Ion Channels and Receptors (Morgan Sheng, lectures 1 and 2)

Importance of ion channels in nervous system and neural signaling

- ion channels are the molecular basis of membrane excitability (synaptic transmission, action potentials, sensory transduction etc)
- ionic basis of excitability inferred for ~50 years, but molecular entities characterized only in last ~15 years
- important target of many clinically used drugs

General classification (traditional):

Ion selectivity K^+, Na^+, Ca^{2+}, Cl^-

Gating Voltage-gated (VG), mechanosensitive, thermosensitive
Ligand-gated ("ionotropic receptor" cf "ligand-gated ion channel")
Ligand: Extracellular (glutamate, GABA);
Intracellular (Ca^{2+}, cyc nucleotide, G-protein α, $\beta\gamma$)

Levels of diversity within each class:

Electrophysiological

- iGluR – fast EPSC (AMPA), slower voltage-dependent EPSC (NMDA)
- AMPA receptors -- rapidly desensitizing, slowly desensitizing
- Voltage-gated K^+ channels -- rapidly inactivating (A-type), non-inactivating
- VG Ca^{2+} channels -- High or low threshold of activation

Pharmacological

- Differential action of drugs: nicotinic vs muscarinic
  AMPA/KA [CNQX] vs NMDA [APV, MK801]
- Increasingly fine distinctions within classes as drugs become more sophisticated
- **Pharmacology is operationally useful**; Specific drugs are often used as *functional* probes that *define* receptor subtypes
- Antagonists generally more useful than agonists in functional definition of channels/receptors

Structural

Primary structure: *digital* information; *finite* repertoire;
the molecular basis of function and pharmacology

Structural diversity can be generated by:
Alternative splicing
RNA editing
Combinatorial (mix and match oligomers) diversity
Post-translational modification – eg phosphorylation, glycosylation, ubiquitination, disulfide bond, fatty acid modification (palmitoylation, etc)

Classifying ion channels by a combination of function / pharmacology / structure
Correlating Function, Pharmacology, Structure
Therapeutic implications

Ascendancy of Primary Structure as the "final" basis of classification:

based on digital information (most definitive of classifications);
potentially comprehensive (post-genomic);
may "predict" tertiary structure;
may offer functional insights (indirect);
reveals evolutionary relationships

But primary structure alone is not sufficient to define functional properties of channels/receptors (e.g. subunit composition, phosphorylation, protein interaction etc further modify activity)

Example of VG calcium channels

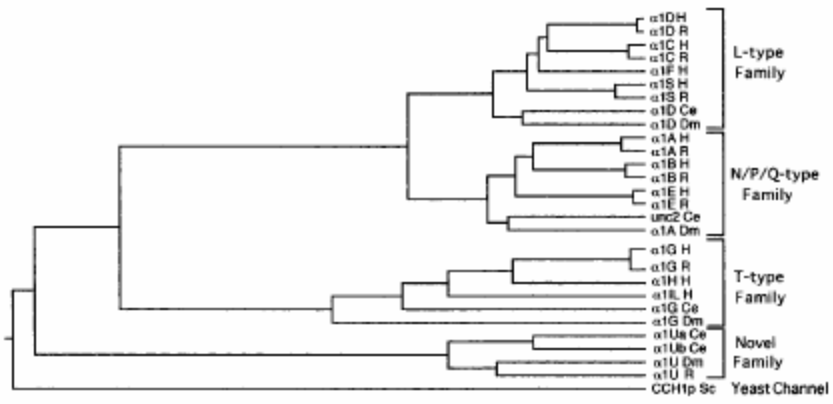


Figure 1. The Voltage-Dependent Calcium Channel Family
A nearest neighbor dendrogram for the voltage-gated calcium channel superfamily is shown. Four calcium channel subfamilies are evolutionarily conserved from invertebrates to mammals. Abbreviations: H, human; R, rat; Ce, *C. elegans*; Dm, *Drosophila melanogaster*.

Membrane topology and domain organization as a means of expressing primary structure

Amino acid – secondary structure – **domain** of tertiary structure – protein – [complex – organelle -- cell – organism]

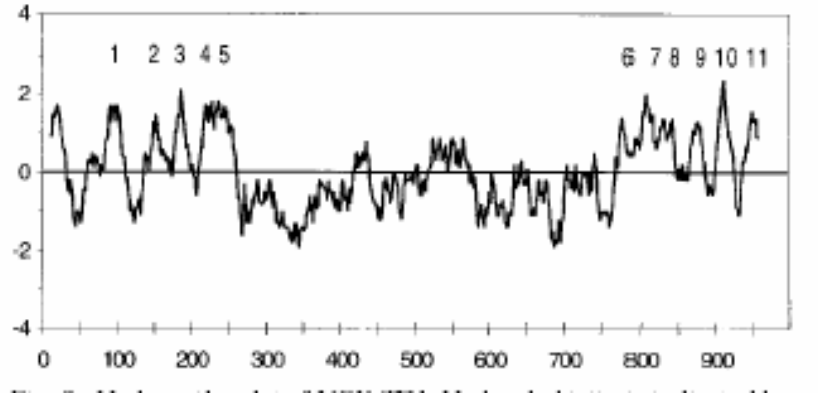
Determination / Prediction of Membrane Topology

Primary structure considerations:
signal sequence – 16-22 hydrophobic residues (clipped off)
identifying and counting transmembrane domains – hydropathy plot

- it takes typically > 20 amino acids in alpha helix to span lipid bilayer of membrane (hydrocarbon core is 3-4 nm thick; each residue adds 0.15 nm to length of α-helix; 25 aa helix extends 3.75nm)
- (α-helices in interiors of proteins are also hydrophobic, but shorter, average length ~10 aa)

- for *single* transmembrane domain, all side chains will face lipid and should be hydrophobic (transmembrane segment often flanked by charged residues)
- hydophobicity index for each amino acid (e.g. Isoleucine 4.5; Valine 4.2; Leucine 3.8; Phenylalanine 2.8; Cysteine 2.5; Methionine 1.9; Alanine 1.8) (Lysine –3.9; Arginine –4.5)
- hydrophobicity plot is a graph of average hydrophobicity over a stretch of amino acids (cf 30 day moving average for a stock price)

Example of hydropathy plot



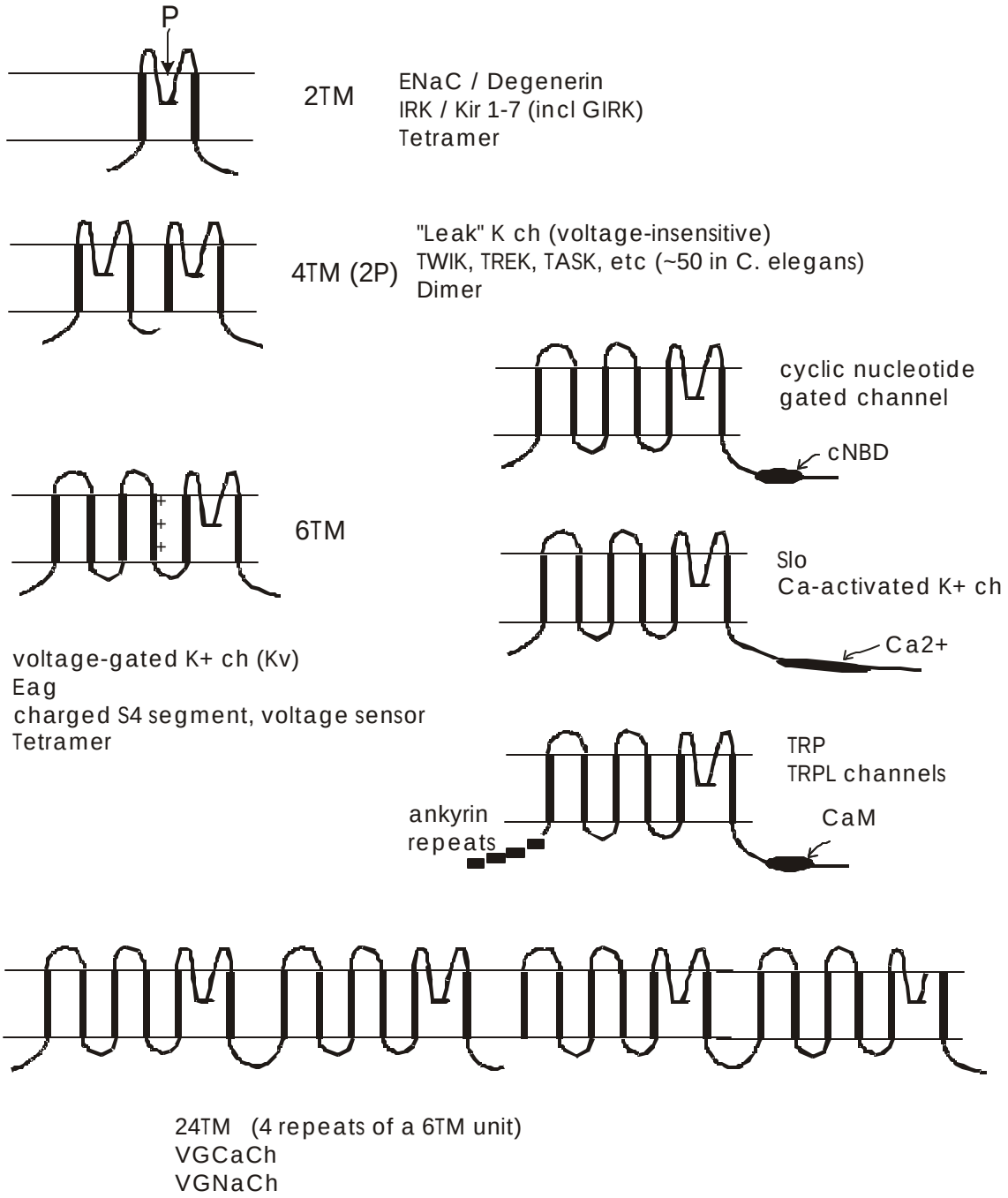
Hydropathy plot of a cardiac Na+/Ca2+ exchanger
Xue, et al *Am. J. Physiol.* 277 (*Cell Physiol.* 46): C693–C700, 1999.

Reference: Kyte and Doolittle, J Mol Biol (1982). A simple method for displaying the hydropathic character of a protein. J Mol Biol. 1982 May 5;157(1):105-32.

