Na^+ channels

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What's your association with Na^+ channels?

- A target for specific, clinically important, local anaesthetic effects in neurons
- Widespread use of local anaesthetics for well over a century

The outline…

Required Readings:

Marban E, Yamagishi T, Tomaselli GF. (1998) Structure and function of voltage-gated sodium channels. J Physiol. 508(3): 647-57.

Goldin Al. (2003) Mechanisms of sodium channel inactivation. Current Opinion in Neurobiology 2003, 13(3): 284–290.

Scheuer T (2011) Regulation of sodium channel activity by phosphorylation. Seminars in Cell & Developmental Biology. 22(2): 160-165. http://www.sciencedirect.com/science/article/pii/S1084952110001643

Ruan Y, Liu N, Priori SG. (2009) Sodium channel mutations and arrhythmias. Nat Rev Cardiol. 6: 337-348.

Further Readings:

Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. (2010) Sodium channels in normal and pathological pain. Annu Rev Neurosci. 33: 325-247.

Dib-Hajj SD, Binshtok AM, Cummins TR, Jarvis MF, Samad T, Zimmermann K. (2009) Voltage-gated sodium channels in pain states: role in pathophysiology and targets for treatment. Brain Res Rev. 60: 65-83.

The outline…

This class will cover:

- Types and structure of Na^+ channels
- Biochemical, molecular and genetic properties
- Physiological roles and regulation
- Pharmacological significance and disorders

Introduction

- Sodium channels are responsible for action potential initiation and propagation in excitable cells, including nerve, muscle, and neuroendocrine cell types.
- They are also expressed at low levels in nonexcitable cells, where their physiological role is unclear.
- Sodium channels are the founding members of the ion channel superfamily in terms of their discovery as a protein and determination of their amino acid sequence.

Introduction

- Sodium channels mediate fast depolarization and conduct electrical impulses throughout nerve, muscle and heart.
- Sodium channels have a modular architecture, with distinct regions for the pore and the gates. The separation is far from absolute, however, with extensive interaction among the various parts of the channel.
- Sodium channels are not static: they move extensively in the course of gating and ion translocation (at a molecular level).
- Sodium channels bind local anaesthetics and various toxins. In some cases, the relevant sites have been partially identified.
- Sodium channels are subject to regulation at the levels of transcription, subunit interaction and post-translational modification (notably glycosylation and phosphorylation).

Introduction

- Sodium channels transmit depolarizing impulses rapidly throughout cells and cell networks, thereby enabling co-ordination of higher processes ranging from locomotion to cognition.
- Na^+ channels are richly concentrated in axons and in muscle, where they are often the most plentiful ion channels.
  - Mammalian heart cells, for example, typically express more than 100 000 Na^+ channels, but only 20 000 or so L-type Ca^{2+} channels and fewer copies of each family of voltage-dependent K^+ channels.
- Na^+ channels consist of various subunits, but only the principal (α) subunit is required.

History

- Sodium channels are of special importance for the history of physiology.
- Elucidation of their fundamental properties in the squid axon launched modern channel theory. In particular, the work of Hodgkin and Huxley on sodium channels revolutionized electrophysiology by elegantly dissecting the elementary processes of gating and permeation.
- Sodium channels first appear phylogenetically in the jellyfish, where they enable the organism to transmit electrical signals efficiently throughout a dispersed neural net.
- Sodium channels were the first voltage-dependent ion channels to be cloned, ushering in the era of heterologous expression and molecular manipulation. The cloning happily coincided with the development of patch-clamp techniques, which enabled single-channel recordings.

Classification of Na^+ channels

- Voltage-gated
- Non-voltage-gated
- Exchangers

Subunits

- Nav1.1
- Nav1.2
- Nav1.3
- Nav1.4
- Nav1.5
- Nav1.6
- Nav1.7
- Nav1.8
- Nav1.9

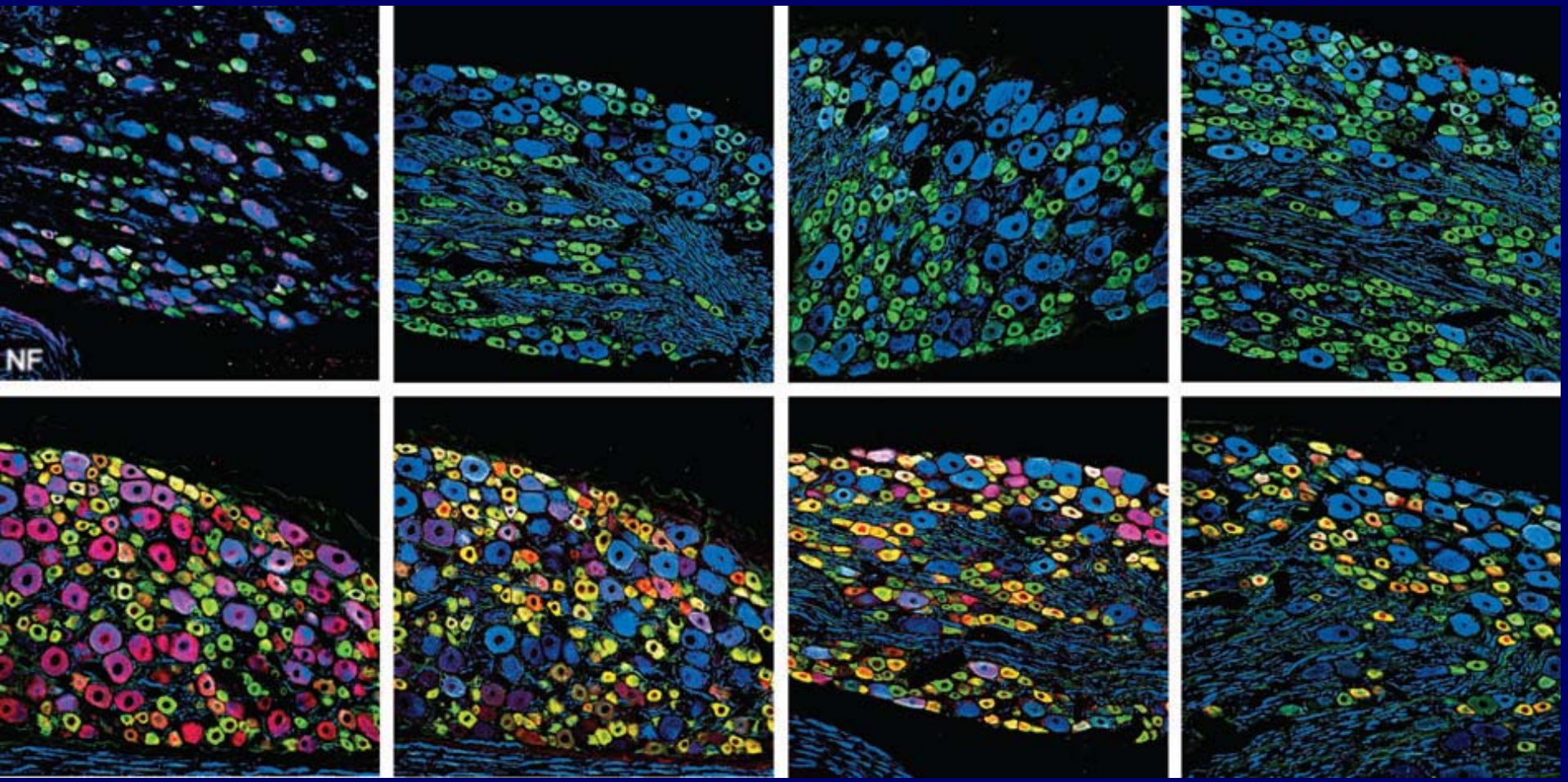
Nomenclature of Na^+ channels

- The functional properties of the known sodium channels are relatively similar.
- It utilizes a numerical system to define subfamilies and subtypes based on similarities between the amino acid sequences of the channels.
- In this nomenclature system, the name of an individual channel consists of the chemical symbol of the principal permeating ion (Na) with the principal physiological regulator (voltage) indicated as a subscript (Na_V). The number following the subscript indicates the gene subfamily (currently only Na_V1), and the number following the full point identifies the specific channel isoform (e.g., $Na_V1.1$). This last number has been assigned according to the approximate order in which each gene was identified. Splice variants of each family member are identified by lowercase letters following the number (e.g., $Na_V1.1a$).
- All of the nine sodium channel isoforms may be considered members of one family.

Phylogenetic relationships of voltage-gated sodium channel α subunits

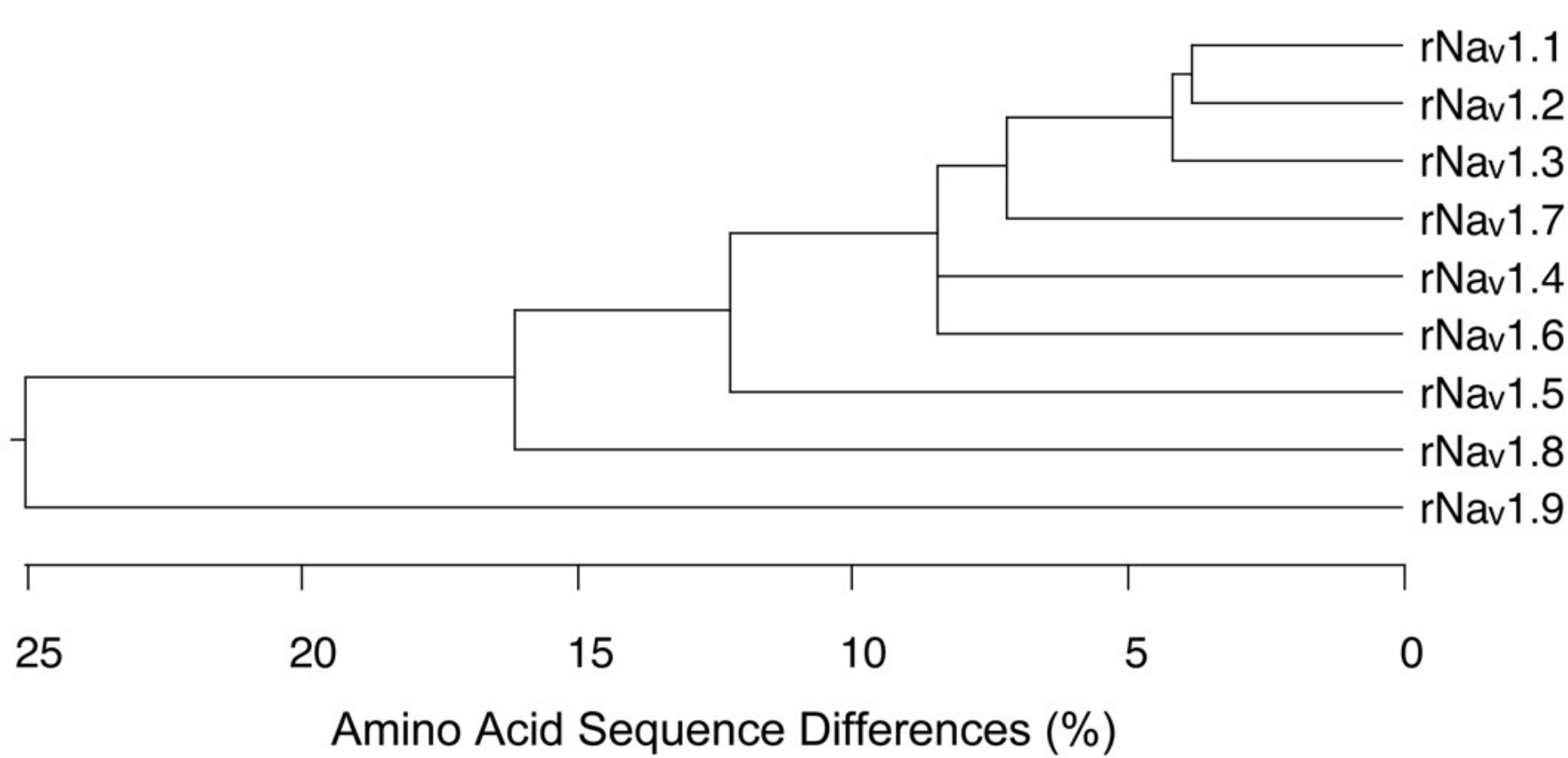
- The nine sodium channel amino acid sequences were aligned and compared for relatedness using a **maximum parsimony procedure** that measured their evolutionary distance by calculating the **number of nucleotide changes** required for the change in codon at each position.
- The phylogenetic tree is consistent the designation of these sodium channels as a single family. $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.7$ are the most closely related group by this analysis. All four of these sodium channels are highly **tetrodotoxin-sensitive** and broadly expressed in neurons. Their genes are all located on human **chromosome 2q23–24**, consistent with a common evolutionary origin.

