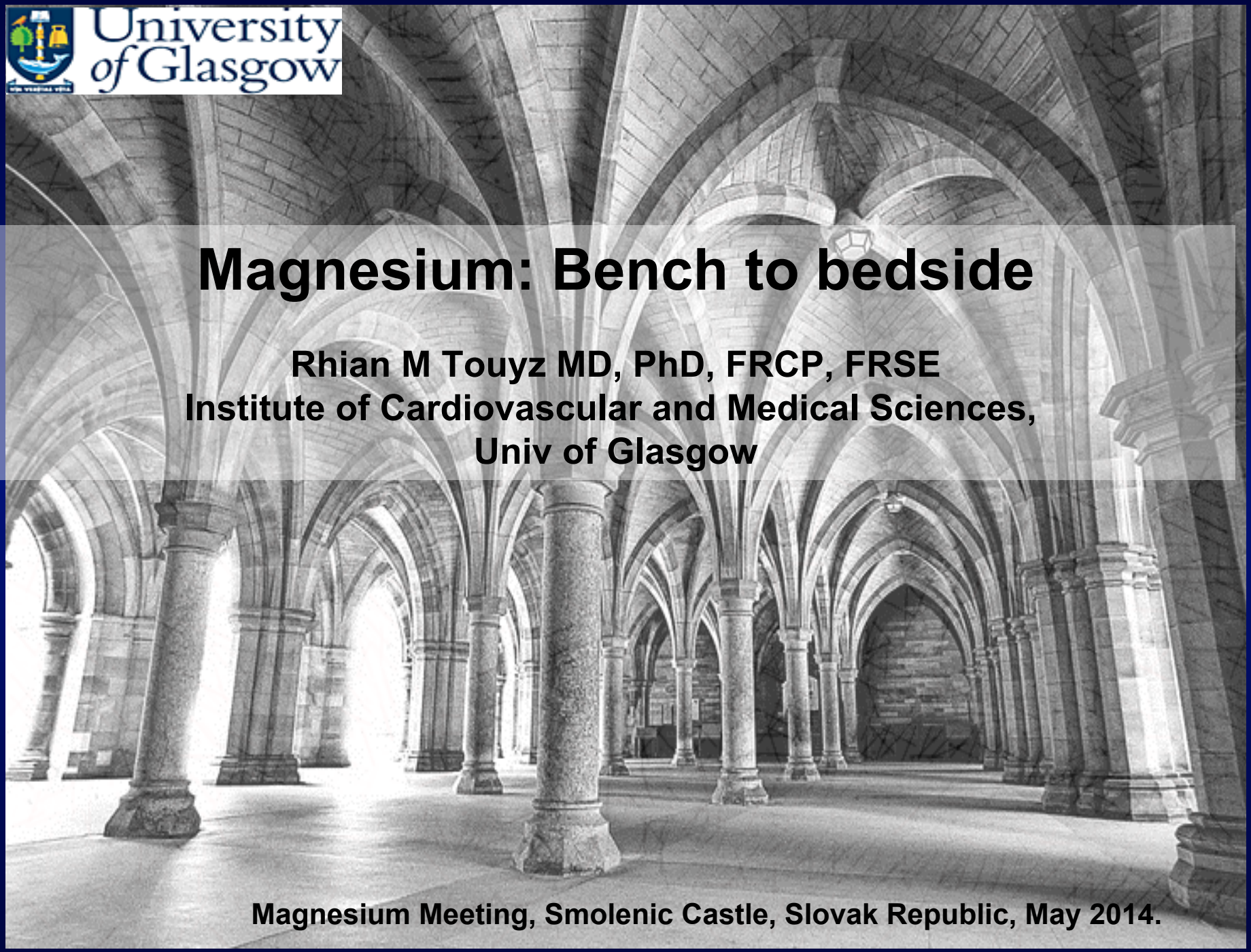


Magnesium: Bench to bedside

Rhian M Touyz MD, PhD, FRCP, FRSE
Institute of Cardiovascular and Medical Sciences,
Univ of Glasgow

Magnesium Meeting, Smolenic Castle, Slovak Republic, May 2014.