Experimental approaches to determining membrane topology

Antibody accessibility of a known epitope (permeabilized and non-permeabilized conditions)
N-glycosylation sites (Asn-X-Thr/Ser) (almost certainly extracellular) add or delete
Phosphorylation sites (almost certainly intracellular)
Epitope accessibility from outside (natural or artificial tags introduced into specific sites of protein) (Enzyme fusions eg alkaline phosphatase)

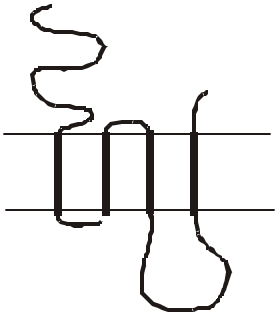
Diagram of membrane topology of classes of ion channels and ionotropic receptors



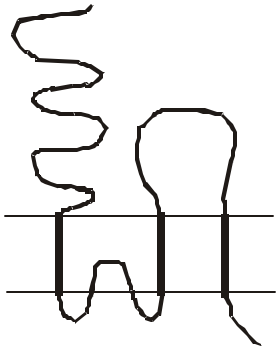
<u>Multisubunit configuration of ion channels</u>

In addition to multimeric assembly, alpha-subunits of ion channels (shown here) are typically associated with accessory subunits (often called beta subunit etc)

Ligand-gated channels / Ionotropic Receptors

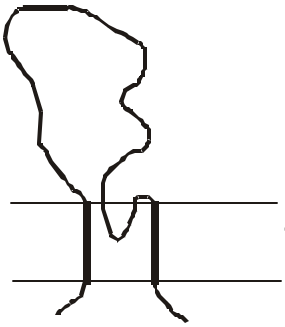


4TM
"ligand-gated superfamily"
nAChR
GlyR
GABA(A)R
5HT3R
Pentamer



3TM plus "P-loop"
Glutamate receptors
NMDA / AMPAR / KAR/ Delta

Tetramer



2TM plus "P-loop"
P2X (ATP) receptor

Trimer

Transmembrane segment M2 forms channel pore of 4TM nAChR superfamily receptors
Membrane re-entrant loop (P-loop) forms channel pore of ionotropic glutamate receptors (cf potassium channels)

Structural features common to all LGICs / ionotropic receptors

- Multisubunit complexes – either homo or hetero-oligomers, but all subunits are homologous
- Subunits organized pseudosymmetrically in the plane of the membrane – ion channel at central axis at the common interface of all subunits
- Neurotransmitter binding site in extracellular domain

Structural features common to members of each family

- Subunit primary structure / membrane topology defines the family
- Oligomeric organization – 5 subunits: nAChR, GABAA, Gly, 5HT3
  4 subunits: GluR (AMPA, KA, NMDA)
- Pore structure -- Transmembrane helix M2: nAChR, GABAA, Gly, 5HT3
  P-loop: GluRs (cf VG channels)
- Agonist binding site – at subunit interfaces: nAChR, GABAA, Gly, 5HT3
  - within single subunit: GluR

"Domain" organization of ion channels / receptors

- Pore: permeability, selectivity (may be part of a transmembrane domain or reentrant P-loop)
- Transmembrane domains that "support" the Pore
- Transmembrane domains that interface with membrane lipid (may be the same)
- Gate: directly controls opening of the pore (implies movement of gate during opening)
- Voltage sensor (transmembrane) : S4, typically charged and in the lipid bilayer [why?]. voltage sensor may be part of gate or be connected to gate – gating charge.
- Ligand-binding domains (extracellular for neurotransmitters, or cytoplasmic for second messengers)
- Catalytic domain of receptors: kinase, phosphatase (pore can be considered catalytic domain of ion channels)
- "Transducing" domains that link ligand-binding domain or voltage sensor to channel/ catalytic activity (may also be a transmembrane domain)
- Inactivation domains: eg N-terminal region in voltage-gated K channels that inactivate by the ball and chain mechanism
- Multimerization / assembly domains – can be intracellular (K+ channels) or extracellular (AMPA receptors)
- Interaction domains (auxiliary subunits, associated proteins): usually intracellular, often C-terminal tails, but also cytoplasmic loops, which bind to specific cytoplasmic proteins, eg through PDZ domain interactions.

The Example of Potassium channels

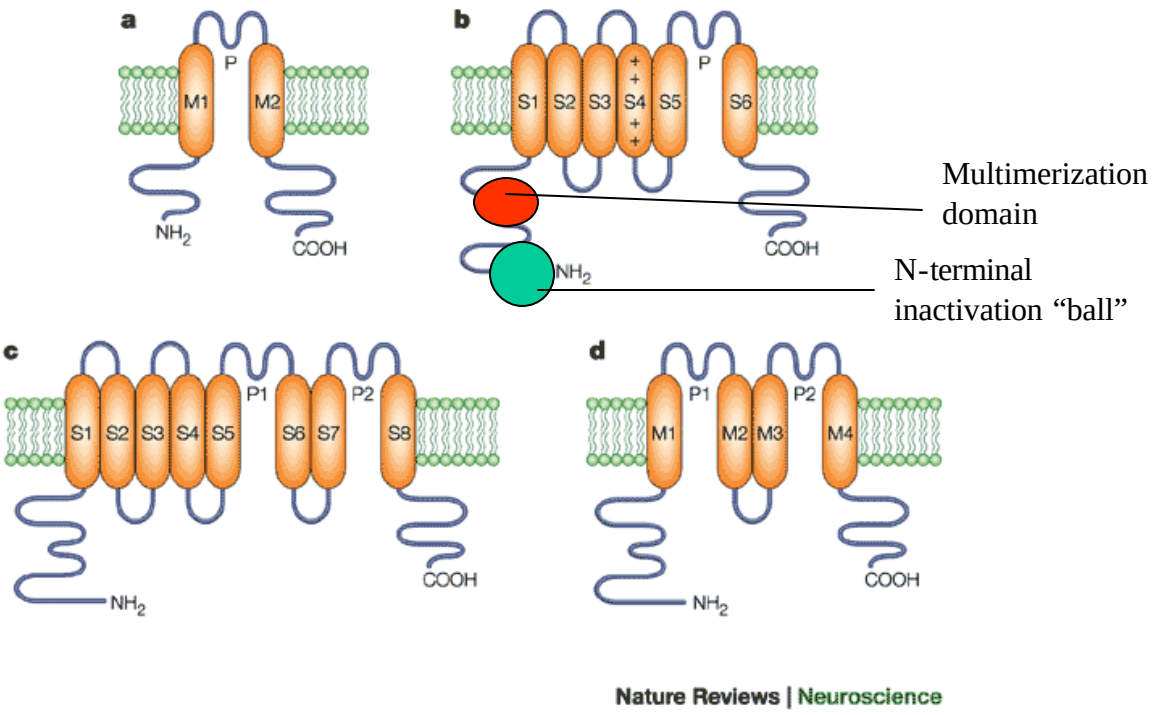
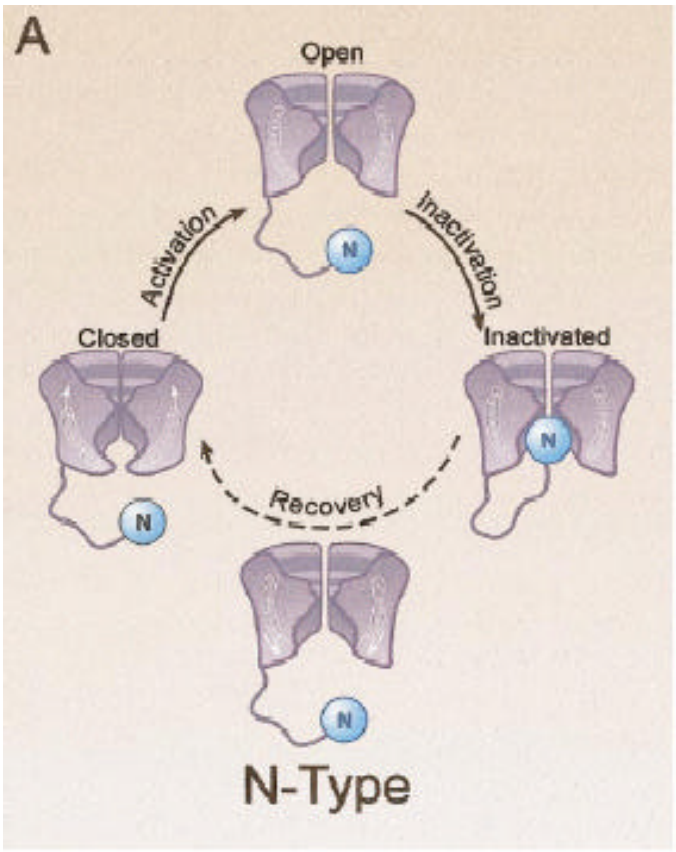


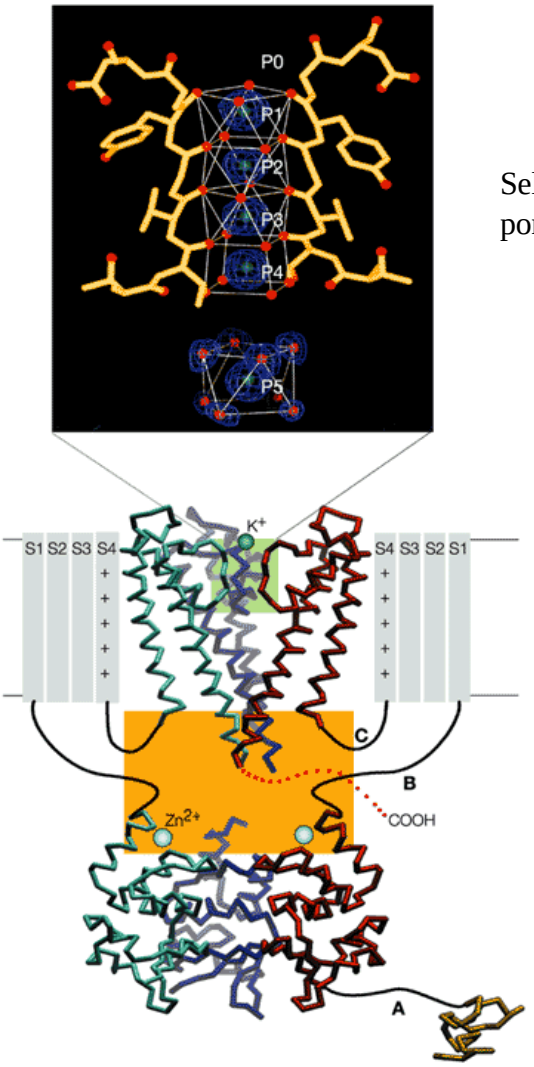
Figure 1 | **The four main classes of potassium channel.** **a** | 2TM/P channels (which consist of two transmembrane (TM) helices with a P loop between them), exemplified by inwardly rectifying K^+ channels and by bacterial K^+ channels such as KcsA. **b** | 6TM/P channels, which are the predominant class among ligand-gated and voltage-gated K^+ channels. **c** | 8TM/2P channels, which are hybrids of 6TM/P and 2TM/P, and were first found in yeast. **d** | 4TM/2P channels, which consist of two repeats of 2TM/P channels. 8TM/2P and 4TM/2P probably assemble as dimers to form a channel. 4TM/2P channels are far more common than was originally thought. These so-called 'leakage' channels are targets of numerous anaesthetics [39]. S4 is marked with plus signs to indicate its role in voltage sensing in the voltage-gated K^+ channels.

Note the various domains such as Pore domain (P), supporting TM domains, S4 voltage sensor, N-terminal and C-terminal cytoplasmic domains that mediate protein interaction, multimerization and inactivation.



N-type inactivation of VG K channels (ball and chain model)

Channel activation is voltage dependent and involves movements of charges intrinsic to the channel molecule, especially the positive charges in the S4 sequence. Panel A, N-type inactivation. N-type inactivation generally follows activation and involves a cytoplasmic “gate” on the amino terminus of the channel subunit. This gate, or ball, carries a net positive charge and interacts with a receptor that is likely to reside in the S4-S5 loop and vestibule. The N-type mechanism therefore has the following properties:
(1) N-type inactivation is lost after N-terminal deletion and restored by exogenous application of short peptides derived from the N-terminal.
(2) Intracellular, but not extracellular, drug binding alters the rate of development of N-type inactivation.
(3) The inactivation rate is unaffected by changes in extracellular K
[See Hoshi et al 1990 Biophysical and molecular mechanisms of Shaker potassium channel inactivation. Science.250:533-8.]