Sodium channels expressed in adult DRG neuron



Blue, neurofilament, a marker of medium and large neurons
Green, redperipherin, a specific small neuron marker
Red, sodium channel isoforms Nav1.1, Nav1.6, Nav1.7, Nav1.8 and Nav1.9

Amino acid sequence similarity of voltage-gated sodium channel α subunits



William et al. 2009, Voltage-Gated Sodium Channels, introductory chapter.

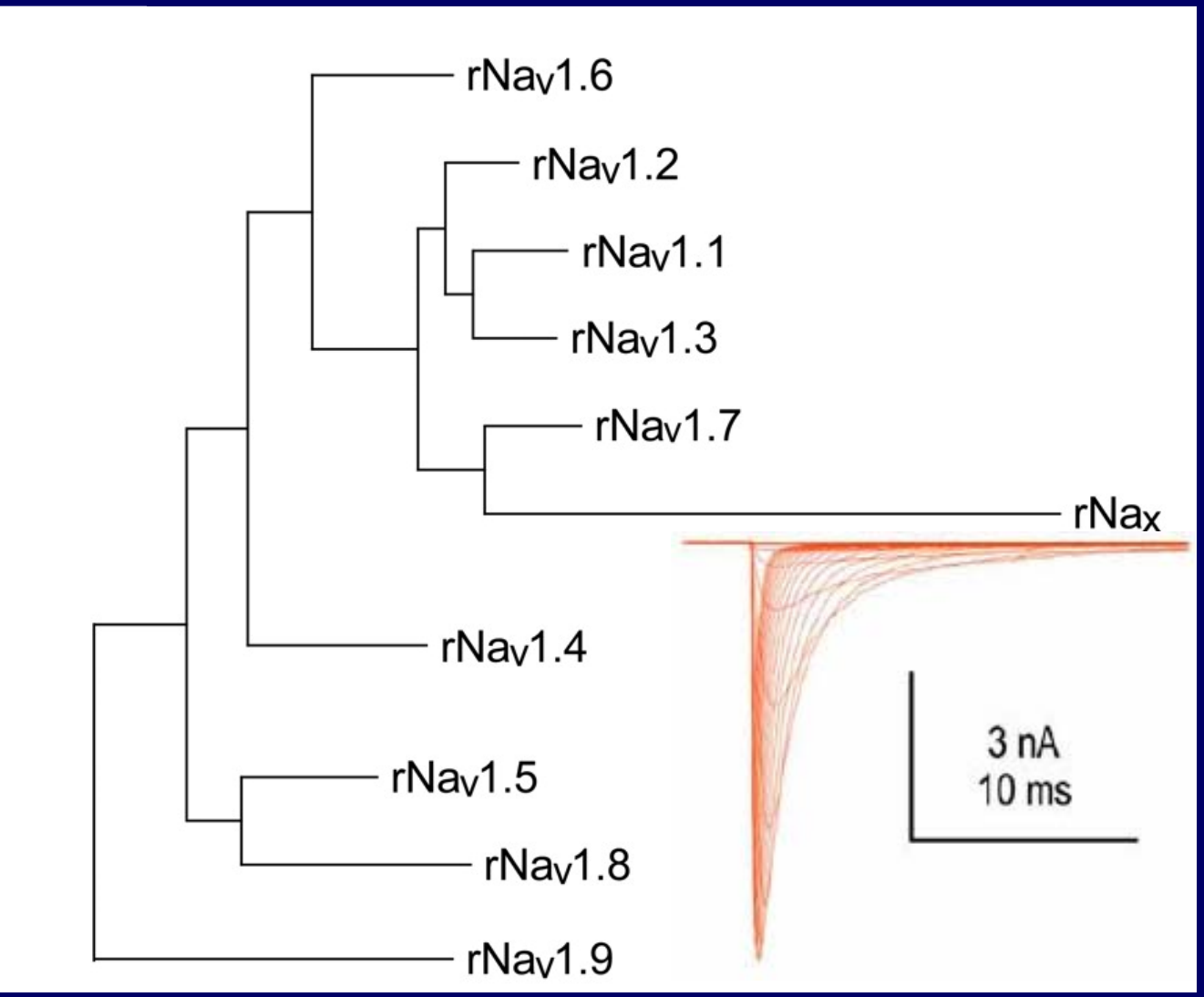
Phylogenetic relationships of voltage-gated sodium channel α subunits

- NaV1.5, NaV1.8, NaV1.9 are also closely related, and their amino acid sequences are greater than 64% identical to those of the four sodium channels encoded on chromosome 2.
- These sodium channels are tetrodotoxin-resistant to varying degrees, due to changes in amino acid sequence at a single position in domain I, and they are highly expresses in heart and dorsal root ganglion (DRG) neurons. Their genes are located on human chromosome 3p21–24, consistent with a common evolutionary origin.
- The isoforms $Na_V1.4$, expressed primarily in skeletal muscle, and $Na_V1.6$, expressed primarily in the CNS, are set apart from these other two closely related groups of sodium channel genes.

Phylogenetic relationships of voltage-gated sodium channel α subunits

- The chromosome segments carrying the sodium channel genes are paralogous segments that contain many sets of related genes, including the homeobox (HOX) gene clusters. These segments were generated by whole genome duplication events during early vertebrate evolution.
- The comparisons of amino acid sequence identity, phylogenetic relationship, and chromosomal relationship lead to the conclusion that all nine members of the sodium channel family that have been functionally expresses are members of a single family of proteins and have arisen from gene duplications and chromosomal rearrangements relatively recently in evolution.

Phylogenetic tree



Based on the evolutionary distance by calculating the number of nucleotide changes required for the change in codon at each position

William et al. 2009, Voltage-Gated Sodium Channels

Voltage-gated sodium channels

Family name	Physiological ion	Official IUPHAR receptor name	Human gene name	Rat gene name	Mouse gene name
Voltage-Gated Sodium Channels	Na^+	$Na_v1.1$	SCN1A	scn1a	scn1a
Voltage-Gated Sodium Channels	Na^+	$Na_v1.2$	SCN2A	scn2a1	scn2a1
Voltage-Gated Sodium Channels	Na^+	$Na_v1.3$	SCN3A	scn3a	scn3a
Voltage-Gated Sodium Channels	Na^+	$Na_v1.4$	SCN4A	scn4a	scn4a
Voltage-Gated Sodium Channels	Na^+	$Na_v1.5$	SCN5A	scn5a	scn5a
Voltage-Gated Sodium Channels	Na^+	$Na_v1.6$	SCN8A	scn8a	scn8a
Voltage-Gated Sodium Channels	Na^+	$Na_v1.7$	SCN9A	scn9a	scn9a
Voltage-Gated Sodium Channels	Na^+	$Na_v1.8$	SCN10A	scn10a	scn10a
Voltage-Gated Sodium Channels	Na^+	$Na_v1.9$	SCN11A	scn11a	scn11a

Sodium channel-like proteins

- Closely related sodium channel-like proteins have been cloned from mouse, rat, and human but have not yet been functionally expressed (Na_x).
- They are approximately 50% identical to the Na_V1 subfamily of channels but more than 80% identical to each other.
- They have significant amino sequence differences in the voltage sensors, inactivation gate, and pore region that are critical for channel function, and have previously been proposed as a distinct subfamily.
- These atypical sodium channel-like proteins are expressed in heart, uterus, smooth muscle, astrocytes, and neurons in the hypothalamus and peripheral nervous system (PNS).

Sodium channel-like proteins

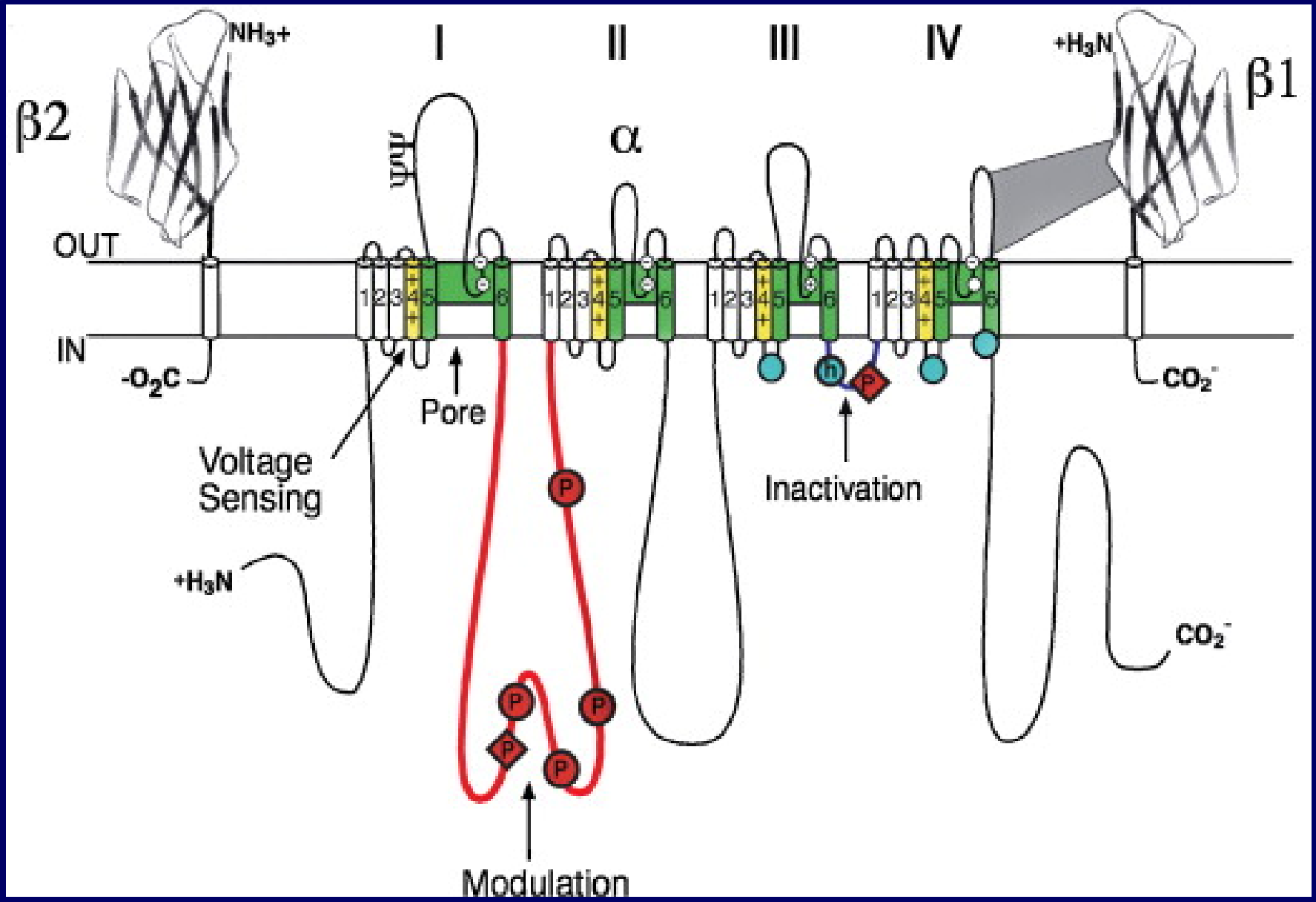
- It is possible that these channels are not highly sodium-selective or voltage-gated.
- They are closely related phylogenetically to the group of sodium channels on human chromosome 2q23–24, where their gene is also located.
- Successful functional expression of these atypical sodium channel-like proteins and identification of additional related sodium channels may provide evidence for a second sodium channel subfamily.

