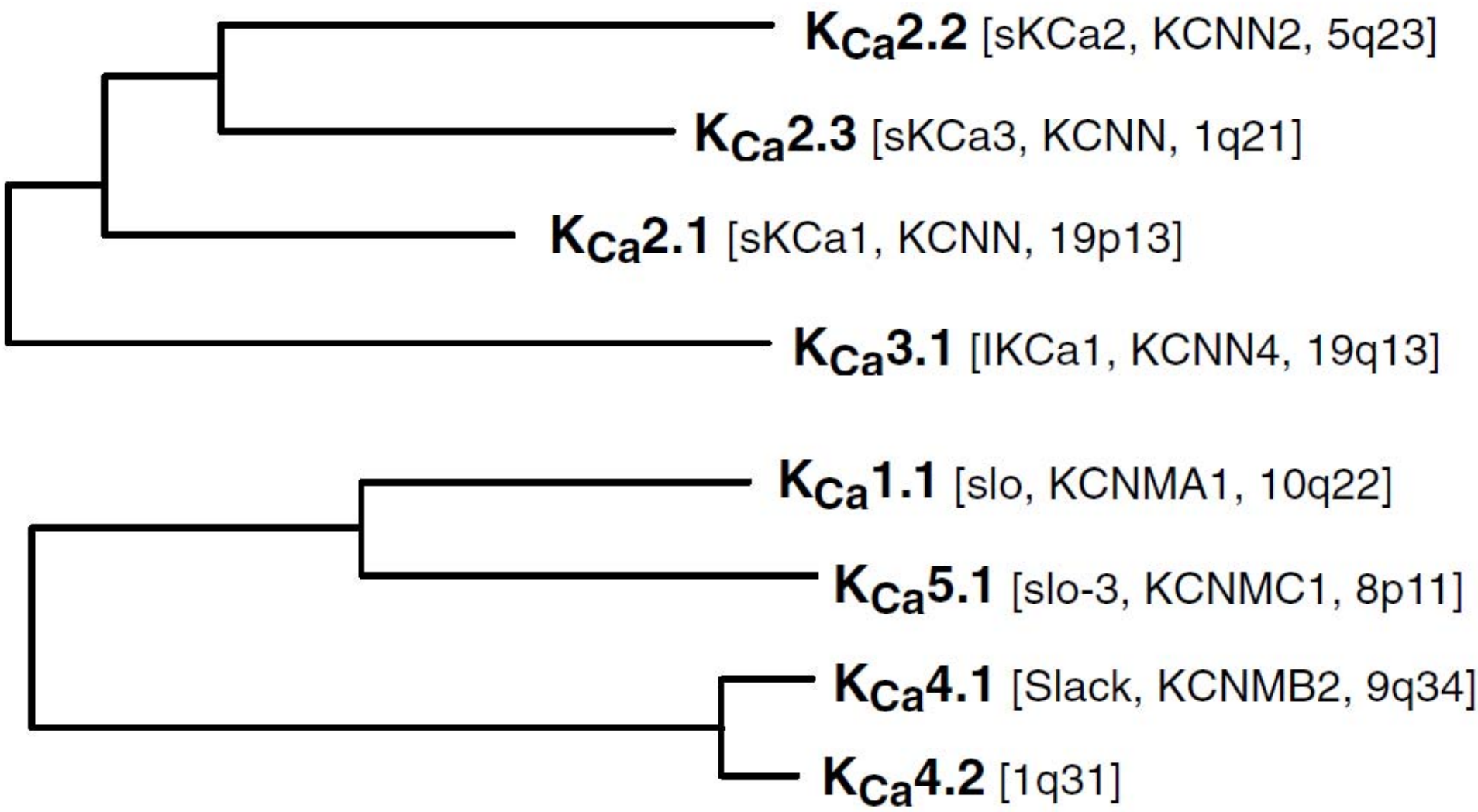
Family name	Physiological ion	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
Calcium-Activated Potassium Channels	K^+	$K_{Ca}1.1$	KCNMA1	kcnma1	kcnma1
Calcium-Activated Potassium Channels	K^+	$K_{Ca}2.1$	KCNN1	kcnn1	kcnn1
Calcium-Activated Potassium Channels	K^+	$K_{Ca}2.2$	KCNN2	kcnn2	kcnn2
Calcium-Activated Potassium Channels	K^+	$K_{Ca}2.3$	KCNN3	Kcnn3	Kcnn3
Calcium-Activated Potassium Channels	K^+	$K_{Ca}3.1$	KCNN4	Kcnn4	Kcnn4
Calcium-Activated Potassium Channels	K^+	$K_{Ca}4.1$	KCNT1	Kcnt1	Kcnt1
Calcium-Activated Potassium Channels	K^+	$K_{Ca}4.2$	KCNT2	Kcnt2	Kcnt2
Calcium-Activated Potassium Channels	K^+	$K_{Ca}5.1$	KCNU1	kcnu1	kcnu1

Other commonly used names of the K_{Ca} group

IUPHAR	HGNC	Other commonly used names
$K_{Ca}1.1$	*KCNMA1*	Slo, Slo1, BK
$K_{Ca}2.1$	*KCNN1*	$SK_{Ca}1$
$K_{Ca}2.2$	*KCNN2*	$SK_{Ca}2$
$K_{Ca}2.3$	*KCNN3*	$SK_{Ca}3$
$K_{Ca}3.1$	*KCNN4*	$IK_{Ca}1$
$K_{Ca}4.1$	*KCNT1*	Slack, Slo2.2
$K_{Ca}4.2$	*KCNT2*	Slick, Slo2.1
$K_{Ca}5.1$	*KCNU1*	Slo3

BK, big-conductance K^+ channel; SK, small-conductance K^+ channel; IK, intermediate-conductance K^+ channel

Phylogenetic tree for K_{Ca} channels



George A et al. 2009, Calcium-activated potassium channels, introductory chapter.

- $K_{Ca}1.1$ (KCNMA1, Slo1) is activated by both voltage and internal Ca^{2+}.
- $K_{Ca}2.1$, 2.2, and 2.3 ("small-conductance" K_{Ca} channels) & $K_{Ca}3.1$ ("intermediate-conductance" channel) are voltage-insensitive and are activated by low concentrations of internal Ca^{2+} (<1.0 μM). They play important roles in many processes involving Ca^{2+}-dependent signaling in both electrically excitable and nonexcitable cells. They do not bind Ca^{2+} directly but rather detect Ca^{2+} by virtue of calmodulin, which is constitutively bound to the C-terminal region. Binding of calcium to this calmodulin results in conformational changes that are in turn responsible for channel gating.
  - $K_{Ca}2.1$, 2.2, and 2.3 are sensitive to block by apamin (100 pM-10 nM), which distinguishes them from all other K_{Ca} channels.
- $K_{Ca}4.1$, 4.2, and 5.2, were all included in the K_{Ca} nomenclature since they all are clearly members of this structurally related group of genes. These three are insensitive to internal Ca^{2+}. $K_{Ca}4.2$ and $K_{Ca}4.1$ are activated by internal Na^{+} and Cl^{-}, and $K_{Ca}5.1$ is activated by internal alkalization (OH^{-}).

Animal research

- Genetic approaches include screening for behavioral changes in animals with mutations in K^+ channel genes. Such genetic methods allowed the genetic identification of the "Shaker" K^+ channel gene in Drosophila before ion channel gene sequences were well known.
- Study of the altered properties of voltage-gated K^+ channel proteins produced by mutated genes has helped reveal the functional roles of K^+ channel protein domains and even individual amino acids within their structures.
- These channels have been studied by X-ray diffraction, allowing determination of structural features at atomic resolution.
- The function of these channels is explored by electro-physiological studies.

Selectivity

- Voltage-gated K^+ channels are selective for K^+ over other cations such as Na^+.
- Channel mutation studies revealed the parts of the subunits that are essential for ion selectivity and showed that the **H5 segment** is essential for K^+ selectivity. H5 includes the amino acid sequence (Thr-Val-Gly-Tyr-Gly) or (Thr-Val-Gly-Phe-Gly) found in all K^+ channels, with only minor changes through evolution.
- As K^+ passes through the pore, **interactions** between potassium ions and water molecules are prevented and the K^+ interacts with specific atomic components of the Thr-Val-Gly-X-Gly sequences from the four channel subunits.
- It seems illogical at first that a channel should be able to allow potassium ions but not the smaller sodium ions through. However in an aqueous environment, potassium and sodium cations are solvated by water molecules.

Selectivity filter

- There is a selectivity filter at the narrowest part of the transmembrane pore.
- The selectivity filter is formed by five residues (TVGYG-in prokaryotic species) in the P loop from each subunit which have their electro-negative carbonyl oxygen atoms aligned towards the centre of the filter pore and form an anti-prism similar to a water solvating shell around each potassium binding site.
- The selectivity filter that determines which cation (e.g., Na^+ or K^+) can pass through a channel is located at the narrowest part.
- When moving through the selectivity filter of the potassium channel, these solvated water molecules are replaced by backbone carbonyl groups of the channel protein.

Selectivity filter

- The diameter of the selectivity filter is ideal for the potassium cation, but too big for the smaller sodium cation. Hence the potassium cations are well "solvated" by the protein carbonyl groups, but these same carbonyl groups are too far apart to adequately solvate the sodium cation.
- The passage of potassium cations through this selectivity filter is strongly favored over sodium cations. Passage of sodium ions would be energetically unfavorable since the strong interactions between the filter and pore helix would prevent the channel from collapsing to the smaller sodium ion size.
- Potassium ion channels remove the hydration shell from the ion when it enters the selectivity filter.

Selectivity filter

- The distance between the carbonyl oxygens and potassium ions in the binding sites of the selectivity filter is the same as between water oxygens in the first hydration shell and a potassium ion in water solution.
- The selectivity filter opens towards the extracellular solution, exposing four carbonyl oxygens in a glycine residue (Gly79 in KcsA). The next residue towards the extracellular side of the protein is the negatively charged Asp80 (KcsA).
- This residue together with the five filter residues form the pore that connects the water filled cavity in the centre of the protein with the extracellular solution.
- The carbonyl oxygens are strongly electro-negative and cation attractive.

Selectivity filter

- The filter can accommodate potassium ions at 4 sites usually labelled S1 to S4 starting at the extracellular side. In addition one ion can bind in the cavity at a site called SC or one or more ions at the extracellular side at more or less well defined sites called S0 or Sext. Several different occupancies of these sites are possible.
- Since the X-ray structures are averages over many molecules, it is, however, not possible to deduce the actual occupancies directly from such a structure. In general, there is some disadvantage due to electrostatic repulsion to have two neighbouring sites occupied by ions.
- The mechanism for ion translocation in KcsA has been studied extensively by simulation techniques.