Composite structure of voltage-gated K^+ channels. Top panel: A backbone diagram of the ion-selectivity filter of KcsA. P1–P5 correspond to five K^+-binding sites that are numbered from the outside (top). The P0 site mentioned in the main text is not shown. Each site is formed by eight oxygen atoms (red) that surround each K^+ ion (green) as it passes through the channel. The P1–P4 sites are formed by oxygens contributed by the channel protein. The P5 site is formed by eight immobilized water molecules. Bottom panel: Composite structure of a voltage-gated K^+ channel. The top half shows the transmembrane (TM) domains, including the 2TM/P core, which, in this case, corresponds to the structure of KcsA[3]. The TM helix S4 — the voltage-sensor — is highly charged with basic residues. The cytoplasmic domains are shown in the bottom half. The amino-terminal tetramerization domain (T1) corresponds to the Kv3.1 T1 domain[24]. Zn^{2+} ions, located between four subunits near the carboxy -terminal ends of the chain, are shown in blue. The structure of the inactivation ball (yellow), connected to the T1 tetramer, corresponds to that derived by NMR[40]. The loops denoted by A–C connect these isolated structures and domains, but their relative dispositions are unknown. The transducer Box (orange) corresponds to the region between the inner leaflet of the membrane and carboxy -terminal side of the T1 tetramer, which constitutes a putative cytoplasmic vestibule. Other components of the 'transducer box' include linkers A–C, and the carboxy -terminal end of S6 (dotted line in red). All of these components are probably involved in transducing conformational changes that underlie voltage-dependent channel gating, inactivation, and protein–protein interactions.

[from Choe 2002 Nature Reviews Neurosci]

Tertiary and Quaternary structure of Receptors / Ion channels

pore-forming subunit

- α-subunit (but may also be called other names etc esp in ionotropic receptors)
- Virtually always form oligomers (typically hetero-oligomeric);
- large families of pore-forming subunits
- often can work as homomeric complexes (e.g. Kv, AMPAR, but not some GIRKs, NMDAR)

auxiliary / accessory subunits

typically called β-subunits in common ion channels

associated proteins

- adaptors; anchoring proteins; scaffolding proteins; modulatory proteins and enzymes
- difference between accessory subunit and associated protein may be semantic (calmodulin as an accessory subunit)

Non-pore-forming subunits/accessory proteins modulate kinetics of gating (activation, inactivation), channel properties, expression levels (stability), surface levels, subcellular targeting

Native ion channel / ionotropic receptor exists in a large protein complex, linked to signaling molecules and cytoskeleton

How were ion channels and receptors cloned?

Genetics

- Drosophila Shaker – (ether sensitive leg-shaking, prolonged NT release at NMJ), positional cloning of gene
- Other ion channels cloned by ts paralysis in Drosophila and shaking phenotype
- TRP (transient receptor potential) channel first cloned as phototransduction mutant in Drosophila; Deg/EnaC channels as mechanosensation mutants in C elegans
- Advantage: can clone channels of low abundance, with no high affinity ligand, but disadvantage is that "channel phenotype" may not be obvious and may be indirectly related to channel itself

Biochemical purification and protein sequencing

Esp *affinity purification* using high affinity ligand (eg antagonist) from rich source of activity

Advantage – can isolate multiple subunits, associated proteins

- Nicotinic acetylcholine receptor of Torpedo electric organ
- VG sodium channel
- VG calcium channel
- GABAA receptor (benzodiazepine): 53 kD alpha, 57 kD beta subunits
- Glycine receptor (strychnine): 48 kD alpha, 58 kD beta subunits, and 93 kD gephyrin

Functional expression in heterologous system

Isolation of cDNA (has to be full length, functional homomer in heterologous cells)
Screen for channel activity by electrophysiological assay
Examples of channels cloned by functional expression:
AMPA and NMDA glutamate receptor
IRK; GIRK
P2XR (ATP), capsaicin receptor (heat and spicy hot activated channel)

Homology to known ion channel cDNAs (increasing popular)

Screening by hybridization, PCR, or database search for similarity

How do you know if you have cloned "the ion channel"?

• "Sufficient" in heterologous expression for channel activity
• "Necessary" for channel activity in loss of function experiments
• "Reconstitution" by purified recombinant protein in lipid bilayer
• Mutation of cDNA affects pore properties of channel
• (Looks like a channel by primary structure)

How many ion channels are there?

	Mammal	Drosophila	C. elegans
VG Na channels α	9	2	**0**
VG Ca channels α	8	4	5
K channels		~30	~90
(Kir)	12	3	3
(TWIK)		11	~50
EnaC/degenerin		24	22
iGluRs	~20	30	15

Table 1. Ion Channel Genes in *Drosophila* and *C. elegans*

Protein Family	*Drosophila* Homologs	*C. elegans* Homologs
Voltage-gated sodium channels		
α subunit	2	0
β subunit	0	0
TipE	2	0
Voltage-gated calcium channels		
α subunit	4	5
β subunit	1	2
α2δ subunit	3	3
γ subunit	?1	0
Potassium channels		
K_v α subunit	5	10
K_v β subunit	1	0
KCNQ α subunit	1	3
MinK β subunit	0	0
EAG α subunit	3	2
MiRP1 β subunit	0	0
slo α subunit	1	2
slo β subunit	0	0
slack α subunit	1	2
SK α subunit	1	4
K_v α subunit	3	3
TWIK α subunit	11	50
Chloride channels	3	6
CNG channels	4	6
Hyperpolarization-activated channels	1	0
Ionotropic glutamate receptors		
AMPA subtype	3	3
kainate subtype	15	4
NMDA subtype	2	2
δ subtype	4	4
divergent	6	2
nACh receptors	10	42
$GABA_A$/glycine receptors	10	37
Trp-like channels	13	11
Amiloride-sensitive sodium channels	24	22
Ryanodine receptor	1	1
IP_3 receptor	1	1
Innexins	8	24

TRP family channels
[Clapham et al 2001 Nature Reviews Neurosci 2, 387-396;
Clapham DE 2003, Nature, 426, 517-524]

6 TM membrane topology; probably tetramers
permeable to monovalent cations and Ca2+; some highly selective for calcium;
opening → depolarization and increased $[Ca2+]_i$
Functions of most TRP channels in mammals still unknown but some are certainly involved in sensory transduction
6 related protein families

- TRP, TRPL: phototransduction mutants in Drosophila – channels that mediate light response
- Nompc: Drosophila mechanosensation (contains large number of N-term ankyrin repeats)
- Vanilloid receptor family: - pain, chilli peppers, hot and cold temperature in mammals.
- **VR1** (also called TRPV1) cloned by functional expression from rat dorsal root ganglion using capsaicin (hot pepper active compound), also gated by heat (>43 C); expressed in sensory neurons; VR1 knockout mice insensitive to nociceptive, inflammatory effects of vanilloid compounds
- OSM9: olfaction in C.elegans
- Polycystin II: calcium permeable channels mutated in dominant forms of polycystic kidney disease

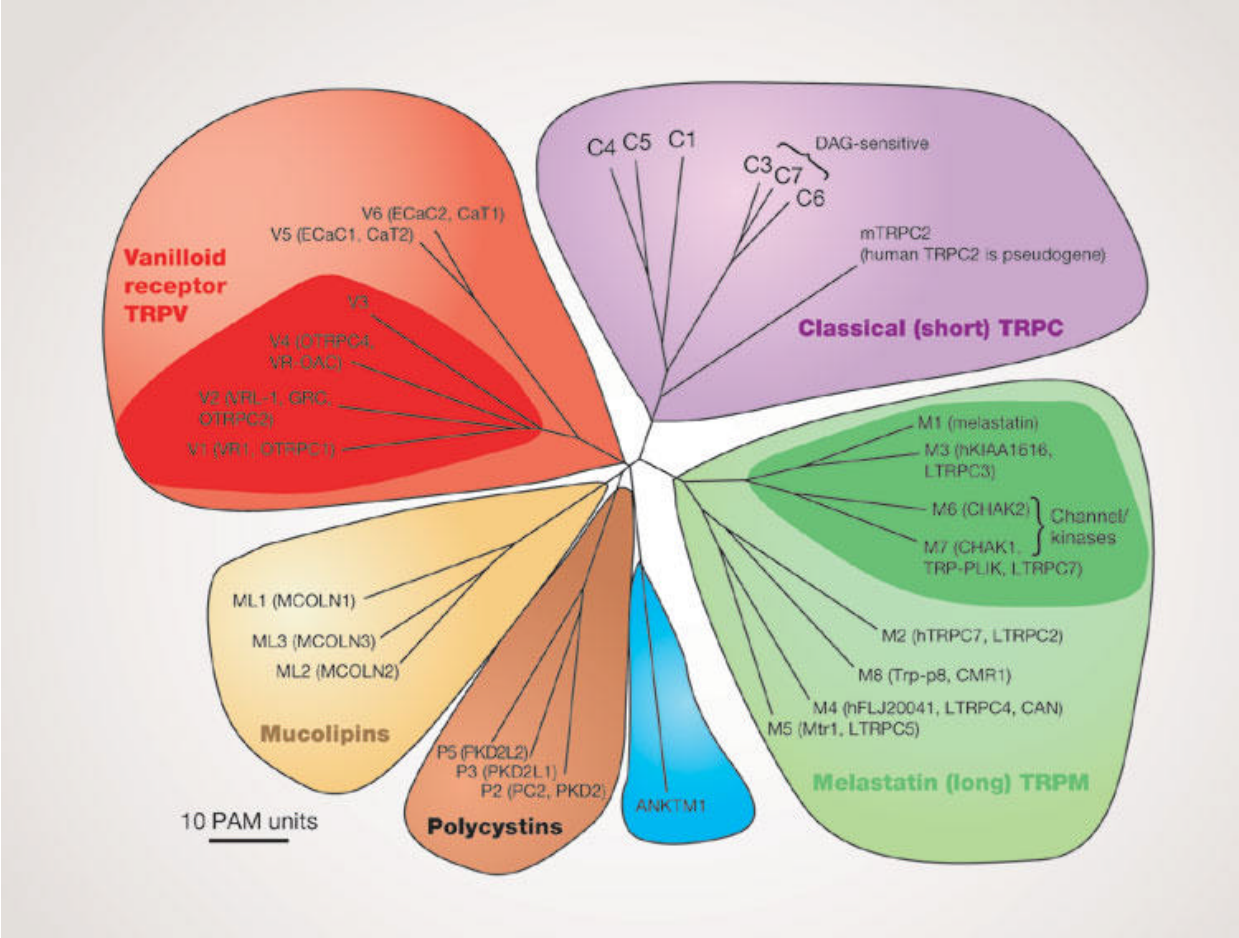
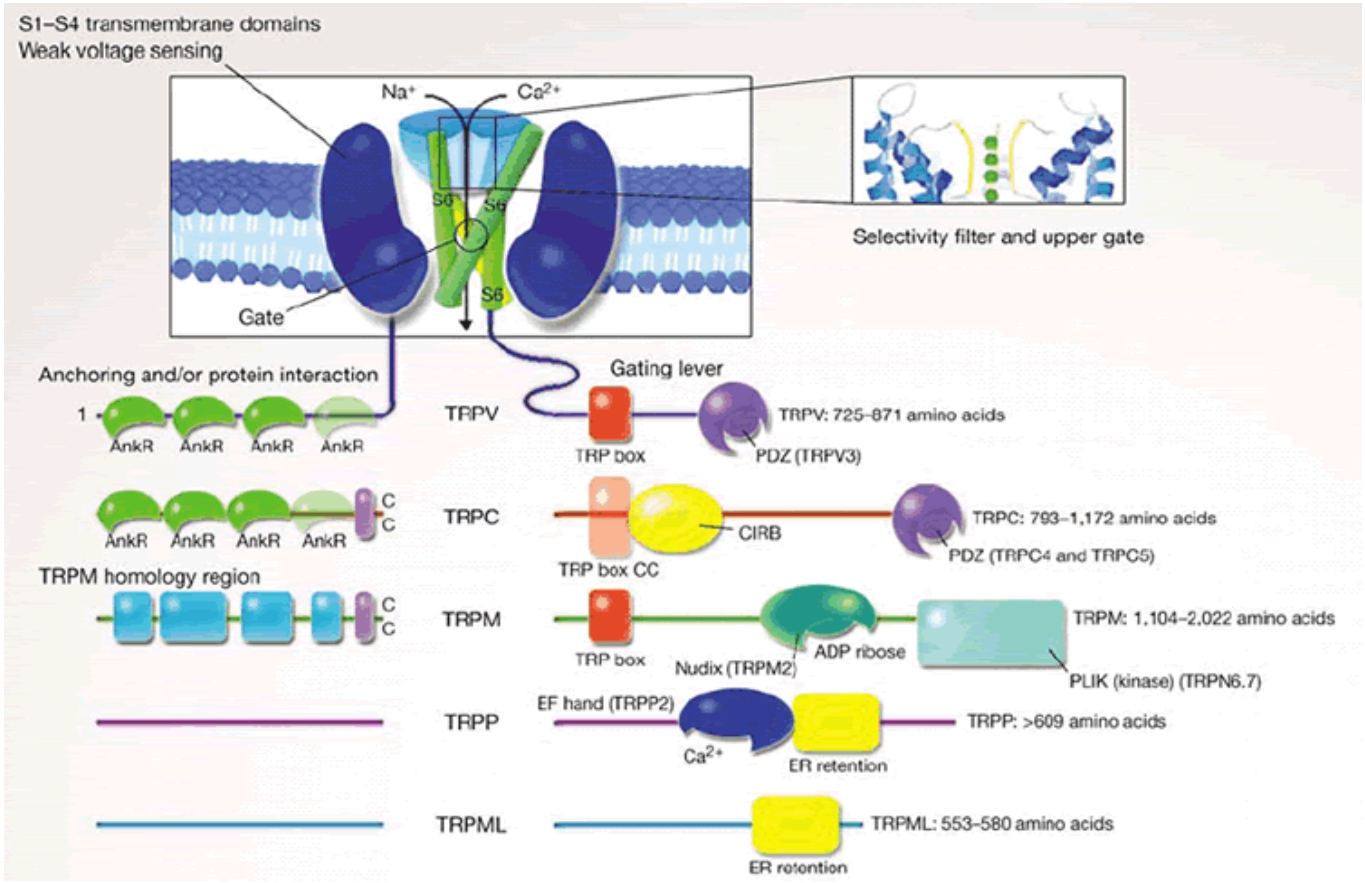


