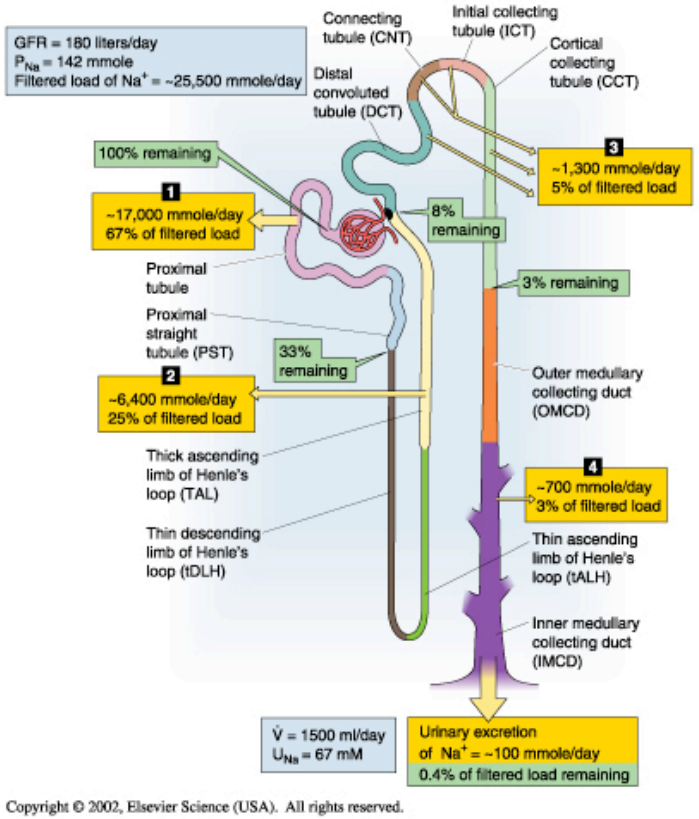
L11: Epithelial Na^+ Channel (ENaC) Structure & Function

Objectives:

- Understand principles of epithelial transport, ENaC-mediated Na^+ absorption and physiological significance of ENaC
- Understand the molecular structure and the general principles of regulation of ENaC
- Can describe the cellular mechanism by which aldosterone upregulates biosynthesis of ENaC
- Can describe proteolytic activation of gating of ENaC
- Can describe physiological significance of proteases in ENaC-mediated Na^+ absorption

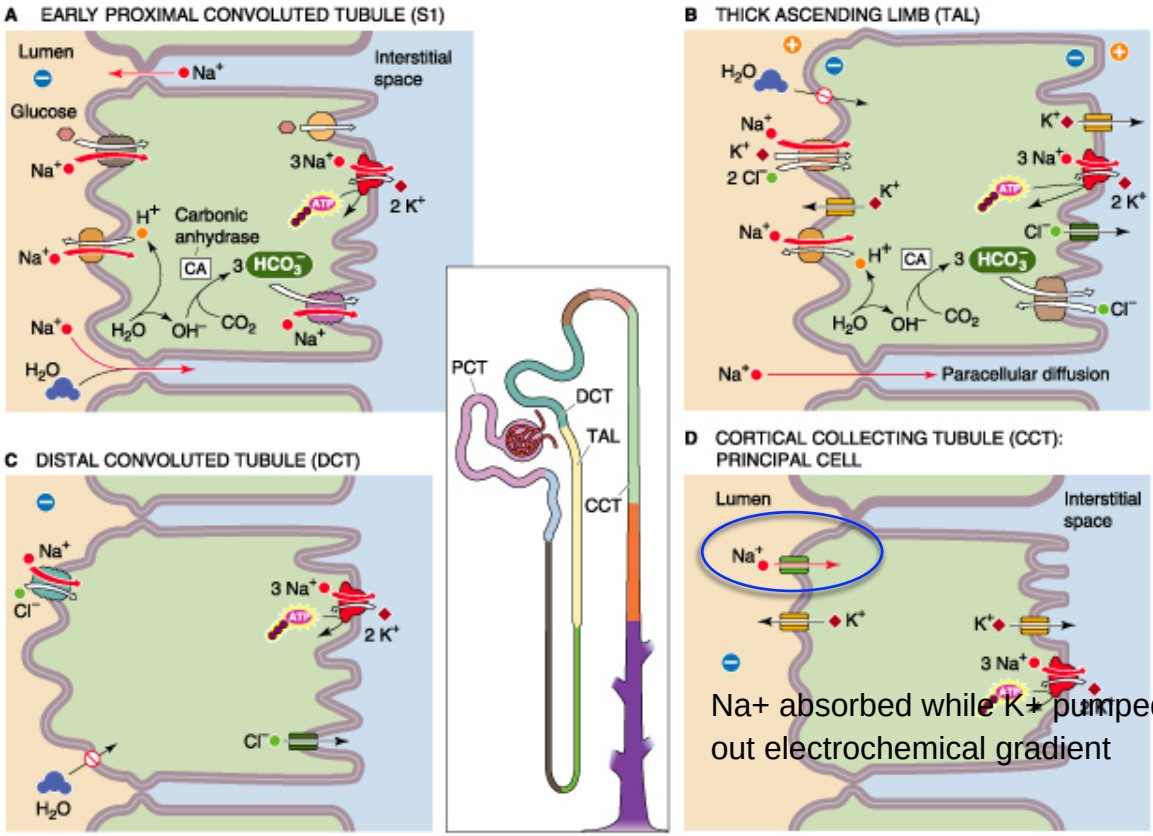
ENaC is very efficient in preserving Na^+



The kidneys absorb 99.6% of filtered Na^+ by the time the urine reaches the renal pelvis.

- ENaC is the last known channel that affects the amount of Na^+ in the urine before the final concentration is established.
- ENaC in the collecting duct can reabsorb from 0 to 100% of Na^+ that enters the distal nephron.
- ENaC is highly regulated.