Selectivity filter

- A complete map of the free energies of the 24=16 states (characterised by the occupancy of the S1, S2, S3 and S4 sites) has been calculated with molecular dynamics simulations resulting in the prediction of an ion conduction mechanism in which the two doubly occupied states (S1, S3) and (S2, S4) play an essential role.
- The two extracellular states, Sext and S0, were found in a better resolved structure of KcsA at high potassium concentration. In free energy calculations the entire ionic pathway from the cavity, through the four filter sites out to S0 and Sext was covered in MD simulations.
- The amino acids sequence of the selectivity filter of potassium ion channels is conserved with the exception that an isoleucine residue in eukaryotic potassium ion channels often is substituted with a valine residue in prokaryotic channels.

Central Cavity

- A **10 Å wide central pore** is located near the center of the transmembrane channel where the energy barrier is highest for the transversing ion due to the hydrophobity of the channel wall.
- The water-filled cavity and the polar C-terminus of the pore helices ease the energetic barrier for the ion. Repulsion by preceding multiple potassium ions is thought to aid the throughput of the ions.
- The presence of the cavity can be understood intuitively as one of the channel's mechanisms for overcoming the dielectric barrier, or repulsion by the low-dielectric membrane, by keeping the K^+ ion in a watery, high-dielectric environment.

Calcium-activated potassium channel

Inwardly rectifying potassium channel

Tandem pore domain potassium channel

Voltage-gated potassium channel

Overview of inward rectification

- A channel that is "inwardly-rectifying" is one that passes current (positive charge) more easily in the inward direction (into the cell). It is thought that this current may play an important role in regulating neuronal activity, by helping to establish the resting membrane potential of the cell.
- By convention, inward current is displayed in voltage clamp as a downward deflection, while an outward current (positive charge moving out of the cell) is shown as an upward deflection.
- At membrane potentials negative to the potassium's reversal potential, inwardly rectifying K^+ channels support the flow of positively charged K^+ ions into the cell, pushing the membrane potential back to the resting potential.
- When the membrane potential is clamped negative to the channel's resting potential (e.g. -60 mV), inward current flows (i.e. positive charge flows into the cell). However, when the membrane potential is set positive to the channel's resting potential (e.g. +60 mV), these channels pass very little charge out of the cell.

Overview of inward rectification

- This channel passes much more current in the inward direction than the outward one. Note that these channels are not perfect rectifiers, as they can pass some outward current in the voltage range up to about 30 mV above resting potential.
- These channels differ from the potassium channels that are typically responsible for repolarizing a cell following an action potential, such as the delayed rectifier and A-type potassium channels. Those more "typical" potassium channels preferentially carry outward (rather than inward) potassium currents at depolarized membrane potentials, and may be thought of as "outwardly rectifying."
- When first discovered, inward rectification was named "anomalous rectification" to distinguish it from outward potassium currents.

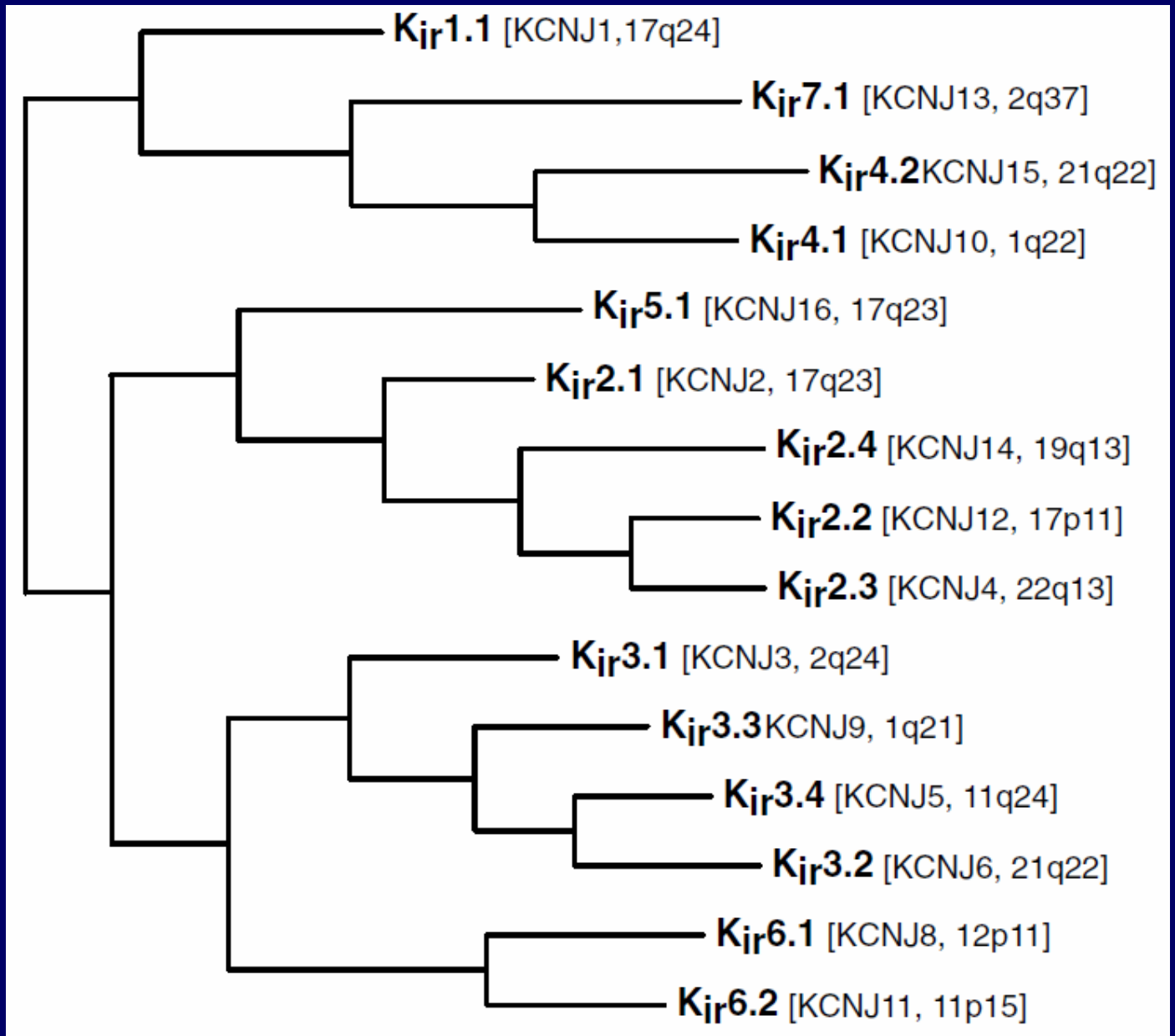
Overview of inward rectification

- Inward rectifiers also differ from tandem pore domain potassium channels, which are largely responsible for "leak" K^+ currents.
- Some inward rectifiers, termed "weak inward rectifiers," carry measurable outward K^+ currents at voltages positive to the K^+ reversal potential (corresponding to, but larger than, the small currents above the 0 nA). They, along with the "leak" channels, establish the resting membrane potential of the cell.
- Other inwardly rectifying channels, termed "strong inward rectifiers," carry very little outward current at all, and are mainly active at voltages negative to the reversal potential, where they carry inward current (the much larger currents below the 0 nA).
- K_{ir} channels are found in multiple cell types, including macrophages, cardiac and kidney cells, leukocytes, neurons, and endothelial cells.

Mechanism of inward rectification

- The phenomenon of inward rectification of K_{ir} channels is the result of high-affinity block by endogenous polyamines, namely **spermine**, as well as **magnesium ions**, that plug the channel pore at positive potentials, resulting in a decrease in outward currents.
- This voltage-dependent block by polyamines causes currents to be conducted well only in the inward direction. While the principal idea of polyamine block is understood, the specific mechanisms are still controversial.

Phylogenetic tree for K_{ir} channels



The newer notable work

- The interaction of $K_{ir}1.1$ with Na^+/H^+ exchange regulatory factor 2 in the postsynaptic density 95/disc-large/zona occludens (PDZ) complex
- The assembly of $K_{ir}2.1$ channels with synapse-associated protein 97 (SAP97), calmodulin-dependent serine protein kinase (CASK), Veli, and Mint1 was shown. $K_{ir}2.1$ contributed to protein trafficking
- $K_{ir}2.2$ in muscle was associated with the dystrophin-glycoprotein complex via α-syntrophin
- $K_{ir}4.1$ was associated with the dystrophin-glycoprotein complex via α-syntrophin and the epilepsy in both causative and protective roles. The loss of K_{ir} 4.1 expression abolishes endocochlear potential and causes deafness in Pendred syndrome.
- The disruption of $K_{ir}6.1$ gene in mice causes phenotypes similar to those of vasospastic (Prinzmetal) angina.
- An activating mutation of $K_{ir}6.2$ causes permanent neonatal diabetes.

Recent developments in the K_{ir} field

- X-ray crystal structures analysis is extremely valuable since it is enable more precise approaches to establishing structure-function relationships.
  - demonstrated that inward rectifier K^+ channels have a long cytoplasmic pore and confirmed the significance of negatively charged amino acids on the wall of the cytoplasmic pore that have been known to play critical roles for inward rectification.
  - provided structure-based clues for the regulation mechanisms of gating by ligands such as G proteins and phosphatidylinositol 4,5-bisphosphate.
- Noteworthy are published studies on the dynamic aspects of channel function using fluorescence resonance energy transfer analysis of fluorescent-labeled molecules. Knowledge of these dynamic aspects of K_{ir} channel function may also be expected to expand in the near future.
- A great deal of additional knowledge on K_{ir} function, structure-function relationships, regulation of expression, and links with diseases has been accumulated.

Biochemistry of K_{ir} channels

- There are seven subfamilies of K_{ir} channels, denoted as K_{ir}1 - Kir7. Each subfamily has multiple members (i.e. K_{ir}2.1, K_{ir}2.2, K_{ir}2.3, etc) that have nearly identical amino acid sequences across known mammalian species.
- K_{ir} channels are formed from as homotetrameric membrane proteins. Each of the four identical protein subunits is composed of two membrane-spanning alpha helices (M1 and M2).
- Heterotetramers can form between members of the same subfamily (i.e. K_{ir}2.1 and K_{ir}2.3) when the channels are overexpressed.