Figure Mammalian TRP family tree.
The evolutionary distance is shown by the total branch lengths in point accepted mutations (PAM) units, which is the mean number of substitutions per 100 residues.

TRP channels have diverse cytoplasmic domains built around a basic 6TM architecture similar to voltage-gated potassium channels

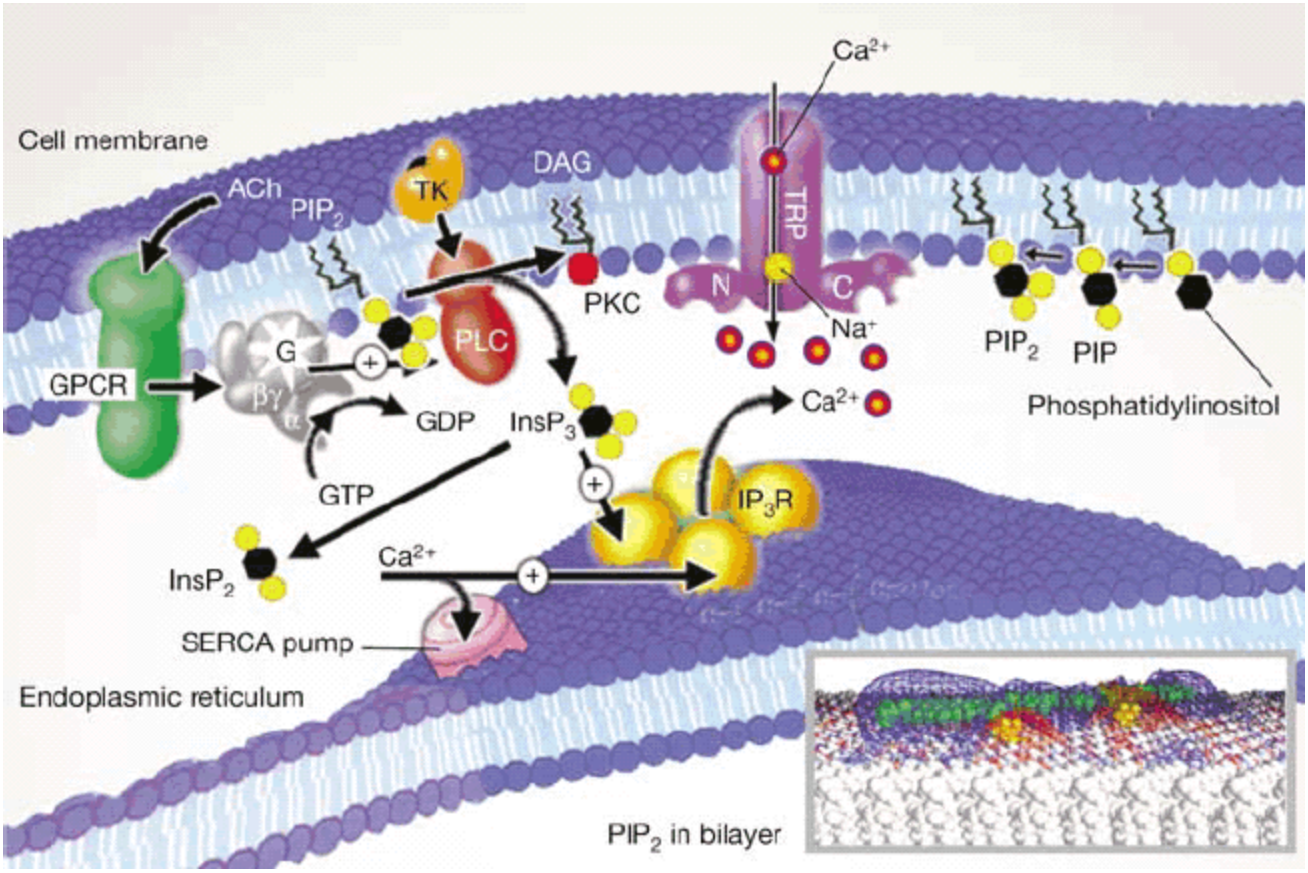
The selectivity filter (light blue and inset) is formed by amino acids that dip into the bilayer (pore loops), one contributed from each of the four subunits. S5 has been removed to emphasize the link between the S6 gating helix and the TRP C-terminal polypeptide chain. The TRP box is EWKFAR in TRPC, but is less conserved in TRPV and TRPM. CC indicates a coiled-coil domain. Ankyrin repeats (AnkR) range from 0 to 14 in number (3 or 4 in TRPV and TRPC, 14 in ANKTM; not shown). Numbers on the right indicate range in length. CIRB, putative calmodulin- and IP_3R-binding domain; EF hand, canonical helix–loop–helix Ca^{2+}-binding domain; PDZ, amino acids binding PDZ domains; PLIK, phospholipase-C-interacting kinase, an atypical protein kinase intrinsic to the TRPM6 and TRPM7 polypeptide chains; Nudix, NUDT9 hydrolase protein homologue binding ADP ribose. The function of the TRPM homology region is not known. Domains not drawn to scale.



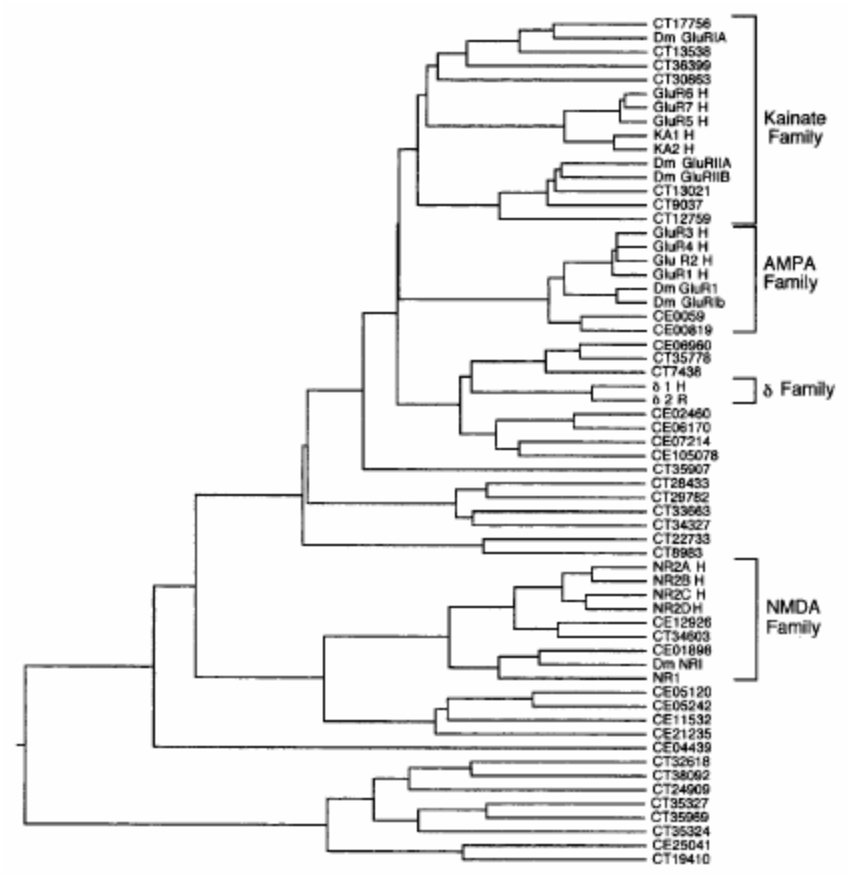
Box 2 Canonical TRP signal transduction

TRP channels are activated primarily by signal transduction pathways. A common pathway that is well established for *Drosophila* TRP activation is outlined in the Box 2 figure (for a review, see ref. 11). In mammalian cells, a GPCR (for example, muscarinic M1 acetylcholine receptor) catalyses G protein nucleotide exchange to form active G_{α} and $G_{\beta\gamma}$ subunits, in turn activating PLCβ. Alternatively, tyrosine kinase (TK) receptors activate PLCγ. PLC hydrolyses an abundant membrane component, PIP_2, into soluble and lipophilic messengers. Diacylglycerol, one product of PIP_2 hydrolysis, remains in the membrane. Soluble $InsP_3$ activates the IP_3R on the endoplasmic reticulum to release intracellular Ca^{2+}. The inset to the Box 2 figure shows PIP_2 (yellow) with its charged head group protruding above the bilayer (cytoplasmic side shown). A hydrophobic peptide (green) with interspersed basic amino acid residues sequesters PIP_2. The +25 mV electrostatic potential for the peptide (blue lines) and the -25 mV electrostatic potential for PIP_2 (red lines) are shown[82] (inset courtesy of S. McLaughlin, SUNY Stony Brook, USA).