Structure of Na^+ channels

Sodium channel subunits

- Sodium channels consist of a highly processed α subunit (~ 260 kDa) associated with auxiliary β subunits.
- The α subunits are organized in four homologous domains (I–IV), which each contain six transmembrane α helices (S1–S6) and an additional pore loop located between the S5 and S6 segments.
- The pore-forming α subunit is sufficient for functional expression.
- The pore loops line the outer narrow entry to the pore whereas the S5 and S6 segments line the inner wider exit from the pore.

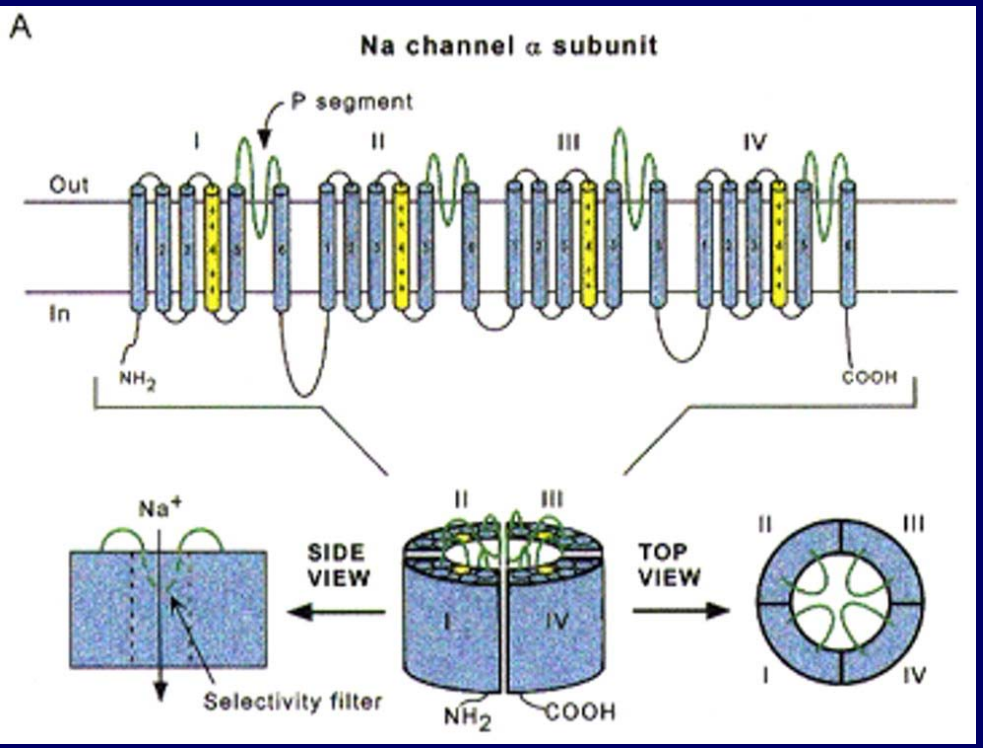
Transmembrane organization of Na^+ channel subunits



Sodium channel subunits

- The S4 segments in each domain contain positively charged amino acid (aa) residues at every third position. These residues serve as gating charges and move across the membrane to initiate channel activation in response to depolarization of the membrane.
- The short intracellular loop connecting homologous domains III and IV serves as the inactivation gate, folding into the channel structure and blocking the pore from the inside during sustained depolarization of the membrane.

Schematic depictions of the Na^+ channel α subunit



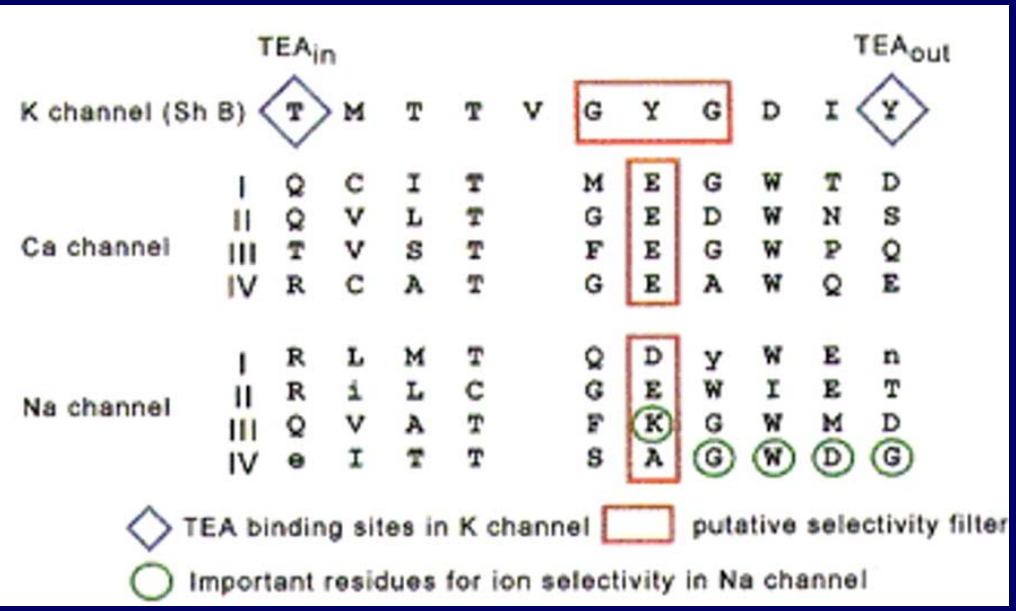
- four internally homologous domains (labelled I-IV)
- each domain contains six transmembrane segments
- four domains fold together so as to create a central pore whose structural constituents determine the selectivity and conductance properties of the channel
- Na^+ channels might have arisen in an analogous manner or, more likely, from mutations in a primitive Ca^{2+} channel.

Yellow: the charged S4 segments

Green: the pore-lining P segments

Marban E et al. J Physiol 1998; 508: 647-657

Primary aa sequences in single-letter code of the P segments



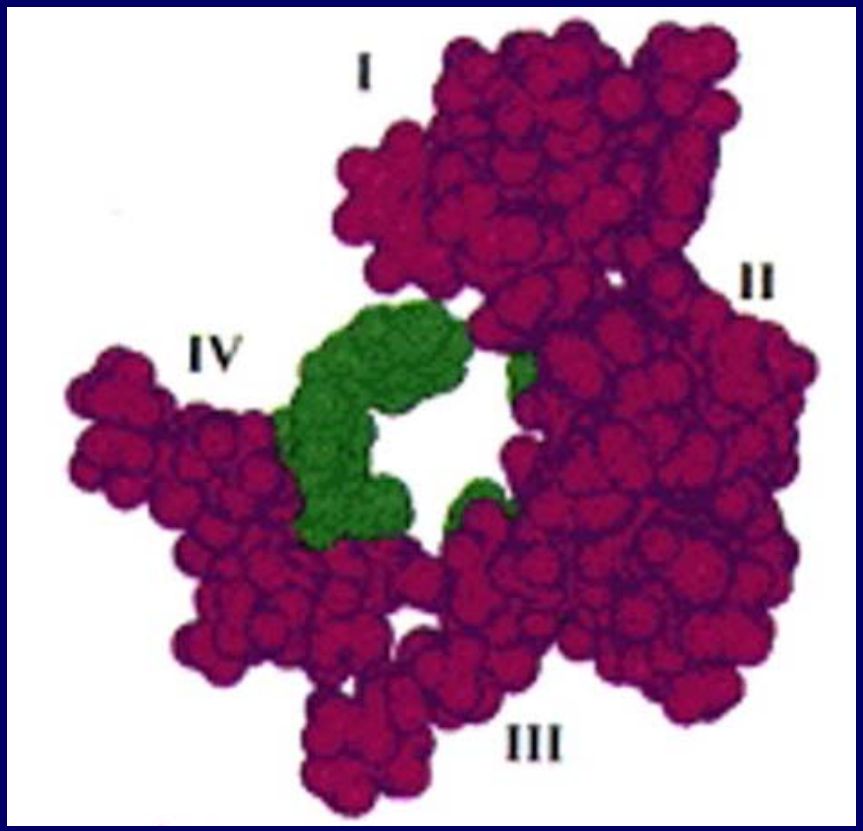
- Aligned primary amino acid sequences in single-letter code of the P segments in a K^+ channel (Shaker B)
- The four domains of the cardiac L-type Ca^{2+} channel
- The four domains of the Na^+ channel
- Residues shown in upper case are highly conserved among voltage-dependent Na^+ channels

Marban E et al.
J Physiol 1998; 508: 647-657

- Evolutionary considerations serve to point out various themes that are general to voltage-dependent ion channels
- The architecture is modular, consisting either of four homologous subunits (in K^+ channels) or of four internally homologous domains (in Na^+ and Ca^{2+} channels).
- The proteins wrap around a central pore. The pore-lining ('P segment') regions exhibit exquisite conservation within a given channel family of like selectivity (jellyfish, eel, fruit-fly and human Na^+ channels have very similar P segments), but not among families with different selectivities.
- The general strategy for activation gating is highly conserved: the fourth transmembrane segment (S4), stereotypically studded with positively charged residues, lies within the membrane field and moves in response to depolarization, somehow opening the channel.

- The S5-S6 linkers or P segments of each domain come together to form the pore.
- Inspection of the primary structures of the linkers in each domain reveals that each is unique.
  - The structural basis of permeation thus differs fundamentally from that of K^+ channels, in which four identical P segments can come together to form a K^+-selective pore.
- Accessibility mapping studies in Na^+ channels have revealed marked asymmetries in the contributions of each domain to the permeation pathway.

