Cl^- channels & ligan-gated channels

Cheng Long, chenglong_scnu@qq.com
School of Life Sciences, South China Normal University
Mar. 27, 2012

The outline…

Required Readings:

Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physiological function of chloride channels. Physiol Rev. 2002, 82(2): 503-568.

Verkman AS, Galietta LJ. Chloride channels as drug targets. Nat Rev Drug Discov. 2009, 8(2): 153-171.

Benarroch EE. (2011) NMDA receptors: recent insights and clinical correlations. Neurology. 76(20): 1750-1757.

Luscher B, Fuchs T, Kilpatrick CL. (2011) GABAA receptor trafficking-mediated plasticity of inhibitory synapses. Neuron. 70(3): 385-409.

Edward O. Mann, Ole Paulsen (2007) Role of GABAergic inhibition in hippocampal network oscillations. Trends in Neurosciences. 30(7): 343-349.

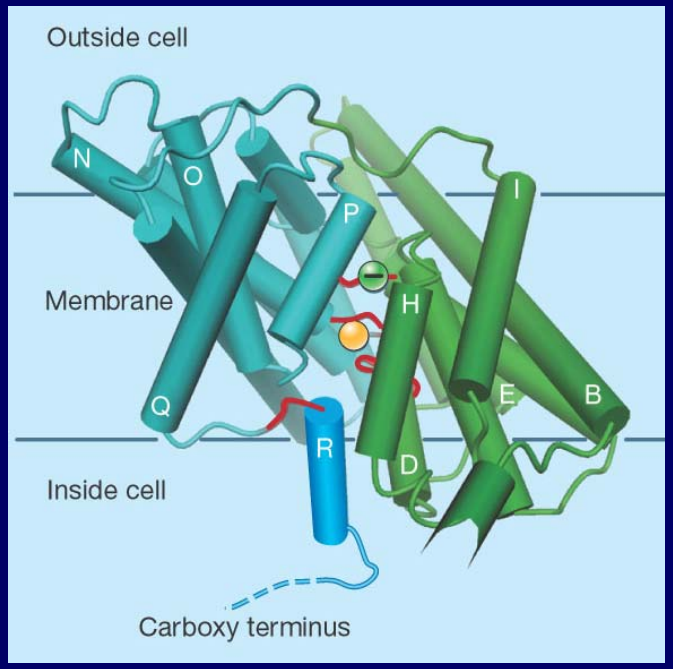
Further Readings:

Duran C, Thompson CH, Xiao Q, Hartzell HC. (2010) Chloride channels: often enigmatic, rarely predictable. Annu Rev Physiol. 72: 95-121.

Lee HK, Kirkwood A. (2011) AMPA receptor regulation during synaptic plasticity in hippocampus and neocortex. Semin Cell Dev Biol. 22(5): 514-520.

The outline…

This class will cover:



- Anion channels
- Types & structure of Cl^- channels
- Function & classification of Cl^- channels
- Regulation & disorders of Cl^- channels
- NMDA
- GABA

Introduction

- **Anion channels are proteinaceous pores in biological membranes that allow the passive diffusion of negatively charged ions along their electrochemical gradient.**
- **Although these channels may conduct other anions (e.g., I^- or NO_3^-) better than Cl^-, they are often called Cl^- channels because Cl^- is the most abundant anion in organisms and hence is the predominant permeating species under most circumstances.**
- **Cl^- channel gating may depend on the transmembrane voltage (in voltage-gated channels), on cell swelling, on the binding of signaling molecules (as in ligand-gated anion channels of postsynaptic membranes), on various ions [e.g., anions, H^+ (pH), or Ca^{2+}, on the phosphorylation of intracellular residues by various protein kinases, or on the binding or hydrolysis of ATP.**

Where are anion channels encountered?

Anion channels were detected almost **everywhere**.

- In **synaptic vesicles** from rat brain and from *Torpedo* electric organ, voltage-dependent anion channels of **intermediate conductance (10–100 pS)** were found. These channels were present in every synaptic vesicle.
- Reconstitution of **endoplasmic reticulum membranes** from rat hepatocytes yielded a **large-conductance (150–200 pS)** anion channel, which was also voltage dependent. A different type of anion channel has been found in sheep brain endoplasmic reticulum membranes, where it is colocalized with calcium release channels.
- An anion channel in the **Golgi complex** was characterized, which was present even in the absence of protein translation, indicating that these channels are not en route to the plasma membrane, but endogenous to this compartment.

Anion exchange proteins

- SLC4A1 (AE1): Erythrocyte band 3 protein
  - Major integral glycoprotein in erythrocyte membrane
  - Polymorphisms determine Diego blood group
  - Diseases: Spherocytosis; Ovalocytosis; Renal tubular acidosis; Hypokalemic periodic paralysis
- Other
  - SLC4A2: Anion exchanger; Choroid plexus, GI & Other
  - SLC4A3: Anion exchanger; Cardiac & Brain
  - SLC4A4: Na Bicarbonate cotransporter; Renal; Renal tubular acidosis, glaucoma, cataracts, & band keratopathy
  - SLC4A5: Na Bicarbonate cotransporter; Pancreas
  - SLC4A6: Na Bicarbonate cotransporter; Retina

Anion exchange proteins

- Other
  - SLC17A5 (Sialin): Salla syndrome (Sialic acid storage)
  - SLC26A3: Down-regulated in adenoma (DRA)
    - Sulfate transporter
    - Congenital chloride diarrhea
  - SLC26A4: Transporter of Chloride & Iodide
    - Non-syndromic deafness, congenital (DFNB4)
    - Pendred syndrome
    - Enlarged vestibular aqueduct syndrome

Voltage dependent anion selective channel proteins (VDAC)

- **Location: Outer mitochondrial membrane, inner mitochondrial membrane, plasma membrane**
- **Functions**
  - **Channels for small hydrophilic molecules**
  - **Translocation of adenine nucleotides through outer mitochondrial membrane**
  - **BCL2 proteins bind to VDAC: Regulate mitochondrial membrane potential & release of cytochrome c during apoptosis**
  - **Mitochondrial binding site for: Hexokinase (HK1); Glycerol kinase**

VDAC

- VDAC1
  - Pathway for movement of adenine nucleotides through outer mitochondrial membrane
  - Mitochondrial binding site for hexokinase and glycerol kinase
- VDAC2
  - Open conformation: At low or zero membrane potential; Weak anion selectivity
  - Closed conformation: At potentials above 30-40 mV; Cation-selective
- VDAC3
  - High expression in testis
  - Null mice
    - Sperm motility: Reduced
    - Muscle: Mitochondria abnormally shaped, Respiratory chain complex activity reduced
- VDAC4

Anion transporter

- Organic anion transporter (OATP)
- Na^+-independent transport of organic anions, e.g. bile acids
- Canalicular multispecific organic anion transporter (cMOAT)
- Dubin-Johnson Syndrome
- Sulfate anion transporter

Chloride channels

- Considerably less attention has been given to chloride channels. This is partly because their identification and analysis has lagged behind those of other targets, and because of technical challenges in screening for chloride channel modulators.
- Electrophysiologists have historically considered anion channel currents as 'unimportant leaks' associated with cation channels in excitable cells.
- Chloride channels display a variety of important physiological and cellular roles that include regulation of pH, volume homeostasis, organic solute transport, cell migration, cell proliferation and differentiation.

Chloride channels

- A large molecular diversity of Cl^- channels. Chloride channels are a superfamily of poorly understood ion channels consisting of approximately 13 members.
- Cl^- channels may be classified as to their localization (plasma membrane vs. vesicular), single-channel conductance, or mechanism of regulation. However, such classification schemes are ambiguous. The most logical classification of Cl^- channels will be based on their molecular structures.
- Based on sequence homology the Cl^- channels can be subdivided into a number of groups.
- The importance of one such group, the CLC family of Cl^- channels, can be seen from the diseases that develop when the channel does not function normally.

Functions of Cl^- channels

- Cl^- channels may perform their functions in the plasma membrane or in membranes of intracellular organelles. On the one hand, these functions are related to the transport of charge, i.e., to the electric current flowing through the channel, and on the other hand to the transport of matter.
- Allow the passive diffusion of negatively charged ions along electrochemical gradient
- Plasma membrane Cl^- currents are important for the regulation of excitability in nerve and muscle. Currents flowing through intracellular Cl^- channels are thought to ensure the overall electroneutral transport of the electrogenic H-ATPase that acidifies several intracellular compartments.

Functions of Cl^- channels

- Chloride channels are important for setting cell resting membrane.
- Bulk flow of chloride is important for cell volume regulation, as well as for transepithelial transport.
- These channels conduct Cl^- as well as other anions such as HCO_3^-, I^-, SCN^-, and NO_3^-. May conduct other anions (e.g., I^- or NO_3^-) better than Cl^-.
- Functions often related to transport of charge.
- The regulation of Cl^- channel activity by anions implies that changes in intracellular Cl^- concentration ($[Cl^-]_i$) may have a regulatory role.
- Cl^- does not seem to play a role as intracellular messenger.

Classification schemes

- **Localization: Plasma membrane *vs.* vesicular**
- **Single-channel conductance**
- **Mechanism of regulation**
- **Molecular structure**

Classification of chloride channels

- cAMP-activated chloride channel (Cystic fibrosis transmembrane conductance regulator, CFTR)
  - activated by cyclic AMP-dependent phosphorylation
- Calcium activated chloride channels (CaCCs)
- Voltage gated chloride channels (ClCs)
- Ligand-gated chloride channels (GABA (γ-aminobutyric acid) and glycine activated)
- Swelling-activated chloride channels (Volume regulated chloride channels)
- Putative intracellular chloride channels

Structural classes of Cl^- channels

- Extracellular ligand-gated Cl^- channels (ELG)
- Cystic fibrosis transmembrane conductance regulator (CFTR)
- Voltage-gated chloride channels (CLC)
- Nucleotide sensitive chloride channel (CLNS1A): Volume sensitive
- Chloride intracellular channels
- Calcium activated: Mediate a calcium-activated chloride conductance

Extracellular ligand-gated Cl^- channels (ELG)

- 4 transmembrane domains in each subunit
- Receptors function as pentamers
- Types
  - Post synaptic
  - GABA & glycine receptors

Voltage-gated chloride channels (ClC)

- Membrane-associated part of ClC channels is composed of **17 α-helices**
  - Helix A is not inserted into the membrane
  - Most of the helices are not perpendicular to the membrane, but severely tilted
  - Helices may not span the width of the bilayer
- Carboxy terminus of eukaryotic ClC proteins has **two CBS domains**
  - Unspecified role in protein-protein interaction
- ClC channels are **dimers**: Each monomer has one pore (double-barreled channels)
- ClC-K proteins
  - Associate with the β-subunit barttin which spans the membrane twice

ClC channel types

- CLCN-1
  - Skeletal muscle, Placenta
  - Voltage stabilization
  - Mutation disorders: Myotonia; Paramyotonia
- CLCN-2
  - Ubiquitous
  - Cell volume regulation
  - Activated by hyperpolarization, cell swelling & acidic pH
  - Mutation disorders: Idiopathic generalized epilepsies
- CLCN-3
  - Brain, Kidney (Type B intercalated cells), Skeletal muscle, Lung, Retina
  - Intracellular
  - Endosomes & Synaptic vesicles
  - Knockout: Degeneration of hippocampus & retina

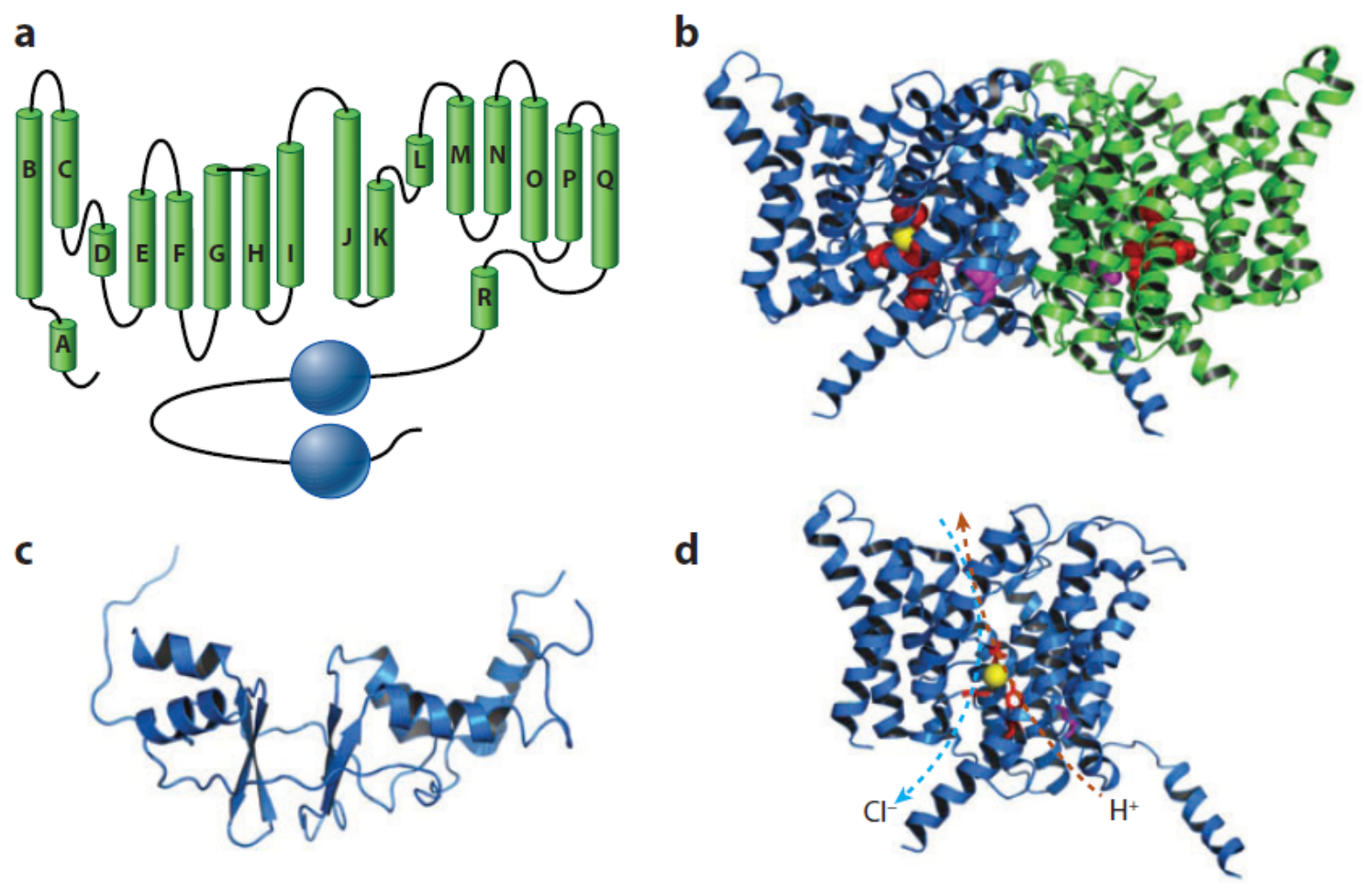
ClC channel types

- CLCN-4
  - Muscle, Brain, Heart, Kidney, Retina
  - Vesicular channel
- CLCN-5
  - Kidney (Type A intercalated cells)
  - Endosomal channel
  - Renal endocytosis
  - ? Cl^- reabsorption
- CLCN-6
  - Ubiquitous
  - Intracellular
  - CLCN6-null mice
    - Reduced pain sensitivity
    - Behavioral abnormalities
    - Enlarged proximal axons with autofluorescent electron-dense material, containing lysosomal proteins

ClC channel types

- CLCN-7
  - Brain; Testes; Skeletal muscle; Kidney
  - Lysosomal
  - Mutations: Osteopetrosis
- ClC-0: *Torpedo* electric organ Cl^- channel
- ClC-K/barttin channels: Transepithelial transport in kidney & inner ear
  - CLC-K1 (ClCN-KA): Kidney
    - Transepithelial Cl^- transport
    - Location: Thin ascending limb of Henle loop in renal inner medulla
    - Plays role in urine concentration
  - CLC-K2 (ClCN-KB)
    - Kidney
    - ? Cl^- reabsorption
    - Bartter syndrome types 3 & 4

ClC protein structure



- Blue: cystathionine-β-synthase (CBS) domains
- Green: membrane segments
- Yellow: bound Cl^-
- Red: the intracellular proton-binding site E203

Chloride intracellular channels

- General
  - Location: Nuclear or plasma membrane
  - No membrane spanning domains
  - Found vacuolar organelles
  - Function: Electrolyte composition & acidification of intravesicular spaces
- CLIC1: Nuclear
- CLIC2: Skeletal muscle; fetal liver
- CLIC3: Plasma membrane; interacts with Erk7
- CLIC4: Brain, heart, placenta, skeletal muscle
  - Role: Development of vascular collaterals in skeletal muscle & brain
- CLIC5: Heart; skeletal muscle

Channels of intracellular organelles

- Anion channels (or transporters) are needed for the passage of anionic substrates like **phosphate** and **sulfate** out of degradative as well as biosynthetic compartments, e.g., lysosomes and the Golgi apparatus.
- A large-conductance anion channel of cardiac sarcoplasmatic reticulum is shown to conduct adenine nucleotides, but the physiological role of this conductance remains elusive.
- Anion channels are implicated in organellar volume regulation. Mitochondria are subject to volume changes, depending on the metabolic state of the cell. This is probably mediated by the flux of K^+ and Cl^- across the inner mitochondrial membrane.