Diversity

Gene	Protein	Aliases	Associated subunits
KCNJ1	$K_{ir}1.1$	ROMK1	NHERF2
KCNJ2	$K_{ir}2.1$	IRK1	$K_{ir}2.2$, $K_{ir}4.1$, PSD-95, SAP97, AKAP79
KCNJ12	$K_{ir}2.2$	IRK2	$K_{ir}2.1$ and $K_{ir}2.3$ to form heteromeric channel, auxiliary subunit: SAP97, Veli-1, Veli-3, PSD-95
KCNJ4	$K_{ir}2.3$	IRK3	$K_{ir}2.1$ and $K_{ir}2.3$ to form heteromeric channel, PSD-95, Chapsyn-110/PSD-93
KCNJ14	$K_{ir}2.4$	IRK4	$K_{ir}2.1$ to form heteromeric channel
KCNJ3	$K_{ir}3.1$	GIRK1, KGA	$K_{ir}3.2$, $K_{ir}3.4$, $K_{ir}3.5$, $K_{ir}3.1$ is not functional by itself
KCNJ6	$K_{ir}3.2$	GIRK2	$K_{ir}3.1$, $K_{ir}3.3$, $K_{ir}3.4$ to form heteromeric channel
KCNJ9	$K_{ir}3.3$	GIRK3	$K_{ir}3.1$, $K_{ir}3.2$ to form heteromeric channel
KCNJ5	$K_{ir}3.4$	GIRK4	$K_{ir}3.1$, $K_{ir}3.2$, $K_{ir}3.3$
KCNJ10	$K_{ir}4.1$	$K_{ir}1.2$	$K_{ir}4.2$, $K_{ir}5.1$, and $K_{ir}2.1$ to form heteromeric channels
KCNJ15	$K_{ir}4.2$	$K_{ir}1.3$	
KCNJ16	$K_{ir}5.1$	BIR 9	
KCNJ8	$K_{ir}6.1$	K_{ATP}	SUR2B
KCNJ11	$K_{ir}6.2$	K_{ATP}	SUR1, SUR2A, and SUR2B
KCNJ13	$K_{ir}7.1$	$K_{ir}1.4$	

Role of K_{ir} channels

in cellular physiology vary across cell types

- **Maintain resting membrane potential near equilibrium potential for K^+ ions**
- **Contribute to cell excitability**
- **Mediate a small hyperpolarizing K^+ current at negative membrane potentials**
- **Mediate inhibitory neurotransmitter responses**
- **Excitable tissues: Heart, brain, skeletal muscle**
- **Non-conducting at positive membrane potentials**

Role of K_{ir} channels

Location	Function
cardiac myocytes	Kir channels close upon depolarization, slowing membrane repolarization and helping maintain a more prolonged cardiac action potential. This type of inward-rectifier channel is distinct from delayed rectifier K^+ channels, which help repolarize nerve and muscle cells after action potentials; and potassium leak channels, which provide much of the basis for the resting membrane potential.
endothelial cells	Kir channels are involved in regulation of nitric oxide synthase.
kidneys	Kir export surplus potassium into collecting tubules for removal in the urine, or alternatively may be involved in the reuptake of potassium back into the body.
neurons and in heart cells	G-protein activated IRKs (Kir3) are important regulators, modulated by neurotransmitters. A mutation in the GIRK2 channel leads to the weaver mouse mutation. "Weaver" mutant mice are ataxic and display a neuroinflammation-mediated degeneration of their dopaminergic neurons. Weaver mice have been examined in labs interested in neural development and disease for over 30 years.
pancreatic beta cells	KATP channels (composed of Kir6.2 and SUR1 subunits) control insulin release.

Diseases related to K_{ir} channels

- Persistent hyperinsulinemic hypoglycemia of infancy is related to autosomal recessive mutations in K_{ir}6.2. Certain mutations of this gene diminish the channel's ability to regulate insulin secretion, leading to hypoglycemia.
- Bartter's syndrome can be caused by mutations in K_{ir} channels. This condition is characterized by the inability of kidneys to recycle potassium, causing low levels of potassium in the body.
- Andersen's syndrome is a rare condition caused by multiple mutations of K_{ir}2.1. Depending on the mutation, it can be dominant or recessive. It is characterized by periodic paralysis, cardiac arrhythmias and dysmorphic features.
- Barium poisoning is likely due to its ability to block K_{ir} channels.
- Atherosclerosis (heart disease) may be related to K_{ir} channels. The loss of K_{ir} currents in endothelial cells is one of the first known indicators of atherogenesis (the beginning of heart disease).

Calcium-activated potassium channel

Inwardly rectifying potassium channel

Tandem pore domain potassium channel

Voltage-gated potassium channel

Two-pore-domain potassium channels

- This family of 15 members form what is known as "leak channels" which possess Goldman-Hodgkin-Katz (open) rectification.
- These channels are regulated by several mechanisms including oxygen tension, pH, mechanical stretch, and G-proteins.
- Their name is derived from the fact that the α subunits consist of four transmembrane segments, each containing two pore loops. As such, they structurally correspond to two inward-rectifier α subunits and thus form dimers in the membrane.

Overview of K_{2P}

- In less than a decade since their discovery, the study of K_{2P} channels has revealed that background leak of potassium ions via dedicated pathways is a highly regulated mechanism to control cellular excitability.
- Potassium leak pathways, active at rest, stabilize membrane potential below firing threshold and expedite repolarization. Although the existence of leak currents was proposed in 1952 by Hodgkin and Huxley, they remained a biophysical curiosity for more than 4 decades.
- Identification of the first molecular correlate of a potassium leak current was preceded by cloning of potassium channels in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* with two pore-forming P loops in each subunit and four or eight transmembrane (TM) domains.

- The distinct 2P/4TM topology can be found in more than 70 predicted homologs in genome databases. Fifteen mammalian genes in the family are designated as *KCNK* genes encoding the K_{2P} channels; most readily reveal ion channel function upon expression.
- K_{2P} channels are under tight control by a plethora of chemical and physical stimuli, including oxygen tension, pH, lipids, mechanical stretch, neurotransmitters, and G protein-coupled receptors; the channels are also the molecular targets for certain volatile and local anesthetics.
- Regulation of K_{2P} channels alters the attributes subject to change in any ion channel: number of pores at the site of operation, open probability, and unitary current.
- Phosphorylation of $K_{2P}2$ endows the open rectifier with sensitivity to voltage, and desumoylation of $K_{2P}1$ (removal of covalently-bound small ubiquitin-modifier protein) relieves chronic silencing of complexes that reside in the plasma membrane, thereby revealing that the protein can function as an ion channel and operates like $K_{2P}Ø$ as an open rectifier.

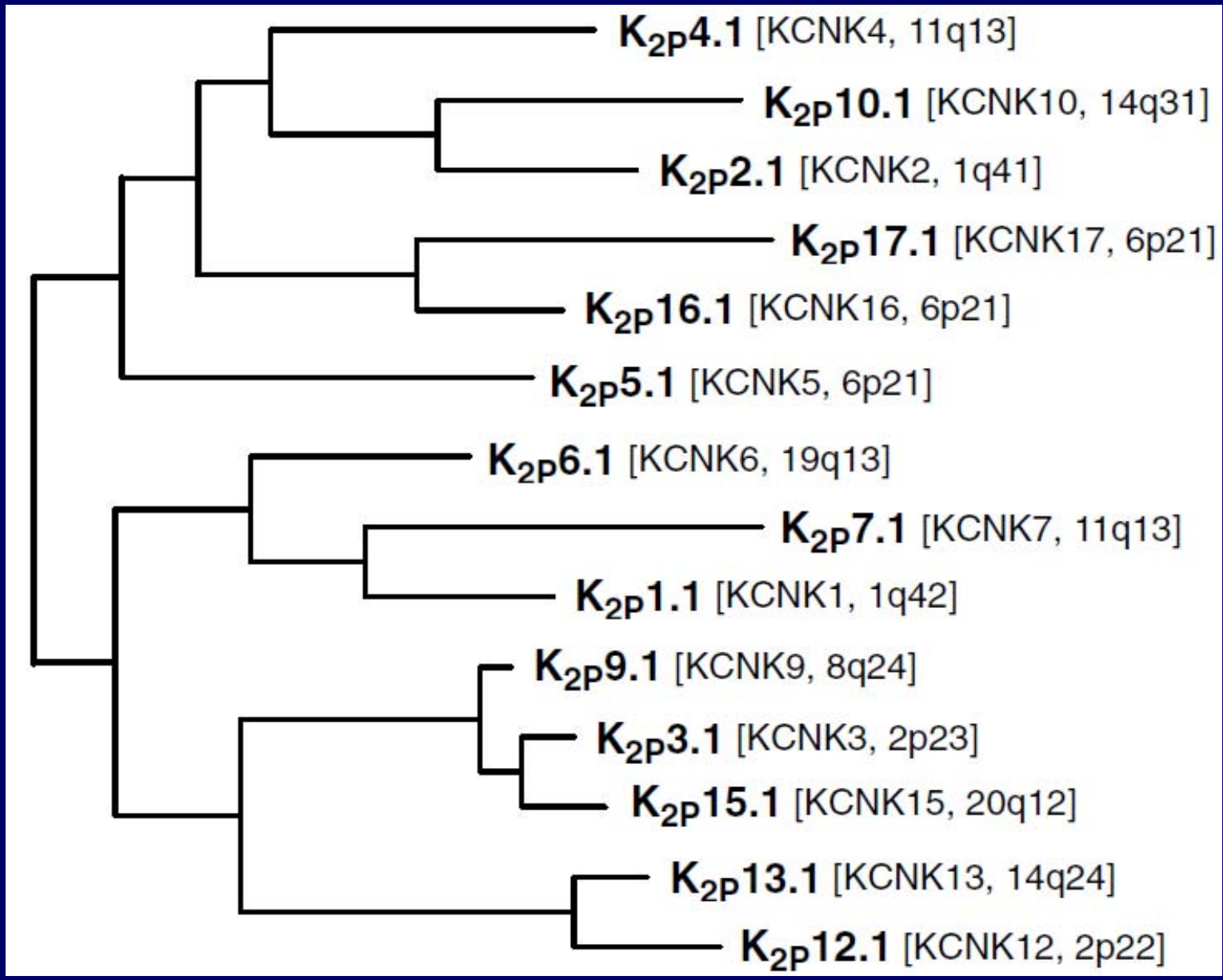
$K_{2P}Ø$

- **$K_{2P}Ø$ was isolated by functional expression cloning from the neuromuscular tissue of *Drosophilia melanogaster*.**
- **Biophysical characterization revealed $K_{2P}Ø$ to be a potassium-selective channel with the predicted attributes of a background conductance, that is, a voltage-independent portal showing Goldman-Hodgkin-Katz (open) rectification.**
- **When the concentration of potassium is symmetrical across the membrane, $K_{2P}Ø$ currents change in a linear manner with voltage; under physiological conditions (high internal and low external potassium), $K_{2P}Ø$ passes greater outward than inward currents.**