Structure for the Na^+ channel pore



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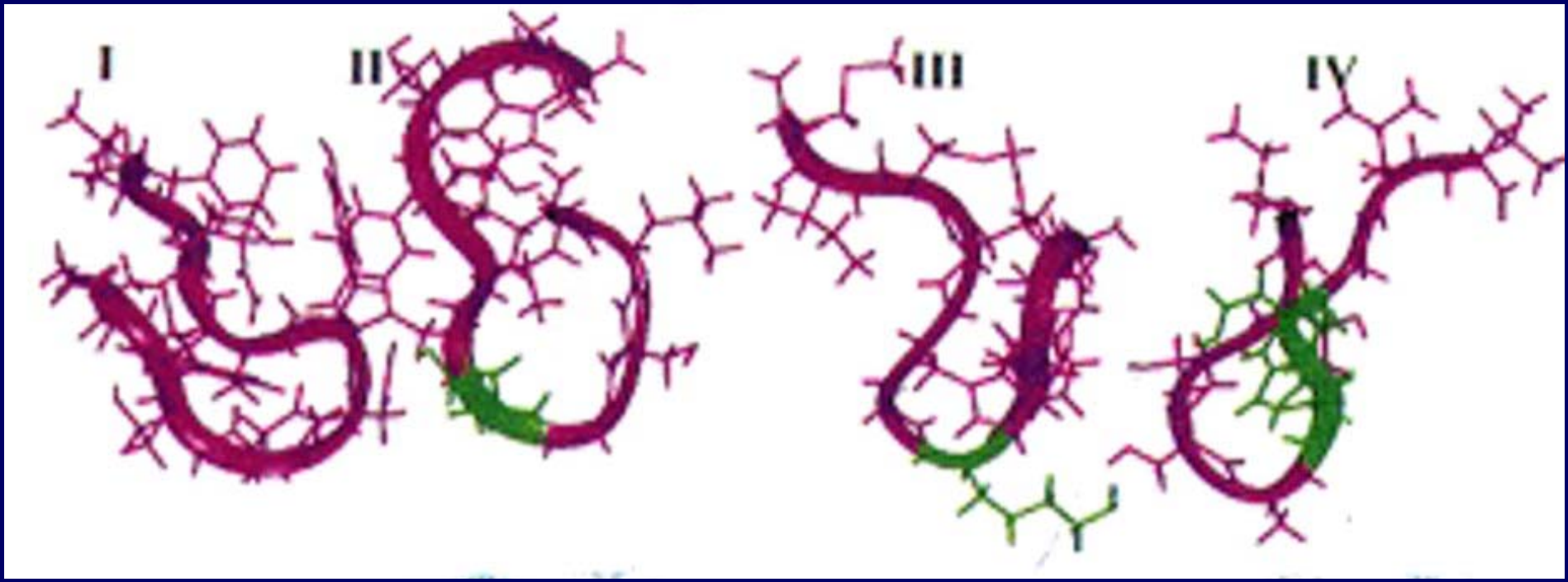
J Physiol 1998; 508: 647-657

- A top view of a space-filling model of the Na^+ channel pore which is consistent with the available mutagenesis data.
- The asymmetry is apparent in top and side views
- Two domains play a particularly prominent role in determining Na^+ selectivity
  - III, in which a lysine (K1237 in the μ1 sequence) is critical for discrimination for Na^+ over Ca^{2+}
  - IV, in which mutations of various contiguous residues (1531–1534) render the channel non-selective among monovalent cations

Structure for the Na^+ channel pore

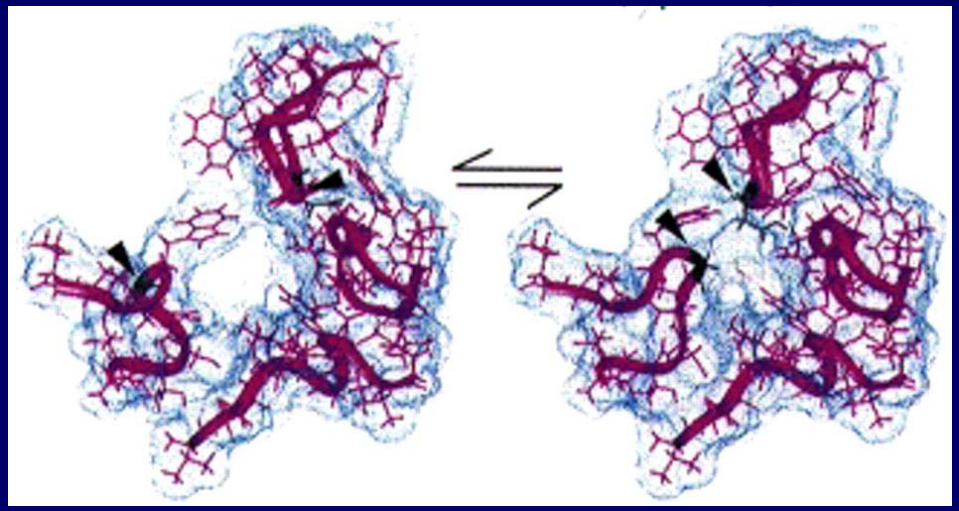
- Residues in domains I and IV which are quite distant from each other in the equilibrium structure can come sufficiently close together to occlude the pore.
- The motions occur over millisecond time scales and may span several nanometres in extreme cases.



Marban E et al. J Physiol 1998; 508: 647-657

Structure for the Na^+ channel pore

- Na^+ channel pore motions illustrated by the pairing of residues D400 and G1530 (arrowheads), which can form an internal disulphide in the pore when substituted with cysteines.
- An internal disulphide crosslink renders channels less selective than those with two reduced sulfhydryls, hinting that flexibility plays an important role in selective ion translocation.



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- One of the seminal contributions of Hodgkin and Huxley was the notion that Na^+ channels transit among various conformational states in the process of opening ('activation'); yet another set of conformations is entered when the channels shut during maintained depolarization ('inactivation').
- The *m* gates that underlie activation, and the *h* gates that mediates inactivation, were postulated to have intrinsic voltage dependence and to function independently.
- While some of the implicit structural predictions of that formulation have withstood the test of time, others have not.

S4 segment

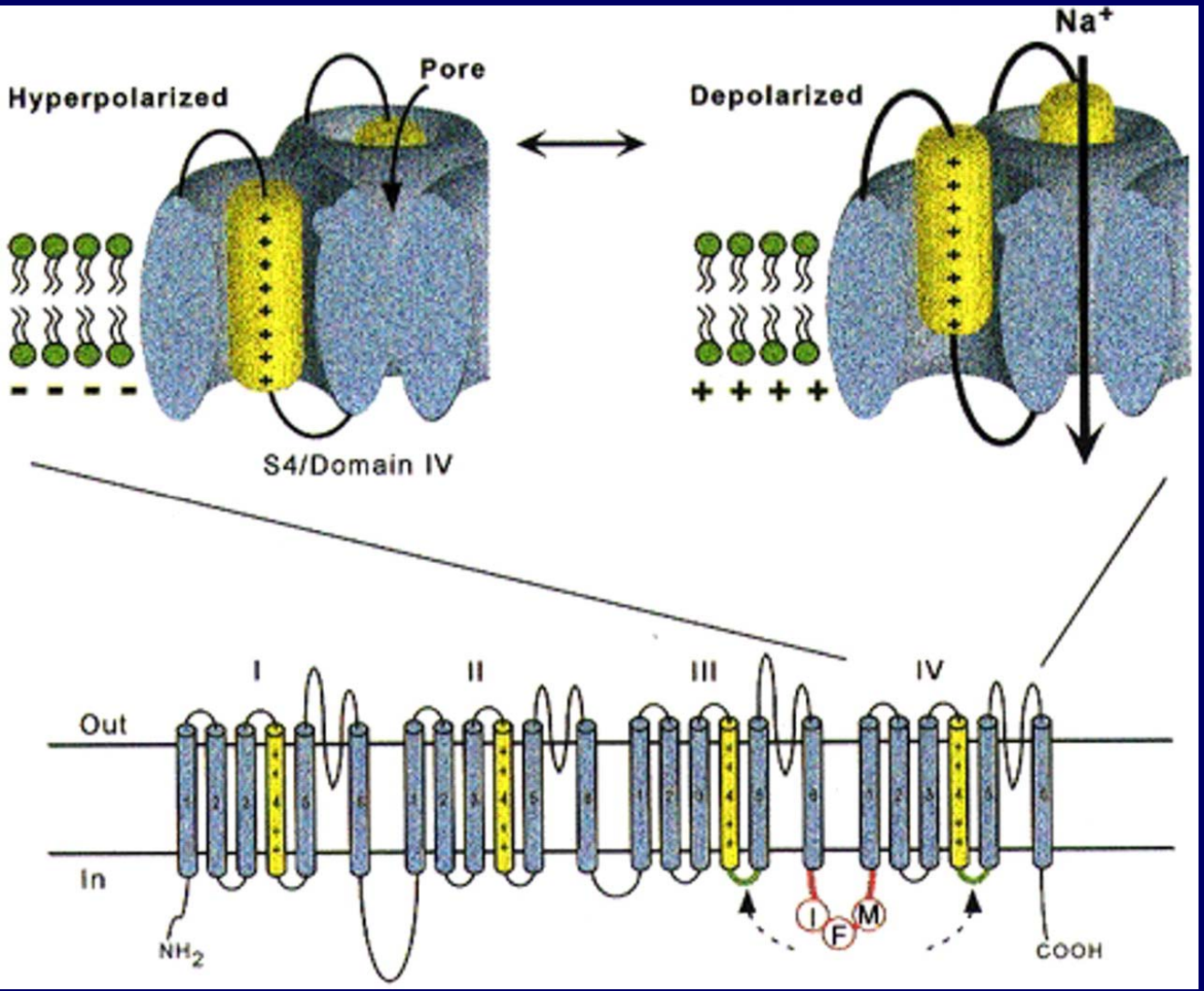
- The four S4 segments are now widely acknowledged to serve as the activation sensors.
- In the process of activation, several charged residues in each S4 segment physically traverse the membrane through a narrow cuff formed by other, as yet unidentified regions of the channel.
- The S4 segment in domain IV undergoes a minimal translocation of 0.5 nm (5 Å) in response to a voltage step, emphasizing once again the importance of internal motions for the function of these proteins.
- The idea that the sensors are equivalent and independent turns out to be incorrect.
- The contributions of each S4 segment to activation are markedly asymmetrical; some of the charged residues play a much more prominent role than others in 'homologous' positions.

- Charge-altering mutations in multiple S4 segments do not exert simply additive effects on gating; there is some co-operativity, the extent of which varies from site to site.
- Gating current and mutagenesis studies have additionally revealed that activation is coupled to inactivation.
- The time course of current decay predominantly reflects the voltage dependence of activation, although microscopic inactivation itself does vary with voltage (particularly in cardiac channels).

S4 segment

- If the S4s are the sensors, where are the activation gates themselves? This crucial question remains unresolved.
- The gates must be on the internal aspect of the permeation pathway, since pore-lining residues remain accessible to externally applied reagents regardless of whether the channels are open, closed or inactivated.
- It is possible that motion of S4 distorts the S4-S5 linker, which may contribute to the inner pore (by analogy to K^+ channels) and serve as a gate.
- Alternatively, S4 motion may displace other parts of the channel.

Schematic depictions of the Na^+ channel α subunit



- Top: the S4 activation sensors
- Bottom: the III-IV linker which contributes to fast inactivation.
- I, F & M: Fast inactivation mediated by the cytoplasmic linker between domains III and IV.

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S6 segment

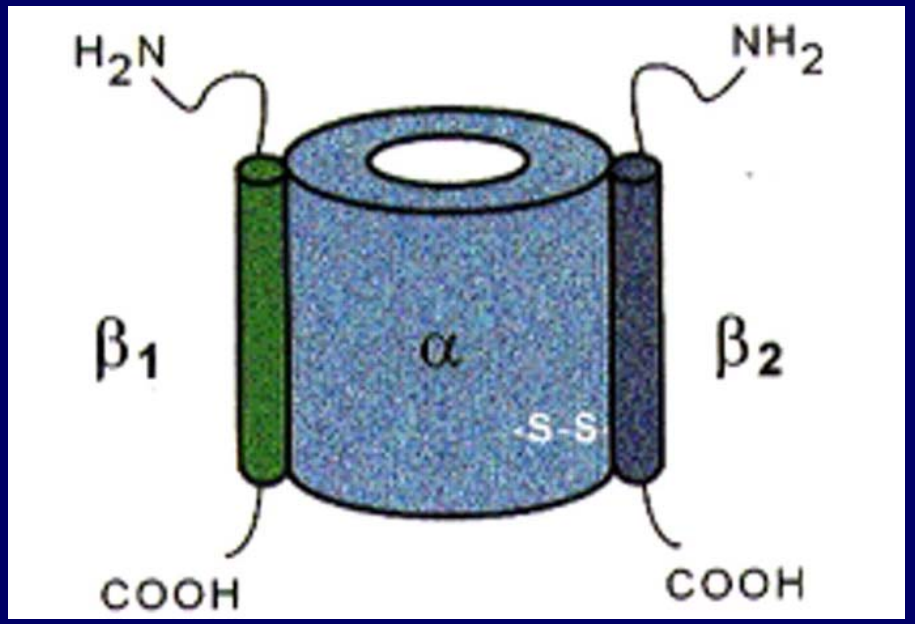
- Mutations in S6 alter Na^+ channel gating as well as block by local anaesthetics. Taken together with the finding in K^+ channels that S6 can physically occlude the inner channel mouth in response to voltage, S6 emerges as the leading contender for the physical activation gate.
- The S6 segment of domain IV has been proposed to contain the receptor for local anaesthetics, which block Na^+ channels in a voltage-dependent manner.
- Block is enhanced at depolarized potentials and/or with repetitive pulsing. These observations are consistent with the idea that local anaesthetics act as allosteric effectors of inactivation gating: when they bind to the channel, they facilitate inactivation.