Channels of intracellular organelles

- A vesicular volume increase is reported to accompany the exocytosis of secretory granules in mast cells and in pancreatic acinar cells, which was also mediated by the uptake of potassium chloride.
- Cl^- channels play an important role in maintaining electroneutrality. Electrogenic uptake of protons or calcium ions into intracellular compartments will very soon create a charge imbalance hampering further uptake. This is true for the Ca^{2+}-ATPase of endoplasmic and sarcoplasmic reticulum as well as for the V-type H-ATPase of the Golgi lamellae as well as endosomal and synaptic vesicles.
- To build up the necessary calcium or proton gradients, the excess positive charge in these organelles has to be neutralized. In principle, this may be achieved either by import of chloride (via anion channels) or by export of potassium (via cation channels).

Channels of intracellular organelles

- **Acidification is more efficient in the presence of extravesicular chloride. In situ studies with secretory and recycling endosomes of the *trans*-Golgi network indicated a dependence of the acidification rate on both potassium and chloride in the cytosol. This demonstrates the requirement for a chloride conductance in the acidification of these intracellular organelles.**

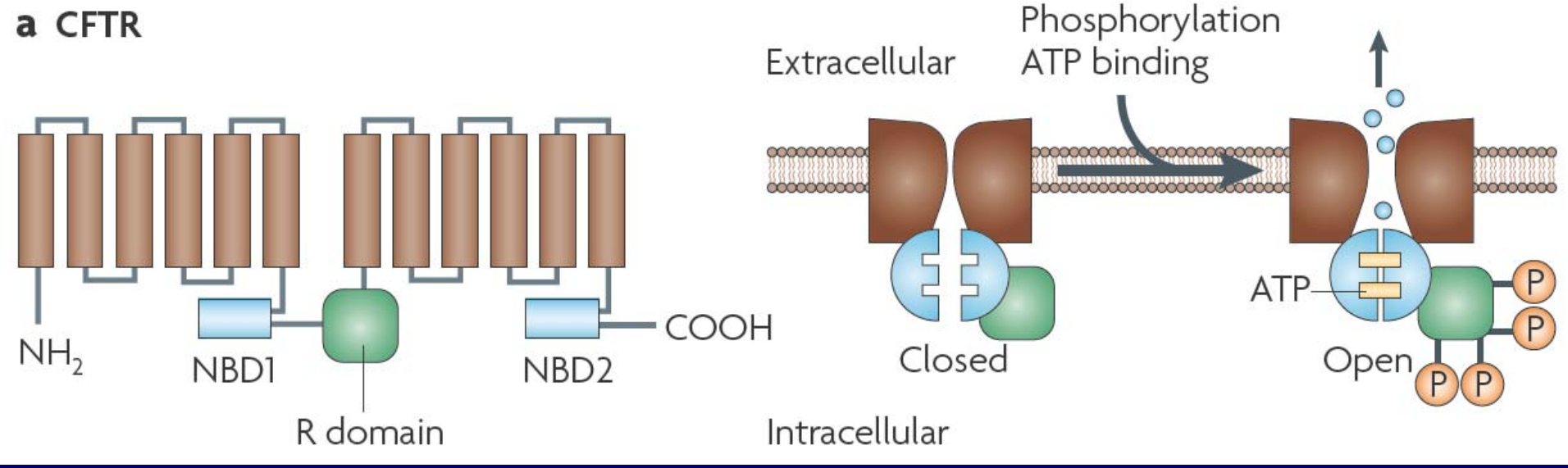
Methods studying for intracellular channels

- The isolation of the membrane i.e. in the form of small vesicles.
  - Tracer-flux assays
  - Lipid bilayer for electrophysiological investigation
  - Patch-clamp techniques
- The purification of the channel protein and subsequent reconstitution is an alternative, but this entails the loss of the native environment and possibly conformational changes of the protein.
- The direct observation of intracellular ion channels in intact membranes has been reported, but this is technically very demanding.
- A much simpler way to study intracellular channels would be to redirect them to the plasma membrane. By overexpressing them, some of the intracellular ClC channels (ClC-3, -4, -5) are incorporated into the plasma membrane, but this does not work for all intracellular channels.

Structure

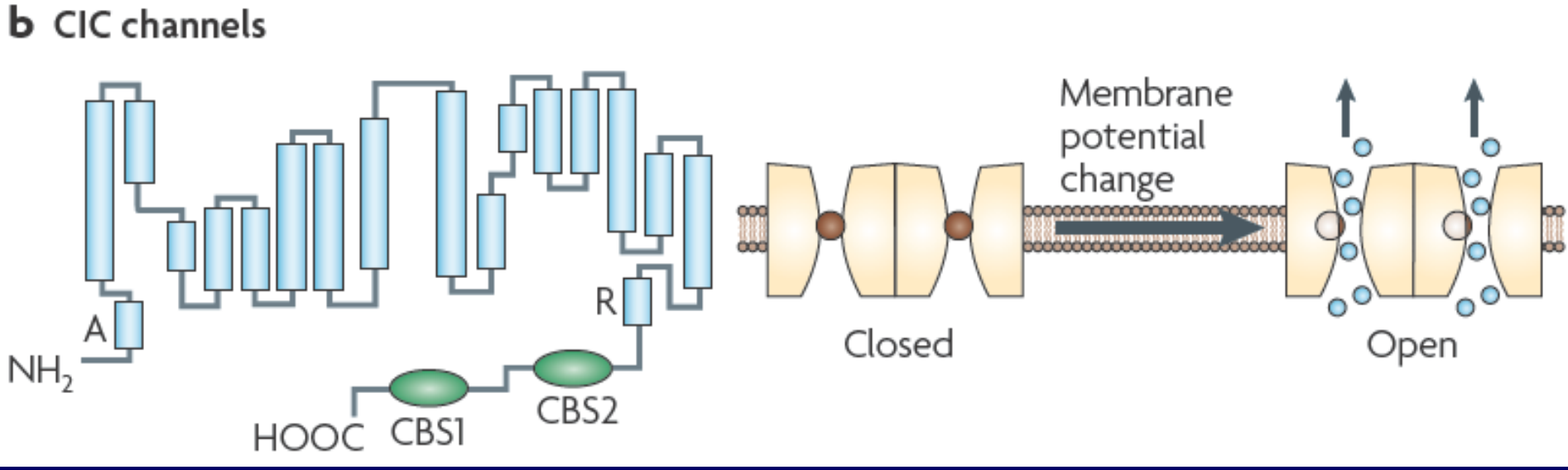
- The structure of these channels are not like other known channels.
- Chloride channel subunits contain between 1 and 12 transmembrane segments. This family of ion channels contains 10 or 12 transmembrane helices. Each protein forms a single pore.
- Some members of this family form homodimers. In terms of primary structure, they are unrelated to known cation channels or other types of anion channels.
- Three ClC subfamilies are found in animals. ClC-1 (UniProt P35523) is involved in setting and restoring the resting membrane potential of skeletal muscle, while other channels play important parts in solute concentration mechanisms in the kidney.
- These proteins contain two CBS domains.
- Some members of this family are activated by voltage, while others are activated by Ca^{2+}, extracellular ligands, and pH among other modulators.

Mechanisms of regulation of Cl^- channels



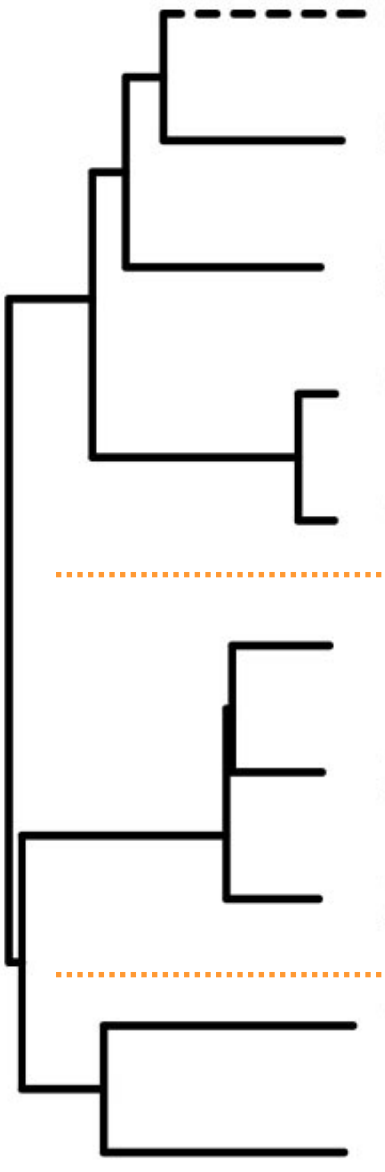
- Cystic fibrosis transmembrane conductance regulator (CFTR). Shown here are 12 membrane-spanning segments of CFTR plus two nucleotide binding domains (NBDs 1 and 2) and a regulatory R domain.
- CFTR activation involves cyclic AMP-dependent phosphorylation and binding of ATP molecules at the NBDs.

Voltage-gated Cl^- channels



- CIC channels has 18 segments (labelled A to R), most of which span the plasma membrane partially and in a strongly tilted configuration.
- Fast gating involves flipping of a pore-lining glutamate side chain into and out of the chloride pathway. Channels are arranged as dimers with a slow gate controlling the activity of both channels simultaneously.
- CBS, cystathione β-synthase-related domain.

The ClC family of Cl^- channels in mammals



	expression	function	disease	KO mouse
ClC-0				
ClC-1 7q35	skeletal muscle	stabilization of plasma membrane V	myotonia congenita	myotonia congenita (adr mouse)
ClC-2 3q27	broad	transepithelial transport? pH,volume regulation?	?	degeneration: testes and retina
ClC-Ka 1p36	kidney, inner ear*	transepithelial transport	? (BSND)*	nephrogenic diabetes insipidus
ClC-Kb 1p36	kidney, inner ear*	transepithelial transport	Bartter's syndrome (BSND)*	-
ClC-3 4q33	broad (brain, kidney, liver ...)	acidification of endosomes, synaptic vesicles	?	degeneration: hippocampus and retina
ClC-4 Xp22.3	broad (brain, muscle...)	?	?	?
ClC-5 Xp11.22	kidney (intestine, liver...)	acidification of endosomes	Dent's disease	defect in renal endocytosis
ClC-6 1p36	broad	?	?	?
ClC-7 16p13	broad	acidification of lysosomes, resorption lacuna of osteoclasts	osteopetrosis	osteopetrosis

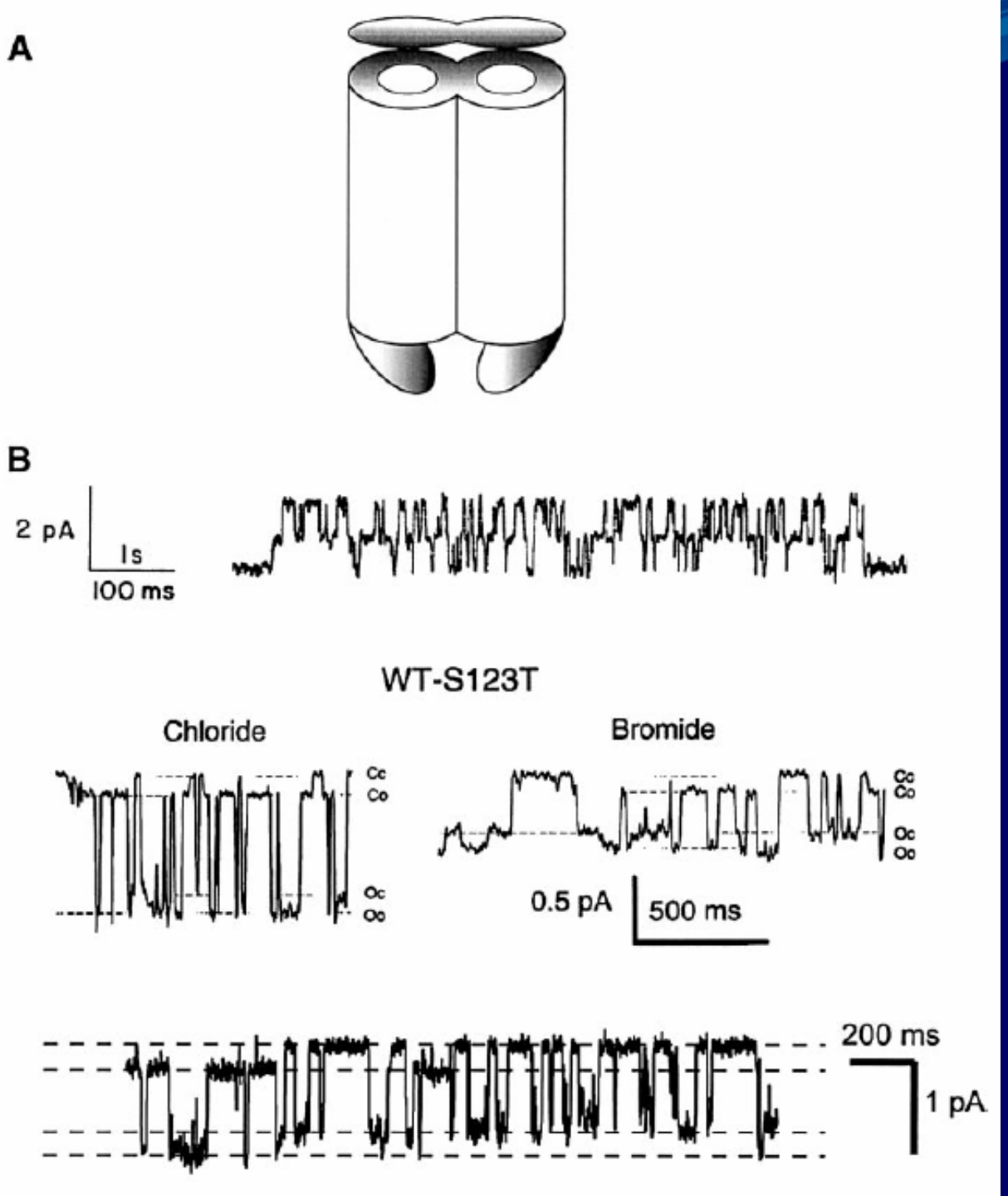
Voltage-gated chloride (ClC) channels

- The ClC-1 Cl channel provides the bulk of resting conductance of the plasma membrane of skeletal muscle. As a consequence, its mutational inactivation leads to myotonia in humans and mice.
- The role of ClC-2 is less clear. The testicular and retinal degeneration resulting from its disruption in mice may suggest a role in transepithelial transport.
- The two renal ClC-K channels function (in a heteromeric complex with barttin) in transepithelial transport across different nephron segments, as demonstrated by Bartter's syndrome in humans and renal diabetes insipidus in mice. In addition, both ClC-Ka/barttin and ClC-Kb/barttin are important for inner ear K secretion. Accordingly, human mutations in barttin lead to Bartter syndrome associated with deafness.

Voltage-gated chloride (ClC) channels

- The knock-out of ClC-3 in mice led to a severe degeneration of the hippocampus and the retina. ClC-3 is present in endosomes and synaptic vesicles, but whether the degeneration is due to the observed impairment of synaptic vesicle acidification is currently unclear.
- Mutations in ClC-5 underlie Dent's disease, an inherited disorder characterized by kidney stones and proteinuria. Both symptoms are a consequence of a reduced proximal tubular endocytosis, as revealed by a recent ClC-5 knock-out (KO) mouse model. Probably similar to ClC-3, ClC-5 provides a shunt for the H-ATPase that is necessary for the efficient acidification of endosomes.
- Mutations in ClC-7 lead to osteopetrosis, as first recognized in a mouse model and then confirmed for humans.

- The double-barreled structure of CIC channels
- A recording from a native CIC-0 channel incorporated into a lipid bilayer
- Excised patch containing a concatemer of a WT and a mutant (S123T) CIC-0 protein
- Registration of a CIC-0/CIC-2 concatemer



Swelling-activated Cl^- channels

- Cells must change their volumes in the face of several external and internal challenges during growth and cell division. Volume regulation is an universal feature of all vertebrate cells.
- Cells are endowed with various ion and organic osmolyte transport proteins that activate upon cell swelling or cell shrinkage.
- In the presence of a significant water permeability of the plasma membrane, water follows osmotically, resulting in a regulated change of cell volume. This is called regulatory volume increase (RVI) and regulatory volume decrease (RVD). RVI most often involves the uptake of Na^+ and Cl^-, for instance, by the concomitant activation of Na^+/H^+ and Cl^-/HCO_3^- exchangers. Na^+ is replaced by K^+ through the Na^+-K^+-ATPase, resulting in a net intracellular accumulation of KCl. In RVD, intracellular KCl may be extruded by KCl cotransporters of the KCC gene family, or by the concerted activation of swelling-activated Cl^- channels and K^+ channels.

Swelling-activated Cl^- channels

- In neurons and other cell types, $[Cl]_i$ is on the order of a few millimolar.
- An exclusive reliance on KCl extrusion would allow these cells to shrink only by a few percent. Much higher volume changes are observed experimentally in neuronal cells. This may be accomplished by the regulated release of intracellular osmolytes like taurine, glutamate, or aspartate, whose intracellular concentration is more abundant.
- A loss of organic osmolytes during RVD is by no means restricted to neurons. In kidney, taurine may be the most abundant intracellular amino acid, and other osmolytes like *myo*-inositol, sorbitol, and betaine play important roles in the hyperosmolar environment of the renal medulla.