Two-pore-domain human potassium channels

Gene	Channel
KCNK1	$K_{2p}1.1$
KCNK2	$K_{2p}2.1$
KCNK3	$K_{2p}3.1$
KCNK4	$K_{2p}4.1$
KCNK5	$K_{2p}5.1$
KCNK6	$K_{2p}6.1$
KCNK7	$K_{2p}7.1$
KCNK9	$K_{2p}9.1$
KCNK10	$K_{2p}10.1$
KCNK12	$K_{2p}12.1$
KCNK13	$K_{2p}13.1$
KCNK15	$K_{2p}15.1$
KCNK16	$K_{2p}16.1$
KCNK17	$K_{2p}17.1$
KCNK18	$K_{2p}18.1$

Phylogenetic tree for K_{2P} channels



Calcium-activated potassium channel

Inwardly rectifying potassium channel

Tandem pore domain potassium channel

Voltage-gated potassium channel

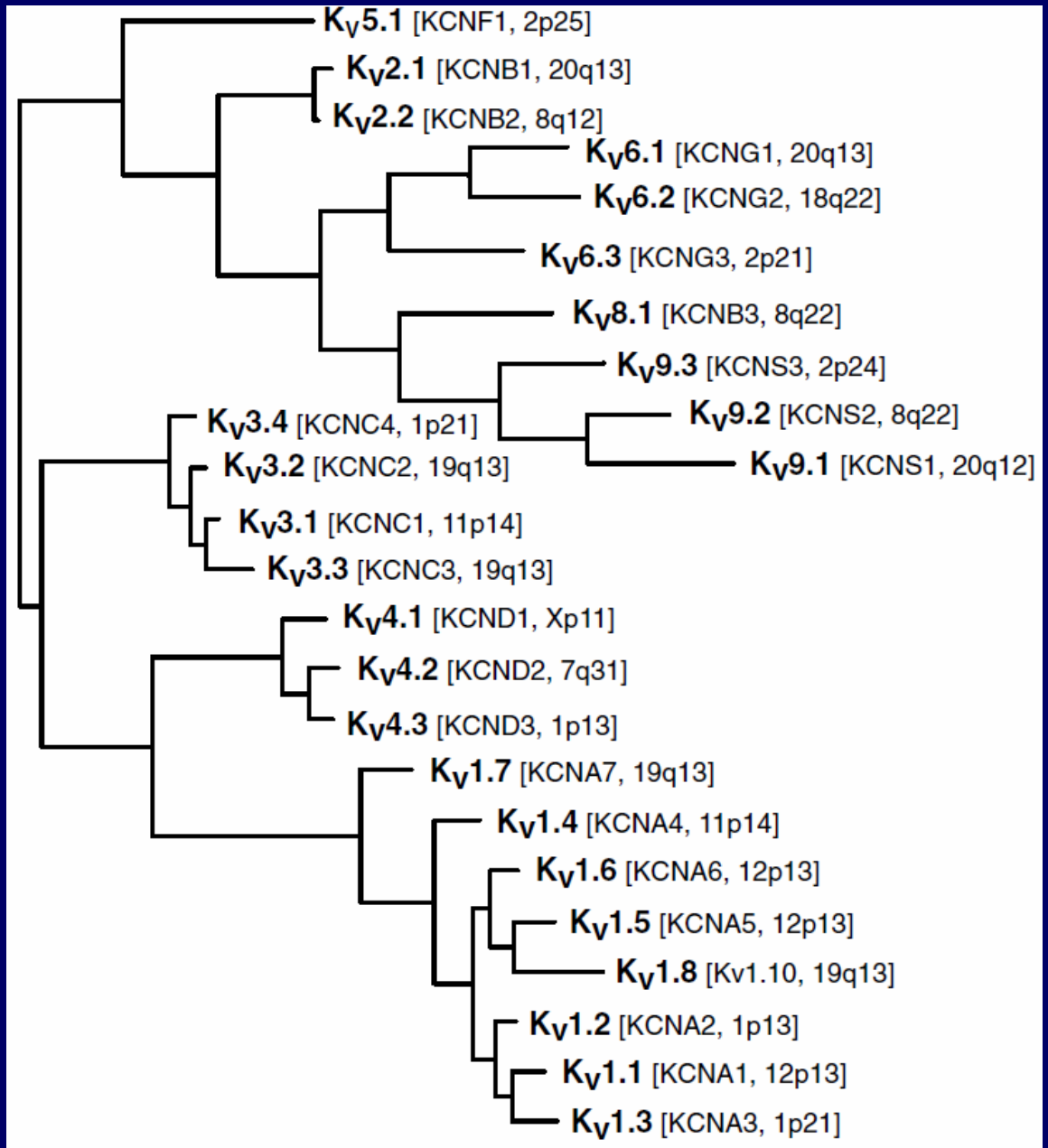
Overview of voltage-gated K^+ channels

- The voltage-gated K^+ channels (K_V), in turn, form the largest family of some 40 genes among the group of human potassium channels.
- K_V channels are central to the function of nerves and muscles. Without them the brain would immediately suffer neural gridlock, and the heart would seize up.
- K_V and K_{Ca} channels together constitute the six/seven-transmembrane group of potassium-selective channels, made up of subunits containing six or seven membrane-spanning domains, including the positively charged S4 segment, which confers on some of these channels their voltage sensitivity.

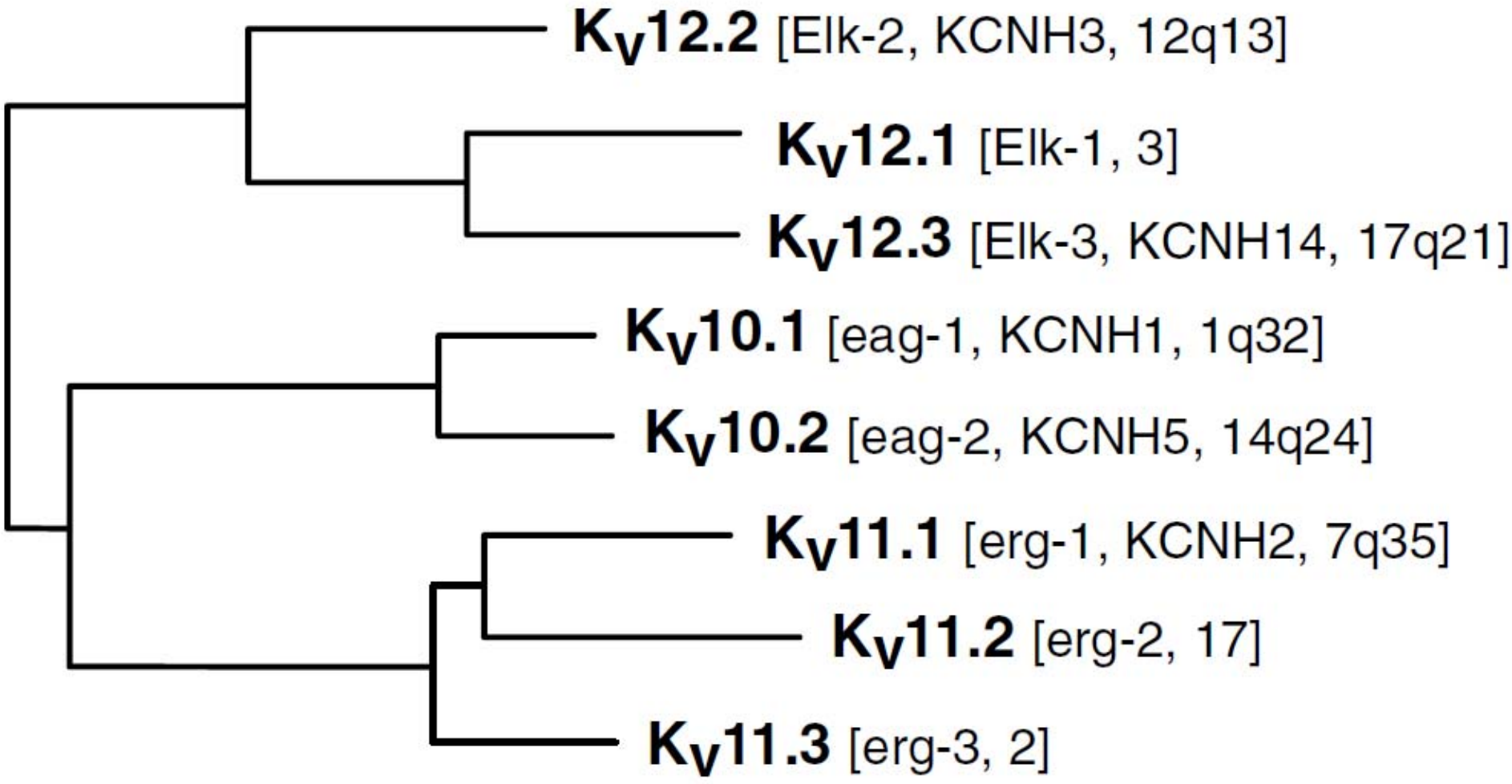
K_V channel families Gene names

IUPHAR	HGNC	Other names
Kv1.1	KCNA1	Shaker-related family
Kv1.2	KCNA2	
Kv1.3	KCNA3	
Kv1.4	KCNA4	
Kv1.5	KCNA5	
Kv1.6	KCNA6	
Kv1.7	KCNA7	
Kv1.8	KCNA10	
Kv2.1	KCNB1	Shab-related family
Kv2.2	KCNB2	
Kv3.1	KCNC1	Shaw-related family
Kv3.2	KCNC2	
Kv3.3	KCNC3	
Kv3.4	KCNC4	
Kv4.1	KCND1	Shal-related family
Kv4.2	KCND2	
Kv4.3	KCND3	
Kv5.1	KCNF1	Modifier
Kv6.1	KCNG1	Modifiers
Kv6.2	KCNG2	
Kv6.3	KCNG3	
Kv6.4	KCNG4	
Kv7.1	KCNQ1	KVLQT
Kv7.2	KCNQ2	KQT2
Kv7.3	KCNQ3	
Kv7.4	KCNQ4	
Kv7.5	KCNQ5	
Kv8.1	KCNV1	Modifiers
Kv8.2	KCNV2	
Kv9.1	KCNS1	Modifiers
Kv9.2	KCNS2	
Kv9.3	KCNS3	
Kv10.1	KCNH1	eag1
Kv10.2	KCNH5	eag2
Kv11.1	KCNH2	erg1
Kv11.2	KCNH6	erg2
Kv11.3	KCNH7	erg3
Kv12.1	KCNH8	elk1, elk3
Kv12.2	KCNH3	elk2
Kv12.3	KCNH4	elk1

Phylogenetic tree for the K_V1–9 families



Phylogenetic tree for the K_v10–12 families



Diversity of K_v channels

K_v channels form an exceedingly diverse group, much more so than one would predict simply based on the number of distinct genes that encode them.