S6 segment

- Whether or not this particular model of drug action turns out to be correct, it is clear that gating interacts with local anaesthetic block so profoundly that it is difficult to interpret at face value the localization of a ‘receptor’ to S6.
- Mutations in S6, at the putative receptor sites, alter gating independent of superimposed drug effects.
- Mutations in distant parts of the molecule can also dramatically alter the phenotype of local anaesthetic block.
- S6 segments appear to play a special role in drug effects in a variety of channels, at least some of which appear to be independent of changes in gating.

Auxiliary subunits of sodium channels

- β subunits are important modulators of Na^+ channel function.
- Three auxiliary subunits of sodium channels have been defined: β1, β2, and β3.
- Not all Na^+ channels include either or both β subunits.



- Sodium channels in the adult central nervous system (CNS) contain β1 (or β3) and β2 subunits whereas sodium channels in adult skeletal muscle have only the β1 subunit.

β subunits

- Antibodies directed to the β1 or β2 subunit will immunoprecipitate the entire brain Na^+ channel complex with a subunit stoichiometry of 1α:1β1:1β2.
- The β1 subunit is non-covalently associated, while β2 is linked by a disulphide bond to the α subunit.
- The β1 and β2 subunits have been cloned and the deduced primary structures indicate that they are unrelated proteins of molecular weights 23 and 21 kDa, respectively.
- The predicted transmembrane topology of the β subunits is similar: each contains a small carboxy-terminal cytoplasmic domain, a single membrane-spanning segment, and a large amino-terminal extracellular domain with several consensus sites for N-linked glycosylation.

β subunits

- The β2 subunit has several unique features, including an extracellular immunoglobulin-like fold with similarity to the neural cell adhesion molecule contactin. Expression of β2 with neuronal α subunits in Xenopus oocytes increases the current amplitude, modulates gating and increases the membrane capacitance.
- Co-expression of β1 subunits with either neuronal or skeletal muscle α subunits in oocytes also produces clear-cut effects on channel function. The current density increases, activation and inactivation gating are accelerated, and the steady-state inactivation curves are shifted in the hyperpolarizing direction.
- The mRNA encoding the β1 subunit appears to be widely expressed and clearly forms an important component of neuronal and skeletal muscle Na^+ channels. However, the functional role of this subunit in the heart is uncertain.

Sodium Channel Molecular Pharmacology

Receptor sites for neurotoxins

- Neurotoxin receptor site 1 binds the nonpeptide pore blockers tetrodotoxin (TTX) and saxitoxin and the peptide pore blocker -conotoxin. The receptor sites for these toxins are formed by amino acid residues in the pore loops and immediately on the extracellular side of the pore loops at the outer end of the pore.
- Neurotoxin receptor site 2 binds a family of lipid-soluble toxins including batrachotoxin, veratridine, aconitine, and grayanotoxin, which enhance activation of sodium channels. Photoaffinity labeling and mutagenesis studies implicate transmembrane segments IS6 and IVS6 in the receptor site for batrachotoxin.
- Neurotoxin receptor site 3 binds the α–scorpion toxins and sea anemone toxins, which slow the coupling of sodium channel activation to inactivation. These peptide toxins bind to a complex receptor site that includes the S3–S4 loop at the outer end of the S4 segment in domain IV.

Receptor sites for neurotoxins

- Neurotoxin receptor **site 4** binds the β-scorpion toxins, which enhance activation of the channels. The receptor site for the -scorpion toxin includes the S3–S4 loop at the extracellular end of the voltage-sensing S4 segments in domain II.
- Neurotoxin receptor **site 5** binds the complex polyether toxins brevetoxin and ciguatoxin, which are made by dinoflaggelates and cause toxic red tides in warm ocean waters. Transmembrane segments IS6 and IVS5 are implicated in brevetoxin binding from photoaffinity labeling studies.
- Neurotoxin receptor **site 6** binds δ-conotoxins, which slow the rate of inactivation like the α-scorpion toxins. The location of neurotoxin receptor site 6 is unknown.

Receptor sites for neurotoxins

- The local anesthetics and related antiepileptic and antiarrhythmic drugs bind to overlapping receptor sites located in the inner cavity of the pore of the sodium channel. Amino acid residues in the S6 segments from at least three of the four domains contribute to this complex drug receptor site, with the IVS6 segment playing the dominant role.

These neurotoxins are widely used and are diagnostic of channel identity and function.

Receptor sites on Na^+ channels

Receptor Site	Toxin or Drug	Domains
Neurotoxin receptor site 1	Tetrodotoxin	IS2–S6, IIS2–S6
	Saxitoxin	IIIS2–S6, IVS2–S6
	μ-Conotoxin	
Neurotoxin receptor site 2	Veratridine	IS6, IVS6
	Batrachotoxin	
	Grayanotoxin	
Neurotoxin receptor site 3	α-Scorpion toxins	IS5–IS6, IVS3–S4
	Sea anemone toxins	IVS5–S6
Neurotoxin receptor site 4	β-Scorpion toxins	IIS1–S2, IIS3–S4
Neurotoxin receptor site 5	Brevetoxins	IS6, IVS5
	Ciguatoxins	
Neurotoxin receptor site 6	δ-Conotoxins	IVS3–S4
Local anesthetic receptor site	Local anesthetic drugs	IS6, IIIS6, IVS6
	Antiarrhythmic drugs	

Na^+ channel toxins

- Pharmacological competition studies and mutagenesis have defined a number of neurotoxin binding sites on the Na^+ channel.
- Tetrodotoxin (TTX), a guanidinium-containing blocker, has contributed the most to our understanding of Na^+ channel structure and function. Externally applied TTX blocks Na^+ channels potently (in the nanomole range) in neural and skeletal muscle isoforms, but block of cardiac channels requires much higher concentrations (~10^{-5} M).
- The identity of one particular residue in the P region of domain I accounts for most of the isoform-specific TTX sensitivity: an aromatic residue at this position (401 in μI) confers high affinity, while its absence renders the channel TTX resistant.
- Many other residues in the outer mouth of the channel contribute to the binding of TTX and the related divalent guanidinium toxin, saxitoxin (STX), suggesting that the toxin has a large footprint on the external surface of the channel.

Na^+ channel toxins

- μ-conotoxins
  - Natural toxins which bind with high affinity to selected Na^+ channels
  - A class of inhibitory peptide toxins that specifically block the skeletal muscle isoform at a site which overlaps partially with that of TTX/STX.
  - These toxins have backbones which are rendered quite rigid by the three internal disulphide bonds. They are particularly useful probes of the outer vestibule of the Na^+ channel. The toxins are amenable to structural characterization by established physical methods such as X-ray crystallography. They can be grown in bacteria and mutated at specific sites adds considerable versatility.
- Na^+ channel toxins from various species of cone snails have been described.

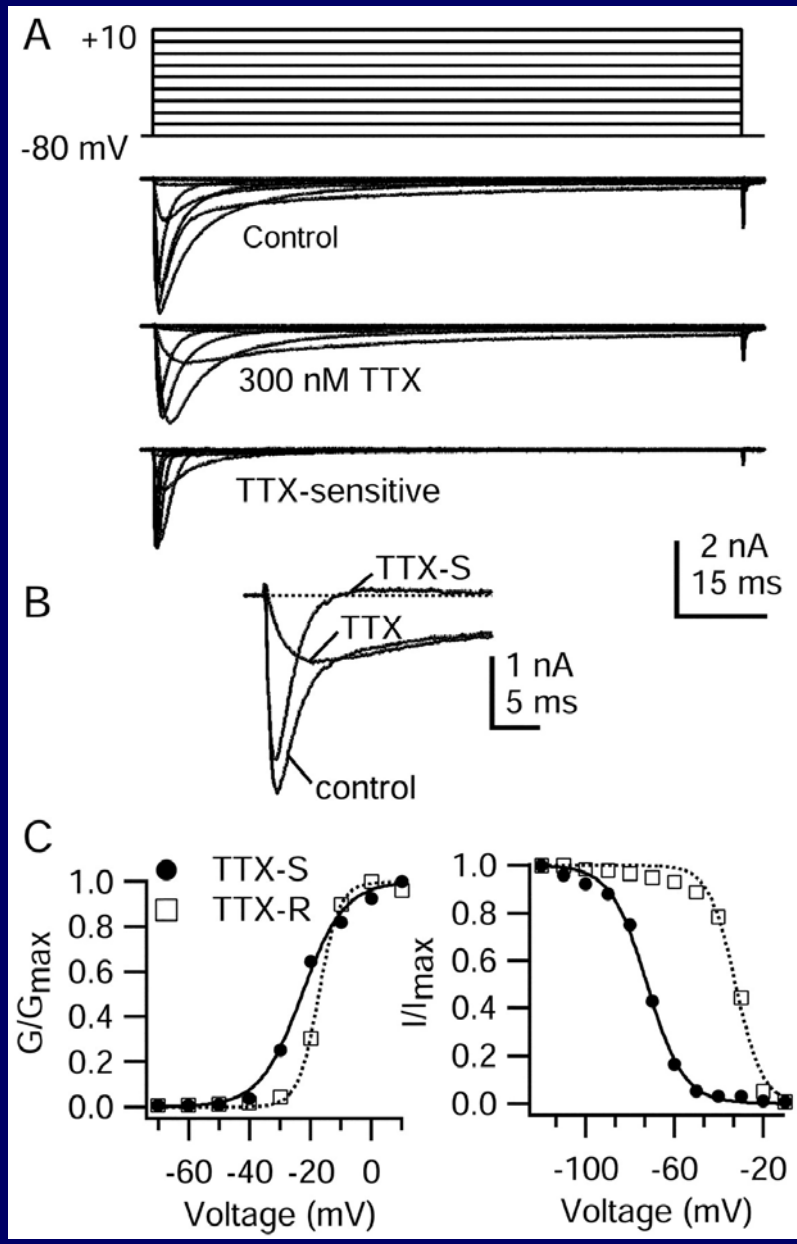
Na^+ channel toxins

- **Sea anemone** (e.g. anthopleurin A and B, ATX II) and **scorpion toxins** inhibit Na^+ channel inactivation by binding to sites that include the S3-S4 extracellular loop of domain IV.
  - These toxins slow current decay by binding to an external site remote from the III-IV linker highlights the importance of disparate regions of the channel in inactivation gating.
- **Other factors** contribute to the functional diversity of Na^+ channels
  - Variable expression of tissue-specific α subunits
  - Differential susceptibility to phosphorylation
  - Glycosylation
  - Presence or absence of ancillary $\beta 1$ and $\beta 2$ subunits

TTX resistant currents

- Most of the neuronal channels are sensitive to nanomolar concentrations of TTX (TTX-S), while the cardiac channel Nav1.5 and the sensory neuron-specific channels Nav1.8 and Nav1.9 are resistant to 100–1000 fold higher concentrations of TTX (TTX-R).
- The peripheral sodium channels Nav1.7, Nav1.8 and Nav1.9 channels produce sodium currents with distinct biophysical properties which enable them to make specific contributions to the electrogenic properties of neurons under normal and pathogenic conditions.

Blair & Bean, 2002, J Neurosci. 22: 10277



Small molecule blockers

- A significant focus on research and development of isoform-specific small molecule blockers or natural toxins that differentially block Nav1.7 and Nav1.8 has begun to yield drug candidates, at least in animal models.
- An alternative strategy is to differentially target nociceptor neurons with existing cell membrane impermeable anesthetic derivatives.