Biophysical characteristics of swelling-activated Cl^--currents

- Cell swelling induces a characteristic anion-selective whole cell conductance, which is commonly called $I_{Cl,swell}$, displays moderate outward rectification, lacks conspicuous time-dependent activation upon depolarization, and shows variable inactivation at voltages more positive than 40 mV. In some cells, $I_{Cl,swell}$ shows less rectification and inactivation, possibly suggesting a molecular diversity of underlying channel proteins.
- It is commonly agreed that $I_{Cl,swell}$ displays an $I^- > Br^- > Cl^- > F^- >$ $glutamate^-$ permeability sequence. Similarly, it also displays a preference of I^- over Cl^- when conductances are compared.
- This anion channel also mediates osmolyte flux. Single-channel recordings showed a significant conductance and permeability for aspartate, glutamate, and taurine.

Biophysical characteristics of swelling-activated Cl^--currents

- Both Cl^- conductance and osmolyte transport were activated with the same time course after hypotonic swelling, $P_{Cl}/P_{taurine}$ remained constant during hypotonic swelling, and there was a similar or identical pharmacological profile of both permeation processes.
- Cl^- and polyol osmolytes may compete for a common binding site, and both Cl^- current and osmolyte efflux depend on intracellular ATP.
- Another study reports a different time course of activation of I^- and taurine efflux in HeLa cells, leading to the suggestion that these are mediated by different proteins.

Regulation of $I_{Cl,swell}$

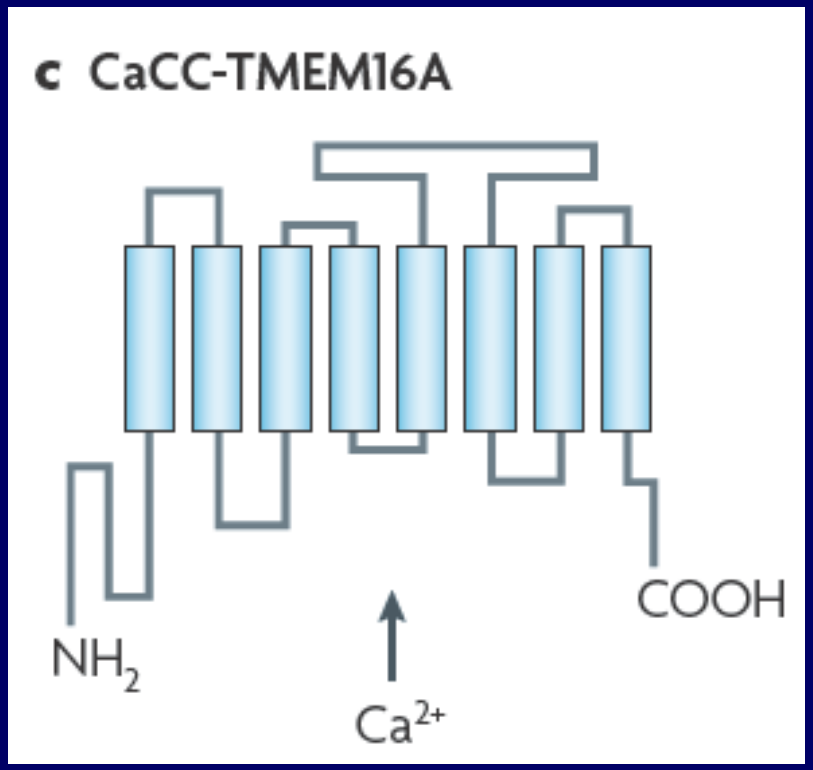
- The activation of $I_{Cl,swell}$ depends on the presence of intracellular ATP.
- No ATP hydrolysis is necessary, as it could be replaced by nonhydrolyzable analogs. The dependence on cytosolic ATP may serve to prevent the loss of metabolically valuable intracellular organic osmolytes during starvation. At low $[ATP]_i$, intracellular Mg^{2+} inhibits $I_{Cl,swell}$. The activation of $I_{Cl,swell}$ is modulated by the $[Cl^-]_i$. High $[Cl^-]_i$ shifted the set-point of activation to larger volumes or decreased the rate of activation.
- There is no direct mechanical gating.
- Unidentified second messengers might be involved. $I_{Cl,swell}$ cannot be activated by raising $[Ca^{2+}]_i$, although a basal level of $[Ca^{2+}]$ is probably needed.

Regulation of $I_{Cl,swell}$

- No evidences for phosphorylation in the activation of $I_{Cl,swell}$
- A role of mitogen-activated and tyrosine kinases in activating $I_{Cl,swell}$ in astrocytes. tyrosine phosphorylation by p56lck kinase is involved: swelling-activated currents could not be elicited in p56lck-deficient cells, but were restored upon transfection of the WT cDNA and by the addition of the kinase to the intracellular side of excised patches.
- Hyposmotic swelling increased the activity of the kinase. In contrast to these observations, a recent report stated that protein phosphotyrosine phosphatase inhibitors suppressed RVD and the volume-sensitive Cl^- conductance in mouse fibroblasts.
- Other proposed mechanisms of activation involve G proteins, lipoxygenases and arachidonic acid metabolites.

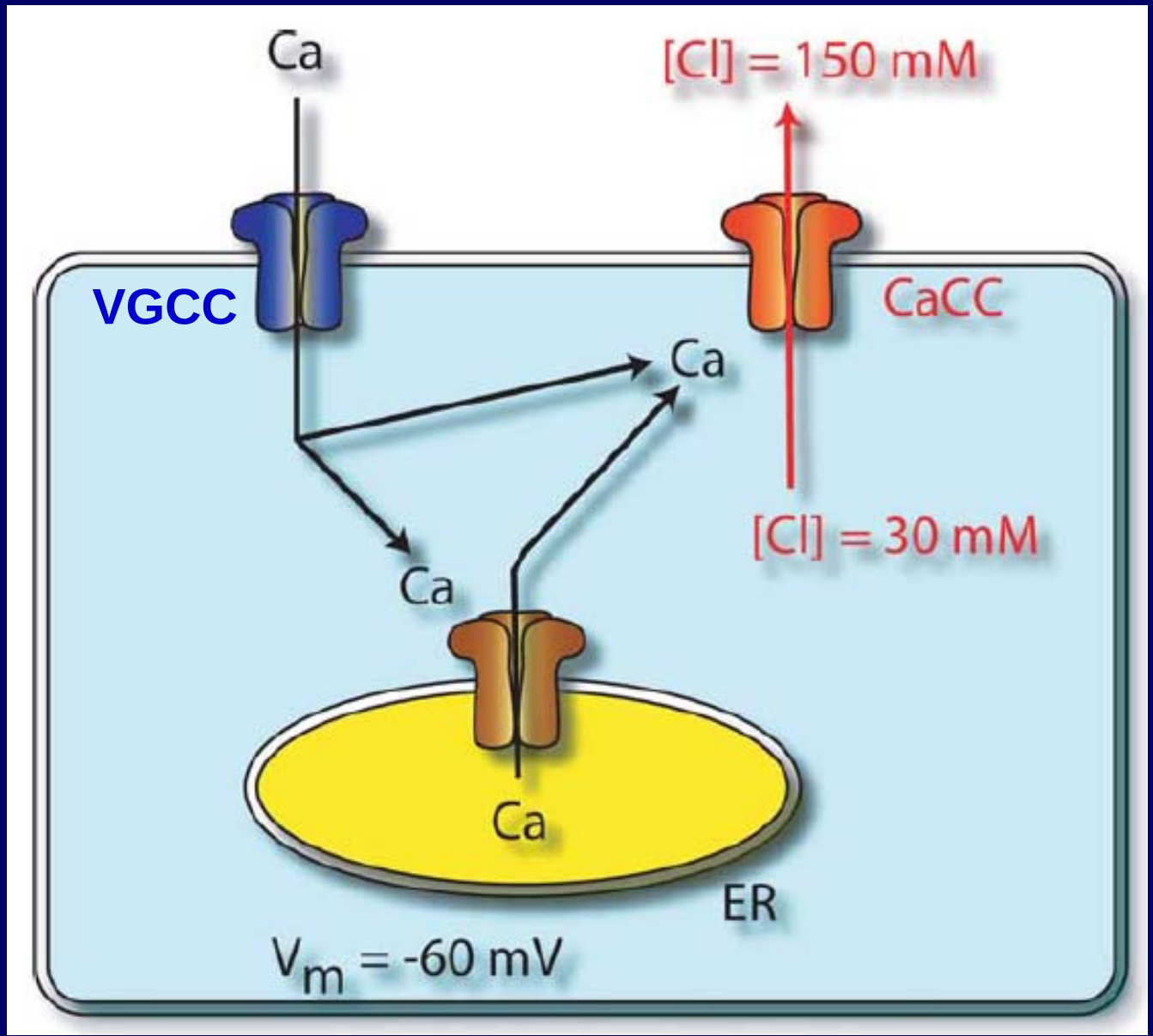
Calcium-activated chloride channel

Calcium-activated chloride channel

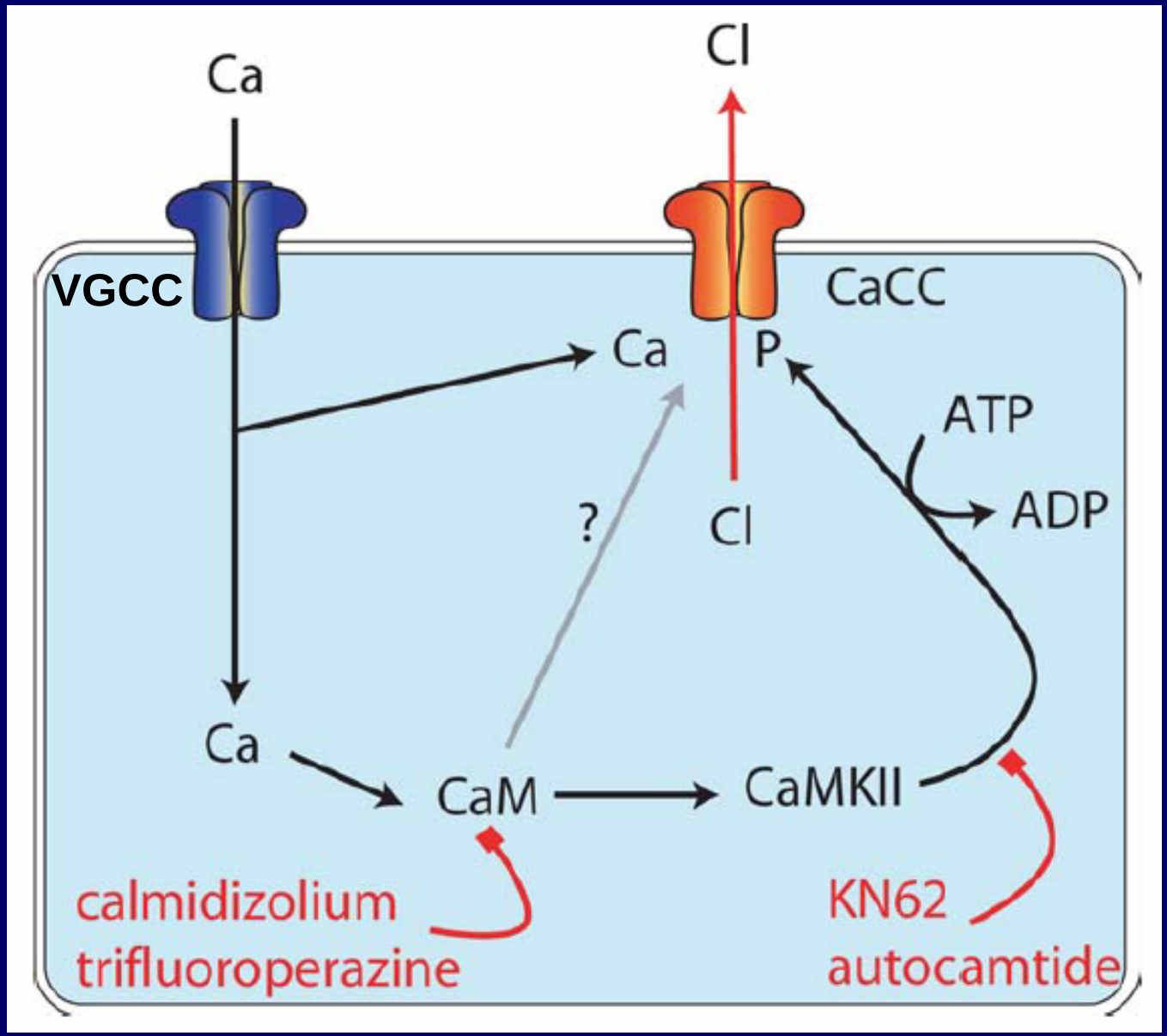


- The calcium-activated chloride channel (CaCC) TMEM16A (anoctamin-1), with predicted topology showing **eight transmembrane segments** with cytosolic amino and carboxy termini.
- The mechanism of calcium activation is unknown.

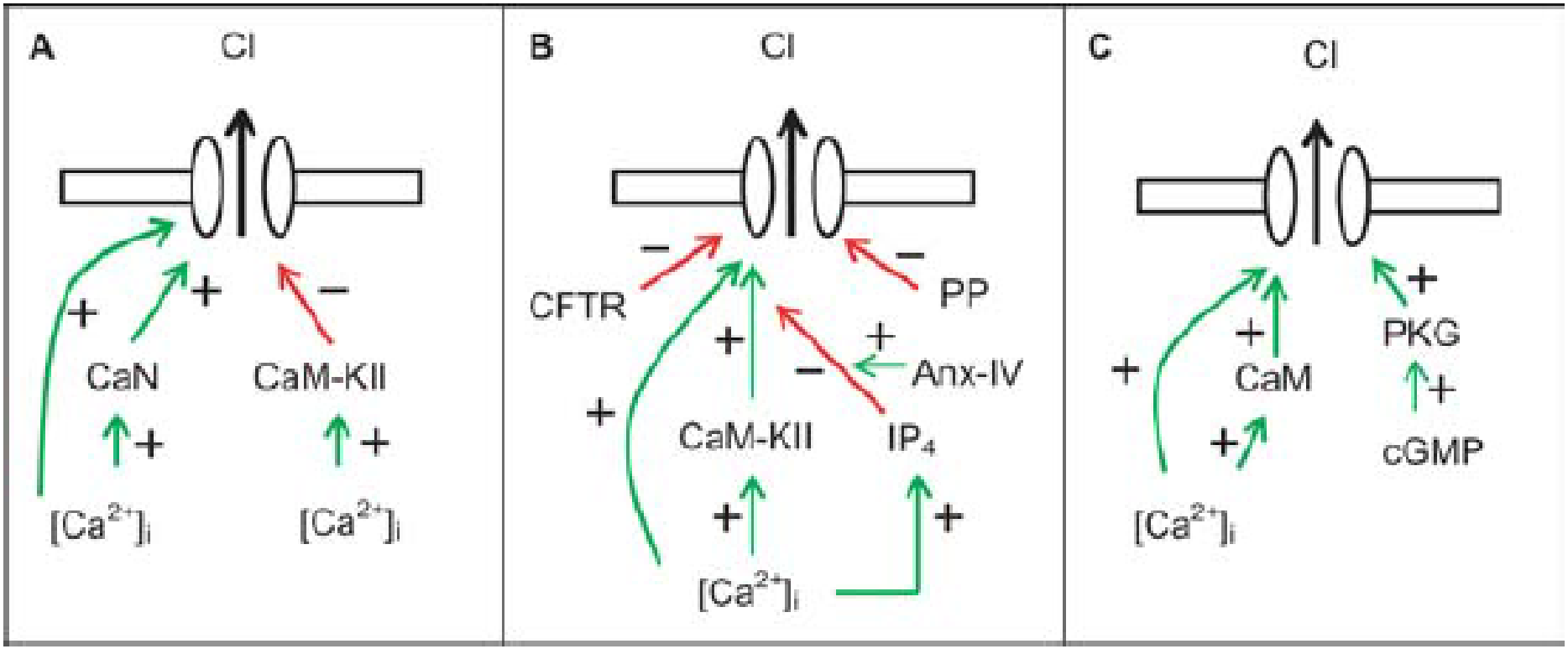
Factors controlling Cl^- flux through CaCC



Pathways regulating CaCC



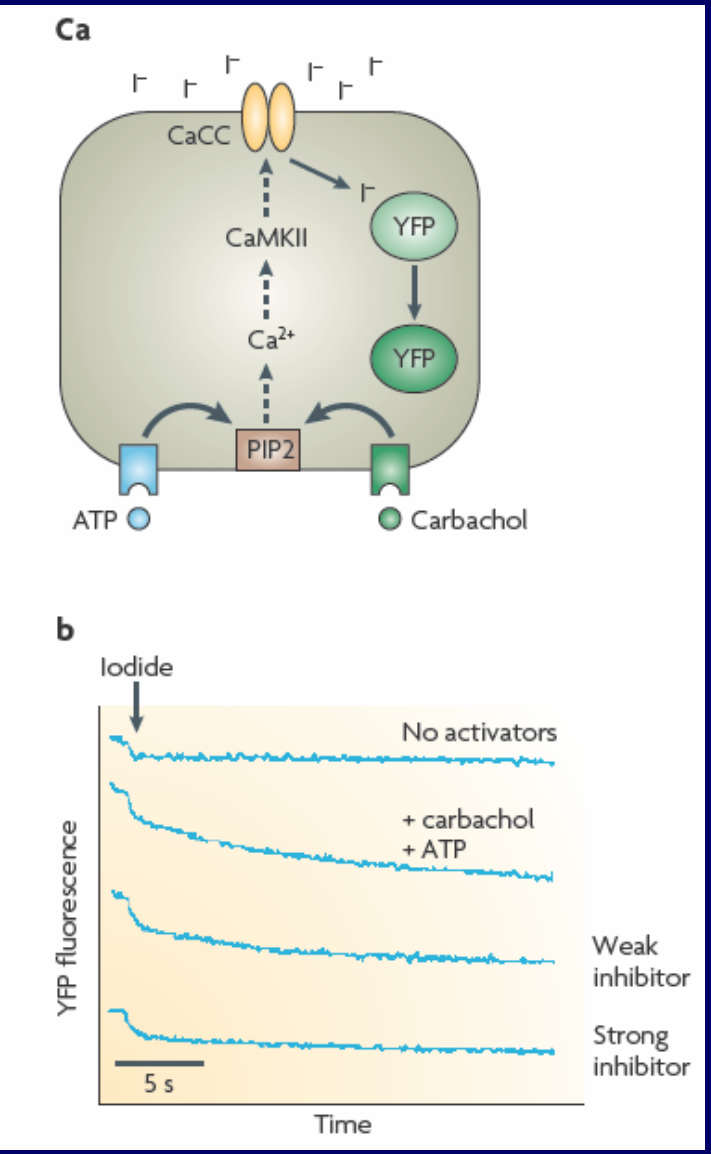
Alternative pathways of CaCC regulation



Calcium activated Cl^- channels

- Mediate a calcium-activated chloride conductance
- CLCA1: Intestinal basal crypt epithelium & goblet cells
  Lung-endothelial cell adhesion molecule-1 (Lu-ECAM-1)
- CLCA2: Lung & trachea
  - Inhibitors: DIDS, Dithiothreitol (DTT), Niflumic acid, Tamoxifen
- CLCA3: ? Does not function as a channel protein

Screen for calcium-activated chloride channel (CaCC) inhibitors



- CaCC halide conduction in human colonic cells expressing native CaCC and transfected with YFP indicator is measured following stimulation by an agonist mixture (ATP, carbachol).
- Iodide influx quenches YFP fluorescence. Fluorescence data showing controls (no activators, no test compounds) and examples of inhibitors.