Joseph Black 1728 - 1799

Discoverer of magnesium as an element

- At the University of Glasgow, he discovered magnesium as an element in 1750-1755.
- Black found out that MgO was a compound of magnesia and that magnesia was not the same as calcium carbonate.
- He submitted this for his MD thesis

A history of magnesium in clinical research

Epson salts

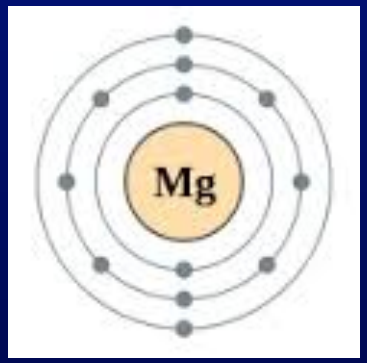
elemental Mg^{2+}

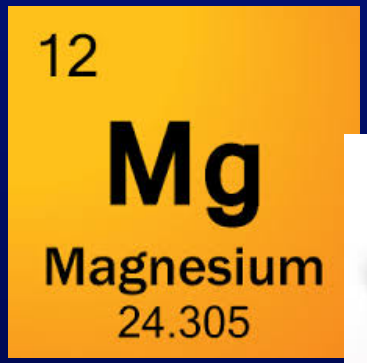
Mg^{2+} Isolation

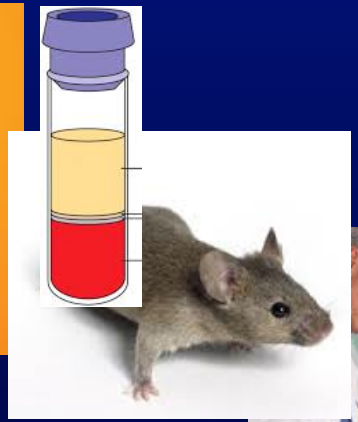
Mg^{2+} in plasma
Mg^{2+} essential for life
Hypomagnesemia in patients