- This diversity arises from several factors:
- *Heteromultimerization*. Each K_v gene encodes a peptide subunit, four of which are required to form a functional channel. K_v channels may be homotetramers but may also be heterotetramers formed between different subunits within the same family (in the case of the K_v1, K_v7, and K_v10 families), and these diverse heterotetramers express properties that may be considerably different from those of any of the homotetramers.
- *"Modifier" subunits.* Four of the K_v families (K_v5, 6, 8, and 9) encode subunits that act as modifiers. Although these do not produce functional channels on their own, they form heterotetramers with K_v2 family subunits, increasing the functional diversity within this family.

Diversity of K_v channels

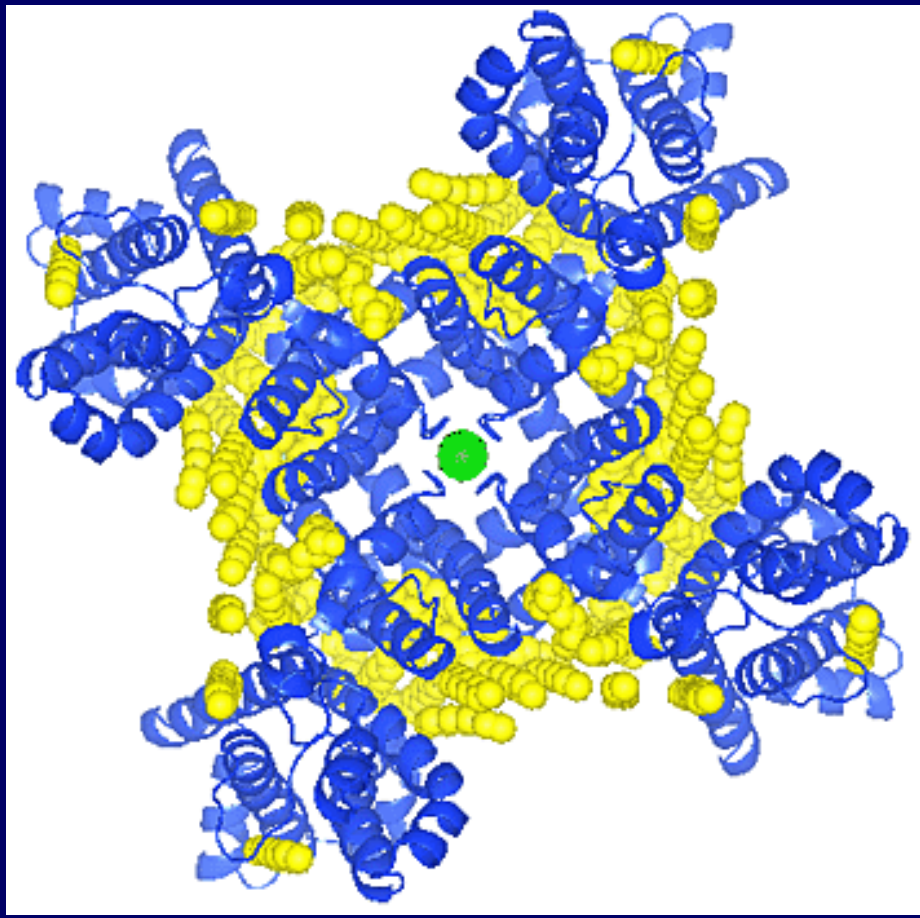
- ***Accessory proteins*. A variety of other peptides has also been shown to associate with K_v tetramers and modify their properties, including several β subunits (which associate with K_v1 and K_v2 channels), KCHIP1 (K_v4), calmodulin (K_v10), and minK (K_v11)**
- ***Alternate mRNA splicing.* A number of K_v channel genes are known to contain intronless coding regions, including all of the K_v1 family genes (with the sole exception of K_v1.7) and K_v9.3. Various members of the K_v3, 4, 6, 7, 9, 10, and 11 gene families have coding regions made up of several exons that are alternately spliced, providing yet another significant source of K_v channel functional diversity.**
- ***Post-translational modification.* Many K_v channels can be post-translationally modified by phosphorylation, ubiquitinylation, and palmitoylation, which in turn modifies channel function.**

Structure of K_v channels

- Voltage-gated K^+ channels of vertebrates typically are tetramers of four identical subunits arranged as a ring, each contributing to the wall of the trans-membrane K^+ pore. Each subunit is composed of six membrane spanning hydrophobic α-helical sequences.
- A high resolution crystallographic structure of the rat K_v α1.2/β2 channel has recently been solved (Protein Databank Accession Number 2A79), and then refined in a lipid membrane-like environment (PDB 2r9r).

Kv channel in its natural habitat

- The green sphere depicts potassium ions in the selectivity filter.
- The protein, depicted as helical ribbons (blue) consists of a central pore surrounded by four voltage sensors.
- The yellow objects represent lipid molecules, which are observed in the crystal structure.



The channel's function is likely to be profoundly influenced by lipid molecules within the cell membrane in which the channel is embedded.

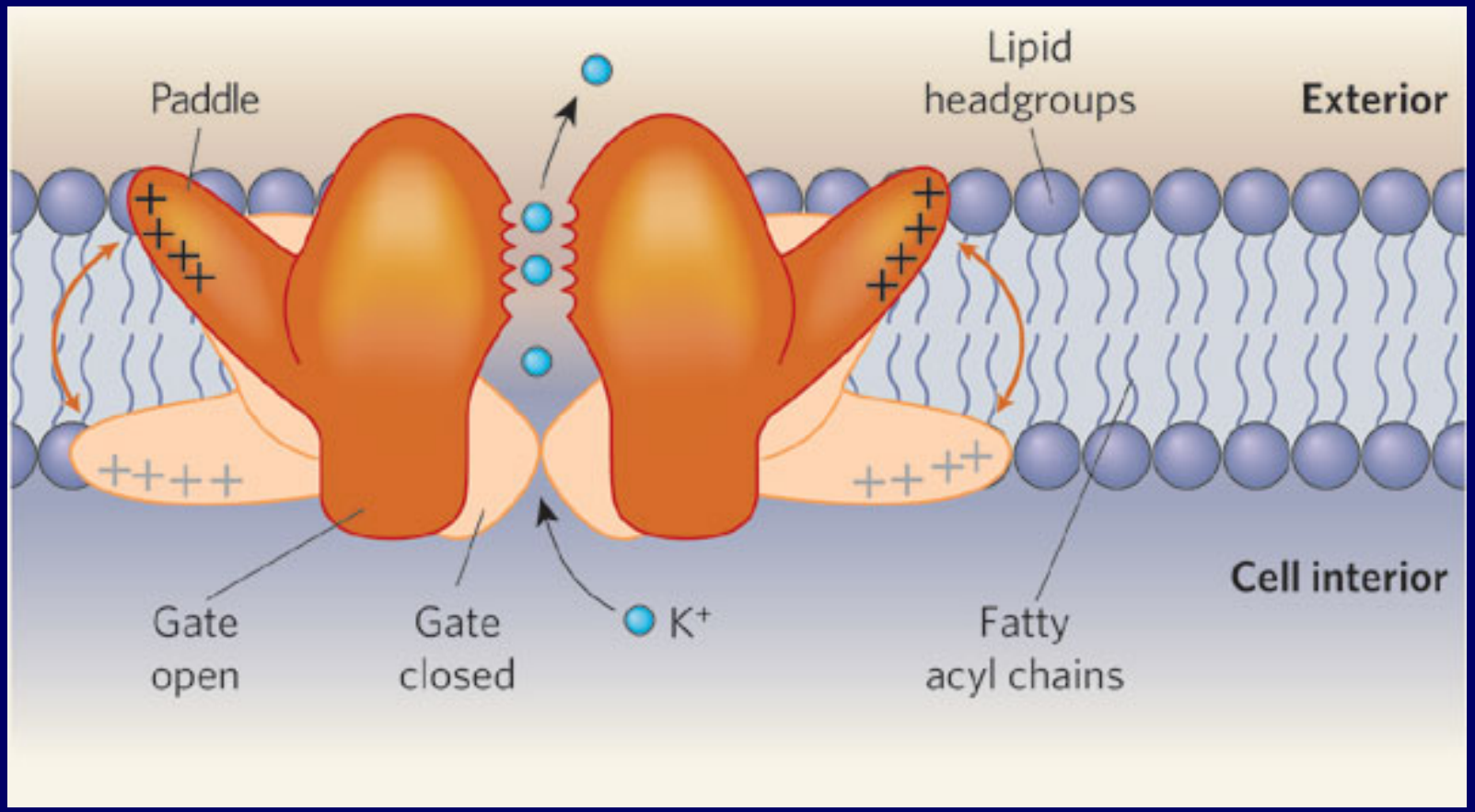
The voltage sensor

- The voltage sensor is the component of the voltage-dependent potassium ion channels that senses changes in voltage.
- The voltage sensor reacts to a change in the membrane electrical polarity to open or close the pore. The structure of the voltage sensor was determined by x-ray crystallography.
  - Protein crystals are bombarded with x-ray beams. As the x-rays pass through and bounce off of atoms in the crystal, they produce a diffraction pattern, which can then be analyzed to determine the three-dimensional shape of the protein.
- The voltage sensors contained a helix-turn-helix structure, which MacKinnon's group has called the voltage sensor paddle. The voltage sensor paddle contains positively charged amino acids that enable the voltage sensor to respond to the membrane's electrical polarity.