Epithelial Na^+ channel (ENaC) mediates Na^+ absorption

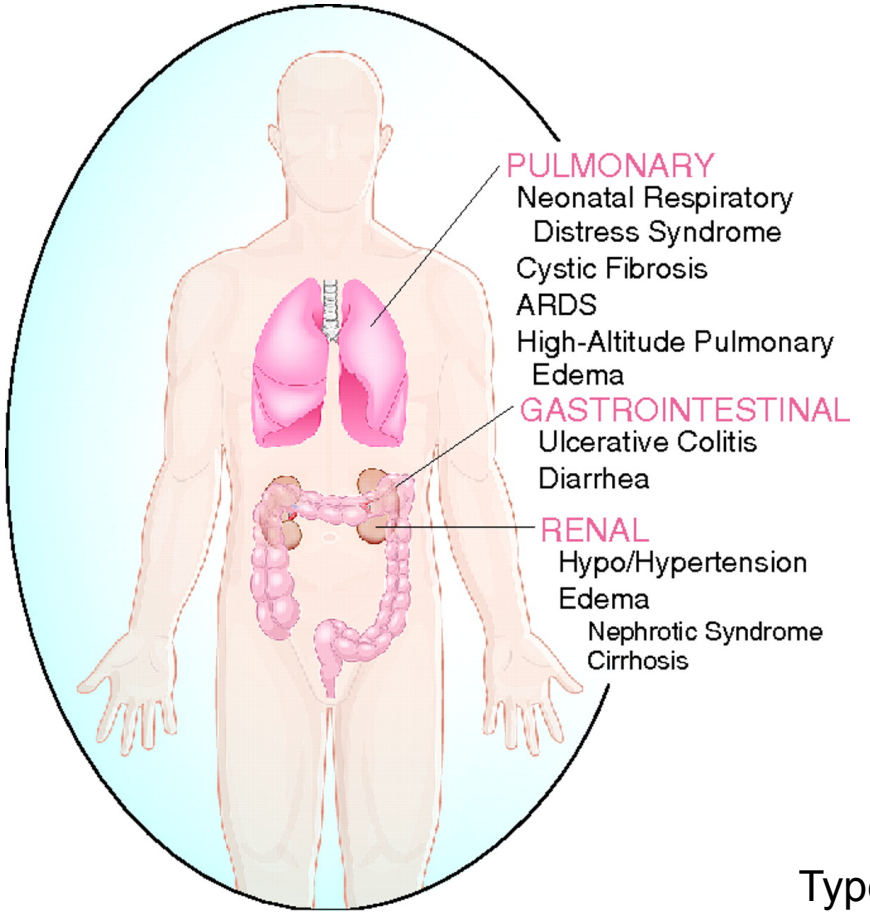


ENaC mediates Na^+ absorption in the distal collecting duct of the kidneys

Na^+ absorbed while K^+ pumped back out to balance out electrochemical gradient

From "Medical Physiology" by Boron & Boulpep

ENaC mediates Na^+ absorption



regulating fluid layer on lung surface - too much ENAc will result in mucous accumulation, making it hard to breathe

↓ Low ENaC activity in the lung of premature infants

↑ Hyperactivity of ENaC in cystic fibrosis lung

in colon to solidify the stool - low ENaC can result in diarrhoea

Type1 Pseudohypoaldosteronism: ↓ ENaC

Liddle's syndrome: ↑ ENaC

Essential Hypertension: ↑ ENaC

Type II diabetes/chronic hyperinsulinemia : ↑ ENaC

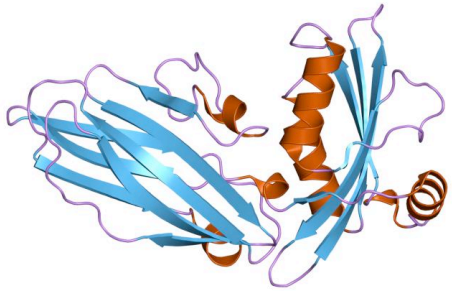
Bhalla V , Hallows K R JASN 2008;19:1845-1854

Physiological roles of ENaC

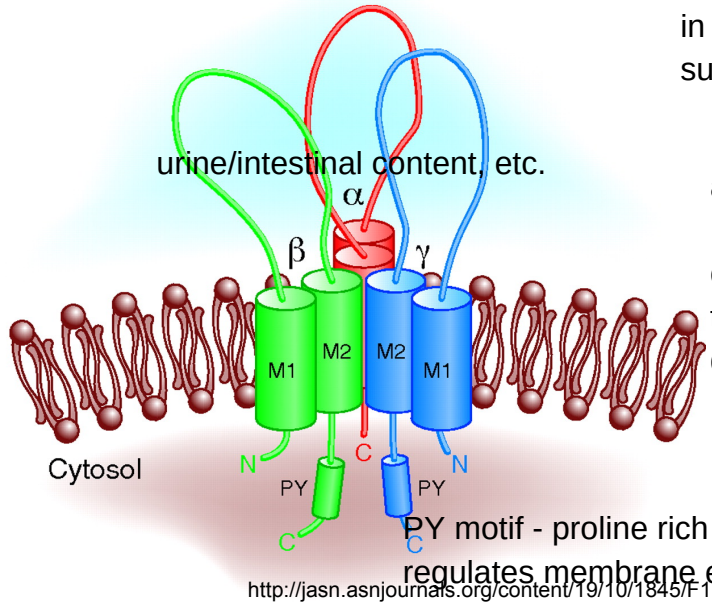
- Kidney: ENaC in the distal nephron is controlled by aldosterone.
  fluid homeostasis on kidney
- Lung: ENaC maintains the composition of the airway surface liquid , contributes to the clearance of fetal lung liquid at birth (respiratory distress syndrome in premature birth) fluid balance in lung, can result in infection if out of balance
- Gastrointestinal tract : Na^+ absorption; salivary duct & distal colon and salt taste. solidify intestinal content
- Skin: ENaC in amphibian skin maintains homeostasis

  Na+ exchange with pond water to blood, amphibians cannot survive without ENaC - many diseases with frogs attack ENaC

Structure: ENaC is composed of 3 homologus subunits



https://en.wikipedia.org/wiki/Epithelial_sodium_channel



http://jasn.asnjournals.org/content/19/10/1845/F1.expansion

in humans we have alpha, beta gamma subunits.

approx 600 amino acids long per ENAc, aroudn 400 are extracellular coil within the membrane to insert into the membrane (transmembrane domains)

PY motif - proline rich motif - in all 3 subunits of ENAc, regulates membrane expression and channel retrieval

ENaC is multimeric - many subunits from separate genes ... subunits are homologous

- ENaC is expressed in the apical membrane of Na^+ absorptive epithelium.
- Subunit composition of active ENaC at the cell membrane: $\alpha_2, \beta_1, \gamma_1$ or $\alpha_3, \beta_3, \gamma_3$???
- αENaC has the ability to generate small Na^+ current (transport Na^+).
- β- and γENaC do not form functional Na^+ channels but greatly potentiate the Na^+ current when co-expressed with αENaC.