For a cell at rest, $[Ca^{2+}]$ is ~20,000 times lower in the cytoplasm than outside the cell. It is maintained at 50–100 nM concentrations by transporters and a wealth of binding proteins. Readily bound by proteins, Ca^{2+} is inherently a localized second messenger. Ca^{2+} escapes the grasp of negatively charged domains for only microseconds before being rebound, dramatically decreasing its effective diffusion coefficient. It is likely that it modulates, either directly or indirectly, all TRP channels. DAG, a diester of glycerol and two fatty acids, is best known for its anchoring and activation of protein kinase C, but it also binds and translocates other proteins (for example, RasGRPs, Munc13 s and DAG kinase γ). DAG kinase (DAGK) phosphorylates DAG to form phosphatidic acid. Several TRP channels are activated by DAG and *Drosophila* TRP channels are constitutively active in DAGK-defective mutants[98]. DAG is also converted into arachidonic acid by DAG lipase. Arachidonic acid, itself a second messenger, is the wellspring of a large cascade of active molecules.



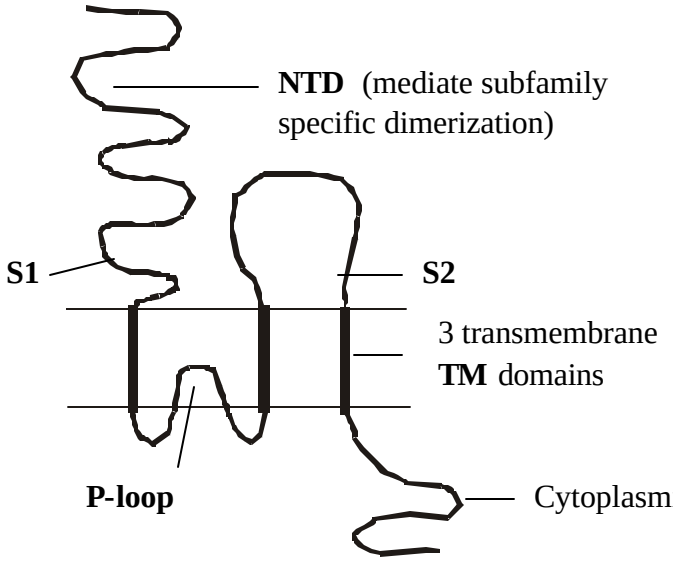
Ionotropic glutamate receptors



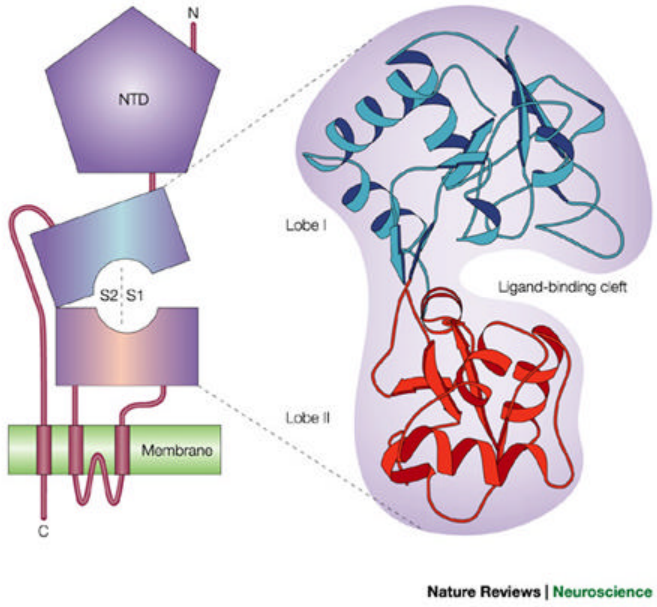
	Mammalian genes	
AMPA receptors	GluR1, 2, 3, 4 (A-D)	Fast synaptic transmission Usually not calcium-permeable (determined by GluR2)
Kainate receptors	GluR5-7, KA1, 2	Fast synaptic transmission Presynaptic modulation
NMDA receptor	NR1, NR2A, B, C, D	Fast synaptic transmission, Coincidence detector for synaptic plasticity (Extracellular Mg2+ block) Ca permeable; glycine coagonist
“orphan”	delta1, delta 2	mouse *lurcher* mutation; cerebellum
“ancient”	GluR0	prokaryotic GluR, lacking NTD

Further diversity by differential splicing (e.g. NR1 has 8); RNA editing (e.g. GluR2 QR site), subunit combination, post-translational modification (e.g. phosphorylation).

The modular nature of iGluRs



P-loop shows sequence conservation with K+ channels, but is less selective in permeability (Na+, K+, even Ca2+)
cf K+ channels (highly selective for K+)

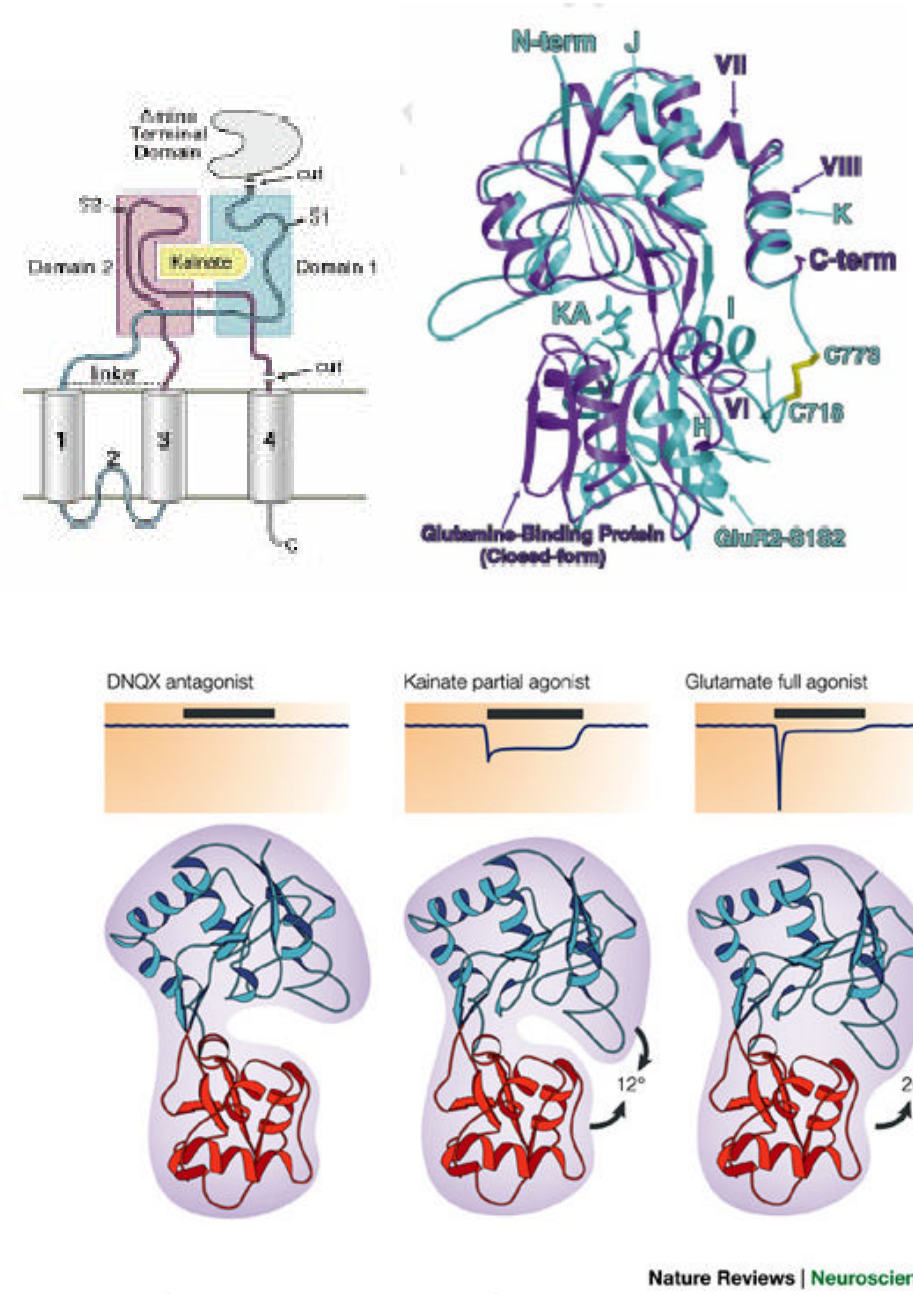


NTD and ligand binding core (S1/S2) are homologous to bacterial periplasmic binding proteins

Diagram from Madden DR Nature Rev Neurosci 2002

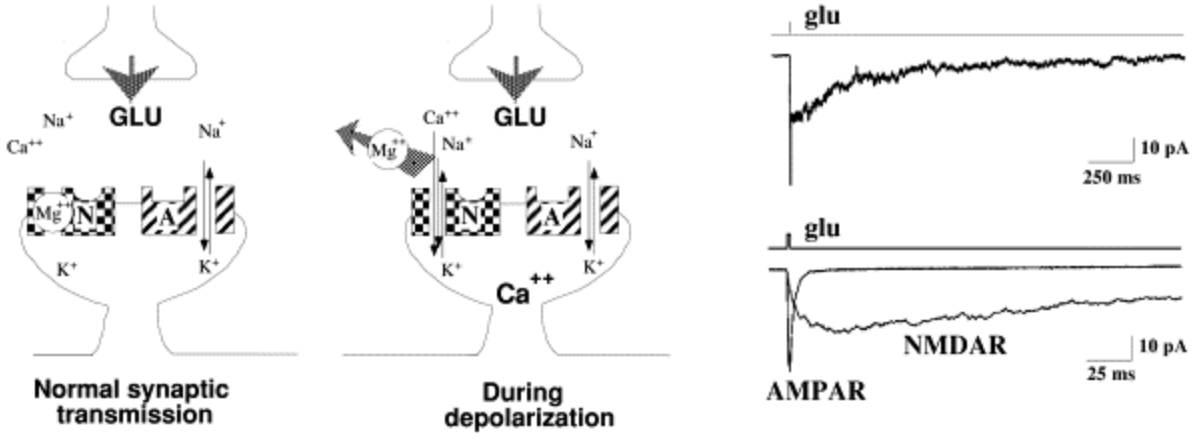
Despite the modular organization, only the ligand binding core is understood at the 3D structure level.
Structure of ligand binding core of AMPA receptor (homologous to periplasmic binding protein of bacteria). Binding of ligand induces clam-like closure of bipartite binding site.