The voltage sensor

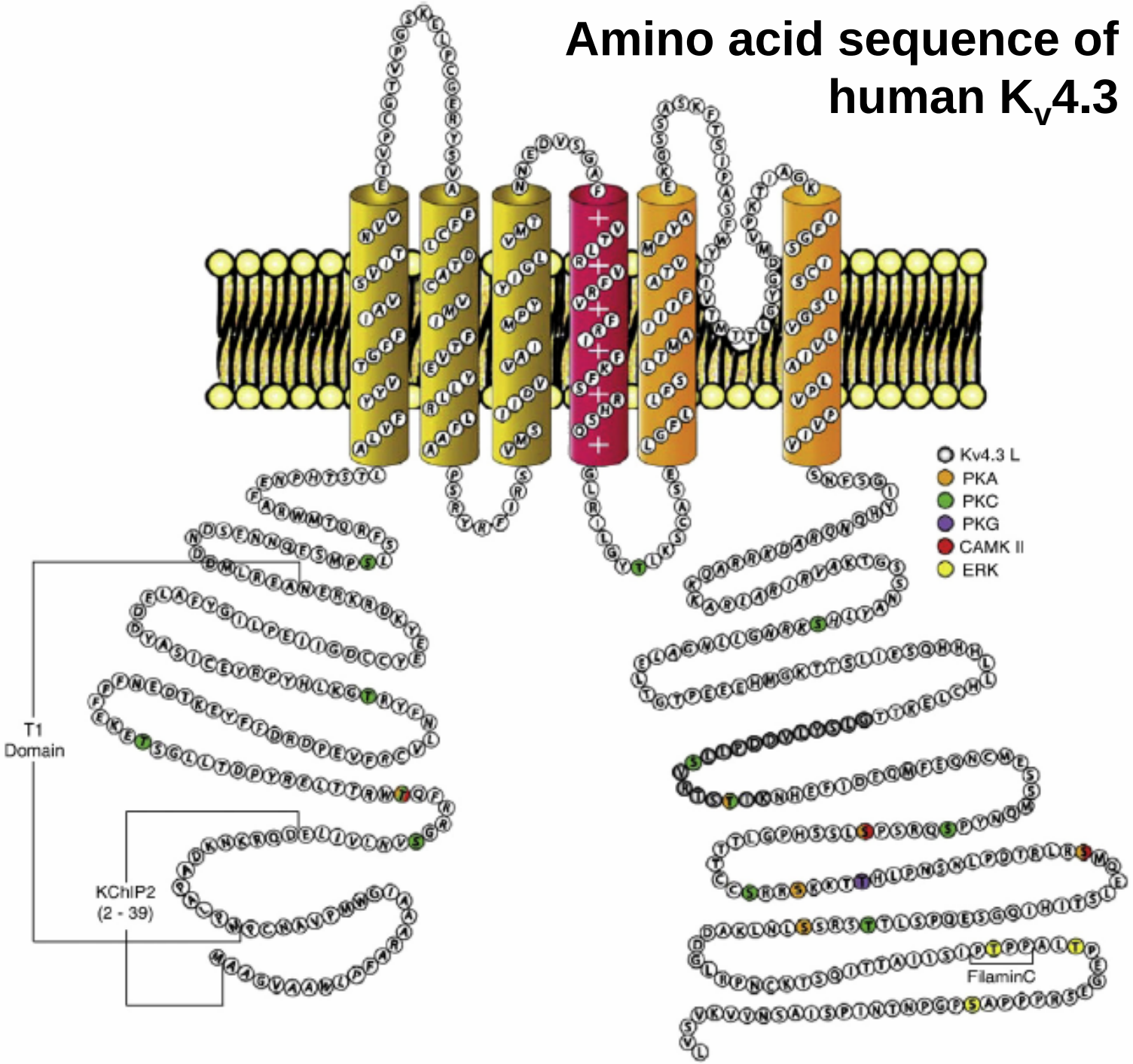
- The positively charged paddle moves within the membrane at the protein-lipid interface.
  - When the membrane becomes positively charged on the inside, the paddles is attracted to move toward the outside and open the channel, allowing potassium to flow out and restoring the membrane charge to its resting state.
  - When the inside of the membrane becomes negatively charged, the paddles move inward snapping the channel shut.
- The voltage sensors are mostly surrounded by lipid membrane, and what that means is that the voltage sensor can't help but be influenced by the lipid. This influence is so profound, that you can't simply say what the properties of a given voltage-dependent channel are without specifying the composition of the surrounding lipid. And what makes this influence of lipid biologically significant is that we know that different cells in the body do not have the same lipid composition. The lipid membrane would influence the channel's function.

The voltage sensor



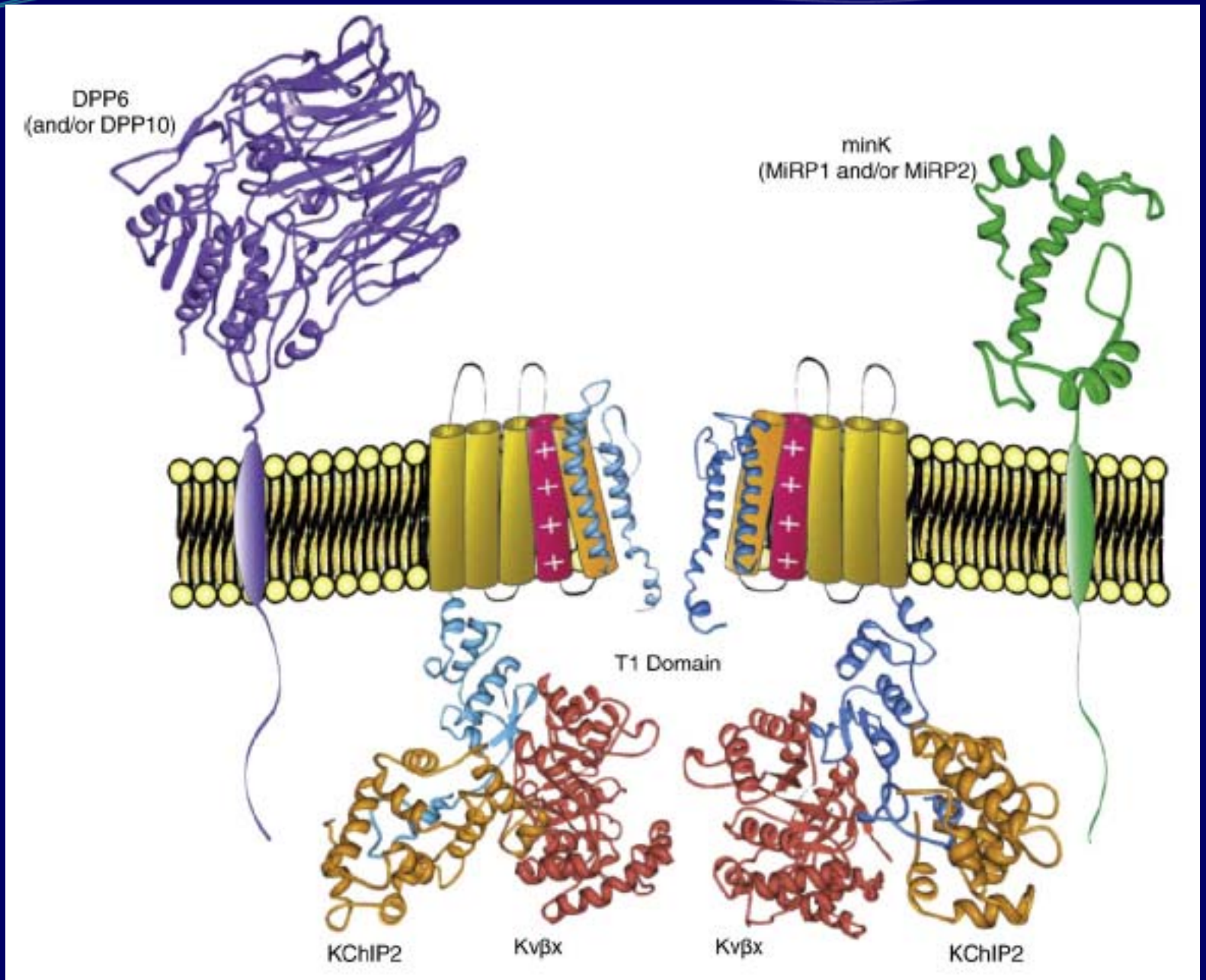
- **paddle: a helix-turn-helix structure, contains positively charged amino acids**

Amino acid sequence of human $K_v4.3$



- T1: tetramerization domain
- CaMKII: calcium–calmodulin-dependent protein kinase II
- ERK: extracellular signal regulated kinase (ERK)

K_v4.3 channel with multiple accessory subunits



Classification of Kv channels

Alpha subunits

- Delayed rectifier
- A-type potassium channel
- Outward-rectifying
- Inward-rectifying
- Slowly activating
- Modifier/silencer

Beta subunits

- Kvβ1 (KCNAB1)
- Kvβ2 (KCNAB2)
- Kvβ3 (KCNAB3) ……

Alpha subunits

- Alpha subunits form the actual conductance pore. Based on sequence homology of the hydrophobic transmembrane cores, the alpha subunits of voltage-gated potassium channels have been grouped into 12 classes labeled K_v α1-12.
- The following is a list of the 40 known human voltage-gated potassium channel alpha subunits grouped first according to function and then subgrouped according to the K_v sequence homology classification scheme:

Delayed rectifier

- Delayed activation; slowly inactivating or non-inactivating
- Allows efficient repolarization after action potential
- K_v α1.x - Shaker-related: K_v1.1 (KCNA1), K_v1.2 (KCNA2), K_v1.3 (KCNA3), K_v1.5 (KCNA5), K_v1.6 (KCNA6), K_v1.7 (KCNA7), K_v1.8 (KCNA10)
- K_v α2.x - Shab-related: K_v2.1 (KCNB1), K_v2.2 (KCNB2)
- K_v α3.x - Shaw-related: K_v3.1 (KCNC1), K_v3.2 (KCNC2)
- K_v α7.x: K_v7.1 (KCNQ1) - K_vLQT1, K_v7.2 (KCNQ2), K_v7.3 (KCNQ3), K_v7.4 (KCNQ4), K_v7.5 (KCNQ5)
- K_v α10.x: K_v10.1 (KCNH1)

A-type potassium channel

- rapidly inactivating
- K_v α1.x - Shaker-related: K_v1.4 (KCNA4)
- K_v α3.x - Shaw-related: K_v3.3 (KCNC3), K_v3.4 (KCNC4)
- K_v α4.x - Shal-related: K_v4.1 (KCND1), K_v4.2 (KCND2), K_v4.3 (KCND3)

Outward-rectifying

- K_v α10.x: K_v10.2 (KCNH5)

Inward-rectifying

- Passes current more easily into the inwards direction (into the cell).
- K_v α11.x - ether-a-go-go potassium channels: K_v11.1 (KCNH2) - hERG, K_v11.2 (KCNH6), K_v11.3 (KCNH7)

Slowly activating

- K_v α12.x: K_v12.1 (KCNH8), K_v12.2 (KCNH3), K_v12.3 (KCNH4)

Modifier/silencer

- Unable to form functional channels as homotetramers but instead heterotetramerize with Kv α2 family members to form conductive channels.
- K_v α5.x: Kv5.1 (KCNF1)
- K_v α6.x: Kv6.1 (KCNG1), Kv6.2 (KCNG2), Kv6.3 (KCNG3), Kv6.4 (KCNG4)
- K_v α8.x: Kv8.1 (KCNV1), Kv8.2 (KCNV2)
- K_v α9.x: Kv9.1 (KCNS1), Kv9.2 (KCNS2), Kv9.3 (KCNS3)

β subunits

β subunits are auxiliary proteins which associate with α subunits, sometimes in a α4β4 stoichiometry. These subunits do not conduct current on their own but rather modulate the activity of Kv channels.