for transport, we need 2 alpha, 1 beta, 1 gamma

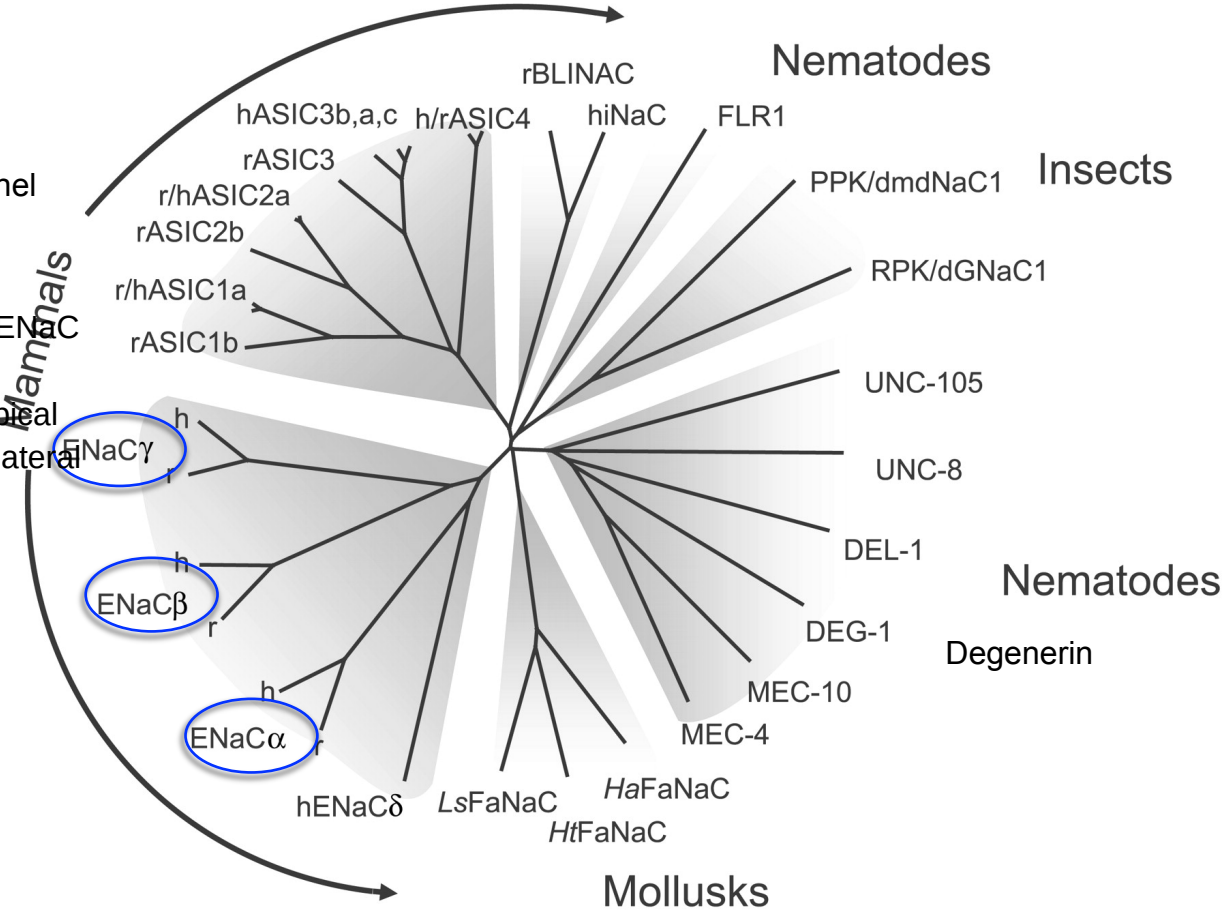
alpha subunit must be important in building transport pathway whilst beta and gamma are important for pore regulation (open and allow passage)- regulatory subunits

alpha subunit by itself can transport, but only <10% activity but gamma and beta subunits have no transport activity at all

ENaC /degenerin family

Acid-Sensing Ion Channel

involved in pain sensation
ENaC alpha, beta, gamma
only eukaryotic cells have ENaC expression...
ENaC always appear on apical membrane, never on basolateral



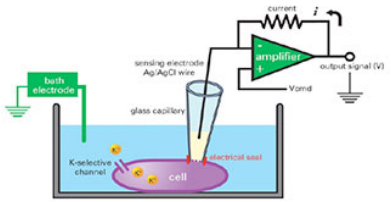
Kellenberger & Schild, Physiological Review (2002) DOI: 10.1152/physrev.00007.2002

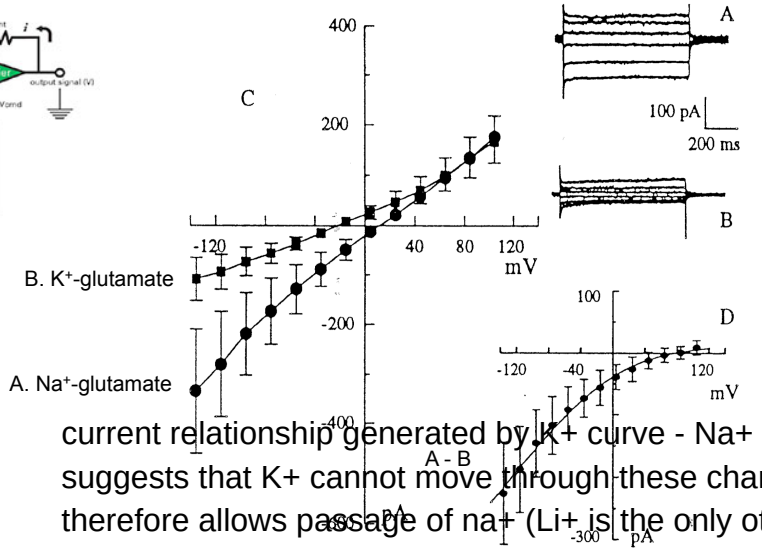
ENaC: Na^+ selectivity, inhibited by amiloride

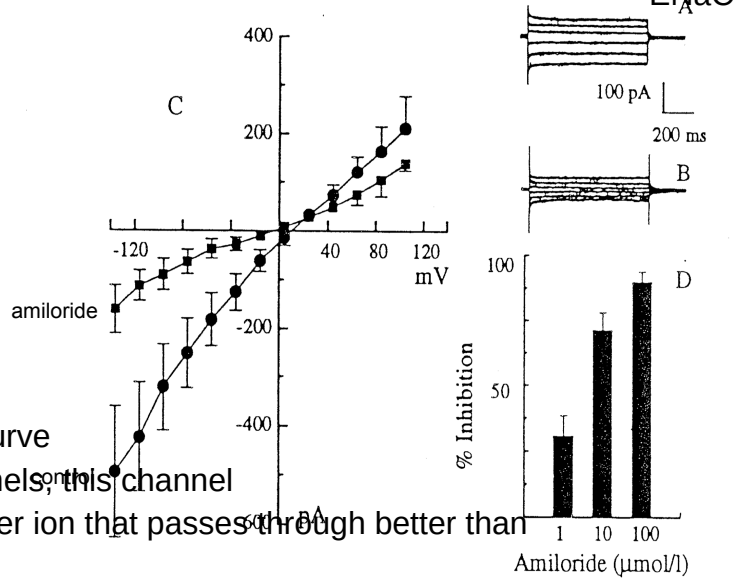
whole-cell patch-clamp
(K-glutamate pipette)