CaMKII, calcium/calmodulin kinase II;

PIP2, phosphatidylinositol 4,5-bisphosphate.

Native Ca^{2+}-activated Cl^- channels

- Cl^- channels activated by intracellular calcium are found in many cell types: epithelial cells, neurons, cardiac and smooth muscle cells, as well as blood cells.
  - In neurons and muscle cells, Ca^{2+}-activated Cl^- channels may modulate excitability, e.g., by generating afterpotentials, regulate the tonus of smooth muscle. In olfactory receptor cells, Ca^{2+}-activated Cl^- channels play an interesting role in signal transduction as they are activated by Ca^{2+} entering through cGMP-activated channels. In epithelial cells, in particular in many acinar glands, they play an important role in transepithelial transport.
- Some Cl^- channels are also dependent on extracellular calcium: the cloned renal Cl^- channel ClC-K1 is activated by extracellular Ca^{2+}; a certain Cl^- channel of *Xenopus* oocytes is inhibited by extracellular calcium.

Ca^{2+}-activated Cl^- channels

- The activation of Cl^- channels by $[Ca^{2+}]_i$ may or may not involve phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII), suggesting an underlying molecular diversity of Ca^{2+}-activated Cl^- channels.
- Ca^{2+}-activated Cl^- channels generally display an $I^- > NO_3^- > Br^- > Cl^- > F^- > CH_3SO_4^-$ permeability sequence and are almost impermeable to glutamate. They are blocked by DIDS or niflumic acid.
- A molecular diversity of Ca^{2+}-activated Cl^- channels by the widely different single-channel conductances:
  - small-conductance (1–3 pS) Ca^{2+}-activated Cl^- channels were found in various cell types; The 10-pS channels were observed in hepatocytes and pulmonary artery endothelial cells. The 14 pS channels were observed in a human biliary cell line. They were inhibited by calmidazolium in the cell-attached configuration.
  - intermediate-sized (50–70 pS) Cl^- channel, is activated by intracellular Ca^{2+}.

Putative Ca^{2+}-activated Cl^- channels

- A functional channel is a disulfide-bonded tetramer of 38-kDa subunits. When reconstituted into lipid bilayers, the protein gave rise to 25- to 30-pS anion channels with an $I^- > Cl^-$ selectivity.
- Disulfide bonds, $^{125}I^-$ uptake and single-channel activity could be inhibited by treatment with dithiothreitol (DTT). The immunoaffinity-purified protein could be phosphorylated by CaMKII in vitro, and channel activity could be increased by Ca^{2+} and CaMKII. This suggested that it may represent a Ca^{2+}-activated Cl^- channel.
- Using an antibody against this 38-kDa protein, Cunningham et al. cloned a cDNA thought to encode this putative channel protein.

Putative Ca^{2+}-activated Cl^- channels

Overexpression results are quite confusing:

- **Upon expression in *Xenopus* oocytes, largely time-independent and outwardly rectified currents were reported. These currents were observed even without raising intracellular Ca^{2+} and were partially inhibited by DIDS and DTT, but not by niflumic acid.**
- **Currents in transfected COS-7 cells were linear and were only observed upon raising $[Ca^{2+}]_i$.**
- **Sequence and hydropathy analysis predicted a cleavable signal peptide and four putative transmembrane domains, whereas other programs suggested only one or two transmembrane spans.**
- **To test the point that a shorter protein of 40 kDa can give rise to channel activity, both the amino and carboxy termini were truncated by mutagenesis to yield a fragment that contained the four putative transmembrane domains. Again, Cl^- currents were observed in *Xenopus* oocytes.**

The ligand-gated ion channel superfamily (LGIC)

- Nicotinic acetylcholine receptors, glycine and GABA receptors
- Members of the LGIC superfamily have a common structure:
  - Five subunits form an ion channel
  - They share both structural and primary sequence homology and are thought to have evolved from a common ancestral receptor subunit.
  - Each subunit consists of a large amino-terminal extracellular domain of 200 amino acids, 4 putative transmembrane domains (TM), and a short extracellular carboxy terminus.
  - The amino-terminal domain contains a conserved motif, the so-called Cys loop. TM3 and TM4 are linked by a sizable cytosolic loop of variable length.

The ligand-gated ion channel superfamily (LGIC)

- There are no three-dimensional crystals available for any of these receptor channels, resulting in a lack of high-resolution structural information.
- Low-resolution three-dimensional images have been obtained from two-dimensional nAChR crystals in a closed channel conformation and in an open conformation.
- These images suggested that only TM2 is α-helical, whereas the other three domains are probably β-sheets. The five-helical TM2 domains, one from each subunit, kink at the center of the membrane to form the ion channel gate.

- These data might be extrapolated to GABA and glycine receptors.

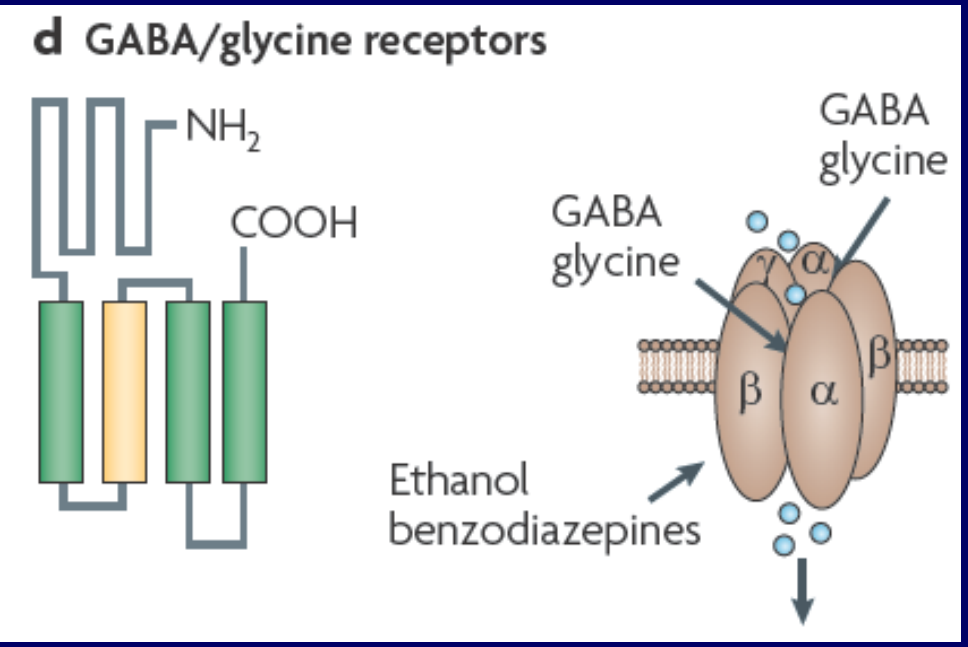
GABA and glycine-gated chloride channels

- Fast inhibitory neurotransmission in the mammalian central nervous system (CNS) is mediated primarily by the neurotransmitters GABA and glycine.
- Glycine is predominantly used in the spinal cord and the brain stem, whereas GABA is more commonly used in the brain. Their binding to their receptors opens intrinsic anion channels. In the adult CNS, this mostly leads to a Cl^- influx, which hyperpolarizes the neuron and thereby inhibits neuronal activity.
- Early in development, GABA and glycine induce a strong depolarizing response that can cause Ca^{2+} influx via voltage-gated Ca^{2+} channels and thus triggers neurotransmitter release. This excitatory action results from a more positive Cl^- equilibrium potential in undifferentiated neurons.
- During further development, the intracellular Cl^- concentration is decreased, in part as a consequence of the upregulation of the cation cotransporter KCC2. This inverts the GABA- and glycine-mediated current from excitatory to inhibitory.

GABA and glycine-gated chloride channels

- Although the physiological relevance of this early excitatory action of GABA and glycine remains unclear in detail, it is believed to be important for neuronal development because it may exert a trophic action through the rise in $[Ca^{2+}]_i$ that is associated with its depolarizing action.
- Because glutamatergic synaptic transmission is first purely *N*-methyl-D-aspartate (NMDA) receptor-based, GABA-induced depolarization may be necessary to relieve the voltage-dependent Mg^{2+} block of NMDA receptors.
- Both GABA and glycine receptors are targets for a wide range of clinically important drugs, including antiepileptic agents, anxiolytics (antianxiety drugs), sedatives, hypnotics, muscle relaxants, and anesthetics.

GABA and glycine-gated chloride channels



- The largest known family of Cl^- channels
- Pentameric channels formed by α, β and γ subunits. Each subunit has four transmembrane segments, with a large extracellular N terminus.
- The second transmembrane segment of each subunit contributes to the formation of the central pore.
- The N termini of the α and β subunits form the ligand binding site.
- They activate upon cell swelling.

GABA receptors

- A pore diameter of GABA receptors was 5.6 Å.
- GABA receptors can be blocked by penicillin. High concentrations of penicillin increased the open frequency, but open times were shortened. The single-channel conductance was not affected.
- Three different types of GABA receptors have been identified on the basis of their pharmacology and electrophysiology.
- The $GABA_A$ and the $GABA_C$ receptors are Cl^- channels, whereas $GABA_B$ receptors are G protein-coupled receptors.
- $GABA_C$ receptors are insensitive to both bicuculline and baclofen. Early studies by Johnston et al. showed that this receptor class was selectively activated by the GABA analog *cis*-4-aminocrotonic acid (CACA).
- $GABA_A$ receptors have modulatory binding sites for benzodiazepines, barbiturates, neurosteroids, and ethanol, whereas $GABA_C$ receptors are insensitive to barbiturates and benzodiazepines.

$GABA_A$ receptors

- 19 mammalian members of this gene family have been isolated, namely, $\alpha1–\alpha6$, $\beta1–\beta3$, $\gamma1–\gamma3$, δ, ε, π, θ, $\rho1–3$. They contain between 450 and 637 amino acid receptors derived from the three ρ-subunits form $GABA_C$ receptors.
- $GABA_A$ receptors are multimeric protein with a total molecular mass of 230–270 kDa. $GABA_A$ receptors are probably pentamers, with α-helical TM2 regions facing the channel pore.
- $GABA_A$ receptors show a permeability sequence $SCN^- > I^- > Br^- > Cl^- > F^-$. They are also permeable to bicarbonate ions, with a permeability amounting to ~20% of Cl^-. The permeability ratio of K^+ to Cl^- (P_K/P_{Cl}) was 0.05.
- $GABA_A$ receptors are antagonized by the convulsant alkaloid bicuculline and are insensitive to activation by the GABA analog baclofen.
- Benzodiazepines, barbiturates and neurosteroids have modulatory effect on a $GABA_A$ receptors.

$GABA_A$ receptors

Family name	Ligand	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
$GABA_A$ receptors*	GABA	α 1	GABRA1	Gabra1	Gabra1
$GABA_A$ receptors*	GABA	α 2	GABRA2	Gabra2	Gabra2
$GABA_A$ receptors*	GABA	α 3	GABRA3	Gabra3	Gabra3
$GABA_A$ receptors*	GABA	α 4	GABRA4	Gabra4	Gabra4
$GABA_A$ receptors*	GABA	α 5	GABRA5	Gabra5	Gabra5
$GABA_A$ receptors*	GABA	α 6	GABRA6	Gabra6	Gabra6
$GABA_A$ receptors*	GABA	β 1	GABRB1	Gabrb1	Gabrb1
$GABA_A$ receptors*	GABA	β 2	GABRB2	Gabrb2	Gabrb2
$GABA_A$ receptors*	GABA	β 3	GABRB3	Gabrb3	Gabrb3
$GABA_A$ receptors*	GABA	γ 1	GABRG1	Gabrg1	Gabrg1
$GABA_A$ receptors*	GABA	γ 2	GABRG2	Gabrg2	Gabrg2
$GABA_A$ receptors*	GABA	γ 3	GABRG3	Gabrg3	Gabrg3
$GABA_A$ receptors*	GABA	δ	GABRD	Gabrd	Gabrd
$GABA_A$ receptors*	GABA	ε	GABRE	Gabre	Gabre
$GABA_A$ receptors*	GABA	θ	GABRQ	Gabrq	Gabrq
$GABA_A$ receptors*	GABA	π	GABRP	Gabrp	Gabrp
$GABA_A$ receptors*	GABA	ρ 1	GABRR1	Gabrr1	Gabrr1
$GABA_A$ receptors*	GABA	ρ 2	GABRR2	Gabrr2	Gabrr2
$GABA_A$ receptors*	GABA	ρ 3	GABRR3	Gabrr3	Gabrr3

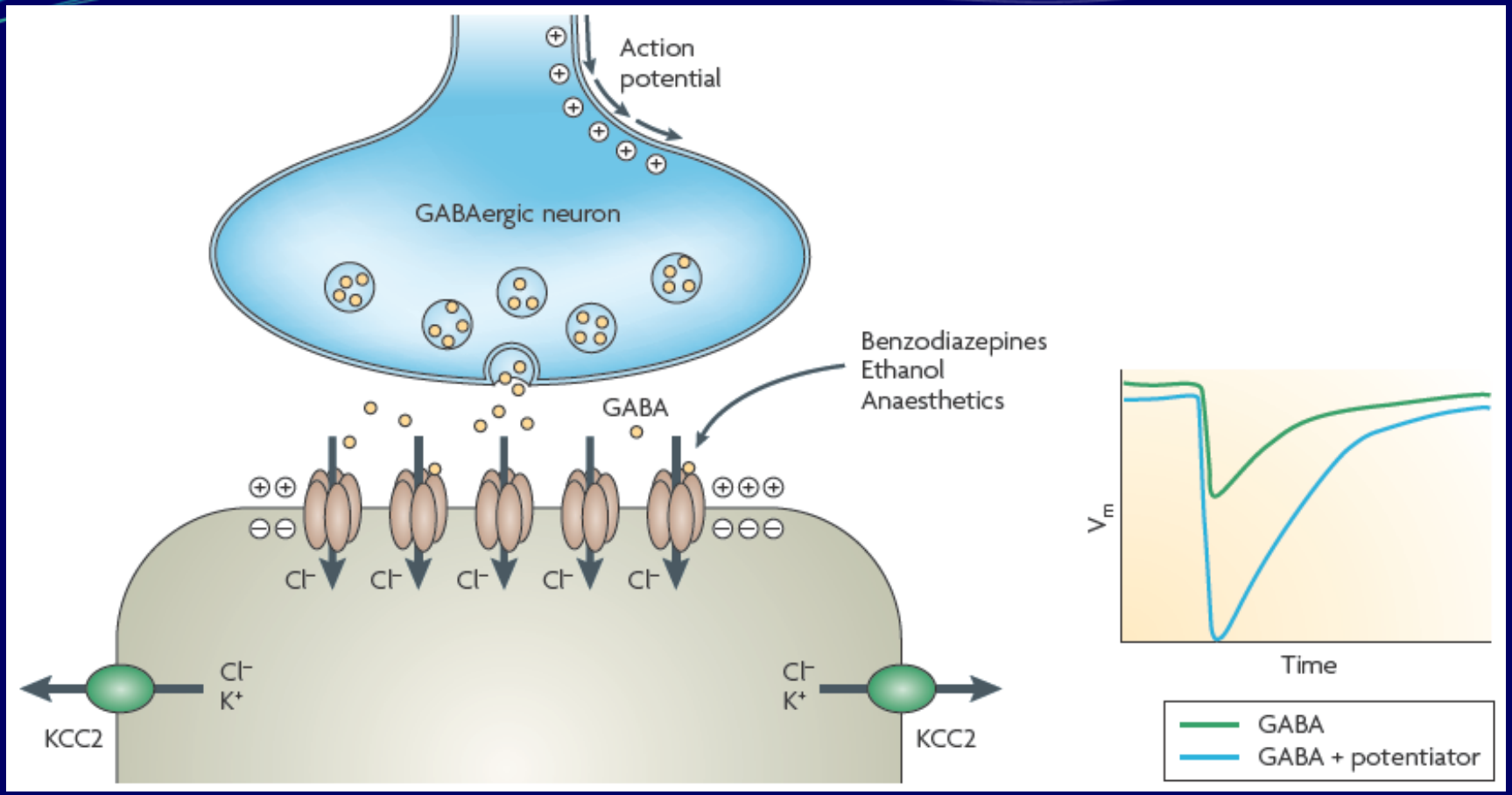
$GABA_C$ receptors

- $GABA_C$ receptors are insensitive to either bicuculline or baclofen, but they have a higher sensitivity to GABA (~10 times more sensitive to GABA than $GABA_A$ receptors).
- $GABA_C$ receptors were first described in interneurons of the spinal cord. $GABA_C$ receptors are also identified in the retina, and are highly enriched in the vertebrate retina.
- Their currents are smaller, and they do not desensitize.
- $GABA_C$ receptors are homo- or hetero-oligomers of ρ-subunits. To date, three different ρ-subunits are known in mammals. They share only 30–38% amino acid sequence identity with the $GABA_A$ receptors subunits. In the rat retina, $GABA_C$ receptors are probably heteromers of ρ1- and ρ2-subunits.
- $GABA_C$ receptors colocalized with the microtubule-associated protein MAP-1B at postsynaptic sites on bipolar cell terminals. ρ-subunits have been localized by immunocytochemistry to axon terminals of bipolar cells.