- Kvβ1 (KCNAB1)
- Kvβ2 (KCNAB2)
- Kvβ3 (KCNAB3)
- minK (KCNE1)
- MiRP1 (KCNE2)
- MiRP2 (KCNE3)
- MiRP3 (KCNE4)
- KCNE1-like (KCNE1L)
- KCNIP1 (KCNIP1)
- KCNIP2 (KCNIP2)
- KCNIP3 (KCNIP3)
- KCNIP4 (KCNIP4)
- Proteins minK and MiRP1 are putative hERG β subunits.

Open and closed conformations

- Attempts continue to relate the structure of the mammalian voltage-gated K^+ channel to its ability to respond to the voltage that exists across the membrane.
- Specific domains of the channel subunits have been identified that are important for voltage-sensing and converting between the open conformation of the channel and closed conformations.
- There are at least two closed conformations; in one, the channel can open if the membrane potential becomes positive inside. Voltage-gated K^+ channels inactivate after opening, entering a distinctive, second closed conformation. In the inactivated conformation, the channel cannot open, even if the transmembrane voltage is favorable. The amino terminal domain of the K^+ channel or an auxiliary protein can mediate "N-type" inactivation. The former has been described as a "ball and chain" model where the N-terminus of the protein forms a ball which is tethered to the rest of the protein through a loop (the chain). The tethered ball is transiently sucked into the inner porehole, preventing ion movement through the channel.

Other K^+ channels

- Na^+ activated K^+ channels
  - Voltage-insensitive
  - Blockers: Mg^{2+}; Ba^{2+}
- Cell volume sensitive K^+ channels
  - Activated by increased cell volume
  - Blockers: Quinidine; Lidocaine; Cetiedil
- Type A K^+ channels: Rapid activation & inactivation
  - Blockers: 4-aminopyridine; Quinidine; Mast cell degranulating peptide; Phencyclidine; Dendrotoxins
  - Regulation of fast repolarizing phase of action potentials: Delay spiking
  - Structure: Tetramer of α-subunits + intracellular β-subunits
  - β-subunits may confer rapid inactivation

Other K^+ channels

- Receptor-coupled K^+ channels
  - Muscarinic-inactivated
    - Slow activation; Non-inactivating; Non-rectifying
    - Openers: Somatostatin; β-adrenoceptor agonists
    - Blockers: Ba^{2+}; Bradykinin
  - Atrial muscarinic-activated
    - Inward rectifying
    - Blockers: Ba^{2+}; Cs^+; 4-aminopyridine; TEA; Quinine
    - Structure: Tetramer of KCNJ3 and KCNJ5

Muscarinic potassium channel

- Some types of potassium channels are activated by muscarinic receptors and these are called muscarinic potassium channels (IKACh).
- These channels are a heterotetramer composed of two GIRK1 and two GIRK4 subunits.
  - Examples are potassium channels in the heart, which, when activated by parasympathetic signals through M2 muscarinic receptors, causes an outward current of potassium which slows down the heart rate.

Potassium channel blockers

- Kv channels
  - 4-aminopyridine; Dendrotoxins; Phencyclidine; Phalloidin; 9-aminoacridine;
- Kir channels
  - LY97241; Gaboon viper venom; Sr^{2+}; Ba^{2+}; Cs^{+}
- ATP-sensitive K^{+} channels
  - Glibenclamide; Tolbutamide; Phentolamine: Ciclazindol; Lidocaine
- High conductance (BK):
  - Iberiotoxin; (+)-tubocurarine; Charybdotoxin; Noxiustoxin; Penitrem-A; TEA
- Intermediate conductance (IK)
  - Cetiedil; Trifluoroperazine; Haloperidol
- Small conductance (SK)
  - Apamin ; Leiurotoxin 1 ; (+)-tubocurarine
- Na^{+} activated K^{+} channels
  - Mg^{2+}; Ba^{2+}
- Cell volume sensitive K^{+} channels
  - Quinidine; Lidocaine; Cetiedil
- Type A K^{+} channels
  - 4-aminopyridine; Quinidine; Mast cell degranulating peptide; Phencyclidine; Dendrotoxins
- Receptor-coupled K^{+} channels Blockers:
  - Ba^{2+}; Bradykinin; Ba^{2+}; Cs^{+}; 4-aminopyridine; TEA; Quinine

Potassium channel openers

Channel Family	Therapeutic Indication(s)	Compounds
KCNQ2/KCNQ3	Epilepsy	Retigabine (also $GABA_A$ agonist)
BK_{Ca}	Cerebral ischemia	BMS-204352
		NS 004 (also inhibits other K^+ channels)
	Coronary disorders	NS 1608, NS1619 (also Ca^{2+} channel inhibitor)
	Antipsychotic	
	Urinary incontinence	NS8
	Pollakisuria	
K_{ATP}	Hypertension,	Pinacidil
	ischemic heart	Diazoxide
	disease,	
	heart failure,	Nicorandil
	Asthma	Aprikalim (RP 52891)
		Bimakalim (EMD52692)
		Celikalim
		Cromakalim
		Emakalim
		NIP121
		RO 316930
		RWJ 29009
		SDZ PCO 400
		Rimakalim (HOE234)
		Symakalim (EMD 57283)
		YM-099, YM-934
	Myocardial ischemia	BMS180448
		U 89232 (BMS 189365)
		(Mito K_{ATP}?)
	Alopecia	P1075, minoxidil
	Urinary incontinence	ZM244085, ZD6169, WAY133537, WAY151616, ZD0947
	Erectile dysfunction	PNU83757

Pathophysiological regulation of K^+ channels

-- genetically linked diseases

- **The genetic linkage analysis → to identify many disease-producing loci**
- **Positional cloning techniques → to identify the location of genetic locus responsible for a given hereditary syndrome without prior knowledge of the biochemical or physiological abnormalities underlying the disease**
- **The candidate gene approach → to examine genetic linkage to the hereditary disease of interest and screened for mutations.**

K^+ channel diseases

- Concurrent with this remarkable progress in our understanding of molecular diversity, structure, and function, a growing number of discoveries have linked K^+ channel gene mutations with various diseases. Such diseases of the heart, kidney, pancreas, and central nervous system involve either mutation(s) in K^+ channel gene(s) and/or altered regulation of K^+ channel function.
- The enhanced understanding of these diseases, facilitated by a combination of genomic and biophysical approaches, has helped our understanding of how various mutations affect channel function, contributes to disease etiology, and rationalizes novel treatment strategies. In this review, we provide a comprehensive overview of our recent understanding of molecular defects of K^+ channels in various diseases and its implications for the development of novel prophylactic or therapeutic approaches targeting distinct types of K^+ channels.

K^+ channels linked diseases

- As K^+ channels play fundamental roles in the regulation of membrane excitability, it is to be expected that both genetic and acquired diseases involving altered functioning of neurons, smooth muscle, and cardiac cells could arise subsequent to abnormalities in K^+ channel proteins.
- Genetically linked diseases of the cardiac, neuronal, renal, and metabolic systems involving members of voltage-gated K^+ channels, inward rectifiers, and channel-associated proteins.

Genetically linked diseases

- Cardiac Diseases
  - Long-QT1 and Long-QT5 Syndromes: KCNQ1 (Kv-LQT1) and minK.
  - Long-QT2 Syndrome and Human ether-a-go-go-Related K1 Channel.
- Neuronal Diseases
  - Episodic Ataxia/Myokymia and $K_v1.1$.
  - Benign Familial Neonatal Convulsions and KCNQ2/KCNQ3.
- Hearing and Vestibular Diseases: Nonsyndromic Dominant Deafness and KCNQ4
- Renal Diseases: Bartter's Syndrome and $K_{ir}1.1$
- Metabolic Diseases: Familial Persistent Hyperinsulinemic Hypoglycemia of Infancy and Sulfonylurea Receptor 1

K^+ channel genes involved in long-QT syndromes

Type	Gene	Current/Channel Type
LQT1	KCNQ1 (KvLQT1)	Component of slowly inactivating delayed rectifier IKs
LQT2	hERG	Delayed rectifier IKr rapidly inactivating cardiac Na^+ channel
LQT3	SCN5A	
LQT4	Chromosome 4q25-27	Subunit involved in regulation of cardiac repolarization?
LQT5	KCNE1 (MinK)	Component of IKs
LQT6	MiRP1	Component of IKr

Potassium Channel Genes and Ancillary Subunits: Localization, Modulators, and Diseases