inside of pipette connects to cells, measure activity of ion channels

amiloride is best ENaC blocker





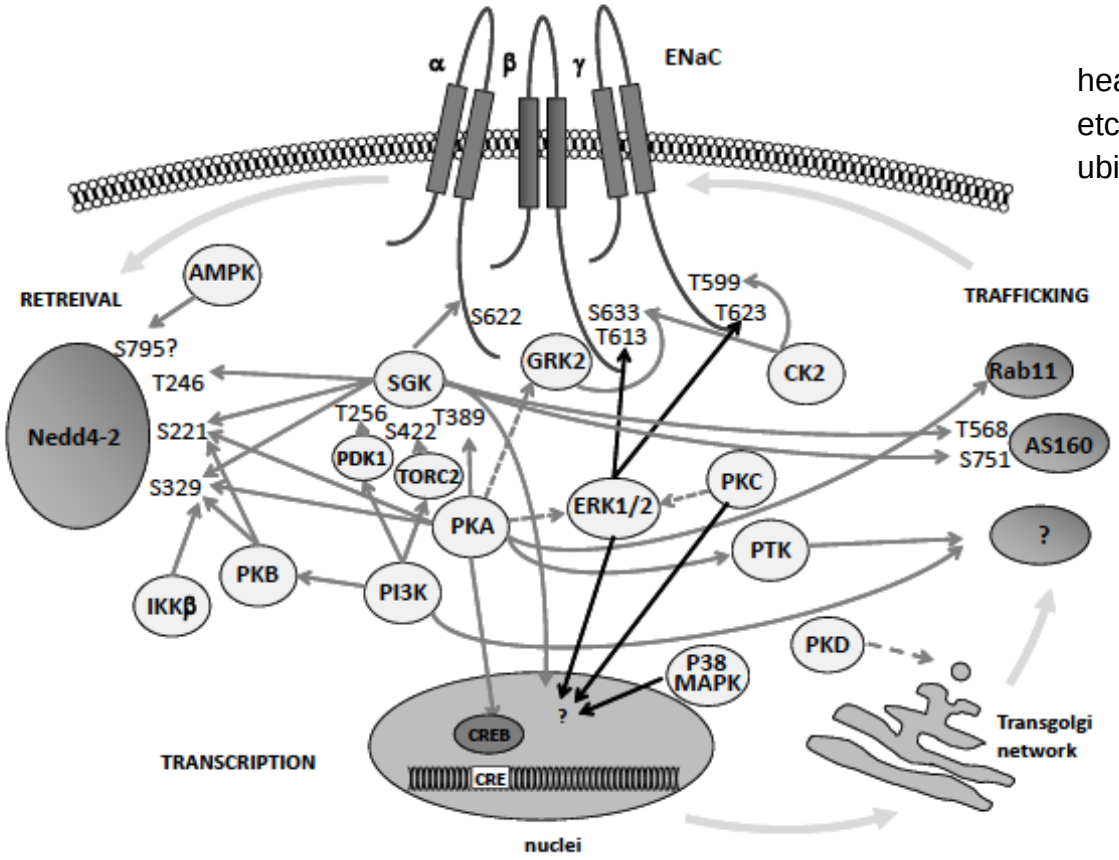


current relationship generated by K+ curve - Na+ curve suggests that K+ cannot move through these channels, this channel therefore allows passage of na+ (Li+ is the only other ion that passes through better than Na)

Dinudom et al (1993) Pfluger Arch, 423: 164-6.

- ENaC is **constitutively active**. It is not sensitive to membrane potential and is activated in the absence of a known stimulus. ENaC opens and closes (gates) with **very low kinetics**
- ENaC is **highly selective for Na^+** over K^+ i.e., K^+ ion does not permeate the channel
- ENaC is **blocked by** the diuretic drugs **amiloride** and its analogs

ENaC is highly regulated

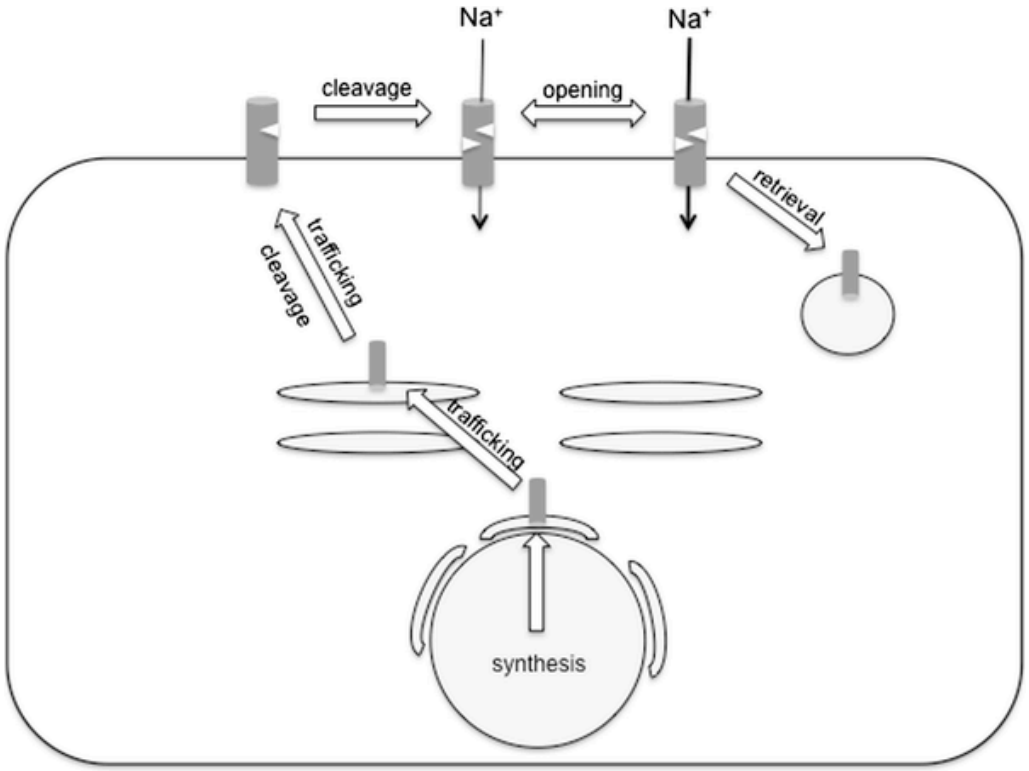


heavily regulated by ERKs, Rap Kinases, etc.

ubiquitin kinase is biggest regulator

ENaC regulation

"Epithelial Na^+ absorption is regulated over a relatively slow time scale of minutes to hours, compatible with mechanisms that alter expression of a protein at the cell surface."



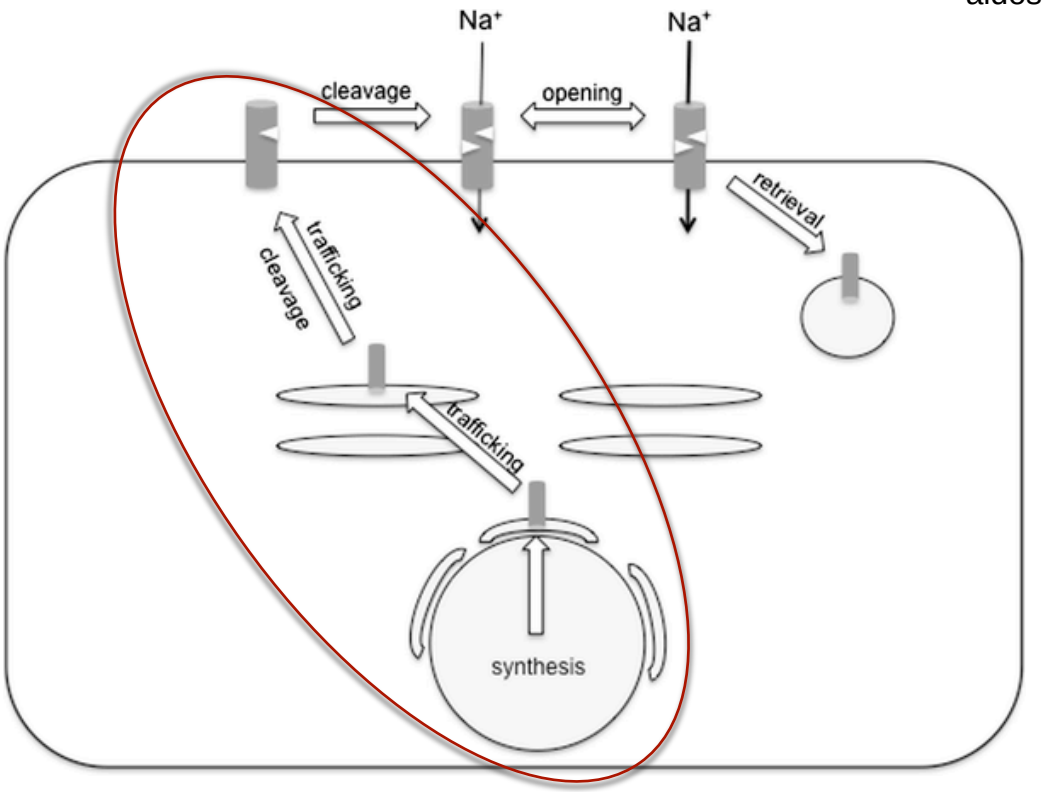
ENaC is regulated a 3 levels:

- Biosynthesis
- Channel gating (open probability)
  ENAc is constitutively active, but rate of open/close is very slow
- Abundance at the cell surface membrane

Palmer et al. (2012) Clin ExP Nephrol. 16: 35-43

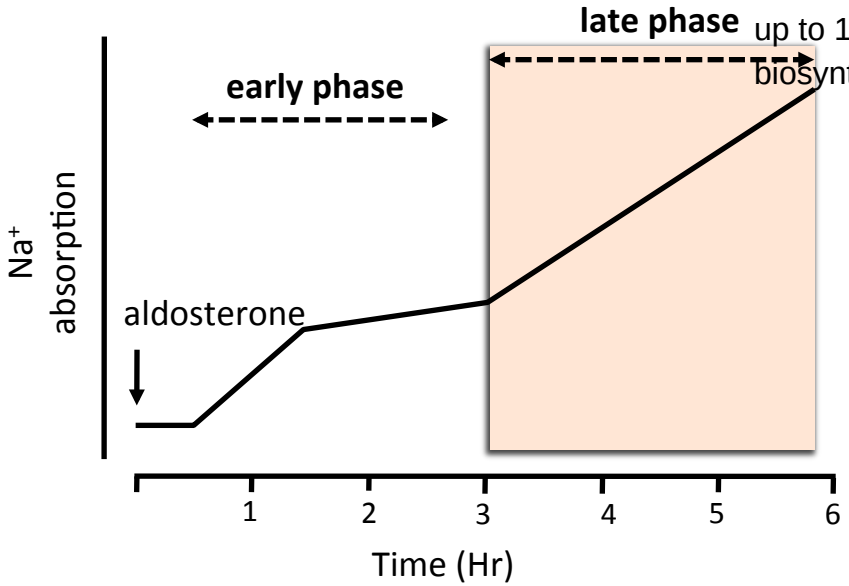
ENaC biosynthesis

within nucleus
aldosterone, insulin regulate ENaC



Aldosterone increases ENaC biosynthesis

Early Phase:
Increases in Na^+ absorption in response to aldosterone begins within 30 min following exposure of the target tissue to the hormone. This early phase DOES NOT result from increased ENaC transcription but is due to the the effect of aldosterone on trafficking and function of ENaC.



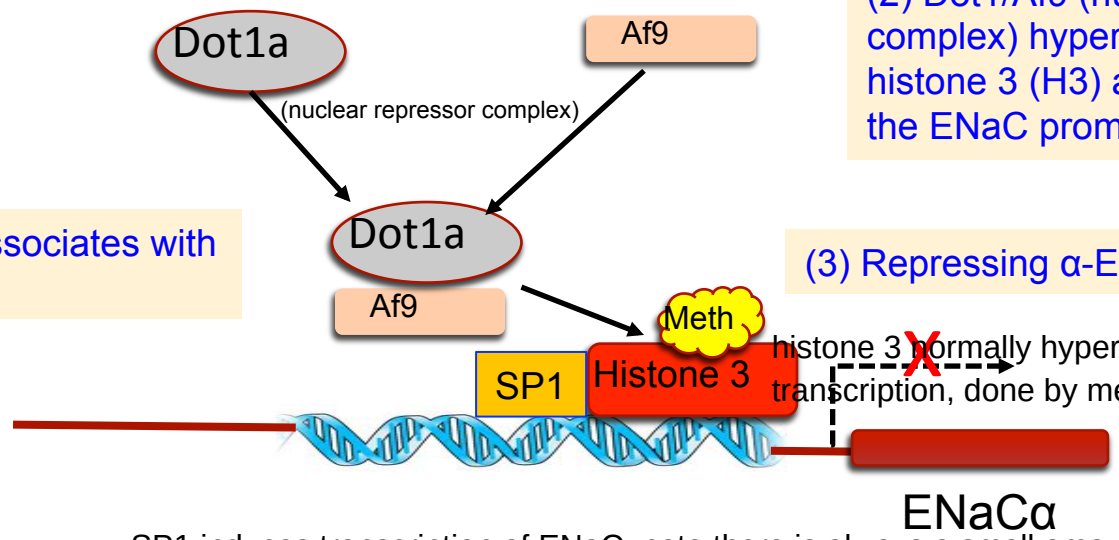
Late Phase:
The effect of aldosterone on BIOSYNTHESIS **of both ENaC and the Na^+/K^+-ATPase** is responsible for the gradual elevation of Na^+ absorption that occurs 3-6 h after exposure to the hormone.

α-ENaC transcription is repressed by Dot1/Af9

(methyltransferase **D**isruptor **O**f **T**elomeric silencing-1)

(**A**LL1-**f**used gene from chromosome **9)**

(2) Dot1/Af9 (nuclear repressor complex) hypermethylates histone 3 (H3) associated with the ENaC promoter