$GABA_C$ receptors

- The electrophysiological properties of native and recombinant $GABA_C$ receptors differ markedly from those of $GABA_A$ receptors.
  - The time constants for activation and inactivation are much larger than those of $GABA_A$ receptors.
  - $GABA_C$ receptors have a smaller single-channel conductance of 7 pS, but longer open times of 150–200 ms.
- $GABA_C$ receptors ideally suited for strong lateral inhibition.
  - The GABA analog CACA is a selective agonist for $GABA_C$ receptors.
  - (1,2,5,6-Tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA) is a potent and highly selective antagonist for $GABA_C$ receptors.
  - ρ1-homomeric receptors are sensitive to picrotoxin, ρ2-homooligomers and native rat $GABA_C$ receptors (that are heteromers of ρ1ρ2-subunits) are rather insensitive to this compound.

Ligand-gated chloride channels



- **Release of GABA from presynaptic membrane triggers the transient activation of GABA receptors.**
- **The low intracellular Cl^- concentration in the postsynaptic neuron, generated by the action of the K^+/Cl^- cotransporter (KCC2), drives Cl^- influx through GABA-activated chloride channels causing membrane hyperpolarization.**
- **Benzodiazepines, anaesthetics, ethanol and other compounds act on GABA receptors to potentiate neurotransmitter effect.**

Molecular structure of glycine receptors

- **The glycine receptor has been purified from rat, porcine, and mouse spinal cord by affinity chromatography on aminostrychnine-agarose columns.**
- **Grenningloh et al. used peptide sequences derived from affinity-purified adult spinal cord glycine receptors to isolate the cDNA of the adult 48-kDa (α1) subunit and the 58-kDa (β) subunit.**
- **Subsequently, cDNA clones corresponding to the embryonic α2- and the adult α3-subunit were cloned by homology screening; a fourth α-subunit has been identified in mouse and chick.**

Molecular structure of glycine receptors

- **When expressed in *Xenopus* oocytes, the α1-subunit forms homomeric channels with properties similar to those from native channels. These homomeric channels can be opened by glycine, taurine, and β-alanine and blocked by strychnine and picrotoxin.**
- **α2 and α3 can also form homomeric channels, but these are not activated by taurine and β-alanine.**
- **Like α1, the mouse α4-subunit formed robust homomeric glycine receptors in *Xenopus* oocytes with properties reminiscent of those formed by the rat α1-subunit.**
- **In contrast, β-subunits are incapable of forming functional glycine receptors by themselves.**

Expression pattern of glycine receptor

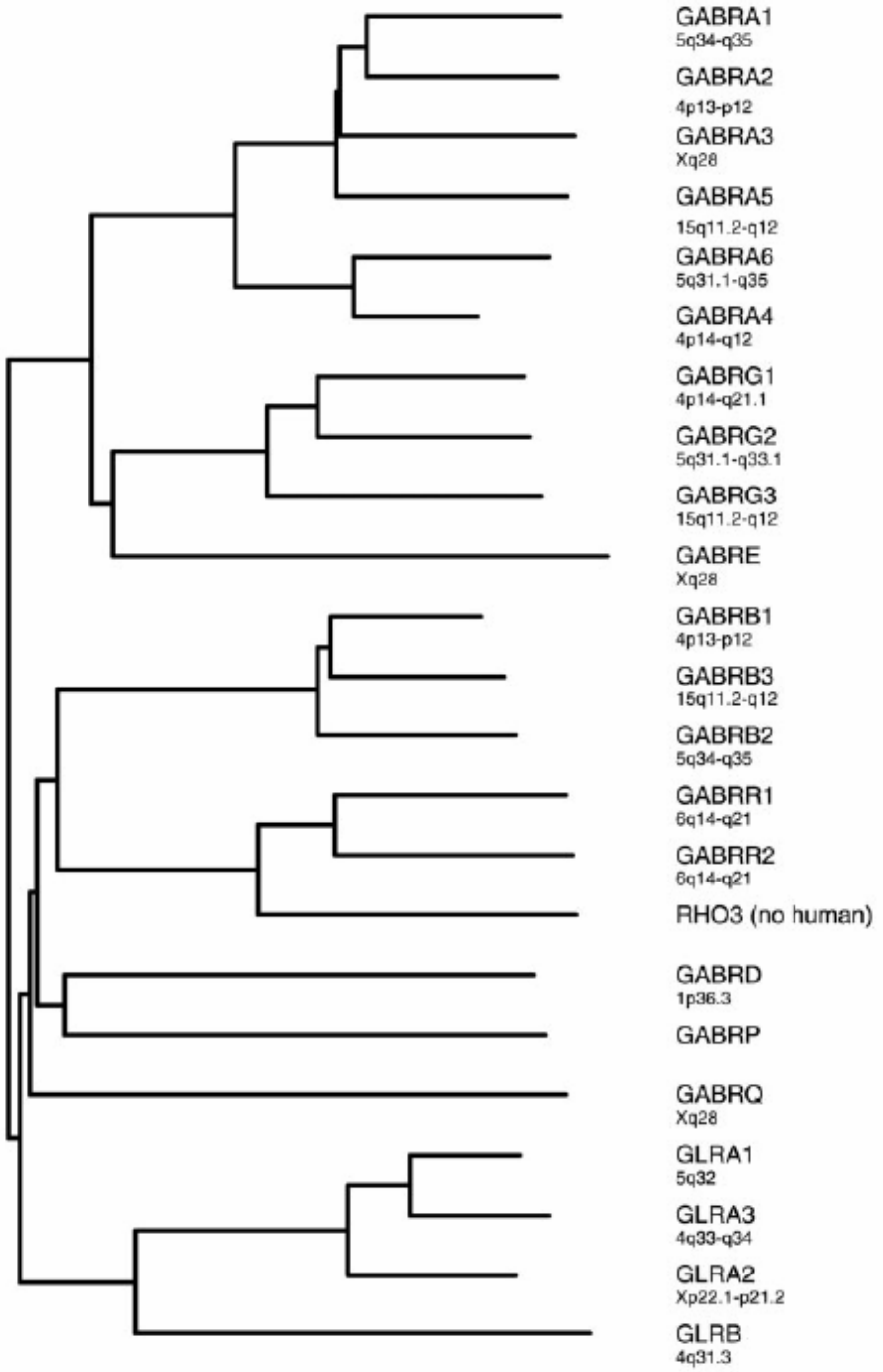
- **Glycine receptor subunits have been localized in the CNS by autoradiography using [^{3}H]strychnine and [^{3}H]glycine binding, and in immunohistochemical studies using monoclonal antibodies against receptor subunits.**
- **They are prominently expressed in the spinal cord and the medulla. Lower levels are found in midbrain and hypothalamus, but they are virtually absent in the higher brain.**
- **Glycine receptors have also been found in the retina, adrenal gland, kidney, liver and sperm.**
- **Glycine and GABA receptors often coexist in spinal cord neurons. Jonas et al. showed that spinal interneurons release both glycine and GABA to activate functionally distinct receptors in their postsynaptic target cells.**

Functional properties of glycine receptor

- A relative **permeability** sequence: $SCN^- > NO_3^- > I^- > Br^- > Cl^- > F^-$, whereas the relative conductances were $Cl^- > Br^- > NO_3^- > I^- > SCN^- > F^-$
- Glycine receptors have **multiple conductance states**:
  - The predominant conductance levels of homomeric ($\alpha1$, $\alpha2$, $\alpha3$) receptors are significantly higher than those of heteromeric ($\alpha1\beta$, $\alpha2\beta$, $\alpha3\beta$) and native glycine receptors.
  - Seven different conductance states of 12–14, 18–23, 24–36, 42–49, 59–72, 80–94, and 105–112 pS have been observed in the various subunit combinations.
- Frequency distribution histograms suggested the existence of at least **three different open states**. The mean open time of $\alpha1$-homomeric channels was much shorter than those of $\alpha2$-homomeric channels, consistent with a reduction in channel open times during development. Raising glycine concentrations did not affect the open time constants but increased the channel open frequency.

Functional properties of glycine receptor

- **Glycine receptors were reported to strongly rectify at voltages more negative than -50 mV both in cultured neurons and upon expression in *Xenopus* oocytes.**
- **Rectification was absent in rat homomeric or heteromeric glycine receptors recombinantly expressed in *Xenopus* oocytes or HEK293 cells.**
- **Glycine receptors desensitize with time, resulting in a transient signal upon agonist binding. Decay time constants generally decreased with increasing agonist concentration.**
- **The time constant varies from 10 ms to 10 s. The shorter time constants correspond to the decay time constant of glycinergic inhibitory postsynaptic potentials.**
- **Receptors recovered completely from desensitization within 60 s.**



Family of ligand-gated chloride channels

- 19 members of the GABA receptor family
- 4 members of the glycine receptor family
- chromosomal localization

Glycine receptors

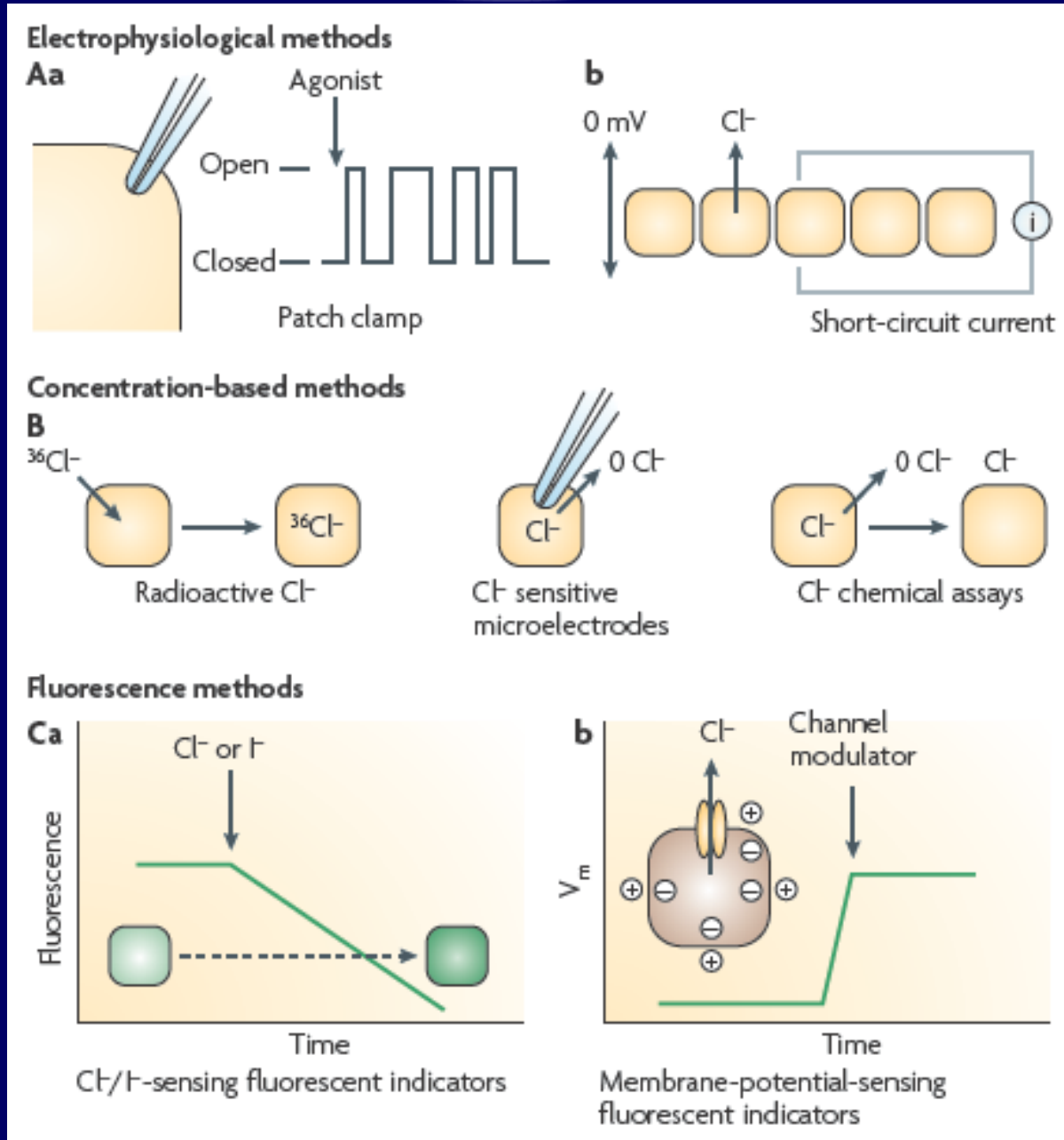
Family name	Ligand	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
Glycine receptors	glycine	α 1	GLRA1	Glra1	Glra1
Glycine receptors	glycine	α 2	GLRA2	Glra2	Glra2
Glycine receptors	glycine	α 3	GLRA3	Glra3	Glra3
Glycine receptors	glycine	α 4 *(pseudogene in humans)*	GLRA4	Glra4	Glra4
Glycine receptors	glycine	β	GLRB	Glrb	Glrb
Glycine receptors	glycine	Glycine Receptor (All subtypes)			

Subtypes, functions and modulators of chloride-channel

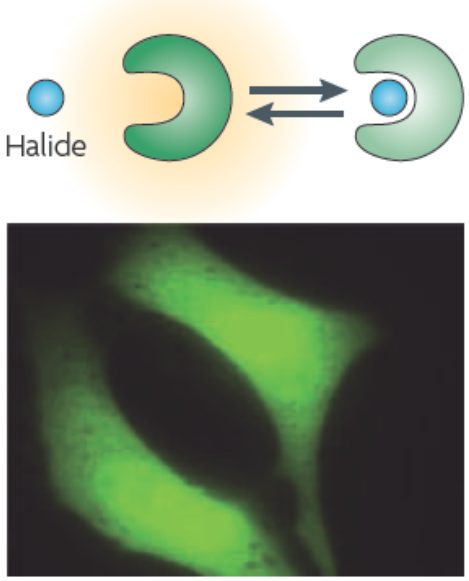
Protein	Mechanism of regulation	Channel properties	Physiological roles	Human diseases	Pharmacological modulators
CFTR	Activated by cyclic AMP-dependent phosphorylation	Linear I–V; $Cl^- > I^-$ permeability	Cl^- secretion by epithelial cells in airways, submucosal glands, pancreas, intestine and testis; Cl^- absorption in sweat glands	Cystic fibrosis	Multiple, nanomolar-potency activators and inhibitors
ClC-1	Activated by depolarization	$Cl^- > I^-$ permeability; double-barrelled pore	Cl^- conductance in skeletal muscle; repolarization after action potential	Myotonia	Weak inhibition by 9-AC, niflumic acid and DPC
ClC-2	Slowly activated by hyperpolarization and cell swelling	Inward rectification of I–V; $Cl^- > I^-$ permeability	Cl^- homeostasis in neurons; cell-volume regulation	Epilepsy (controversial)	Weak inhibition by classical Cl^- channel blockers, inhibited by Zn^{2+}
ClC-4 and ClC-5	None identified	Electrogenic H^+/Cl^- exchange; strong outward rectification	Intracellular Cl^- channels facilitating endosomal and synaptic vesicle acidification	ClC-5: Dent's disease (proteinuria and kidney stones)	No known inhibitors
ClC-7	Requires OSTM1 for membrane expression	Electrogenic H^+/Cl^- exchanger	Acidification of resorption lacuna in osteoblasts; lysosomal acidification	Osteopetrosis; lysosomal storage disease	No known inhibitors
ClC-Ka and ClC-Kb	Weak voltage-dependence; both require barttin for membrane targeting	Moderate outward rectification of I–V; $Cl^- > I^-$ permeability	Transepithelial Cl^- transport in kidney tubules and inner ear	ClC-Kb: Bartter's syndrome (deafness when barttin affected)	Inhibited by phenylbenzofuran carboxylic acids
Bestrophins	Activated by elevated cytosolic Ca^{2+}	$I^- > Cl^-$ permeability	Cl^- transport in retinal pigment epithelium; Ca^{2+}-stimulated Cl^- secretion in epithelia	Best vitelliform macular dystrophy	Weakly inhibited by niflumic acid and stilbenes
TMEM16A (anoctamin-1)	Activated by elevated cytosolic Ca^{2+}	$I^- > Cl^-$ permeability	Ca^{2+}-stimulatd Cl^- secretion in epithelia; smooth-muscle contraction	Not known	Strongly inhibited by niflumic acid and NPPB
GABA receptor	Activated by GABA	$I^- > Cl^-$ permeability	Inhibitory synaptic transmission in the brain	Epilepsy	Potentiated by benzodiazepines and barbiturates
Glycine receptor	Activated by glycine, β-alanine and taurine	$I^- > Cl^-$ permeability	Inhibitory synaptic transmission in the spinal cord	Hyperekplexia	Inhibited by strychnine and picrotoxin