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms
Voltage-gated K^+ channels (*Shaker*)	*KCNA1*	Kv1.1	12p13	Neurons, heart, retina, pancreatic islet	Blocker: α-DTX, HgTX1, MgTX	Episodic ataxia/myokymia syndrome Missense mutations (11 variants)
	KCNA2	Kv1.2	1	Brain, heart, pancreatic islet	Blocker: CTX, α-DTX, HgTX1, MgTX, NxTX	
	KCNA3	Kv1.3	1p21-p13.3	Lymphocyte, brain, lung, thymus, spleen	Blocker: AgTX2, α-DTX, HgTX1, KTX, MgTX, CTX, NxTX, UK78282, WIN 17317-3, correolide	
	KCNA4	Kv1.4	11q13.4-q14.1	Brain, heart, pancreatic islet	Blocker: UK78282	
	KCNA5	Kv1.5	12p13	Brain, heart, kidney, lung, skeletal muscle	Blocker: 4-AP, clofilium, loratadine, perhexiline	
	KCNA6	Kv1.6	12p13	Brain	Blocker: α-DTX	
	KCNA7	Kv1.7	19q13.3	Heart, pancreatic islet, skeletal muscle	Blocker: 4-AP, capsaicin, tedisamil, NxTX, MgTX	
Voltage- and cGMP-gated K^+ channel	*KCNA10*	Kv1.10	1p13.1	Aorta, brain, kidney	a. Blocker: 4-AP, CTX, ketoconazole, pimozide b. Opener: cGMP	
β-subunits for Kv channels	*KCNAB1*	Kvβ1	3q26.1	Brain (Kvβ1.1) Heart (Kvβ1.2)		
	KCNAB2	Kvβ2	1p36.3	Brain, heart		
	KCNAB3	Kvβ3	17p13.1	Brain		
Shab	*KCNB1*	Kv2.1-Kv2.2	20q13.2	Brain, heart, kidney, skeletal muscle, retina	Blocker: hanatoxin, TEA	
Shaw	*KCNC1*	Kv3.1	11p15	Brain, muscle, lymphocyte	Blocker: 4-AP	
	KCNC2	Kv3.2	19q13.3-q13.4	Brain	Blocker: 4-AP	
	KCNC3	Kv3.3	19q13.3-q13.4	Brain, liver	Blocker: 4-AP	
	KCNC4	Kv3.4	1p21	Brain, skeletal muscle	Blocker: 4-AP	
Shal	*KCND1*	Kv4.1	Xp11.23-p11.3	Heart, brain, liver, kidney, lung, placenta, pancreas	Blocker: 4-AP	
	KCND2	Kv4.2	7q31-32	Brain	Blocker: 4-AP, PaTX	
	KCND3	Kv4.3	1p13.2	Heart, brain	Blocker: 4-AP, PaTX	
		Kv5.1		Brain		
		Kv6.1		Brain		

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms
	KCNF2	Kv6.2	18q22-18q23	Heart		
		Kv8.1	8q22.3-8q24.1	Brain		
	KCNS1	Kv9.1		Brain		
	KCNS2	Kv9.2	8q22	Brain		
	KCNS3	Kv9.3	2p24	Lung, brain, artery		
	KCNF1	KH1	2p25	Heart, skeletal muscle		
	KCNG1	KH2	20q13	Brain, placenta, skeletal muscle		
Ether-a-go-go	*KCNH1*	EAG	1q32-q41	Brain		
Human *ether-a-go-go*	*KCNH2*	hERG	7q35-q36	Brain, heart	Blocker: clofilium, dofetilide, E4031, LY97241, terfenadine, sertindole	LQT2 a. Missense mutations (7 variants) b. Deletion (2 variants) c. Substitution in intron 3
	KCNH3	BEC1	12q13	Brain		
	KCNH4	BEC2		Brain		
MinK	*KCNE1*	MinK	21q22.1-q22.2	Kidney, uterus, heart, cochlea, retina		LQT5 Missense mutations (5 variants)
	KCNE2	MinK-related peptide I (MiRP1)	21q22.1			LQT6 Missense mutations (3 variants)
	KCNE3	MiRP2		Small intestine, colon, kidney		
KvLQT1	*KCNQ1*	KvLQT1	11p15.5	Heart, cochlea, kidney, lung, placenta, colon	a. Blocker: chromanol-293B b. Opener: L-364,373, stilbenes, fenamates	LQT1; Jervell-Lange Neilsen syndrome a. Missense mutations (17 variants) b. Deletions (6 variants) c. Insertions (2 variants) d. Insertion/deletion (1 variant) e. Splice variant (1)
	KCNQ2	KvLQT2	20q13.3	Brain, neuron	a. Blocker: TEA, linopirdine, XE991, L-735,821 b. Opener: retigabine	Benign neonatal epilepsy a. Missense mutation (2 variants) b. Insertion (1 variant) c. Deletion (1 variant)
	KCNQ3	KvLQT3	8q24	Brain, neuron	a. Blocker: TEA, linopirdine, XE991 b. Opener: retigabine	Benign neonatal epilepsy Missense mutation (2 variants)

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms
	KCNQ4	KvLQT4	1p34	Outer hair cells, inner ear, central auditory pathway		Hearing loss a. Missense mutation (6 variants) b. Deletion (1 variant)
	KCNQ5	KvLQT5	6q14	Brain, skeletal muscle	Blocker: linopirdine	
Inward rectifier	*KCNJ1*	Kir1.1-Kir1.3	11q24	Kidney, pancreatic islets	Blocker: Ba^{2+}	Bartter's syndrome, type 2; Bartter's syndrome, antenatal onset a. Missense mutation (9 variants) b. Deletion (2 variants)
	KCNJ2	Kir2.1		Heart, brain, smooth muscle, skeletal muscle, lung, placenta, kidney	Blocker: Ba^{2+}, spermine, spermidine, Mg^{2+}	
	KCNJ3	Kir3.1	2q24.1	Heart, cerebellum	Blocker: Ba^{2+}	
	KCNJ4	Kir2.3	22q13.1	Heart, brain, skeletal muscle	Blocker: Ba^{2+}	
	KCNJ5	Kir3.4	11q24	Heart, pancreas	Blocker: Ba^{2+}, Cs^{+}	
	KCNJ6	Kir3.2	21q22.1-q22.2	Cerebellum, pancreatic islet	Blocker: Ba^{2+}, Cs^{+}	Mouse *weaver* Missense mutation (1 variant)
	KCNJ8	Kir6.1/ uK_{ATP}-1	12p11.23	Various	Blocker: Ba^{2+}, Cs^{+}	
	KCNJ9	Kir3.3	1q21-23	Brain	Blocker: Ba^{2+}, Cs^{+}	
	KCNJ10	Kir4.1	1q	Glia	Blocker: Ba^{2+}, Cs^{+}	
	KCNJ11	Kir6.2 (subunit of K_{ATP} channel)	11p15.1	Various		Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) a. Nonsense mutation (1 variant) b. Missense mutation (1 variant)
	KCNJ12	Kir2.2	17p11.2-p11.1	Atrium, ventricle	Blocker: Ba^{2+}, Cs^{+}	
	KCNJ13	Kir7.1	2q37	GI, kidney, cerebellum, hippocampus, thyroid	Blocker: Ba^{2+}, Cs^{+}	

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms
	KCNJ14	Kir2.4	19q13	Brain, retina	Blocker: Ba^{2+}, Cs^{+}	
	KCNJ15	Kir4.2 Kir5.1	21q22.2	Kidney, lung, brain Brain, periphery		
Sulfonylurea receptor	*SUR1*	Sulfonylurea receptor 1 (subunit of K_{ATP} channel)	11p15.1	Pancreas, neurons, skeletal muscle	a. Blocker: glyburide, tolbutamide, glipizide, ciclazindol b. Opener: diazoxide	PHHI a. Missense mutations (11 variants) b. Deletion (4 variants)
	SUR2	Sulfonylurea receptor 2 (2A, 2B) (subunit of K_{ATP} channel)	12p12.1	2A: Heart, skeletal muscle 2B: Brain, liver, skeletal and smooth muscle	a. Blocker: glyburide, ciclazindole b. Opener: P1075, pinacidil, cromakalim	
Large conductance Ca^{2+}-activated	*KCNMA1*	*Slo* (BK_{Ca} channel α-subunit)	10q23.1	Brain, smooth muscle, cochlea, pancreatic islets	a. Blocker: IBTX, CTX, TEA b. Opener: NS1619, NS8, BMS-204352, dehydrosoyasaponin I	
	KCNMB1	BK_{Ca} β1-subunit	5q34	Smooth and skeletal muscle		
	KCNMB2	BK_{Ca} β2-subunit		Kidney, heart, uterus, small intestine		
	KCNMB3	BK_{Ca} β3-subunit	3q26.3-q27	Testis		
	KCNMB4	BK_{Ca} β4-subunit	12q14.1-q15	Brain, heart, kidney, lung		
Small conductance Ca^{2+}-activated	*KCNN1*	SK1	19p13.1	Brain, heart	a. Blocker: tubocurarine, dequalinium, UCL 1848	
	KCNN2	SK2		Brain, adrenal gland, Jurkat T cells	a. Blocker: apamin, ScTX, d-tubocurarine, 4-AP b. Opener: chlorzoxazone, zoxazolamine, 1-EBIO	
	KCNN3	SK3	1q21.3	Brain, heart, liver	Blocker: apamin	

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms
Intermediate conductance Ca^{2+}-activated	*KCNN4*	IKCa1	19q13.2	T lymphocytes, colon, smooth muscles, prostate, red blood cells, neurons, placenta, thymus	a. Blocker: CTX, clotrimazole, nitrendipine, miconazole, cetiedil, econazole, TRAM-34 b. Opener: 1-EBIO, chlorzoxazone, zoxazolamine	
Two-pore K^+ channel	*KCNK1*	TWIK1	1q42-q43	Brain, kidney, heart	Blocker: Ba^{2+}, quinidine, quinine	
	KCNK2	TREK	1q41	Brain, lung	Potentiated by arachidonic acid, riluzole, chloroform, lysophosphatidylcholine, diethyl ether, halothane, isoflurane	
	KCNK3	TASK	2p23	Heart, brain, pancreas, placenta	a. Sensitive to external pH b. Activated by halothane, isoflurane	
	KCNK5	TASK2	6p21	Kidney	a. Sensitive to external pH b. Blocker: quinine, quinidine	
	KCNK6	TWIK2, TOSS	19q13.1	Eyes, lung, stomach, embryo	Sensitive to internal pH	
	KCNK7		11q13			
		TRAAK	11q13	Brain, spinal cord, retina	a. Blocker: gadolinium b. Opener: unsaturated fatty acid (arachidonic acid), riluzole, lysophosphatidylcholine c. Stretch-activated	
		CTBAK-1		Heart, brain, kidney	Blocker: Ba^{2+}	

4-AP, 4-aminopyridine; AgTX2, angiotoxin 2; CTX, charybdotoxin; α-DTX, α-dendrotoxin; HgTX1, hongotoxin 1; IbTX, iberiotoxin; KTX, kaliotoxin; MgTX, margatoxin; NxTX, no
scyllatoxin.

See you next week!