Armstrong et al. Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature. 1998 Oct 29;395(6705):913-7.
Armstrong and Gouaux. Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. Neuron. 2000 Oct;28(1):165-81.



From D. R. Madden, Nature Reviews Neurosci 2002

Figure 2 | **Cleft closure mirrors extent of activation.** Upper panel: schematic current traces indicating the response of non-NMDA (*N*-methyl-D-aspartate) ionotropic glutamate receptors to the antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; left), the partial agonist kainate (middle) and the full agonist glutamate (right)[62]. Lower panel: corresponding structures of the S1S2 domain. DNQX binding to the S1S2 domain stabilizes the open, apo conformation of the domain, which is associated with the closed state of the channel (left). Binding of kainate (middle) induces a ~12° cleft closure in the S1S2 domain, and leads to smaller peak currents and lower levels of channel desensitization than does glutamate binding (right), which causes a ~20° cleft closure in the S1S2 domain[4].



AMPA (A) and NMDA receptors (N) in an excitatory spine synapse. Left panel, during normal synaptic transmission, glutamate activates mainly AMPA receptors which in most synapses are impermeable to Ca^{2+}. The NMDA receptors are blocked by Mg^{2+} at the neuron's resting potential. After depolarization, NMDA receptor activation leads to a transient increase in Ca^{2+} within the spine. Right panel, time-course of excitatory postsynaptic current (EPSC) mediated by AMPA and NMDA receptors simulated by a 1-ms application of glutamate (1 mM). The fast component mediated by AMPA receptors has been pharmacologically dissected from the slow component in the lower graph (by NMDA receptor antagonist APV). Note that the NMDA receptor-mediated current still rises to peak after the fast component has returned to baseline.

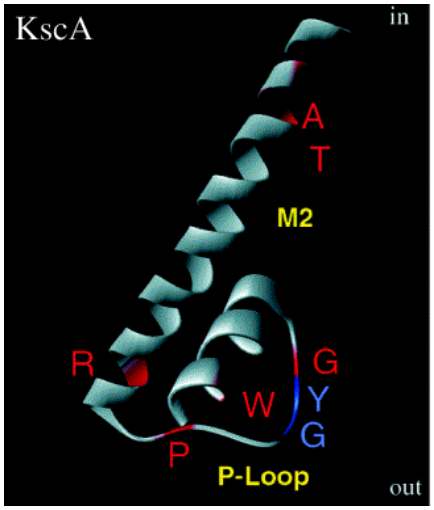
Operationally, AMPA and NMDA receptors are defined by pharmacology.

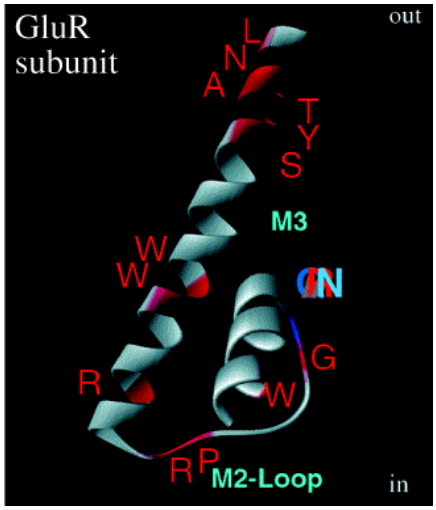
RNA editing of receptor-channels (example of ionotropic glutamate receptors)

AMPA receptors are the best example of neuronal receptor-channel altered by RNA editing.

GluR2 subunit (or GluRB in Germany) determines low Ca-permeability of AMPA receptors.
GluR2 incorporation renders heteromeric AMPA receptors Ca-impermeable.
GluR2-lacking AMPA receptors are calcium permeable.
GluR2 generally expressed highly in principal neurons and low in inhibitory interneurons.

Structure of the pore loop and selectivity filter, modeled after the P-loop of K+ channel KcsA.





Structure of the pore loop and selectivity filter. The critical channel position is indicated by Q/R/N according to the amino acid residues occupying this position in NMDA and AMPA/kainate receptor channels. Gray sticks represent the protein backbone with carbonyl groups red, amino groups blue and thiols yellow. Image rendered with SwissPDBViewer from a molecular model of glutamate receptor channels (Guy and Kuner, unpublished).

Q/R/N site represents critical channel pore residue that is altered by RNA editing.

The genomic exonic sequence in the GluR2 gene specifies that GluR2, like GluR1, 3, 4 subunits, should feature Q (glutamine) at the critical channel site. However, 99% of the GluR2 protein carries R (arginine) instead *due to RNA editing*.

RNA editing: deamination of the central adenosine (A) in the CAG codon for Q to inosine (I). The translational machinery cannot distinguish G (guanosine) from I (i.e. reads I as G) and hence, the critical channel position in GluR2 becomes occupied by R, encoded by the unusual arginine codon CIG.

RNA editing also occurs in other brain transcripts, including kainate receptor subunits (also in channel pore). Probably affects many genes.

Converting Q → R in the genome: Homozygous *GluR*-2$^{R/R}$ mice indistinguishable from wild-type littermates, thus the expression of unedited GluR2(Q) is not required for normal development and postnatal life.

Mechanisms of deamination
Intron following edited exon contains a short sequence which is complementary to the exonic sequence and forms with it an imperfect double-stranded RNA structure. This structure is recognized by an RNA-dependent adenosine deaminase that mediates the codon switch. The *cis*-acting intronic sequence, termed ECS (exon complementary sequence) is essential for Q/R site

editing of GluR2. The same basic mechanism appears to also hold for all the other A to I editings in transcripts found to date.

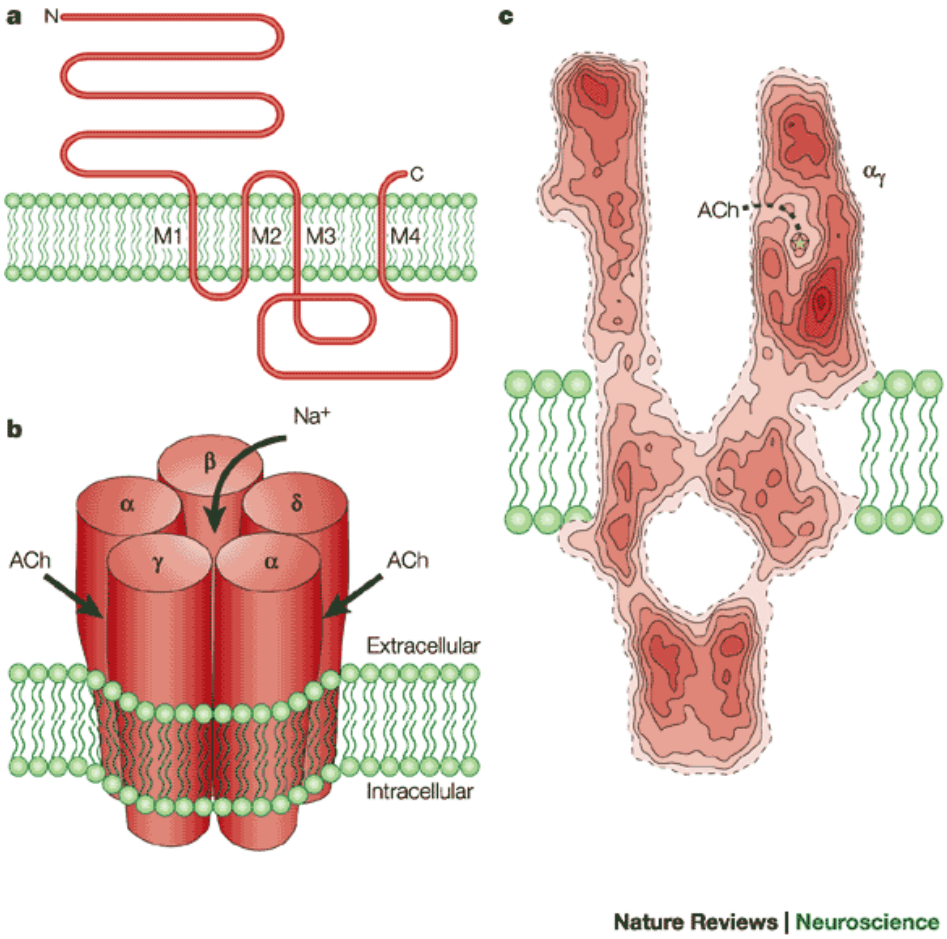
Heterozygous mice carrying one copy of GluR2 gene in which ECS is deleted (*GluR2*$^{\Delta_{ECS}}$) die by 3 weeks age suffering from epilepsy. Thus Q/R site-editing of GluR2 is essential for brain function and survival, presumably because the edited GluR-B version prevents Ca^{2+} entry through AMPA receptors in pyramidal cells (which leads to *excitotoxicity*) and lowers the single-channel conductance by a factor of ~3.

RNA-dependent adenosine deaminases – 3 genes currently known.
KO Mice deficient in ADAR2 show neurological phenotype similar to heterozygous *GluR2*$^{\Delta_{ECS}}$. ADAR2 deficiency is rescued by *GluR*-2$^{R/R}$, implying that GluR2 is the most important target of ADAR2.

The 3D Structure of Ion Channels and Receptors

Nicotinic Acetylcholine Receptor

Most extensively studied ligand-gated channel.
First receptor recognized and named; first receptor studied electrophysiologically; first characterized biochemically



From A. Karlin
Nature Reviews
Neurosci 2002

Figure 1 | **Structure of the nicotinic acetylcholine receptors.** **a** | The threading pattern of receptor subunits through the membrane. **b** | A schematic representation of the quaternary structure, showing the arrangement of the subunits in the muscle-type receptor, the location of the two acetylcholine (ACh)-binding sites (between an α- and a γ-subunit, and an α- and a δ-subunit), and the axial cation-conducting channel. **c** | A cross-section through the 4.6-Å structure of the receptor determined by electron microscopy of tubular crystals of *Torpedo* membrane embedded in ice. Dashed line indicates proposed path to binding site. Part **c** reproduced with permission from Ref. 22 © 1999 Academic Press.

AchR:

Toyoshima C and Unwin N. 1988. Ion channel of acetylcholine receptor reconstructed from images of postsynaptic membranes. Nature 336:247-250.

Toyoshima C and Unwin N. 1990. Three-dimensional structure of the acetylcholine receptor by cryoelectron microscopy and helical image reconstruction. J Cell Biol. 111:2623-35.