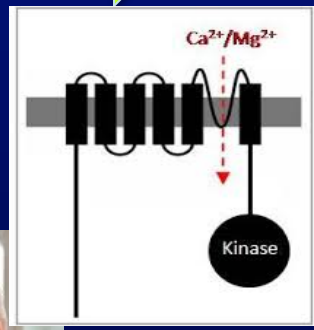
Mg^{2+} transporters

1600 1700 1800 1900 2000

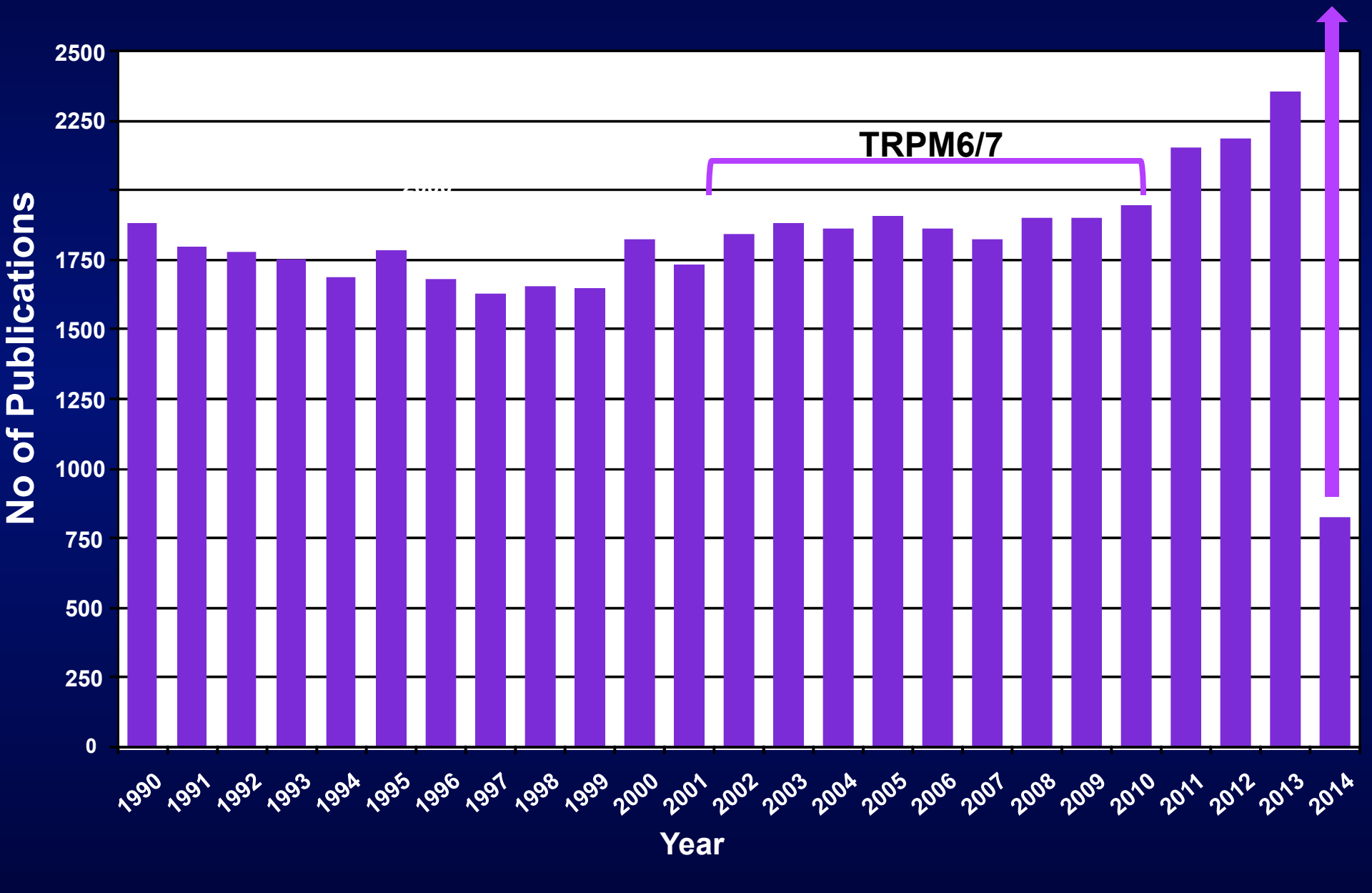






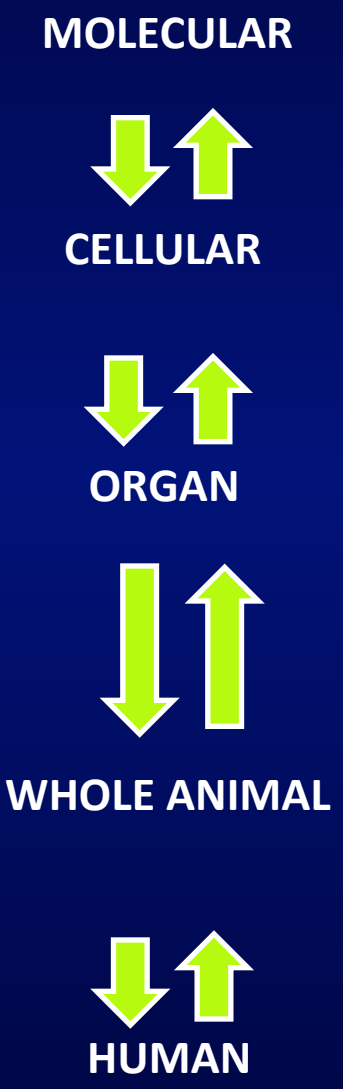


Magnesium Publications 1990-2014



Outline

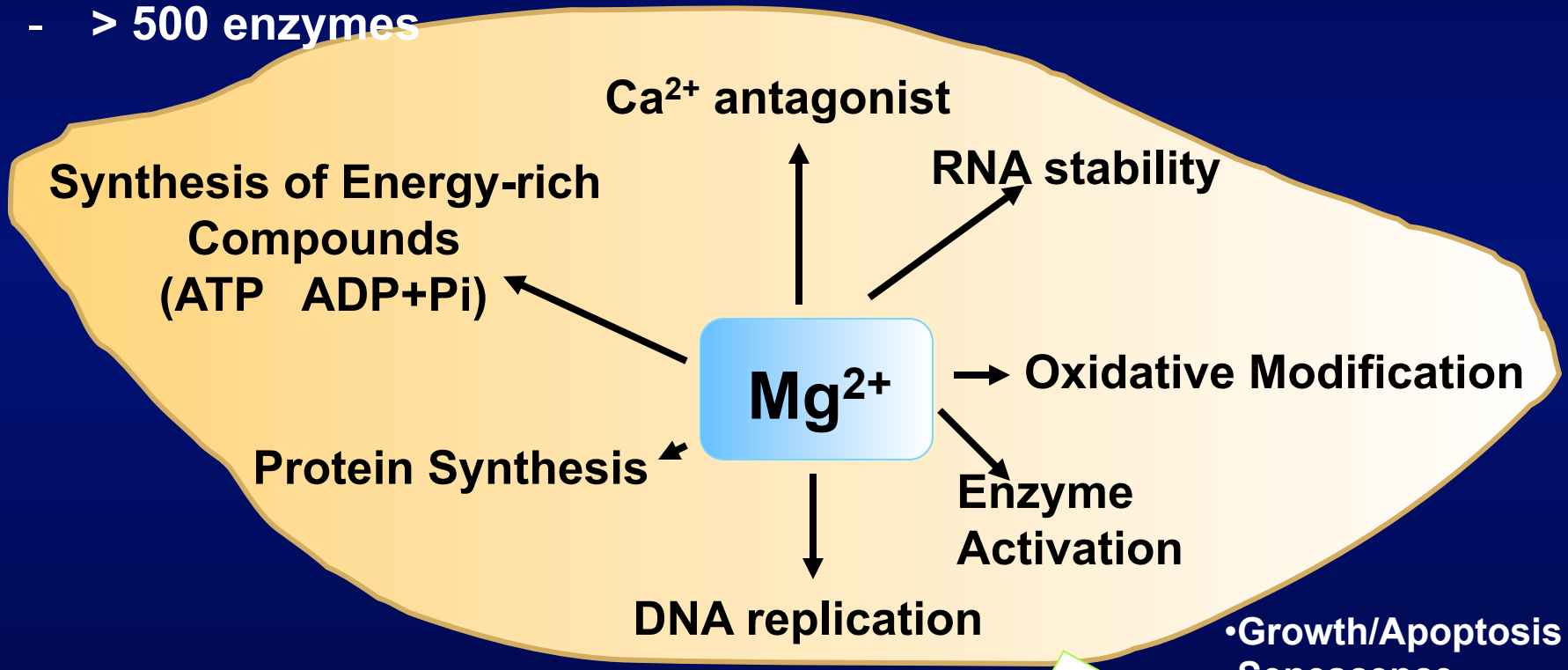
- Biochemistry and cell function
- Mg^{2+} and intracellular signaling
- Mg^{2+} transporters and cell biology
- Physiology of Mg^{2+}
- Mg^{2+} in the clinic



Major Biochemical Properties of Mg^{2+}

Magnesium:

- Second most abundant intracellular cation
- Most abundant cellular divalent cation
- > 500 enzymes