Transcription

- Control of excitability can occur at the genomic level by the regulation of transcription of channel genes. The expression of Na^+ channels is developmentally regulated and tissue restricted.
  - Patterns of electrical activity can also feed back upon and influence transcription: for example, seizures alter Na^+ channel gene expression in the brain.
  - Denervation induces the expression of the cardiac isoform of the channel in skeletal muscle, while transiently suppressing expression of the mature skeletal muscle isoform.
  - Chronic exposure to antiarrhythmic drugs which block Na^+ channels can increase the steady-state levels of Na^+ channel mRNA, in a manner that would tend to counteract the effects of channel blockade.

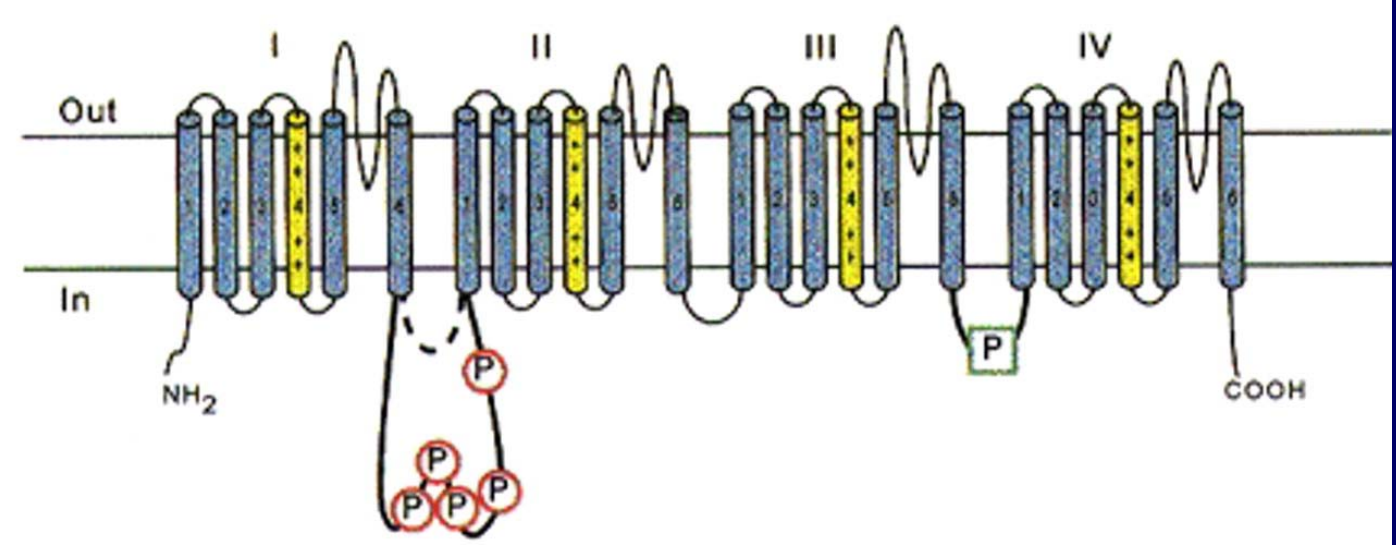
Transcription silencer: REST

The mechanisms controlling Na^+ channel gene expression are only just beginning to be understood.

- Expression of the brain type II Na^+ channel is restricted to neurons by a transcription silencer known as REST.
- REST is a transcription factor with C_2H_2 zinc finger motifs homologous to the Drosophila repressor Krüppel that binds to a specific silencer element (RE-1) in the promoter of the brain II channel.
- REST is found in most tissues; its absence in neurons is what permits expression of the brain II isoform.

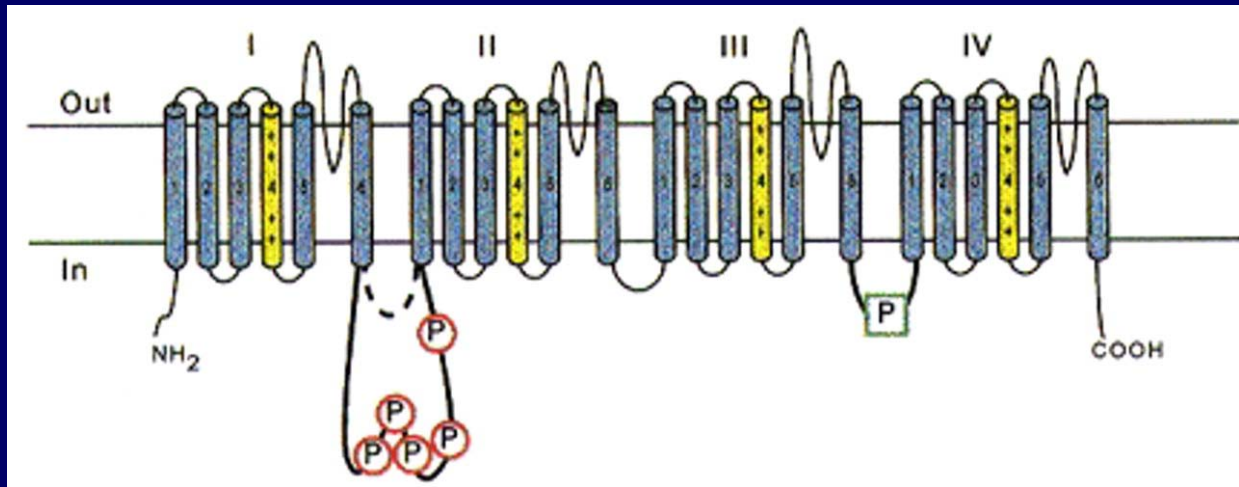
Phosphorylation

- Regulation of the Na^+ channel by phosphorylation is complex. Isoforms of the Na^+ channel α subunit fall into one of two groups, long (neuronal and cardiac) and short (skeletal muscle and eel).
- Phosphorylation of sites in the I-II linker of the brain channel reduces current amplitude without significantly affecting gating.



Phosphorylation-PKA

- The neuronal isoforms have a substantially larger intracellular linker between domains I and II, which contains five consensus sites for cyclic AMP-dependent protein kinase (PKA) phosphorylation. PKA modulates the function of expressed neuronal and cardiac Na^+ channels.
- The cardiac channel has eight candidate consensus PKA phosphorylation sites of the form KRXXS, RXXS or RXS in the I-II linker, all of which are distinct from the neuronal channels. In vitro studies of the expressed cardiac channel demonstrate cyclic AMP-dependent phosphorylation on only two of these serines.

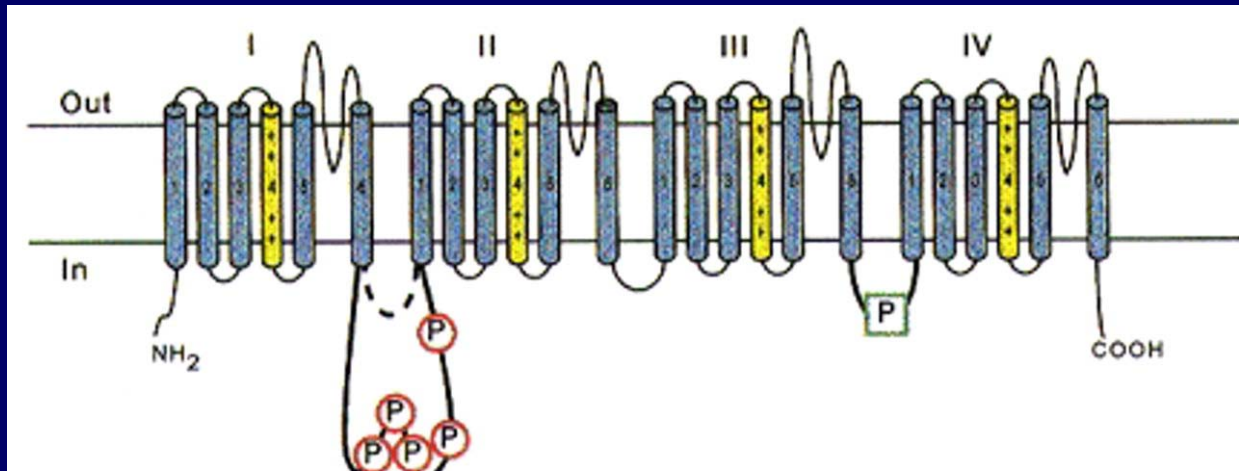


Phosphorylation-PKA

- When the cardiac channel is phosphorylated by PKA, the whole-cell conductance increases, suggesting the specific pattern of phosphorylation is responsible for the functional effect.
- The function of the skeletal muscle isoform of the Na^+ channel is not affected by PKA, despite the fact that this channel is an excellent substrate for PKA-mediated phosphorylation.
- The importance of Na^+ channel phosphorylation is not always clear, and caution should be exercised when attempting to relate phenomena in heterologous expression systems to more physiological settings (and vice versa).

Phosphorylation-PKC

- Protein kinase C (PKC) alters the function of all of the mammalian Na^+ channel isoforms.
- The PKC effect is largely attributable to phosphorylation of a highly conserved serine in the III-IV linker.
- PKC reduces the maximal conductance of the channels and alters gating in an isoform-specific fashion. The macroscopic current decay of neuronal channels is uniformly slowed by PKC.



Phosphorylation

- Disparate effects have been described in skeletal muscle and cardiac channels including acceleration of the current decay and a hyperpolarizing shift in the steady-state availability curve.
- The skeletal muscle Na^+ channel is selectively phosphorylated by human myotonin protein kinase (HMPK).
- Absent or reduced phosphorylation of skeletal muscle Na^+ channels by mutant HMPK may underlie the altered excitability of muscle in myotonic dystrophy, but, curiously, the genetic alterations do not appear to alter kinase function.

Glycosylation

- All of the subunits of the Na^+ channel are modified by glycosylation.
- The $\beta 1$, $\beta 2$ and brain and muscle α subunits are heavily glycosylated, with up to 40 % (eel electroplax α subunit) of the mass being carbohydrate. In contrast, the cardiac α subunit is only 5 % sugar by weight.
- Sialic acid is a prominent component of the N-linked carbohydrate of the Na^+ channel. The addition of such a highly charged carbohydrate has predictable effects on the voltage dependence of gating through alteration of the surface charge of the channel protein.
- Neuraminidase treatment to remove sialic acid from expressed skeletal muscle channels produces a depolarizing shift of steady-state inactivation. Local surface charge is also importantly influenced by charged amino acid residues which stud the outer mouth of the pore, although the predominant effects in this case are on permeation rather than gating.

Glycosylation

- Co-translational glycosylation is essential for the maintenance of cell surface expression of the Na^+ channel in neurons and Schwann cells.
- Inhibition of glycosylation by tunicamycin reversibly decreases the number of STX binding sites on neuroblastoma cells. Tunicamycin also inhibits palmitation, sulphation and disulphide attachment of the $\beta 2$ subunit, preventing the assembly of functional Na^+ channels.

Function

- Generate current to overcome membrane capacitance & resistance
- Generate (Upstroke) & propagate self-regenerating action potential
  - in excitable cells in response to membrane depolarization. In a simplified scheme, sodium channels have these distinct states: resting (closed), activated (open), inactivated (closed) which itself exists as fast-inactivated (within milliseconds) and slow-inactivated (seconds), and recovering from inactivation (repriming) which is a period in which the channel is not available to open in response to a depolarization.

Inactivation

- Inactivation turns out to be a much more arcane process than originally envisioned by Hodgkin and Huxley. Not only is there loose coupling to activation, but there are also multiple inactivation processes.
- These are distinguishable by their recovery kinetics at strongly negative potentials: repriming from the traditional 'fast' inactivation occurs over tens of milliseconds, while recovery from 'slow' inactivation can require tens of seconds or longer.
- Fast inactivation is at least partly mediated by the cytoplasmic linker between domains III and IV (the crucial residues are labelled IFM), which may function as a hinged lid docking onto a receptor formed by the S4-S5 linkers of domains III and IV.