(1) Dot1/Af9 complex associates with chromatin

(3) Repressing α-ENaC transcription

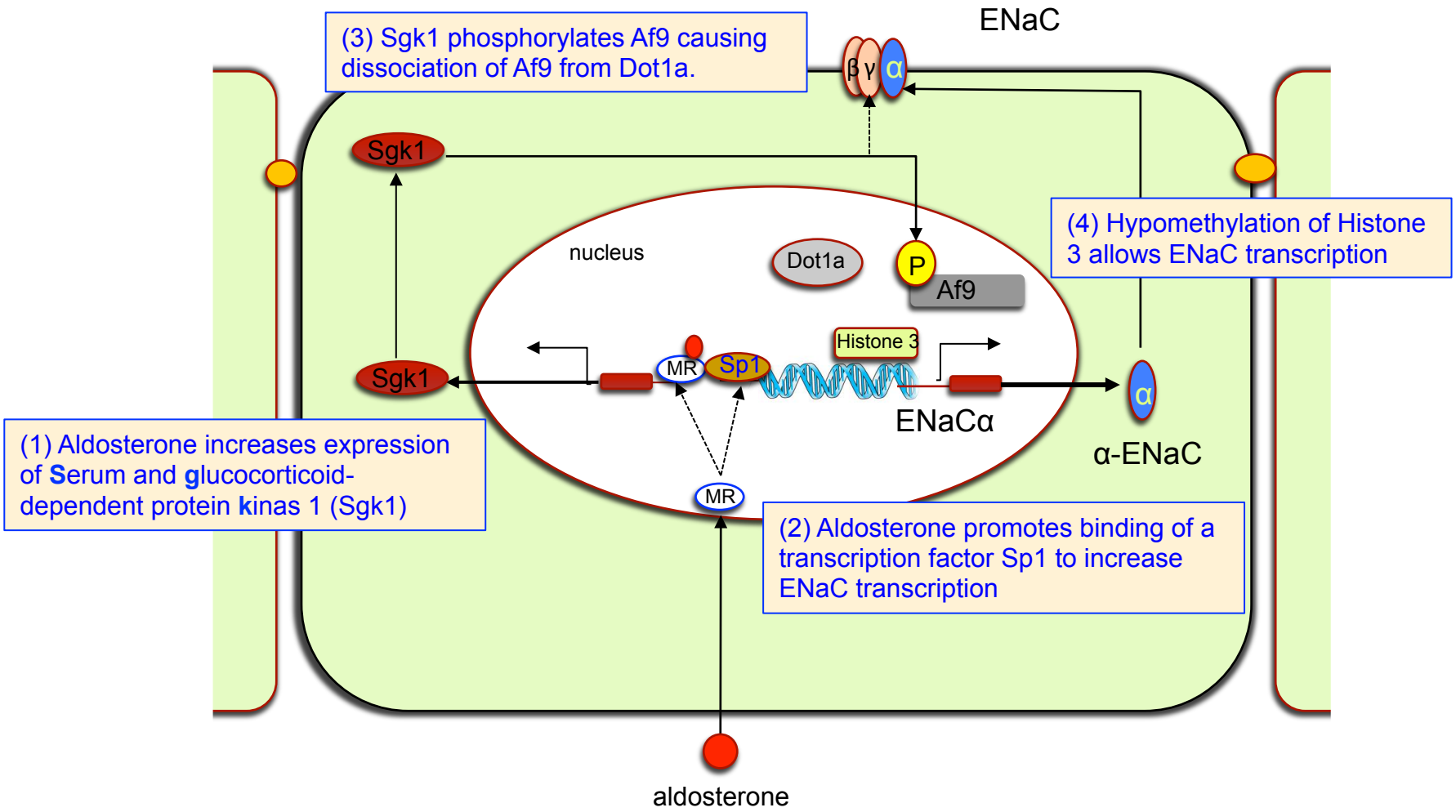
histone 3 normally hypermethylated - does not allow transcription, done by methltrnsfrase1

SP1 induces transcription of ENaC, note there is always a small amoutn of ENaC at any time

Zhang et al (2007) J. Clin. Inves. 117: 773-783

Dot1a + Af9 = methylation of histone 3 (keep it hypermethylated) = prevents gene transcription

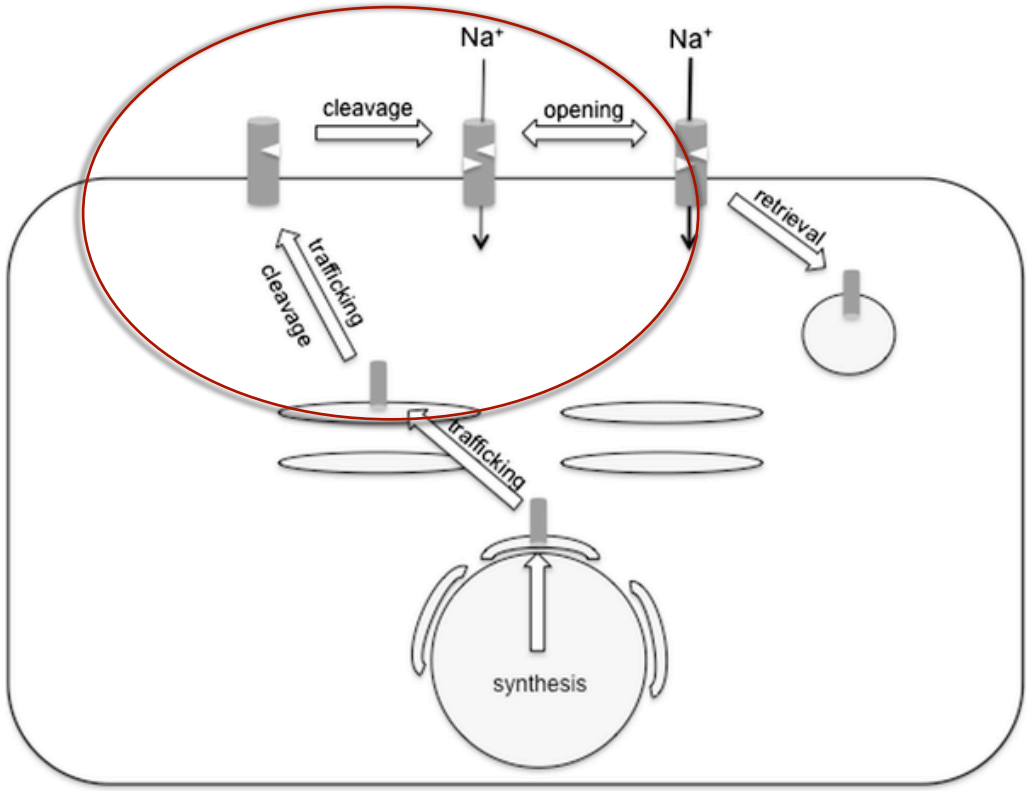
Aldosterone regulates transcription of ENaC gene



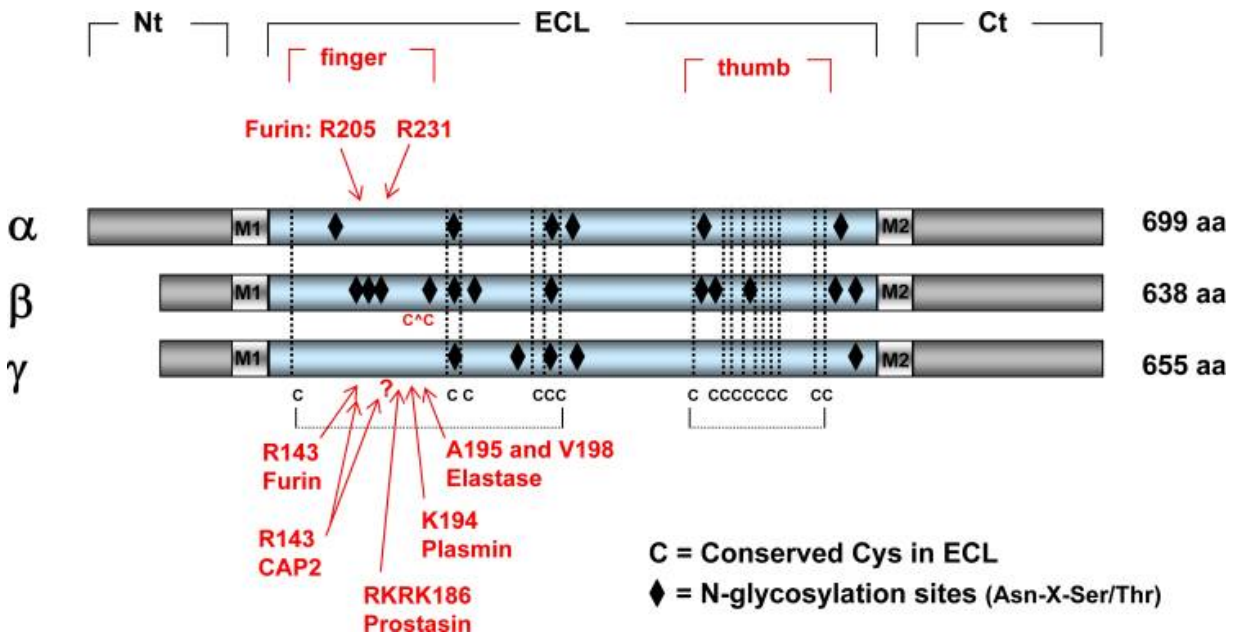
Perce & Kleyman (2007) J Clin. Invest. 117:592-595