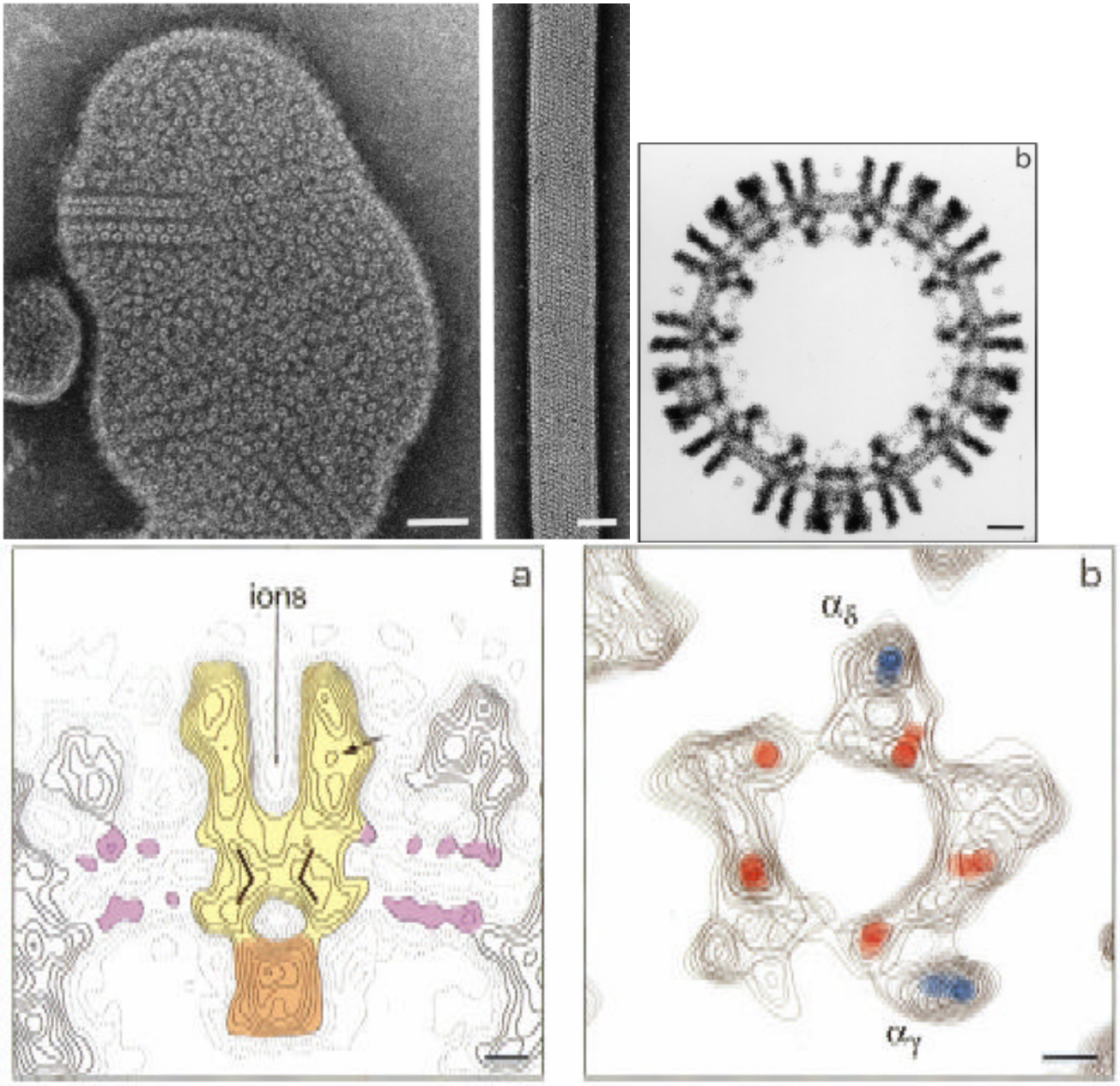
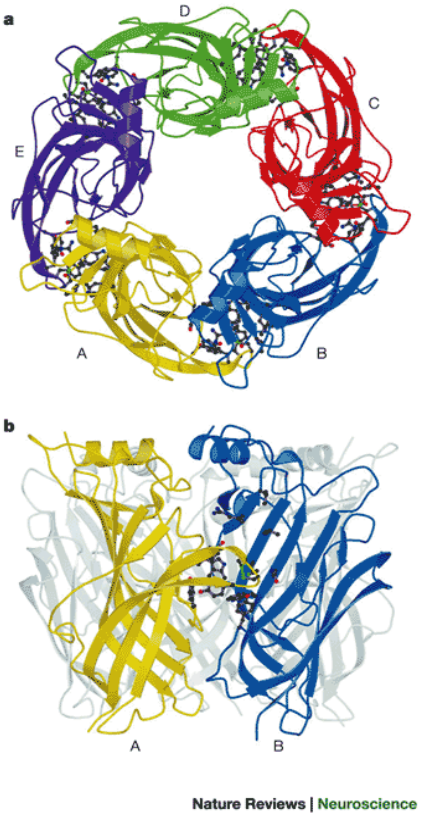


FIG. 4. Elements of secondary structure revealed at 9 Å resolution: (a) in a section normal to the tube axis; (b) in a 12-Å-thick slab at a level −30 Å from the membrane (short arrow in (a)). (a) ACh receptor (yellow) in relation to the membrane (phospholipid head groups in purple) and the clustering protein, rapsyn (orange). The receptor is about 120 Å long and protrudes −65 and −20 Å from the extracellular and intracellular membrane surfaces, respectively. The narrow membrane-spanning pore is framed by two −20-Å-diameter entrances and shaped by a ring of five bent α-helical rods (bars). Side-chains projecting inward from the bends in these rods may form the gate of the channel. (b) is made by stacking sections at 2-Å intervals on top of one another. Each of the subunits at this level contains three short rods; the red disks trace one set of rods. Internal cavities shaped by the three rods in the two α subunigits (α_γ and α_δ) may be the ACh-binding pockets. The outermost rods in these subunits (blue) are tilted at different angles, indicating that α_γ and α_δ have distinct conformations before ACh binds. Scale bars, 20 Å.

From N. Unwin. The nicotinic acetylcholine receptor of the Torpedo electric ray.
J Struct Biol. 1998;121(2):181-90. Review.

No crystal structure of AchR



Crystal structure of ACh binding protein (AChBP) from snail *Lymnaea stagnalis* – secreted by glial cells into cholinergic synapses where it modulates synaptic transmission by binding to Ach. Also pentamer, binds Ach at subunit interfaces

The acetylcholine-binding protein. **a** | The acetylcholine-binding protein (AChBP) viewed down the fivefold axis. Each of the five identical subunits is rendered in a different colour and labelled A, B, C, D or E. A ligand-binding site is formed at each interface, with A forming the (+) side and B forming the (-) side, and so on for B–C, C–D, D–E and E–A. **b** | The view perpendicular to the fivefold axis, showing one ligand-binding site as a ball-and-stick representation.

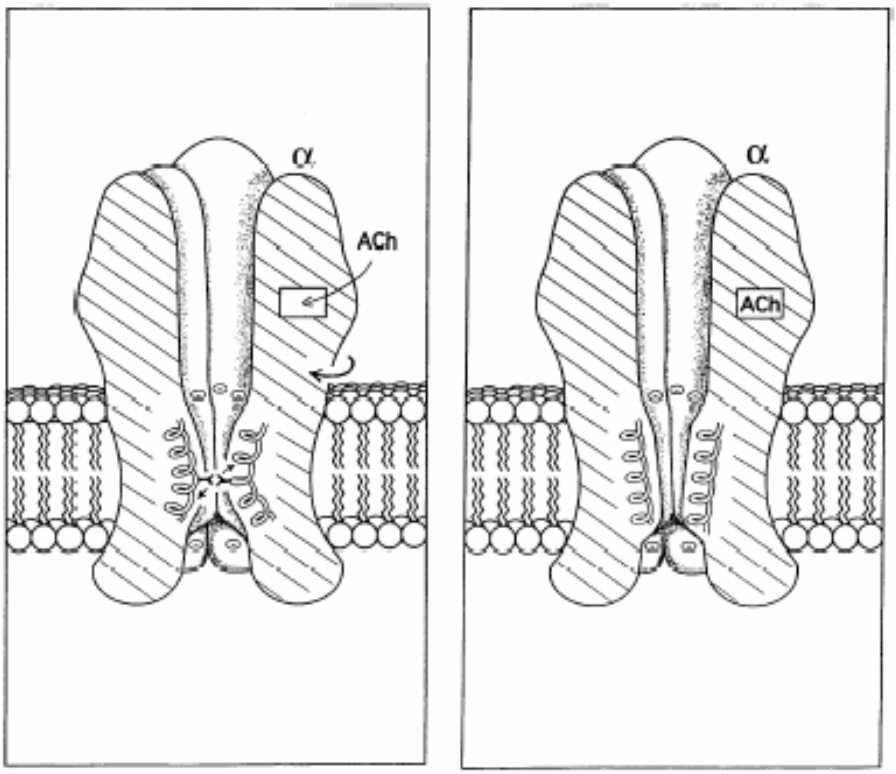


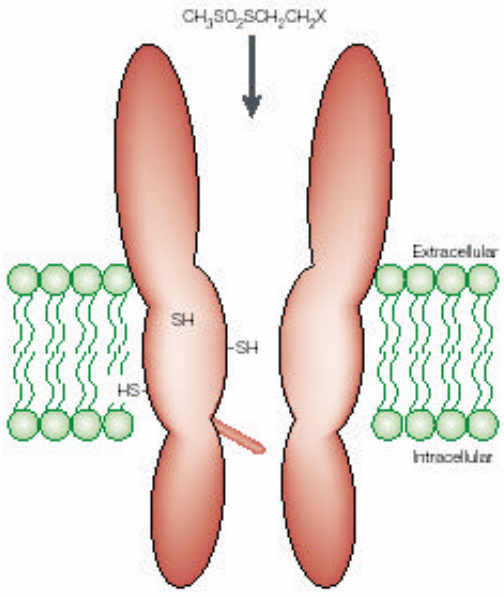
FIG. 8. Simplified diagram of the opening mechanism, suggested by the structural results. An ACh molecule first enters a binding site in one of the α subunits (rectangle), but significant displacements are blocked by the neighboring subunit, lying between the αs. Interaction of another ACh molecule with the other site then attempts to draw the neighboring subunit out of the way. A concerted localized disturbance thereby takes place, which initiates small rotations of the subunits along the shaft to the membrane. The rotations disrupt the gate by destabilizing the "ring" mode of association of α-helices around the pore (left), and the helices switch over to the alternative "twisted-barrel" mode of association, creating a widened, polar pathway for the ions (right).

From N. Unwin. The nicotinic acetylcholine receptor of the Torpedo electric ray.
J Struct Biol. 1998;121(2):181-90. Review.

Other approaches to study ion channel structure

Box 1 | **The substituted-cysteine accessibility method**

The substituted-cysteine accessibility method (SCAM) is an approach to the characterization of channel[101,131,163] and binding-site structures[62,164,165] that probes the environment of any residue by mutating it to cysteine, and by characterizing the reaction of the cysteine with sulphydryl-specific reagents. Among these reagents, the methanethiosulphonates are attractive because of their small size and their specificity for sulphydryls[166]. The reactions of charged and polar methanethiosulphonates, such as those shown in the figure, are directed to cysteines at the water-accessible surface of proteins, both because of the hydrophilicity of the reagent and because these reagents react at least ten orders of magnitude faster with ionized thiolates than with unionized thiols[167]. Cysteines that substitute for residues in the membrane-embedded segments of a channel protein are either buried in the protein interior, exposed to lipid, or exposed to water (see figure). It is assumed that the only water-accessible residues in the membrane domain are exposed to water in the channel lumen. In the case of the acetylcholine (ACh) receptor, the positively charged methanethiosulphonate ethylammonium (MTSEA) and methanethiosulphonate ethyltrimethylammonium (MTSET) are conducted by the open channel[131], and so have access to all exposed residues. The reaction of a methanethiosulphonate with a substituted cysteine in the channel can be sensitively monitored electrophysiologically by the effect of the reaction on ACh-induced current in the heterologously expressed mutant. Fortunately, cysteine substitution is very well tolerated[102,168].