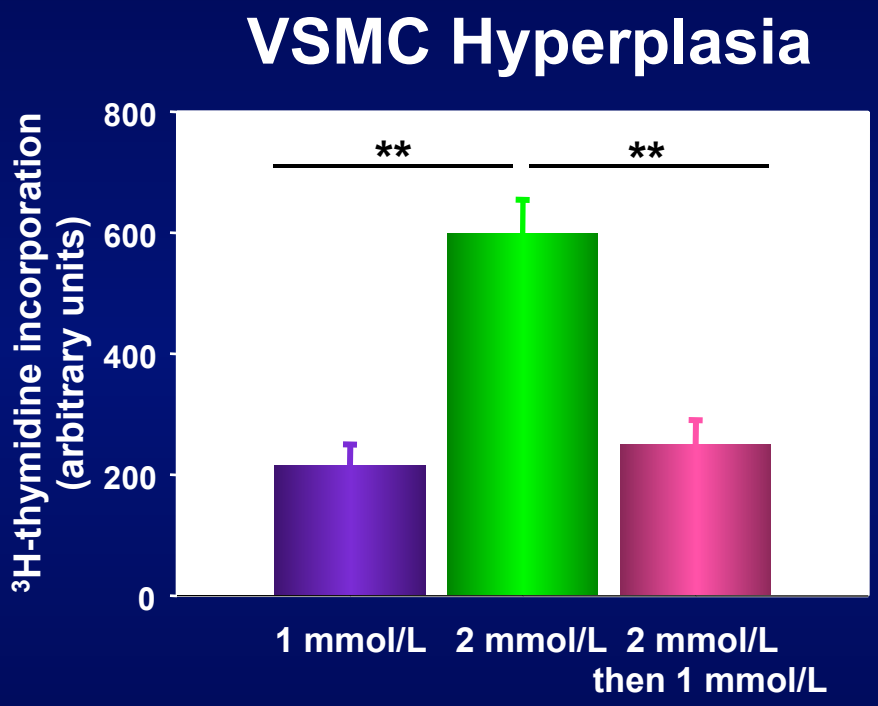
- Growth/Apoptosis
- Senescence
- Differentiation
- Contraction/dilation
- Inflammation, Fibrosis
- ……

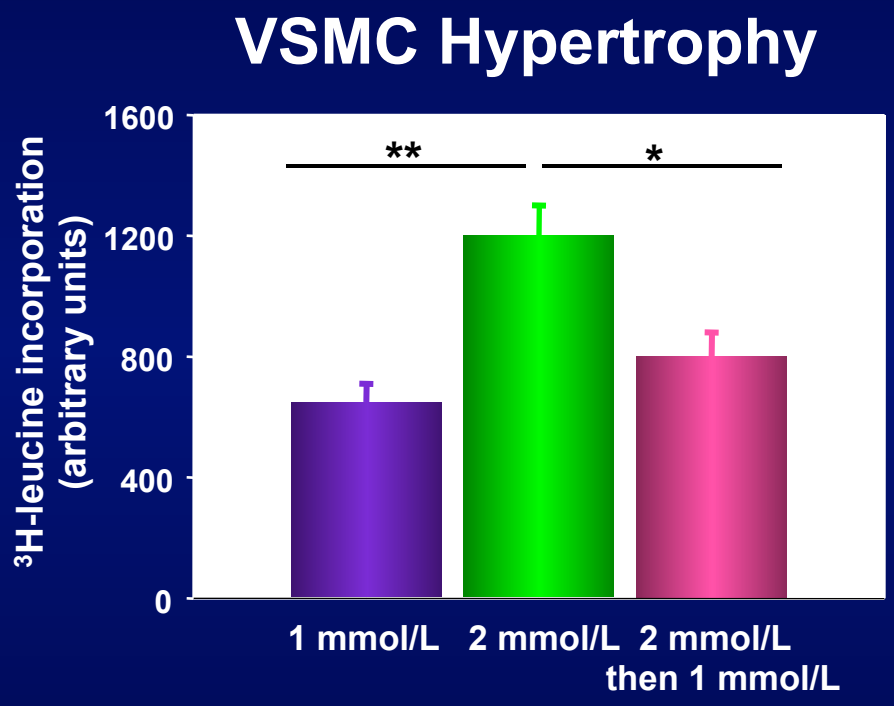
$$ATP + H_2O \xrightleftharpoons[Mg^{2+}]{ATPase} ADP + Pi$$