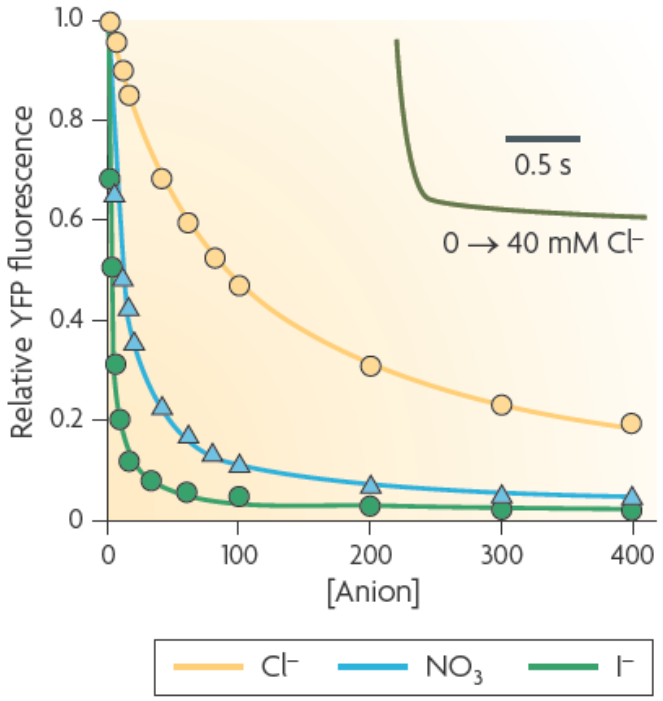
Methods to assay chloride-channel activity

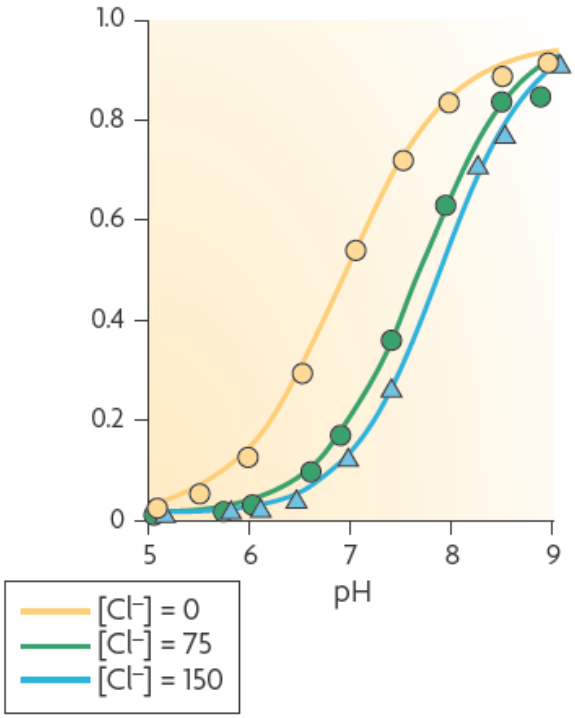
- cell-attached patch-clamp configuration
- changes in intracellular or extracellular chloride concentration in response to imposed chloride gradients
- fluorescent protein are particularly useful for chloride-channel screening assays



Fluorescent-protein-based screening method







- reduced yellow fluorescence protein (YFP) fluorescence following halide binding
- cells expressing YFP in a cytoplasmic pattern
- titration of YFP-H148Q/I152L fluorescence with chloride, iodide and nitrate at pH 7.4
- rapid indicator response following an increase in Cl^- concentration from 0 mM to 40 mM
- pH titration at Cl^- concentration of 0 mM, 75 mM and 150 mM

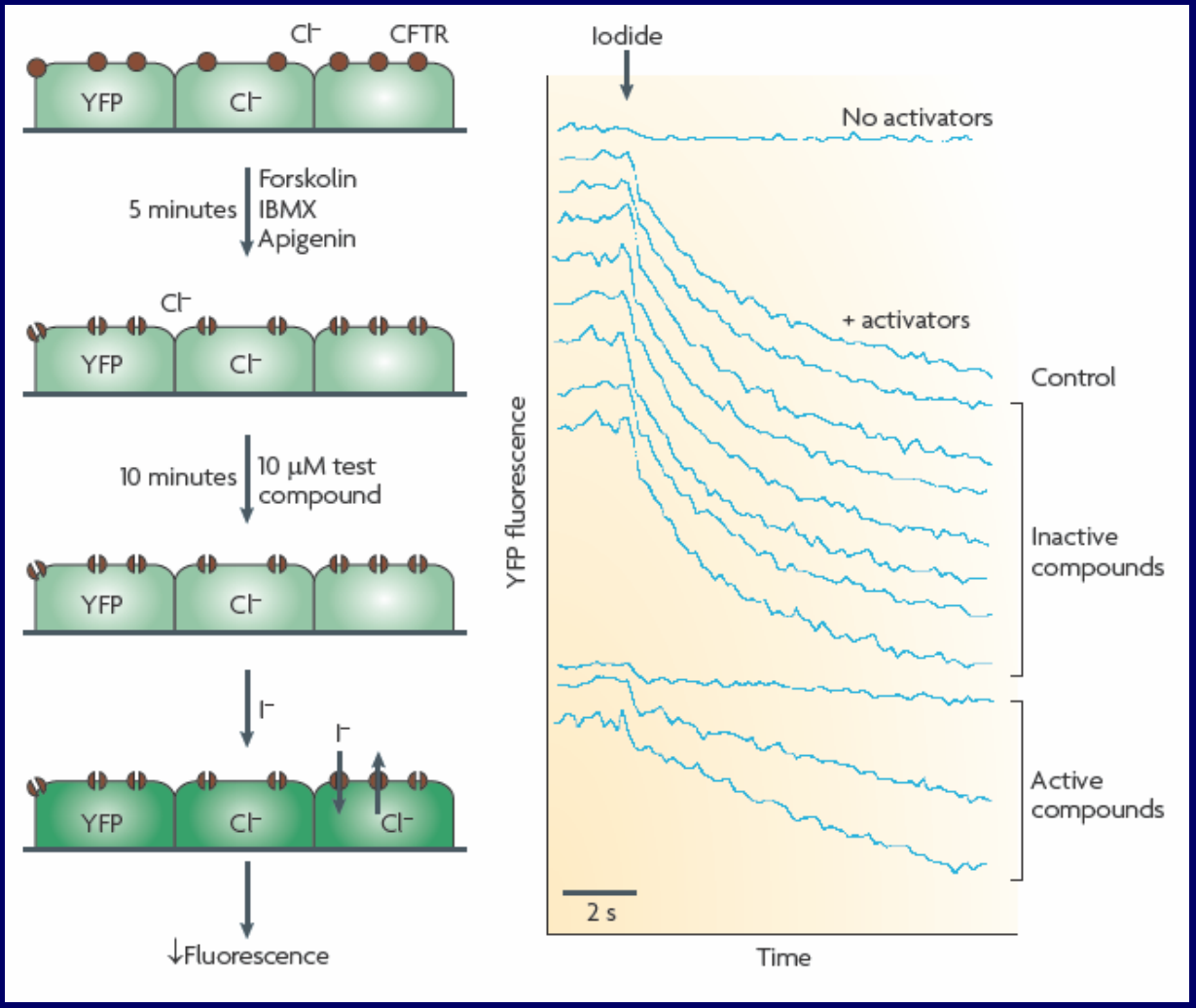
Cystic fibrosis transmembrane conductance regulator (CFTR)

- Family: ATP-binding cassette (ABC) transporters
- Transmembrane domains: 2 Sets of 6
- Sets separated by a cytoplasmic region with nucleotide binding fold (NBF1) & regulatory R domain
- Channel opening is controlled by
  - Intracellular ATP
  - Phosphorylation by cAMP- or cGMP-dependent kinases

CFTR

- CFTR is now known to be a **voltage-independent** anion channel, which requires the presence of hydrolyzable nucleoside triphosphates for efficient activity.
- In symmetrical Cl^- concentrations, CFTR has a linear current-voltage (***I-V***) **relationship**, but in asymmetrical Cl^- concentrations the *I-V* relationship is rectified.
- The single-channel conductance of CFTR is between 6 and 10 pS.
- The anion **permeability** sequence of CFTR in whole cell patch experiments is $Br^- > Cl^- > I^- > F^-$. In single-channel measurements, Tabacharani et al. found a higher permeability for I^- than for Cl^-, but as I^- blocks the pore, Cl^- is conducted better.
- A mechanistic link between ATP hydrolysis and a specific step in CFTR gating is still missing.

Screening protocol for CFTR inhibitors



- CFTR halide conductance in cells co-expressing CFTR and YFP indicator
- Stimulation of an agonist mixture (forskolin, 3-isobutyl-1-methylxanthine (IBMX), apigenin)
- After addition of test compound, iodide influx is measured by YFP fluorescence
- Single-well fluorescence data showing controls and test wells

Cellular regulation of CFTR activity

- CFTR is the substrate for protein kinases, most importantly protein kinase C. It enhances the effect of PKA-mediated phosphorylation.
- CFTR activation appears to be a multistep process that requires the activity of protein kinase C, PKA, and a high ATP/ADP ratio to achieve maximal activity.
- The main "switch" for the cell to turn CFTR on or off seems to be phosphorylation by PKA, which can be substantially increased by a rise in intracellular cAMP concentration and which is kept in a dynamic state by a high phosphatase activity associated with the CFTR protein.
- Up to 15 phosphorylation sites may be involved in the PKA-dependent activation, not all of which are likely to be phosphorylated at the same time.
- CFTR regulation: the effect of the added negative charge primes the channel for gating activity.

ClC-K in Fish

- ClC-K channels from *Xenopus laevis* and *Oeochromis mossambicus* (a fish called tilapia that can adapt to both sea and fresh water) have been cloned. One ClC-K channel has been cloned from each species.
- OmClC-K, an *Oeochromis mossambicus* ClC-K channel, has been localized to the basolateral plasma membrane of distal tubules, and colocalization with Na/K-ATPase channels has been observed.
- The tilapia nephron lacks a loop of Henle; however, tilapia distal tubules share similar properties with the TAL in mammals, such as active Na^+ and Cl^- transport through the apical Na/K/2Cl cotransporter, basolateral Na/K-ATPase, and a lack of water permeability, suggesting that this is a nephron segment to generate free water. Tilapia can adapt to sea water and fresh water.
- Thus if OmClC-K has a role in generating free water, it should be active when tilapia are in freshwater because they must conserve NaCl and secrete water in fresh water conditions. Miyazaki et al. have shown that OmClC-K is expressed only when tilapia are in freshwater, which suggests that the ClC-K channel evolved to enable tilapia to tolerate fresh water.

ClC-K in Fish

- **ClC-K2 in mammals might be akin to ClC-K in tilapia. It is possible that gene duplication resulted in ClC-K1 in mammals, thus enabling even greater urine concentrating ability.**
- **Intrarenal localization of *Xenopus* ClC-K has been reported by Maulet et al. following immunohistochemical staining of ClC-K at the basolateral surface of proximal tubules and the apical surface of distal nephrons. However, this observation of intrarenal and cellular localization of *Xenopus* ClC-K does not fit with the above observations regarding the role of ClC-K channels in mammals and fish.**
- ***Xenopus* ClC-K is present in pronephric distal tubule and duct but not in the proximal tubules. Further characterizarion of *Xenopus* ClC-K may be necessary.**

Pharmacology of Cl^- channels

Inhibitor Type	Substance	ClC-1	ClC-2	CFTR	Cl(Ca)	Cl(Vol)	CLIC
Disulfonic stilbenes (irreversibly binding)	DIDS, SITS		o	−/+*	o	+	o
Disulfonic stilbenes (reversibly binding)	DNDS			−/o*	−	−	o
Arylaminobenzoates	DPC	o	o	o		o	
	NPPB			+	+	+	
Fenamates	FFA			o	+	o	
	NFA	o		o	+	o	
Anthracene carboxylates	9-AC	+	o	o	o	o	
Indanylalkanoic acids	IAA-94			−		o	+
Clofibric acid derivatives	Clofibric acid, CPP	+		o			
Sulfonylureas	Glibenclamide, tolbutamide			+	Varies	o	
Other compounds	*ts-tm*-Calix(4)arene			−			++
	Suramin			−/++*			
	Tamoxifen					++	
Metal ions	Zn^{2+}	o	+				
	Cd^{2+}		o				

- **++, $IC_{50} \leqslant 5\ \mu M$; +, $5\ \mu M < IC_{50} \leqslant 100$ M; o, $100\ \mu M < IC_{50} \leqslant 2$ mM; -, $IC_{50} > 2$ mM. * Potency upon extracellular/intracellular application.**
- **DPC, diphenylaminecarboxylate;**
- **NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid;**
- **FFA, flufenamic acid;**
- **NFA, niflumic acid;**
- **9-AC, anthracene-9-carboxylate;**
- **IAA, indanyloxyacetic acid;**
- **CPP, 2-(*p*-chlorophenoxy)propionic acid.**

Pathology

- **Bartter's syndrome**, which is associated with renal salt wasting and hypokalemic alkalosis, is due to the defective transport of chloride ions and associated ions in the thick ascending loop of Henle. CLC-Kb has been implicated.
- **Dent's Disease**, an inherited disease that affects the kidney organs, is characterised by low molecular weight proteinuria and hypercalciuria where mutations in CLC-5 are implicated.
- **Thomsen disease** is associated with dominant mutations and Becker disease with recessive mutations in CLCN1.
- **Cystic fibrosis** is caused by a mutation in the DF508 region of the CFTR gene, which prevents the proper folding of the protein and subsequent degradation, resulting in decreased numbers of chloride channels in the body. This causes the build up of mucus in the body and chronic infections.

Disorders

- Myotonia congenita (CLC-1)
  - Dominant (Thompsen)
  - Recessive (Becker)
- Myotonic Dystrophy (DM1; DM2): Expanded CUG or CCUG repeats
  - Retained in nucleus
  - Disrupt splicing of chloride channel (ClC-1) pre-mRNA

Disorders

- Epilepsy
  - CLC-2
    - Absence epilepsy: Childhood & Juvenile
    - Myoclonic epilepsy：Juvenile
    - Epilepsy with grand mal seizures on awakening
  - Gamma-aminobutyric acid (GABA) receptors

    γ -2 subunit (GABRG2);
    - Chromosome 5q31.1-q33.1; Dominant
    - Generalized epilepsy with febrile seizures plus, type 3
    - Childhood absence epilepsy
    - Febrile seizures

Disorders

- Renal tubular disorders (CLC-5)
  - Hypercalciuric nephrolithiasis
  - X-linked recessive Nephrolithiasis
  - Dent disease
- Nephrogenic diabetes insipidus (Mouse): (CLC-KA)
- Bartter's syndrome (CLC-KB)
- Cystic fibrosis (Epithelial chloride channel)
- Osteopetrosis, infantile, malignant: CLC-7
- Angleman or Prader-Willi: GABAAB3 receptor subunit
- SLC26A4: Transporter of chloride & iodide
  - Non-syndromic deafness, congenital
  - Pendred syndrome

Disorders

- Alcohol non-tolerant rat: GABAα6 receptor subunit
- Glioma
  - Cl^- channels upregulated in glioma cells
  - High grade (poorly differentiated) tumors also lose Na^+ channels Toxin: Chlorotoxin (Scorpion)

Disorders of Cl^- transport

Human disease	Protein/gene	Defective function
Anderman syndrome	KCC3/SLC12A6	Neural development
Bartter syndrome type I	NKCC/SLC12A1	Renal salt balance
Bartter syndrome type III	ClC-Kb/CLCNKB	Renal salt balance
Bartter syndrome type IV with deafness	Barttin/BSND	Renal salt loss, endolymph secretion
Congenital chloride diarrhea	SLC26A3	Intestinal fluid secretion
Deafness	KCC4/SLC12A7	Potassium recycling
Dent's disease	ClC-5/CLCN5	Endosomal acidification
Distal renal tubule acidosis	AE1/SLC4A1	Renal pH balance
Epilepsy	$GABA_A$ receptor $\gamma 2$	Synaptic inhibition
Hyperekplexia	Gycine receptor/GLRA1	Synaptic inhibition
Idiopathic generalized epilepsy[a]	CLC-2/CLCN2	Cl^- channel deactivation
Juvenile myoclonus epilepsy	$GABA_A$ receptor $\alpha 1$	Synaptic inhibition
Myotonia cogenita	ClC-1/CLCN1	Membrane potential
Neuronal ceroid lipofuscinosis	CLC-7/CLCN7	Lysosomal dysfunction
Osteopetrosis	CLC-7/CLCN7	Acid secretion by osteoclasts
	Ostm1/OSTM1	
Spherocytosis	AE1/SLC4A1	Membrane stability
Vitelliform macular dystrophy	Bestrophin 1/VMD2	Retinal pigment epithelium Cl^- transport?