SCAM has been used to identify channel-lining residues, to determine the potentially different environments of these residues in the open and closed states of the channel, to assess secondary structure, to locate selectivity filters and gates, to map the binding sites of channel blockers, and to estimate the electrostatic potential in the channel[163]. These uses require the determination of the reactivity of the cysteines; that is, the reaction rate constant for each cysteine and for each reagent used. The rate constant for the reaction of a given cysteine with a methanethiosulphonate (or other reagent) depends on the intrinsic reactivity of the reagent, on rates of reagent transport to and from the target cysteine, and on the reactivity of the cysteine sulphydryl[132]. Rates of reagent transport depend on steric and electrostatic factors along the pathways and at the reaction site. The reactivity of the target cysteine itself depends on local steric factors and, crucially, on the extent of deprotonation of the cysteine sulphydryl[133]. The individual determinants of the reaction rate can be estimated by taking the ratio of rate constants for reactions that differ only in that one determinant. We located the resting gate in the ACh receptor by taking the ratio of the rate constant for the reaction of MTSEA added to one side of the membrane to the rate constant for the reagent added to the other side, for a sequence of substituted cysteines that spanned the gating region[123]. We have also estimated the intrinsic electrostatic potential at a given cysteine by taking the ratio of the rate constants for the reactions of methanethiosulphonates that differ only in their charge[62,132,133].

These methods allow specific and sensitive probing of femtomolar quantities of heterologously expressed channels in intact cells.

from A. Karlin, Nature Reviews Neuroscience 2002

K+ channel (KcsA):

Doyle DA…..MacKinnon R. 1998. Structure of the potassium channel: molecular basis of potassium conduction and selectivity. Science 280:69-77.

Yellen G. 1999. The bacterial K+ channel structure and its implications for neuronal channels. Curr Opin 9:267-73. Review

Box 1

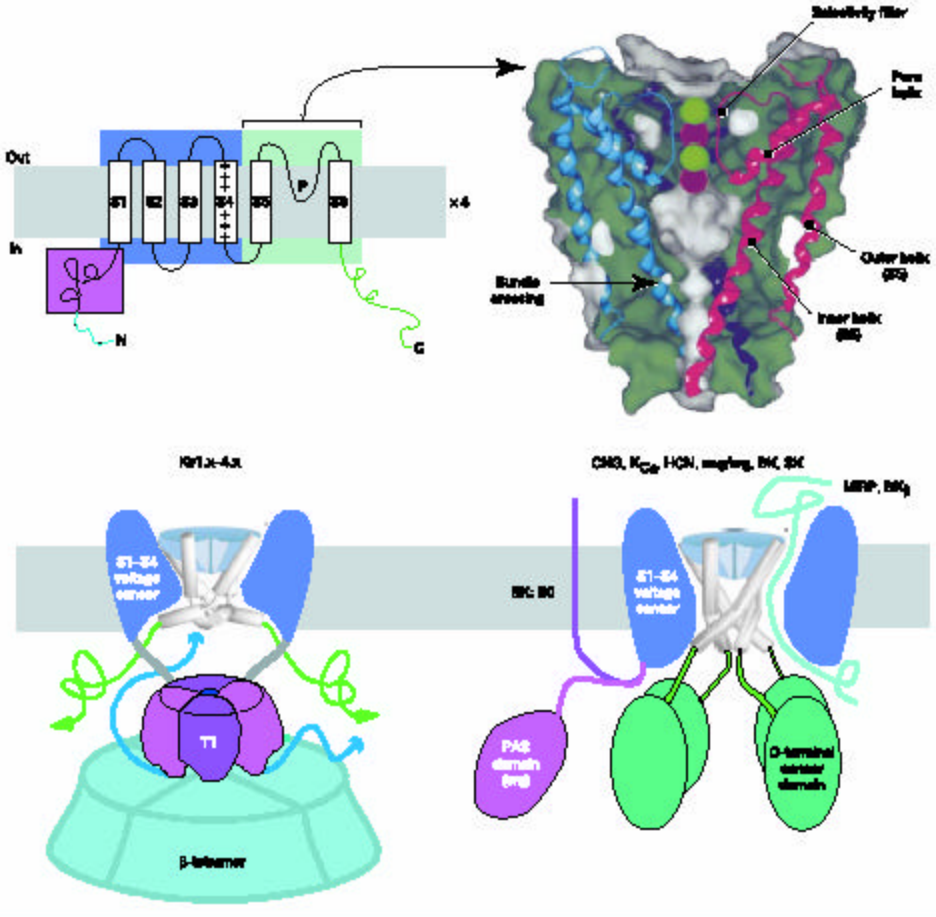
The tetrameric 6TM architecture of the K^+ channel family

The typical voltage-gated K^+ channel is an assembly of four identical (or similar) transmembrane subunits surrounding a central pore. Each subunit has six transmembrane crossings (S1–S6), with both N- and C termini on the intracellular side of the membrane (top left panel). The narrowest part of the pore, the selectivity filter, is formed by a loop between S5 and S6; the voltage sensor includes the S4 region with its multiple positive charges.

The bacterial KcsA K^+ channel (top right panel, in cross-section) is the prototype for the pore-forming domain of the channel. It has only two transmembrane helices: the outer helix is homologous to S5 and the inner helix to S6. The interior of the protein is dark green, with secondary structure shown as ribbons for three of the four subunits, and the water-exposed surface of the protein is grey. The narrow selectivity filter is seen at the top, with the extended selectivity filter loop supported by the pore helices. The four spheres mark the four K^+ ion binding sites; these are typically occupied by alternating K^+ ions and water molecules[5]. The four inner helices cross to constrict the channel at the bottom (the 'bundle crossing'). Between the selectivity filter and bundle crossing is the water-filled 'cavity'.

In the K^+ channel family there are two basic patterns for organizing the structural domains of the channels. All have the central 6TM structure, with a pore domain formed by S5–P–S6 and a voltage sensor comprising S1–S4. The core Kv1.x–Kv4.x subfamily (lower left panel) has at its N terminus a 'tetramerization domain' (T1, purple)[73,74] that determines the specificity of subunit assembly[75] and also serves as a platform for attachment of the optional β-subunits[74] and for other protein–protein interactions. These interactions probably modulate channel activity and may also provide an avenue for the channels to signal directly to intracellular signalling processes[76]. The extreme N terminus can provide the auto-inhibitory 'ball' for channel inactivation (see text). The C termini (green) have no obvious domain structure in this subfamily, although there is commonly a PDZ-binding motif[77] that determines the physical localization of the channel and its association into large signalling complexes.

Many other tetrameric 6TM channels have a different domain structure, characterized by the absence of a T1 domain and the presence of a sensor domain in the C terminus (lower right panel). These include the voltage-gated KCNQ and eag/erg channels, as well as channels gated coordinately by voltage and ligand binding (BK Ca^{2+}-activated channels and HCN hyperpolarization and nucleotide-gated channels), and channels gated exclusively by intracellular ligands (CNG channels and SK channels). In some cases the C-terminal sensor domains adopt a four-fold symmetrical organization[47], whereas in other cases they may function as a dimer of dimers[52]. At the N terminus some family members have an additional transmembrane region (S0)[78] or an additional sensor domain[79]. The auxiliary subunits known to be associated with this family (minK, MiRP, BK_β) are transmembrane; they appear to be intimately associated with the pore domain[80,81] and may displace the voltage-sensor domain or interact with it directly.



Regulation of Ion Channels

Phosphorylation
(universal regulation); applies to all channels/receptors
eg VG Na channels, [Cantrell and Catterall 2001 Nature Reviews Neurosci 2, 397]

Trafficking
Probably also widespread mode of regulation of ion channel function
eg: AMPA receptors

G-protein regulated inward rectifier K channel (GIRK)
Direct interaction with Gβγ (released from activated heterotrimeric G-protein) activates (gates) GIRK channel

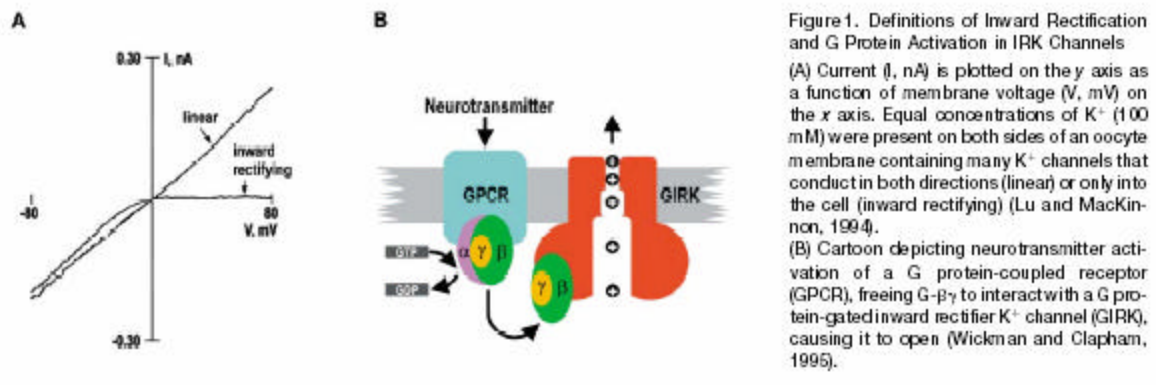


Figure 1. Definitions of Inward Rectification and G Protein Activation in IRK Channels
(A) Current (I, nA) is plotted on the *y* axis as a function of membrane voltage (*V*, mV) on the *x* axis. Equal concentrations of K^+ (100 mM) were present on both sides of an oocyte membrane containing many K^+ channels that conduct in both directions (linear) or only into the cell (inward rectifying) (Lu and MacKinnon, 1994).
(B) Cartoon depicting neurotransmitter activation of a G protein-coupled receptor (GPCR), freeing G-βγ to interact with a G protein-gated inward rectifier K^+ channel (GIRK), causing it to open (Wickman and Clapham, 1995).

[see Nishida and MacKinnon Structural Basis of Inward Rectification. Cytoplasmic Pore of the G Protein-Gated Inward Rectifier GIRK1 at 1.8 A Resolution. Cell. 2002 Dec 27;111(7):957-65.]

Other protein interactions
e.g. Calmodulin etc

Reading for Student presented papers

K channel 3D structure (Feb 12)

Primary article:
Doyle D et al. The structure of a potassium channel: molecular basis of K+ conductance and selectivity. Science 280, 69-77 (1988)
Background and review:
Choe S. Potassium channel structures. Nature Reviews Neurosci 3, 115-121 (2002)

G-protein coupled receptor (Feb 12)

Primary article
He L et al. Regulation of Opioid receptor trafficking and morphine tolerance by receptor oligomerization. Cell 108, 271-282.
Background and review
Pierce KL, Premont RT, Lefkowitz RJ. Nat Rev Mol Cell Biol 3, 639-50 (2002)