Inactivation

- This notion fits nicely with venerable observations that fast inactivation can be disrupted by internal proteases.
- It is increasingly clear that mutations scattered widely throughout the channel affect inactivation gating, undermining somewhat the primacy of the III-IV linker.
- The structural determinants of slow inactivation are even less localized than those of fast inactivation.
- Mutations in the P region of domain I affect both activation gating and slow inactivation, while various widely scattered disease mutations as identified in paramyotonia congenita and other skeletal myopathies suppress slow inactivation.

Inactivation

- Inactivation is a fundamental property of sodium channels that is crucially important.
- There are many different types of inactivation, including fast, slow and ultra-slow inactivation.
- Each of these can be modulated by cellular factors or accessory subunits.
- Subtle defects in either inactivation process can lead to debilitating human diseases, including periodic paralyses in muscle, ventricular fibrillation and long QT syndrome (delayed cardiac repolarization) in the heart, and epilepsy in the CNS.

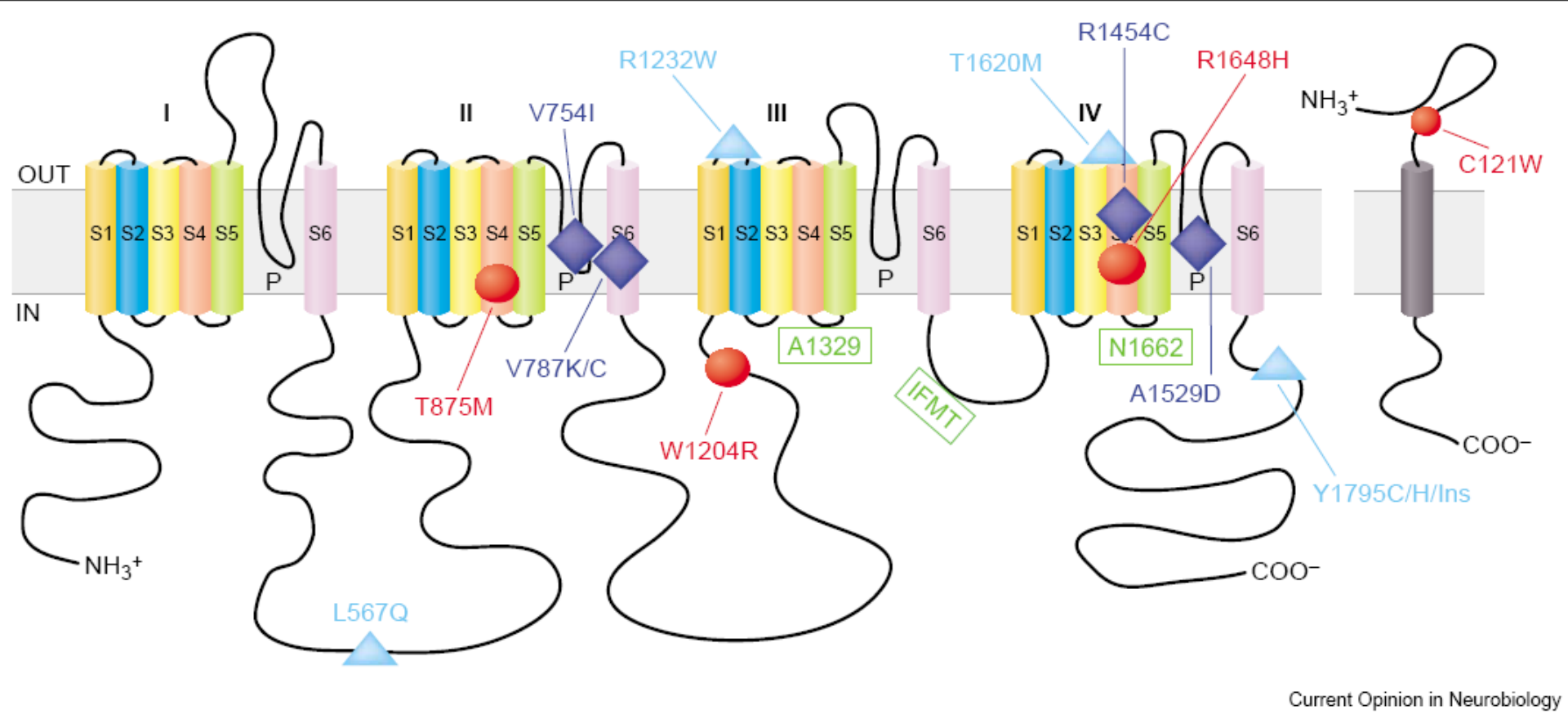
Fast inactivation

- Rapid inactivation of sodium channels is crucial for the normal electrical activity of excitable cells.
- Fast inactivation in voltage-gated sodium channels occurs by a 'ball-and-chain' or 'hinged lid' mechanism, in which a cytoplasmic region (the inactivating particle) occludes the pore by binding to a region nearby (the docking site). It occurs for a period of time in the range of milliseconds.
- The inactivating particle consists of a portion of the cytoplasmic linker connecting domains III and IV, with the crucial region centering on a four amino acid stretch consisting of isoleucine, phenylalanine, methionine and threonine (IFMT).
- The docking site consists of multiple regions including the cytoplasmic linkers connecting segments 4 and 5 (S4-S5) in domains III and IV and the cytoplasmic end of the S6 segment in domain IV.

Slow inactivation

- Slow inactivation, which occurs when the membrane is depolarized for hundreds of milli seconds and is sustained for a time period in the range of seconds, is a separate process that does not involve the III-IV linker inactivation particle.
- One hypothesis to explain slow inactivation is that it results from a structural rearrangement of the pore, similar to the mechanism for C-type inactivation in potassium channels.
  - Ong et al. (2000) demonstrated that long depolarizations that resulted in slow inactivation decreased the accessibility of an engineered cysteine residue in the pore region of domain III.
  - Struyk and Cannon (2002) observed no changes in the modification rates of engineered cysteine residues in the pore regions of the four domains after slow inactivation, indicating that the cysteines were equally accessible to the modification reagent before and after slow inactivation.
- The mouth of the pore does not close during slow inactivation.

- Many local anesthetic, anti-arrhythmic and anti-convulsant drugs modulate sodium channel inactivation, it is likely that a better understanding of these processes will make it possible to identify or design drugs that are more specific and effective in treating disorders of sodium channel abnormalities.



- The green rectangles indicate the amino acids important for fast inactivation, specifically the crucial IFMT motif in the inactivating particle and the docking site residues A1329 and N1662.
- The dark blue diamonds represent the mutations in Nav1.4 that affect slow inactivation (V754I, V787K/C, R1454C and A1529D).
- The light blue triangles indicate the mutations in Nav1.5 that cause long QT or Brugada syndrome (L567Q, R1232W and Y1795C/H/ insertion).
- The red circles represent the mutations that cause GEFSþ (C121W in the in the β1 subunit and T875M, W1204R and R1648H in the Nav1.1 α subunit).

Clinical effects of abnormal inactivation

- Sodium channel mutations cause human diseases of skeletal muscle, cardiac muscle and the CNS, and most of these mutations alter some aspect of channel inactivation.
- Mutations in Nav1.4 cause periodic paralysis, paramyotonia congenita and the potassium-aggravated myotonias, all of which involve delayed muscle relaxation.
- Mutations in Nav1.5 cause long QT type-3, which predisposes to ventricular tachycardia (torsades de pointes), and Brugada syndrome, which is manifested as ventricular fibrillation.
  - Long QT syndrome results from mutations that cause a gain of sodium channel function, whereas Brugada syndrome mutations reduce sodium channel function. Although generally correct, this hypothesis cannot explain the effects of all of the cardiac sodium channel mutations. For example, idiopathic ventricular fibrillation can be caused by either decreased or increased sodium channel inactivation.

Clinical effects of abnormal inactivation

- Mutations in CNS sodium channels cause several types of epilepsy, including generalized epilepsy with febrile seizures plus (GEFSt).
- Destabilization of inactivation (accelerated recovery and slower onset) results from mutations in IIIrd domain S1-S2 (R1232W) and IVth domain S3-S4 (T1620M), whereas acceleration of inactivation results from a mutation in the linker between domains I and II (L567Q).
- Altering a single residue can have opposite effects. Substitution of histidine (H) for Y1795 in the carboxy-terminus accelerates inactivation, whereas substitution of C for Y1795 slows inactivation. The same mutation can even have opposite effects, with insertion of D after position 1795 both increasing sodium channel function by disrupting fast inactivation and decreasing function by augmenting slow inactivation.

Na^+ channelopathies

- Alteration of ion channel function is an important pathophysiological mechanism of various familial diseases of muscle.
- Na^+ channel mutations underlie the aberrant excitability characteristic of some skeletal muscle myotonias and paralysis, as well as the chromosome 3-linked long-QT syndrome, an inherited cardiac arrhythmia. In general, these mutations disable inactivation of the Na^+ channel, producing either repetitive action potential firing (myotonia) or electrical silence (flaccid paralysis) in skeletal muscle.
- A similar defect in the cardiac Na^+ channel produces action potential prolongation and a predisposition to repetitive electrical activity (polymorphic ventricular tachycardia) in the heart.

Sodium channel disorders

Skeletal Muscle

- SCN4A, α-subunit
- Hyperkalemic periodic paralysis
- Hypokalemic periodic paralysis
- Paramyotonia congenita
- Myotonia Fluctuans
- Myotonia Permanens
- Acetzolamide-responsive myotonia
- Malignant hyperthermia
- Myasthenic syndrome
- Monensin overdose: Rhabdomyolysis
- SCN8A (PN4)
- Diseases: motor endplate disease (med) mouse; dmu mouse

Sodium channel disorders

In the CNS

- An alteration in the balance of CNS sodium channel activity can lead to epilepsy.
  - Increased sodium channel activity that results from accelerated recovery from inactivation or a larger persistent current leads to seizures, presumably by causing hyperexcitability.
  - Decreased sodium channel activity resulting from enhanced slow inactivation also causes epilepsy. <= a loss of function from one SCN1A allele.