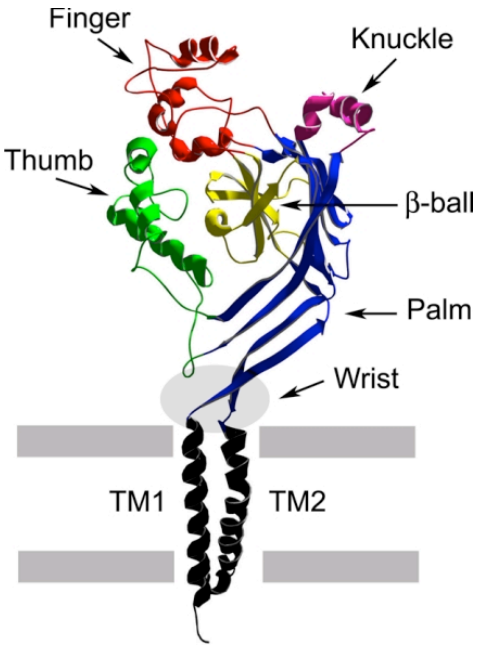
Regulation of ENaC gating

- Proteolysis

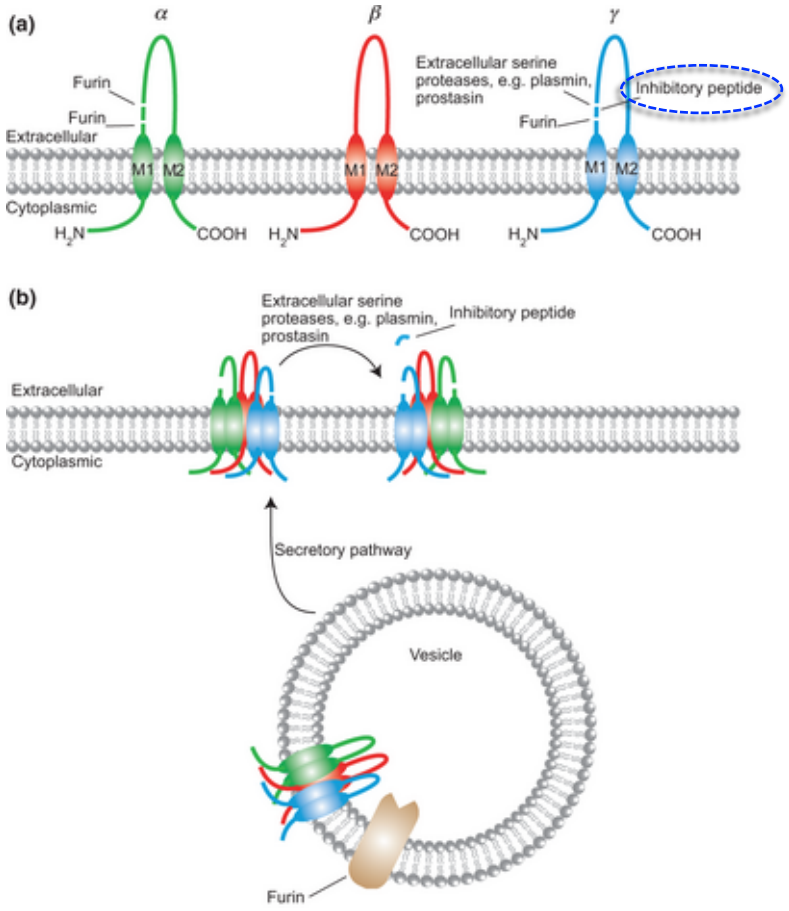


Cleavage sites in α- and γ-ENaC



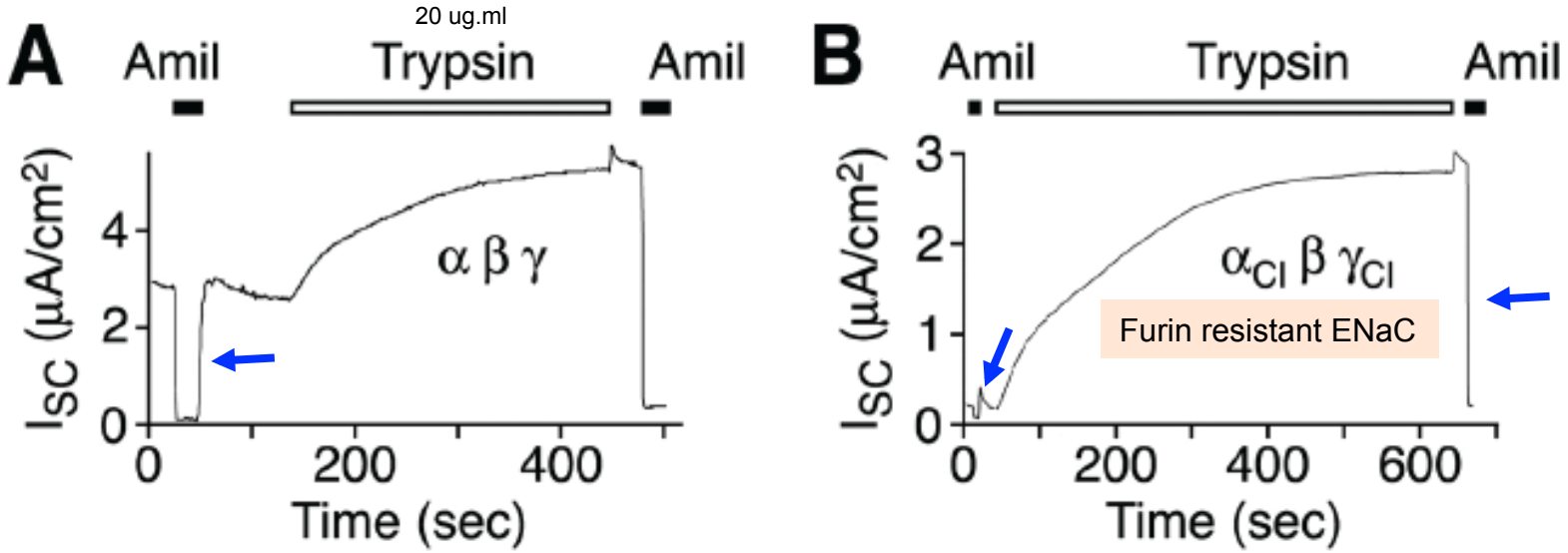


Proteases release inhibitory peptides from ENaC



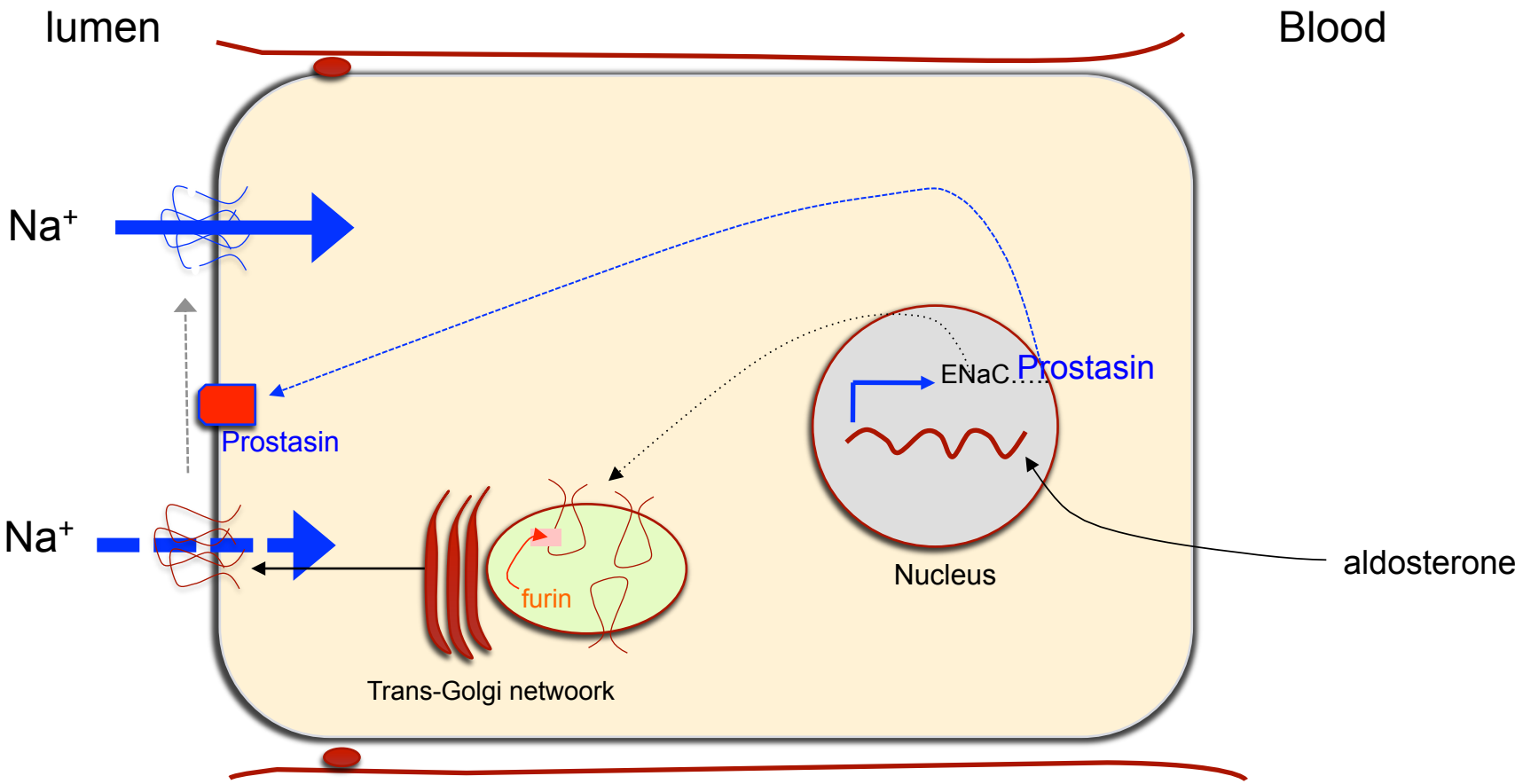
Sevenningsen P., Acta Physiol (2013), 207: 536-45.

Intracellular furin protease activates ENaC

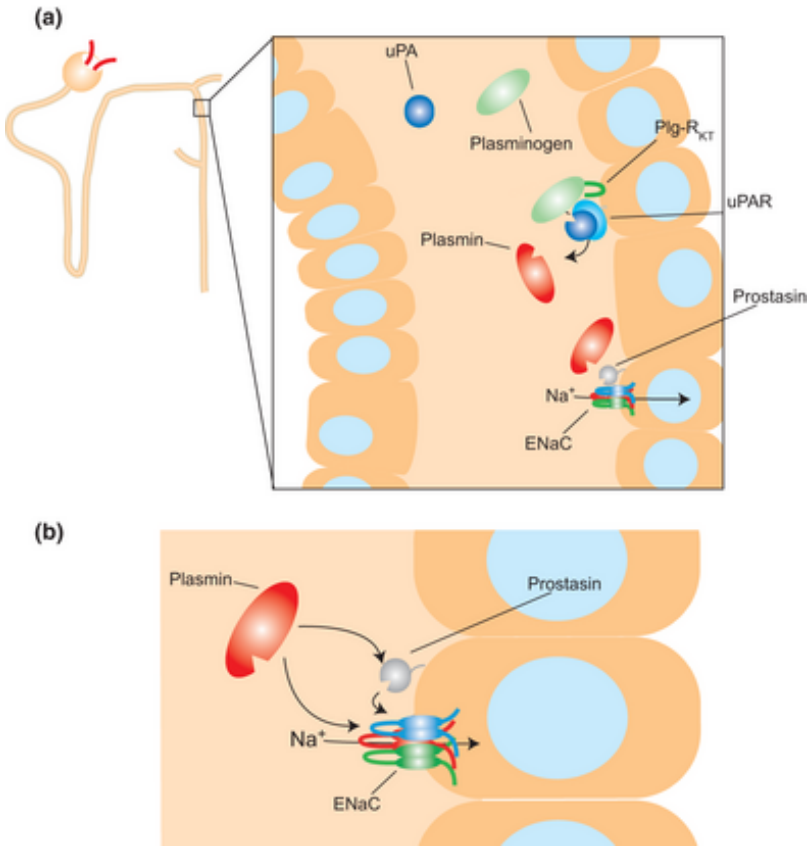


Kabra R et al. J. Biol. Chem. 2008;283:6033-6039

Aldosterone increases proteolysis of ENaC



Proteinuria: plasmin increases proteolysis of ENaC



Proteinuria: Pre-eclampsia, nephrotic syndrome

- oedema
- hypertension
- low plasma aldosterone

- NaCl retention due to high ENaC activity.
- **Minimum change in ENaC abundance**.
- **High plasminogen in urine**.

Plasminogen is activated to plasmin

- cleaves ENaC
- activates prostasin

uPA = urokinase-type plasminogen activator
uPAR = urokinase-type plasminogen activator receptor
Plg-RKT = plasminogen receptor

Sevenningsen P., Acta Physiol (2013), 207: 536-45.

Regulation of ENaC membrane expression