[a]Susceptibility gene.

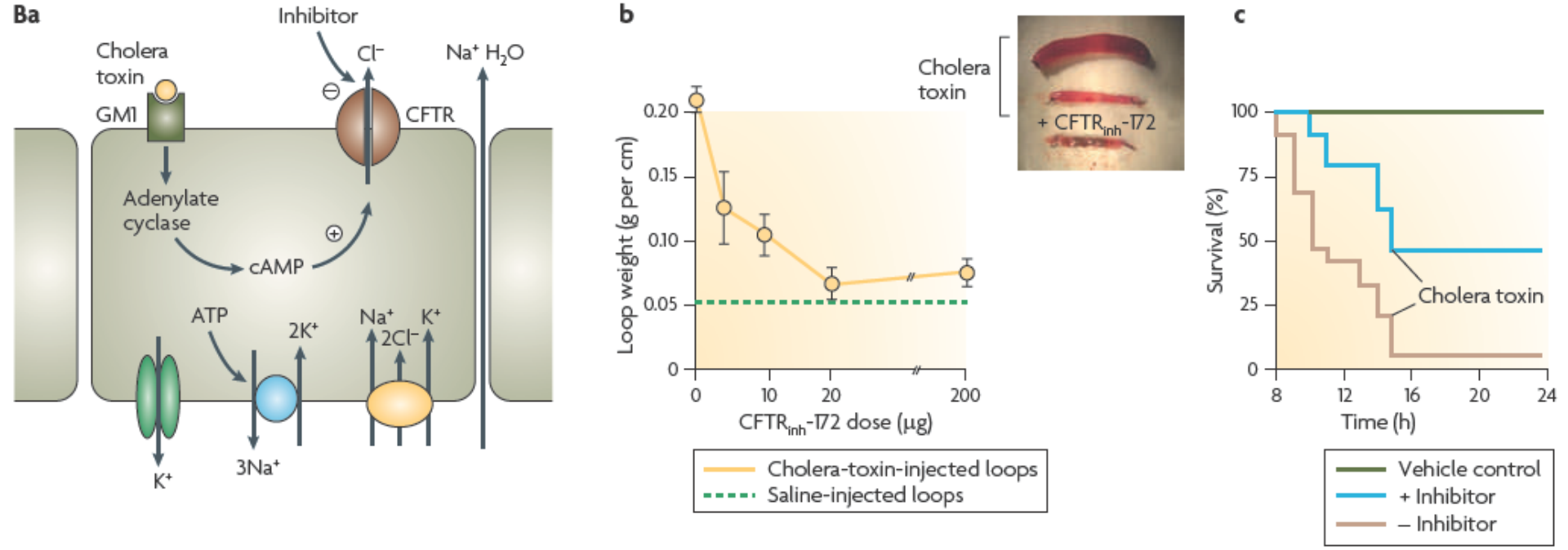
Clinical use

- **Modulators of ligand gated chloride channels, such as barbiturates and benzodiazepines for $GABA_A$ gated chloride channels, are in clinical use.**
- **Several compounds are in Phase II clinical trials for cystic fibrosis, including potentiators of mutant CFTRs and activators of CaCCs, which act by elevating cytoplasmic calcium.**
- **Candidate inhibitors of CFTR, CaCCs and ClCs have also been identified and are in various stages of preclinical development.**

Commercial applications

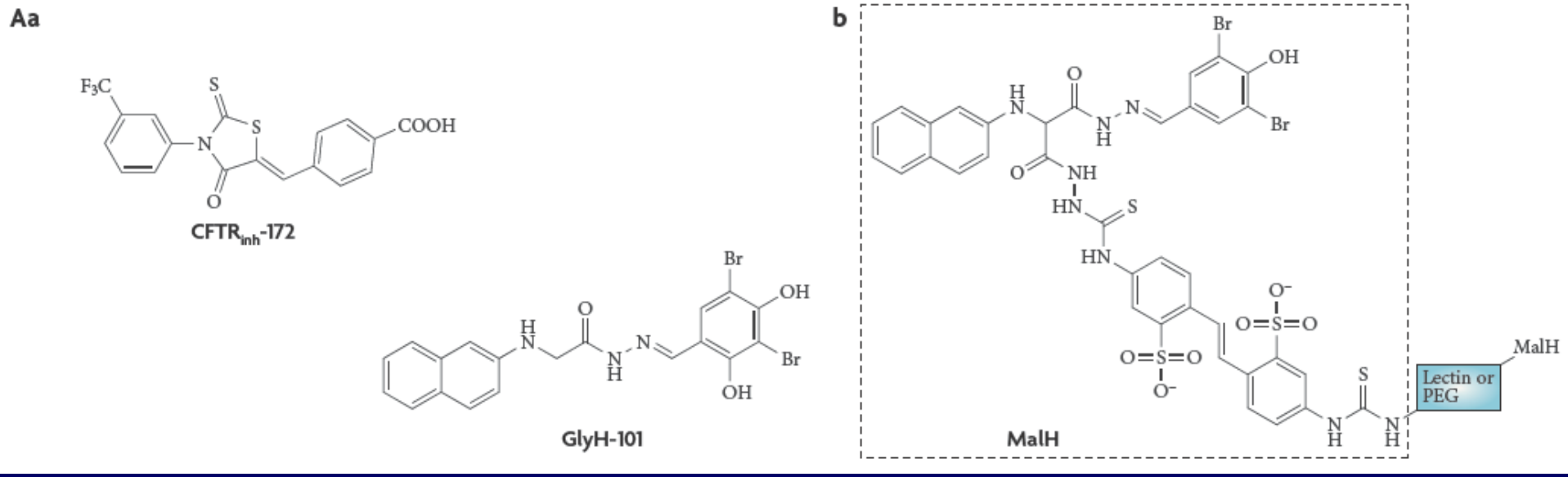
- Some organic materials disrupt chloride channels in fleas, causing death.
- Selamectin is the active ingredient in Revolution, a topical insecticide and antihelminthic used on dogs and cats. Selamectin works by replacing glutamate which normally interacts with receptors that open chloride channels at muscle synapses found in parasites. Unlike glutamate, selamectin activates the chloride current without desensitization, thereby producing prolonged hyperpolarization and impaired muscle contraction.

Intestinal fluid secretion in diarrhoea



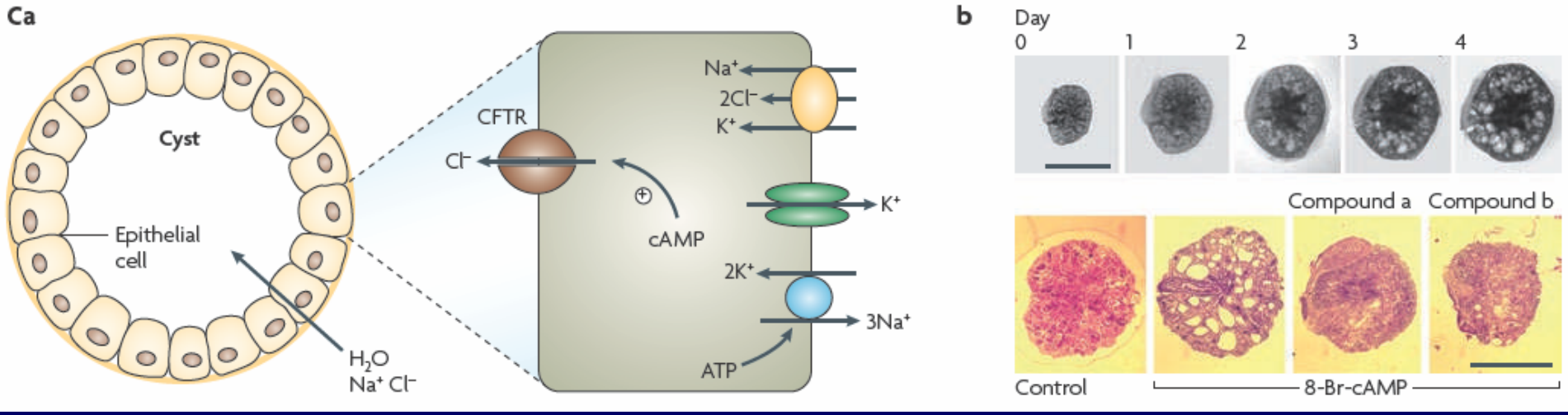
- CFTR chloride secretion following choleratoxin-induced cyclic AMP elevation. Sodium and water follow passively.
- CFTRinh-172 inhibits intestinal fluid accumulation in closed mouse ileal loops.
- Survival of suckling mice following oral administration of cholera toxin with or without CFTR inhibitor MalH–lectin

CFTR inhibitors



- Structures of thiazolidinone CFTR inhibitor CFTRinh-172 and glycine hydrazide GlyH-101
- Malonic acid hydrazide (MalH) conjugated to a macromolecular backbone (lectin or polyethylene glycol (PEG)).

Cyst fluid secretion in polycystic kidney disease

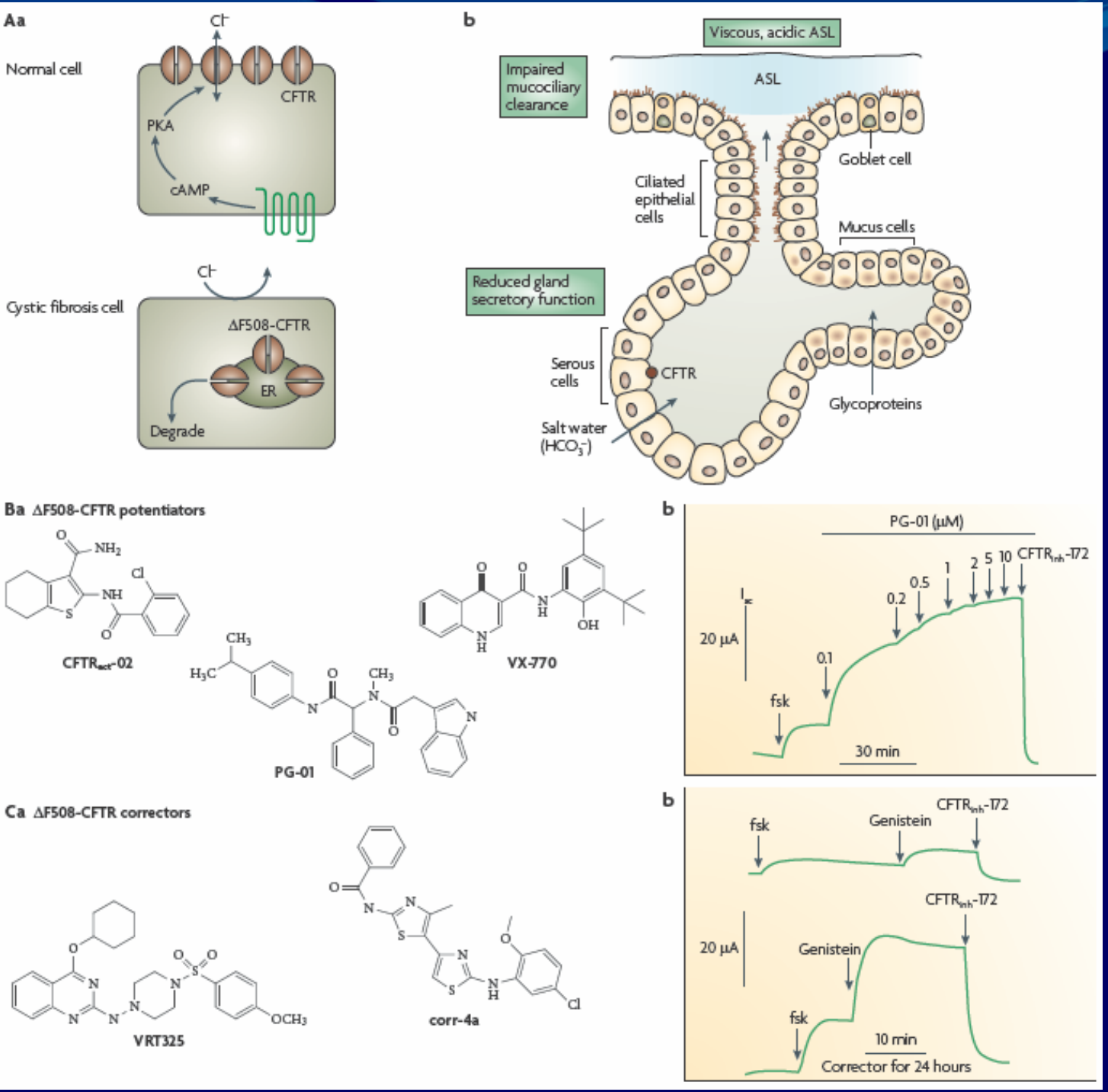


- Mechanism of fluid secretion into cysts, involving CFTR-dependent chloride secretion.
- CFTR inhibitors cause slowing of cyst expansion in embryonic kidney organ culture.

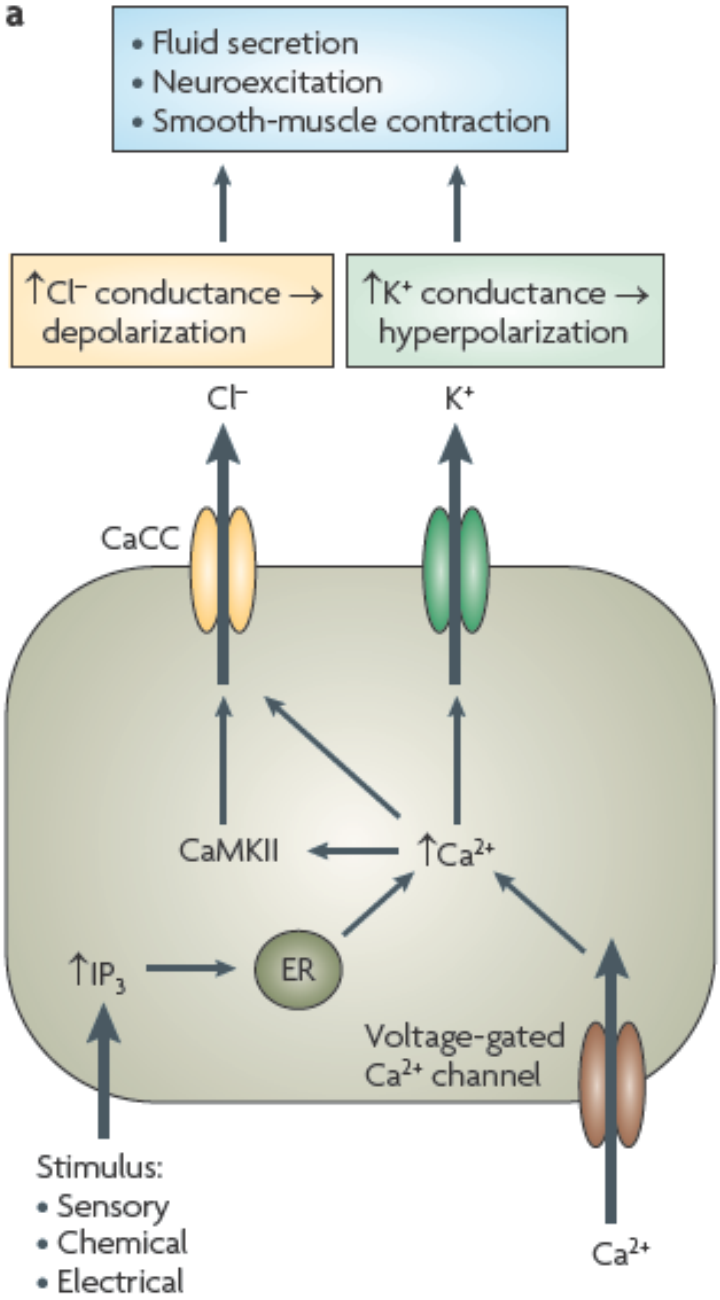
compound a: a tetrazolo-derivatized thiazolidinone analogue