Sodium channel disorders

In the CNS

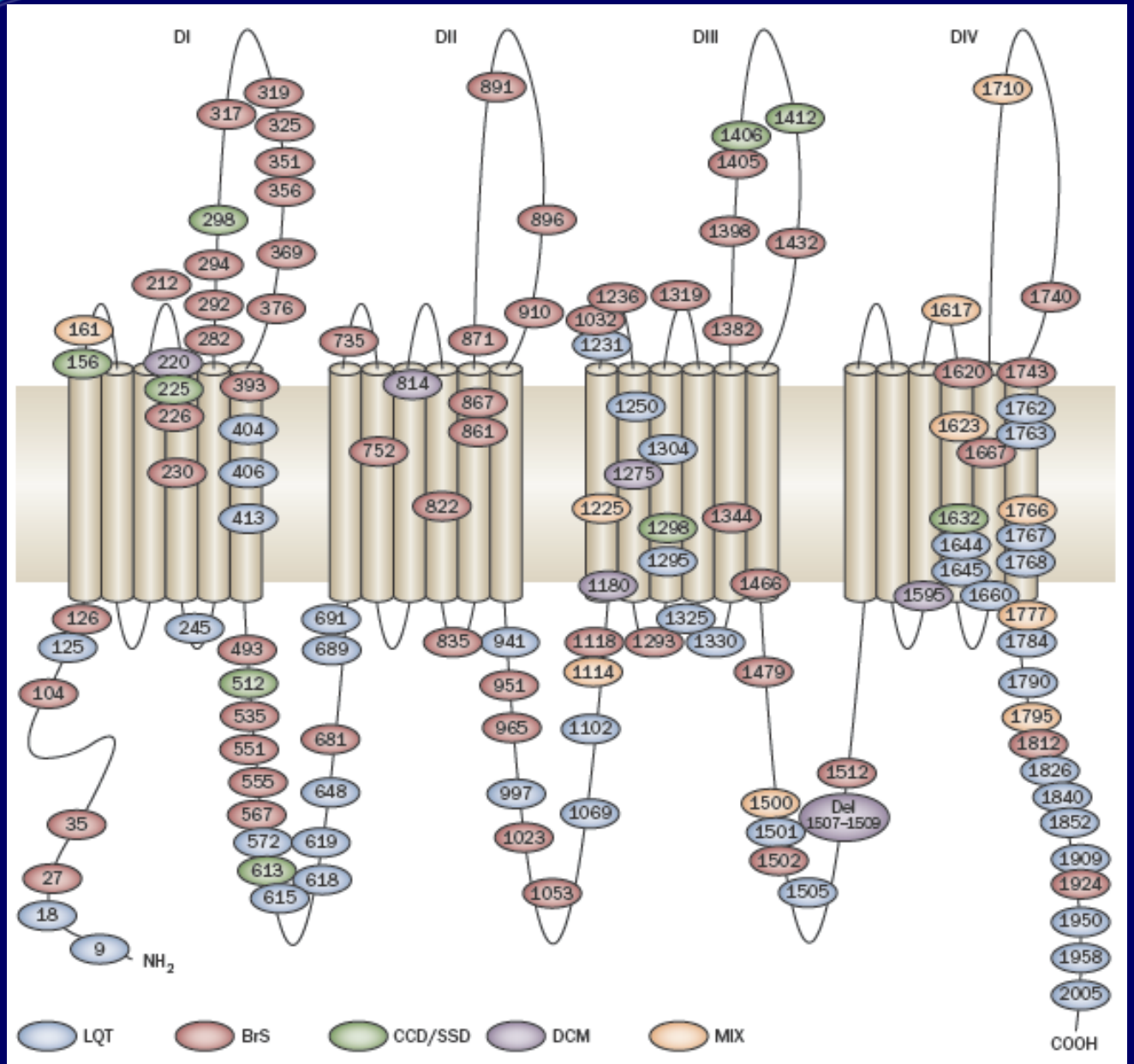
- One mutation that causes GEFSt1 has been identified in the SCN1B gene that encodes the β1 subunit, and 10 mutations that cause GEFSt2 have been identified in the SCN1A gene that encodes the Nav1.1 a subunit (SCN1A).
- Severe myoclonic epilepsy in infancy, the most severe form of GEFSt, results from a haploinsufficiency (functional loss of one allele) of SCN1A, and mutations that cause GEFSt have been identified in the SCN2A gene that encodes the Nav1.2 a subunit.

Sodium channel disorders

Cardiac - α subunit: SCN5A

- Long QT Syndrome (LQT3)
- Idiopathic ventricular fibrillation (IVF)
- Progressive cardiac conduction defect (PCCD2; Lenegre disease)
- Clinical syndrome: Syncope; Right bundle branch block
- Genetics
- DelG5280
- Other locus: Chromosome 19q13.3
- Non-progressive congenital heart block

SCN5A-encoded protein with localization of the mutations and associated phenotypes



- LQT: long QT syndrome
- BrS: Brugada syndrome
- CCD: cardiac conduction defect
- SSD: sick sinus node syndrome
- DCM: dilated cardiomyopathy
- MIX: mixed phenotype

Gain-of-function mutations

- The biophysical changes observed in LQt3 mutations can all be defined as gain-of-function mutations, as they all cause small increases in net inward current over the voltage range and time course of the action potential plateau to result in prolongation of the action potential.
- Gain-of-function mutations disturb the delicate balance between inward and outward current during the plateau phase of the action potential and prolong the repolarization process.
- In the presence of prolonged repolarization, cardiac myocytes have a propensity to develop early after depolarizations that induce torsades de pointes.

Sodium channel disorders

Peripheral nerve

- Localization of voltage-gated Na^+ channels
- RI: Soma
- RII: Axonal initial segment & nodes of Ranvier
- Hereditary
- SCN9A : Familial erythermalgia
- Immune
- Anti-GM1 ganglioside antibodies
- Multifocal Motor Neuropathy
- Acute motor axonal neuropathy
- Guillain-Barré & CIDP
- Nerve injury
- Neuromas: Accumulation of Na+ channels on axons in neuromas
- Nerve transection
- Down regulation:SCN10A; SCN11A
- Up regulation: SCN3A
- Contributes to hyperexcitability (Allodynia; Hyperesthesia)
- Na^+ channel toxins

Sodium channel disorders

Brain & Spinal Cord

- α subunit: SCN1A : Fever associated seizures
- Generalized epilepsy with febrile seizures plus, Type 2 (GEFS+2)
- Mutations
- Missense
- Location: S4 & S5 transmembrane segments
- Mutation action: Disrupts channel inactivation
- Inheritance: Dominant
- Clinical: Mild seizure disorder
- Myoclonic Epilepsy of Infancy: Severe (SMEI)
- Chromosome 2q24; Dominant
- Most mutations
- De novo
- Truncating type
- Disease mechanism: ? Haploinsufficiency
- Clinical

Sodium channel disorders

Brain & Spinal Cord

- Ataxia
- Severe seizures: Onset 2 to 6 months
- Mental retardation
- Infantile spasms
- α subunit: SCN2A1
- Seizure disorders
- Benign familial neonatal-infantile
- Febrile & Afebrile
- Seizure disorder in mice
- Mutation action: Disrupts channel inactivation
- α subunit: SCN8A
- Motor endplate disease (med) in mice
- β subunit: SCN1B
- Generalized epilepsy with febrile seizures + (GEFS+1)
- Mutation action: Disrupts channel inactivation

Sodium channel disorders

- Epithelial, Nonvoltage-gated, α & β subunits
  - SCNN1A & SCNN1B
  - Pseudohypoaldosteronism (Liddle's syndrome; Hereditary hypertension)
- Thyroid
  - Na^+/I^- symporter (SLC5A5) : Congenital hypothyroidism
- Endocrine
  - Sodium-glucose transporter 1 (SLC5A1) : Glucose/galactose malabsorption
- Neoplasms: Voltage gated Na^+ channels
  - Present in small cell lung cancer cell lines
  - Associated with invasion by prostate cancer cells in vitro.

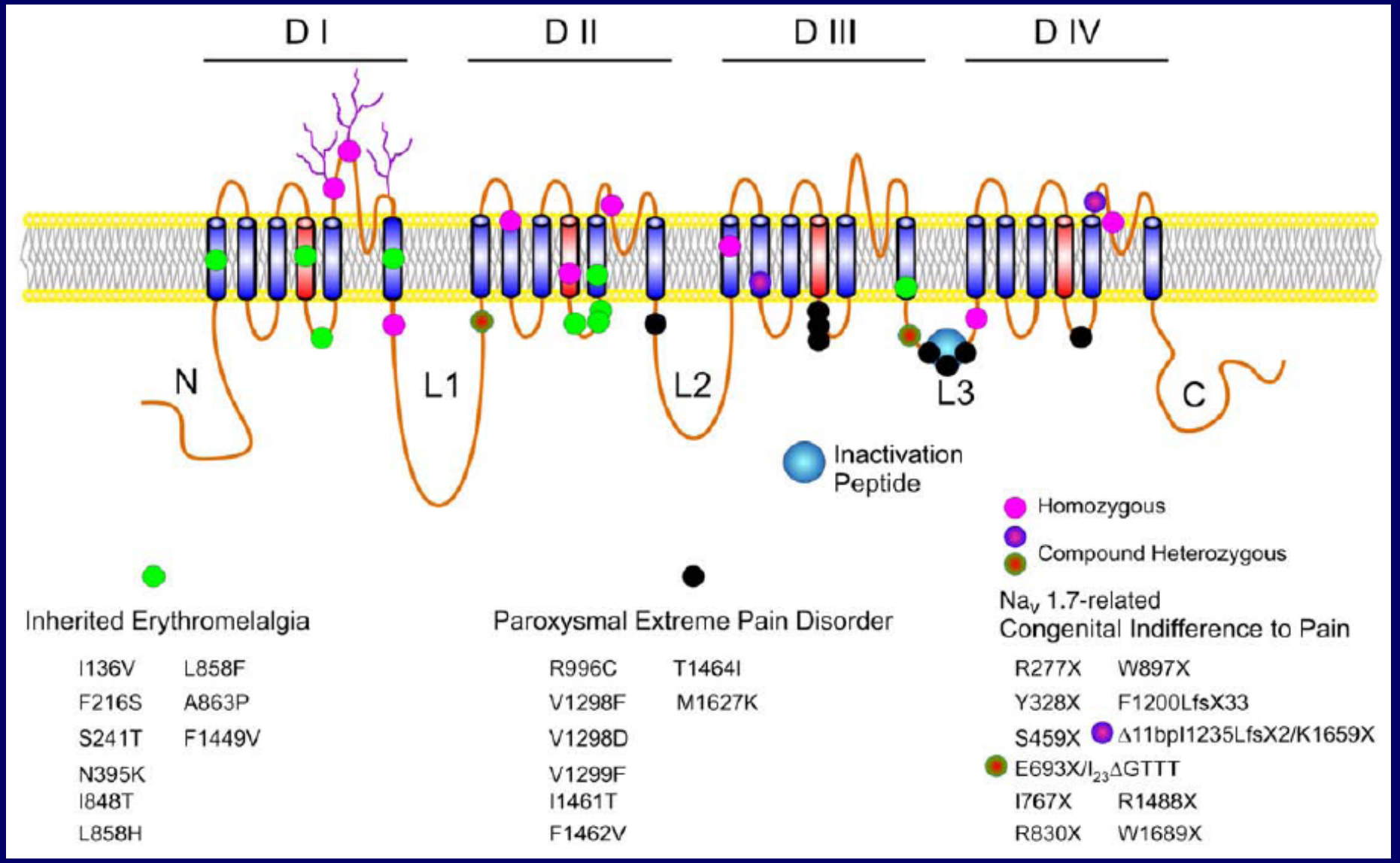
Sodium channels and pain

- Pain is a major unmet medical need which has been causally linked to changes in sodium channel expression, modulation, or mutations that alter channel gating properties or current density in nociceptor neurons.
- There is a link between sodium channel activity and sensory neuron hyperexcitability producing pain. Abnormal increases in sodium conductance can lead to inappropriate, repetitive firing.
- Voltage-gated sodium channels activate (open) then rapidly inactivate in response to a depolarization of the plasma membrane of excitable cells allowing the transient flow of sodium ions thus generating an inward current which underlies the generation and conduction of action potentials (AP) in these cells.
- Activation and inactivation, as well as other gating properties, of sodium channel isoforms have different kinetics and voltage-dependent properties, so that the ensemble of channels that are present determine the electrogenic properties of specific neurons.

Sodium channels and pain

- Biophysical and pharmacological studies have identified the peripheral-specific sodium channels Nav1.7, Nav1.8 and Nav1.9 as particularly important in the pathophysiology of different pain syndromes, and isoform-specific blockers of these channels or targeting their modulators hold the promise of a future effective therapy for treatment of pain.
- Recent studies have begun to elucidate the role of Nav1.7 and Nav1.8 in specific pain syndromes and a better understanding of their role in action potential firing in nociceptive neurons, and their injury-mediated modulation may yield and validate targets for pharmacotherapy.
- Mutations in Nav1.7 have been linked to three pain syndromes
- The presence of Nav1.8 in dorsal root ganglion (DRG) neurons and its absence from sympathetic neurons, for example superior cervical ganglion (SCG), have been linked to hyperexcitability in DRG and hypoexcitability of SCG neurons. Nav1.8 has also been shown to be essential for cold-induced pain.

Locations of the known mutations in Nav1.7-related inherited pain disorders



See you next week!