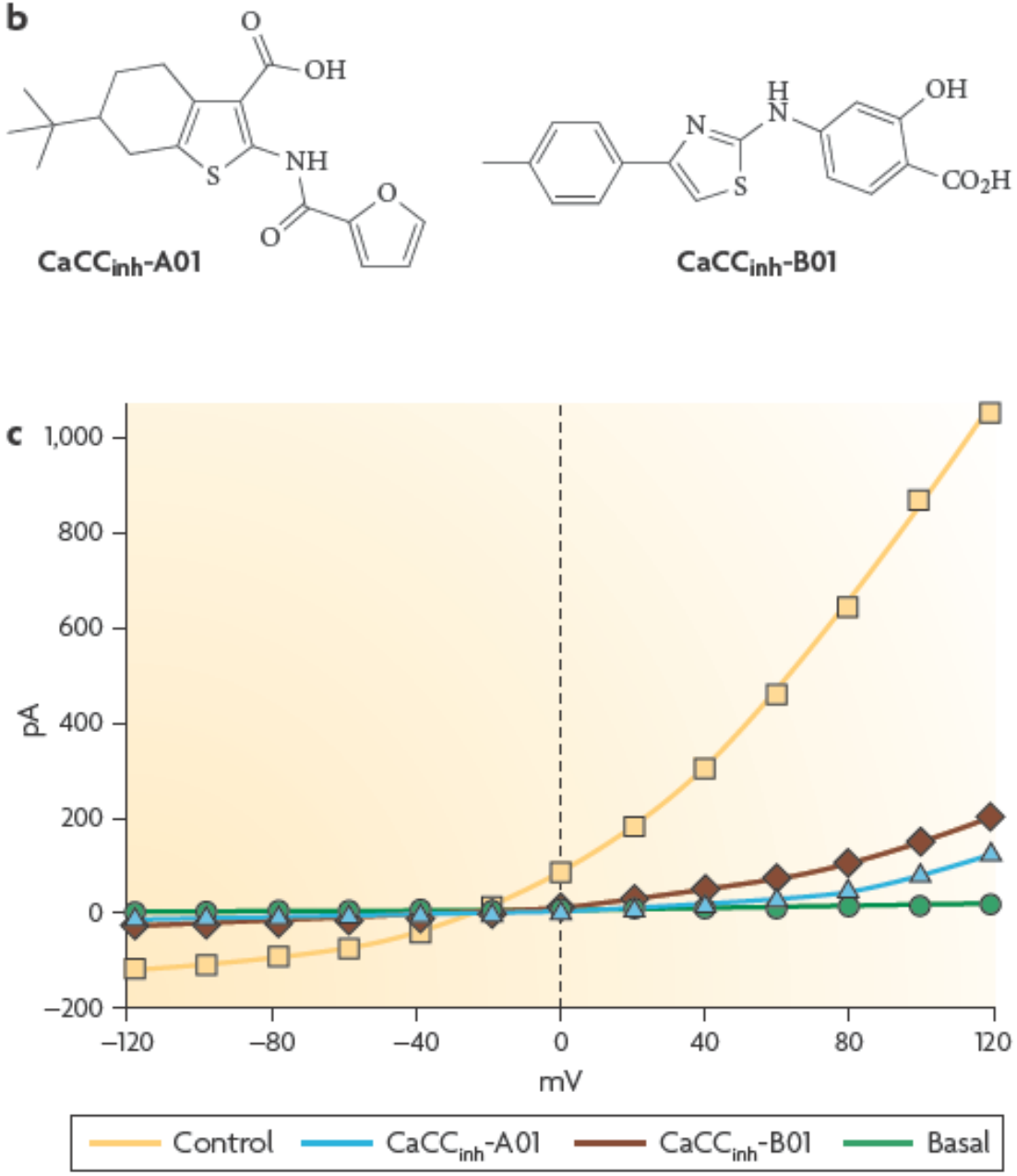
compound b: an absorbable, phenyl-derivatized glycine hydrazide analogue

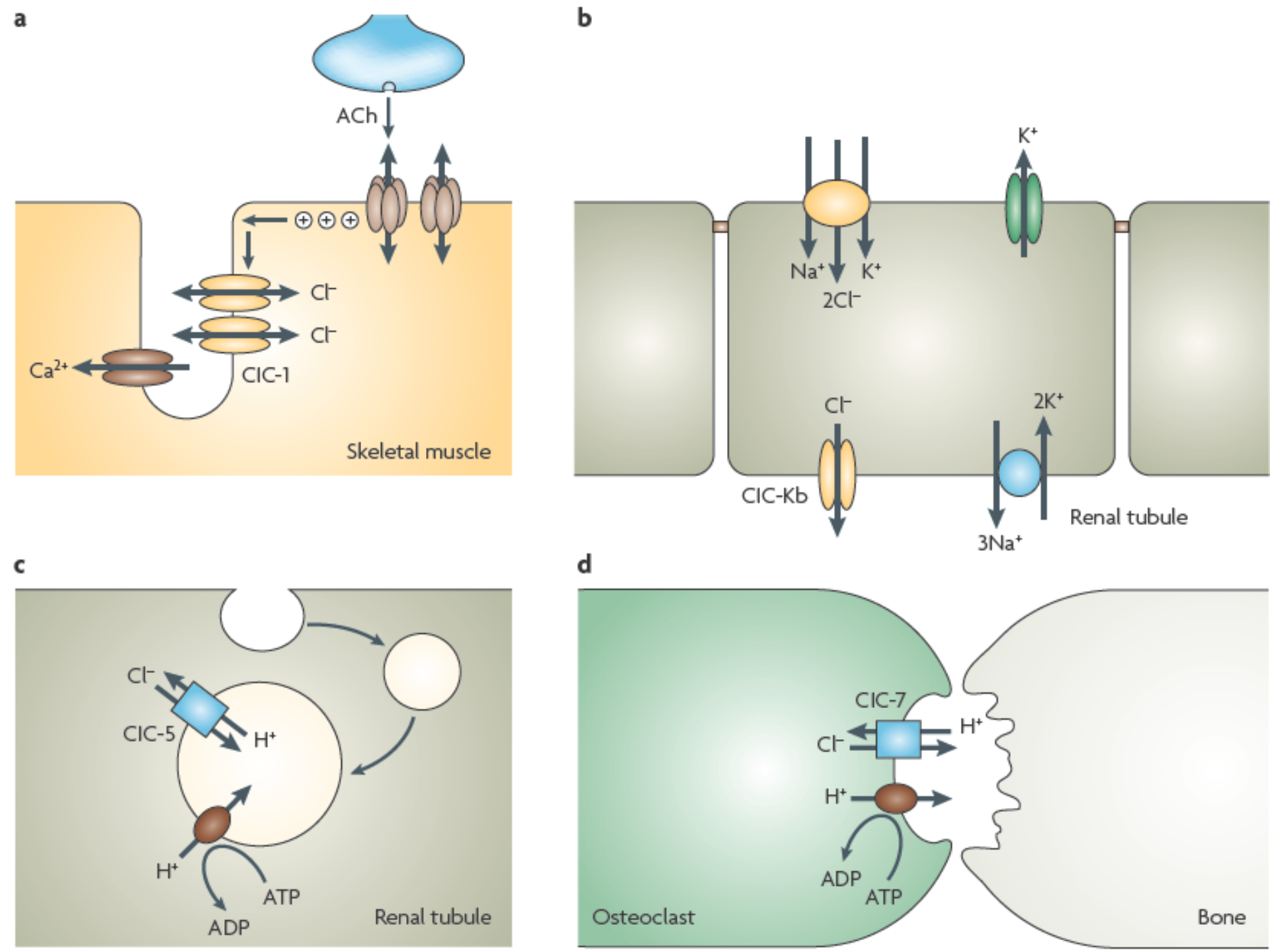
Lung pathophysiology in cystic fibrosis (CF) and activators of ΔF508-CFTR, the most common cF-causing mutation



Cellular physiology of calcium-activated Cl^- channels and small-molecule inhibitors






a
ACh
Cl⁻
Cl⁻
Ca²⁺
ClC-1
Skeletal muscle
b
K⁺
Na⁺
K⁺
2Cl⁻
Cl⁻
2K⁺
ClC-Kb
3Na⁺
Renal tubule
c
Cl⁻
ClC-5
H⁺
H⁺
ATP
ADP
Renal tubule
d
ClC-7
Cl⁻
H⁺
H⁺
ADP
ATP
Osteoclast
Bone

5-HT_3 receptors

Family name	Ligand	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
5-HT_3 receptors	5-hydroxytryptamine	5-HT3A	HTR3A	Htr3a	Htr3a
5-HT_3 receptors	5-hydroxytryptamine	5-HT3B	HTR3B	Htr3b	Htr3b
5-HT_3 receptors	5-hydroxytryptamine	5-HT3C	HTR3C		
5-HT_3 receptors	5-hydroxytryptamine	5-HT3D	HTR3D		
5-HT_3 receptors	5-hydroxytryptamine	5-HT3E	HTR3E		
5-HT_3 receptors	5-hydroxytryptamine	5-HT_3AB			
5-HT_3 receptors	5-hydroxytryptamine	5-HT_3A			

Nicotinic acetylcholine receptors

Family name	Ligand	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
Nicotinic acetylcholine receptors	acetylcholine	α 1	CHRNA1	Chrna1	Chrna1
Nicotinic acetylcholine receptors	acetylcholine	α 2	CHRNA2	Chrna2	Chrna2
Nicotinic acetylcholine receptors	acetylcholine	α 3	CHRNA3	Chrna3	Chrna3
Nicotinic acetylcholine receptors	acetylcholine	α 4	CHRNA4	Chrna4	Chrna4
Nicotinic acetylcholine receptors	acetylcholine	α 5	CHRNA5	Chrna5	Chrna5
Nicotinic acetylcholine receptors	acetylcholine	α 6	CHRNA6	Chrna6	Chrna6
Nicotinic acetylcholine receptors	acetylcholine	α 7	CHRNA7	Chra7	Chrna7
Nicotinic acetylcholine receptors	acetylcholine	α 9	CHRNA9	Chrna9	Chrna9
Nicotinic acetylcholine receptors	acetylcholine	α 10	CHRNA10	Chrna10	Chrna10
Nicotinic acetylcholine receptors	acetylcholine	β 1	CHRNB1	Chrnb1	Chrnb1
Nicotinic acetylcholine receptors	acetylcholine	β 2	CHRNB2	Chrnb2	Chrnb2
Nicotinic acetylcholine receptors	acetylcholine	β 3	CHRNB3	Chrnb3	Chrnb3
Nicotinic acetylcholine receptors	acetylcholine	β 4	CHRNB4	Chrnb4	Chrnb4
Nicotinic acetylcholine receptors	acetylcholine	γ	CHRNG	Chrng	Chrng
Nicotinic acetylcholine receptors	acetylcholine	δ	CHRND	Chrnd	Chrnd
Nicotinic acetylcholine receptors	acetylcholine	ε	CHRNE	Chrne	Chrne

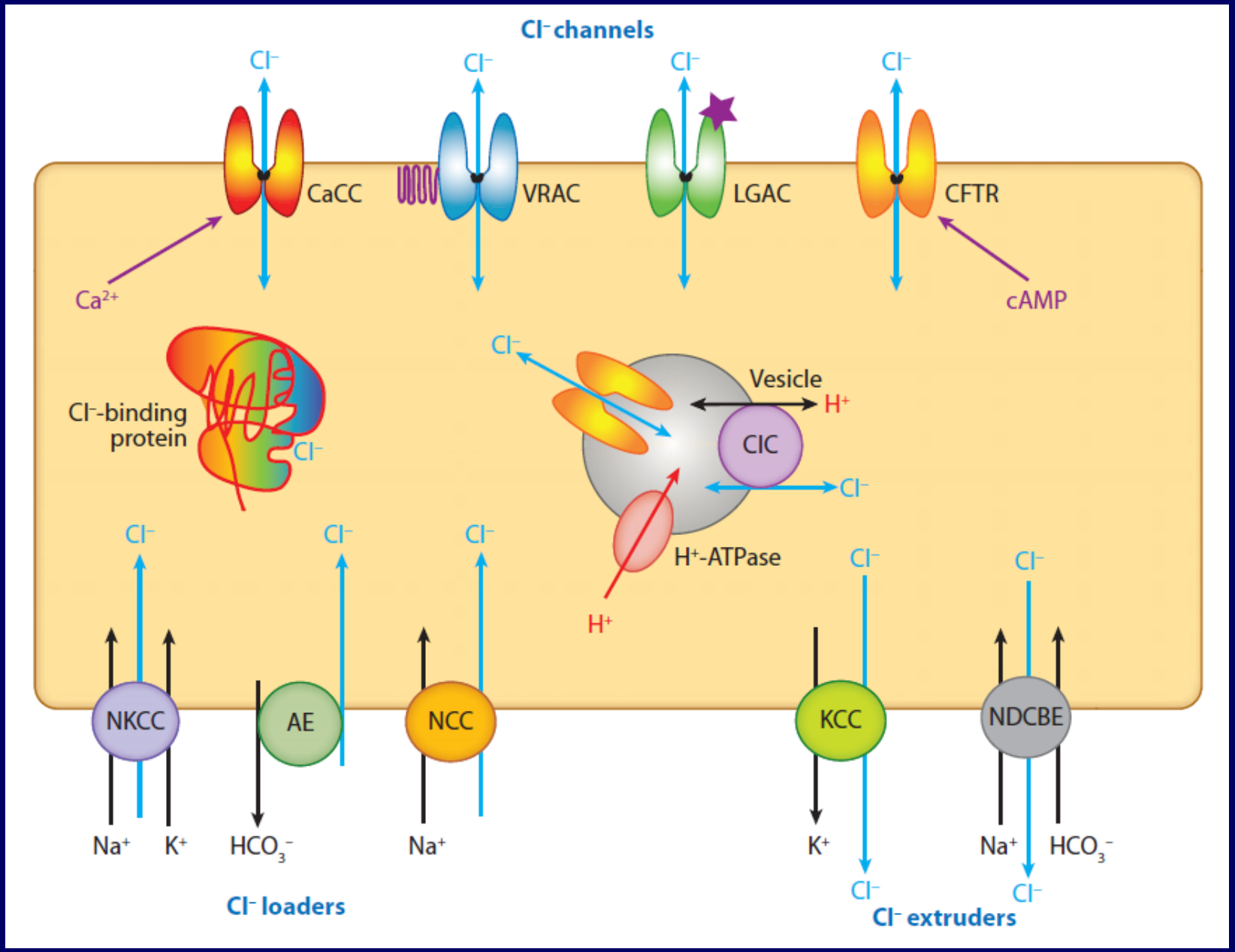
P2X receptors

Family name	Ligand	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
P2X receptors	ATP	P2X1	P2RX1	P2rx1	P2rx1
P2X receptors	ATP	P2X2	P2RX2	P2rx2	P2rx2
P2X receptors	ATP	P2X3	P2RX3	P2rx3	P2rx3
P2X receptors	ATP	P2X4	P2RX4	P2rx4	P2rx4
P2X receptors	ATP	P2X5	P2RX5	P2rx5	P2rx5
P2X receptors	ATP	P2X6	P2RXL1	P2rxl1	P2rxl1
P2X receptors	ATP	P2X7	P2RX7	P2xr7	P2rx7

ZAC

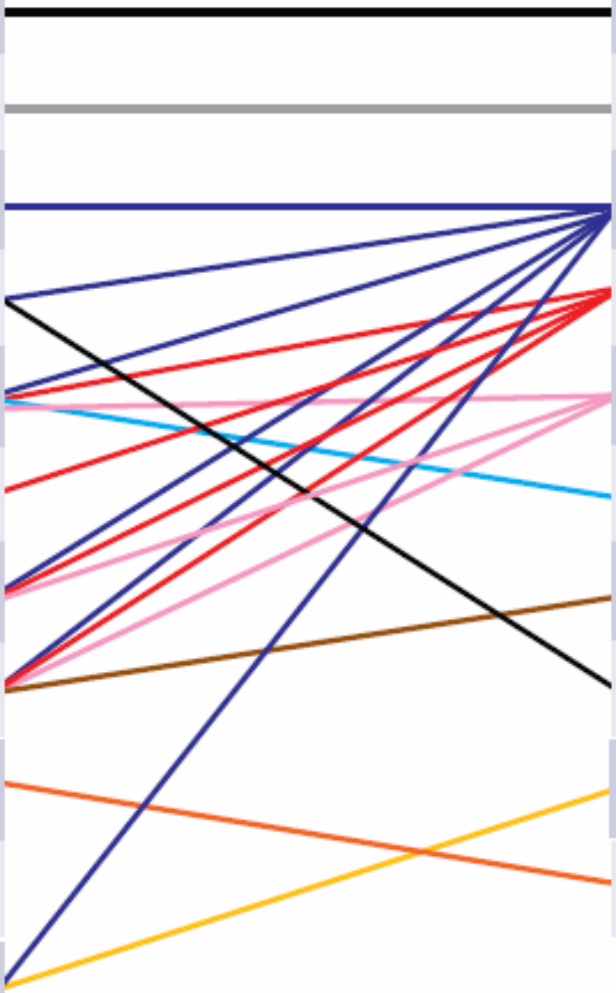
Family name	Ligand	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
ZAC	Zn^{++}	ZAC	ZACN		

Cellular Cl^- signaling



Relationship of biophysically identified Cl^- channels to genes

Biophysically identified	Mammalian candidate genes
Ligand-gated	$GABA_A$, glycine receptors
PKA-activated	CFTR
Voltage-gated	ClCs (9 genes)
Acid-activated	Bestrophins (4 genes)
Ca^{2+}-activated (CaCC)	Anoctamins (10 genes)
cGMP-dependent CaCC	CLCAs (4 genes)
Volume-regulated (VRAC)	CLICs (6 genes)
Intracellular	SLC26A7
Large conductance	Glutamate transporters
ORCC	Tweety
Transporter-associated	



See you next week!