Mg^{2+} and cell function

- Survival
- Apoptosis
- Growth
- Proliferation
- Migration
- Differentiation
- Inflammation

Effects of Mg^{2+} on VSMC Growth

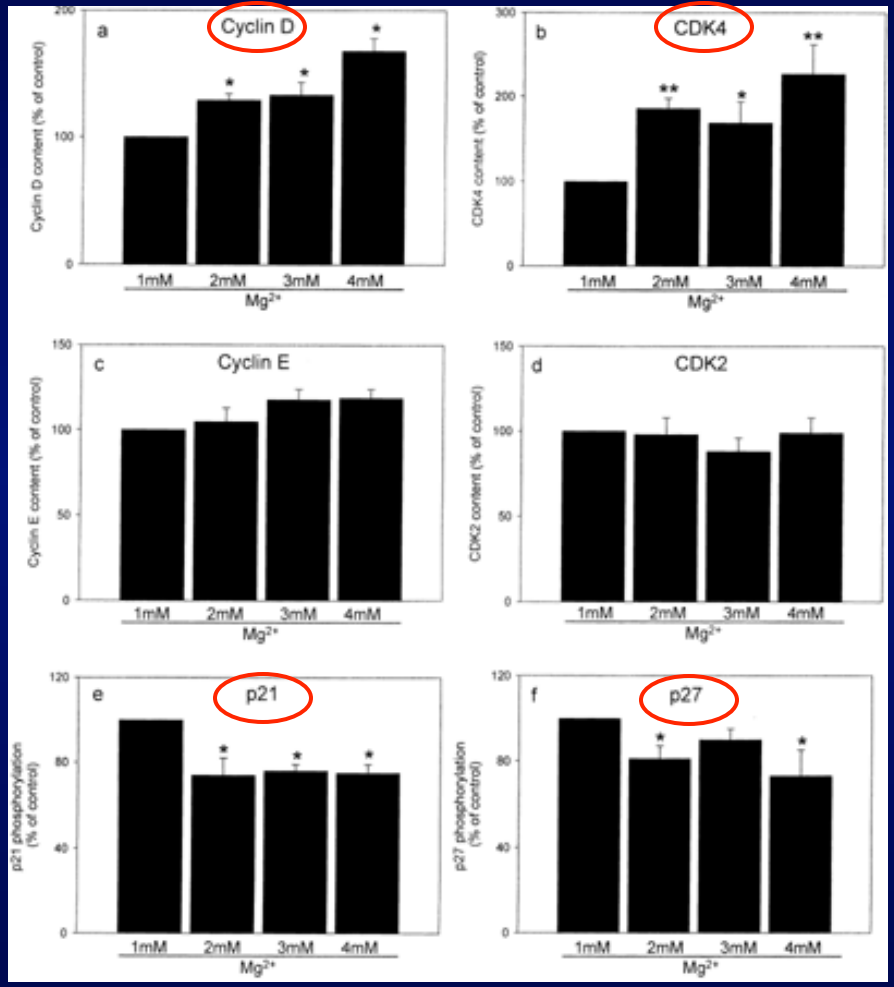




*p<0.05, **p<0.01

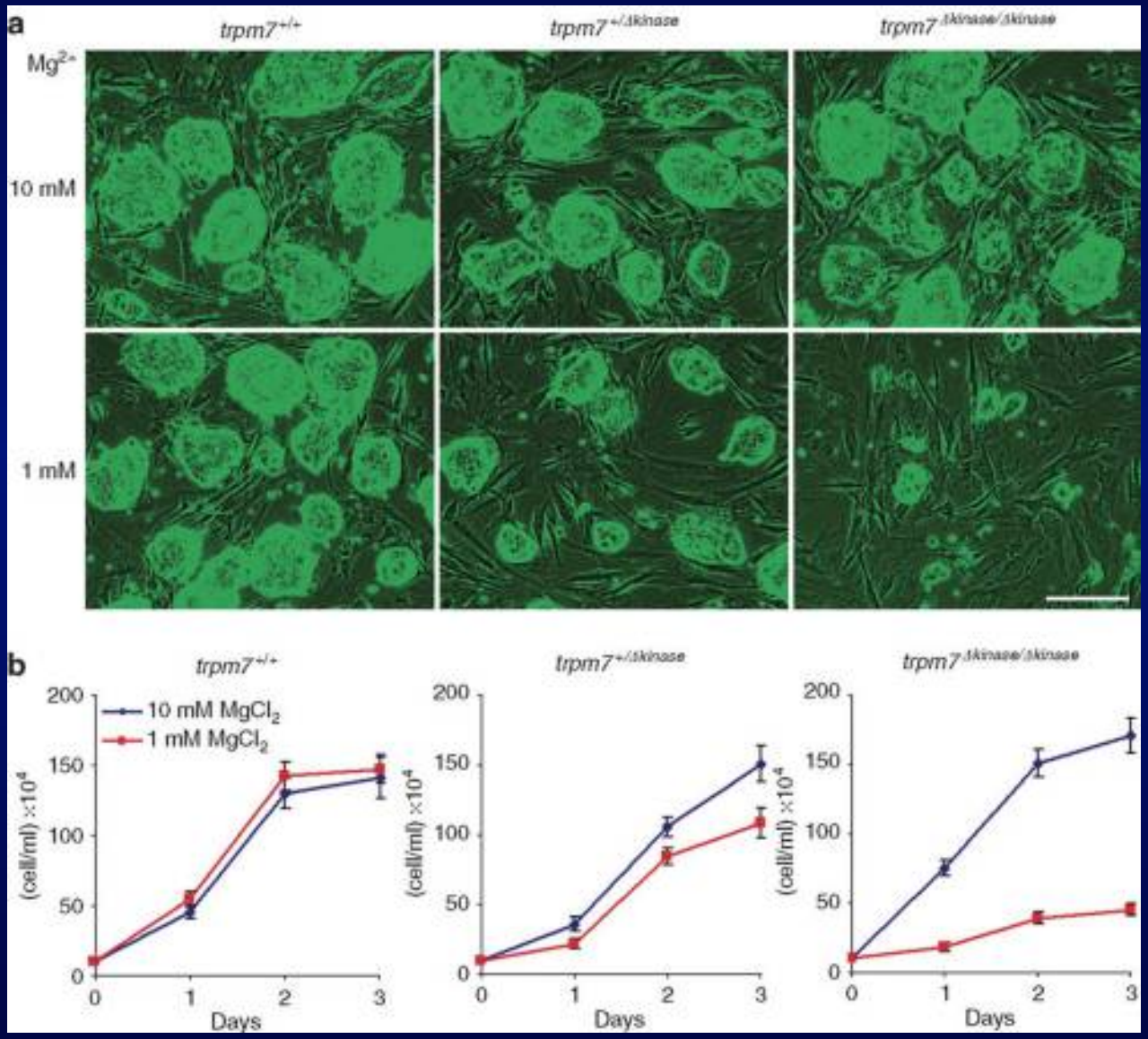
J Cell Physiol. 2003;197(3):326

Mg^{2+} Modulates Cell Cycle Regulators in VSMCs



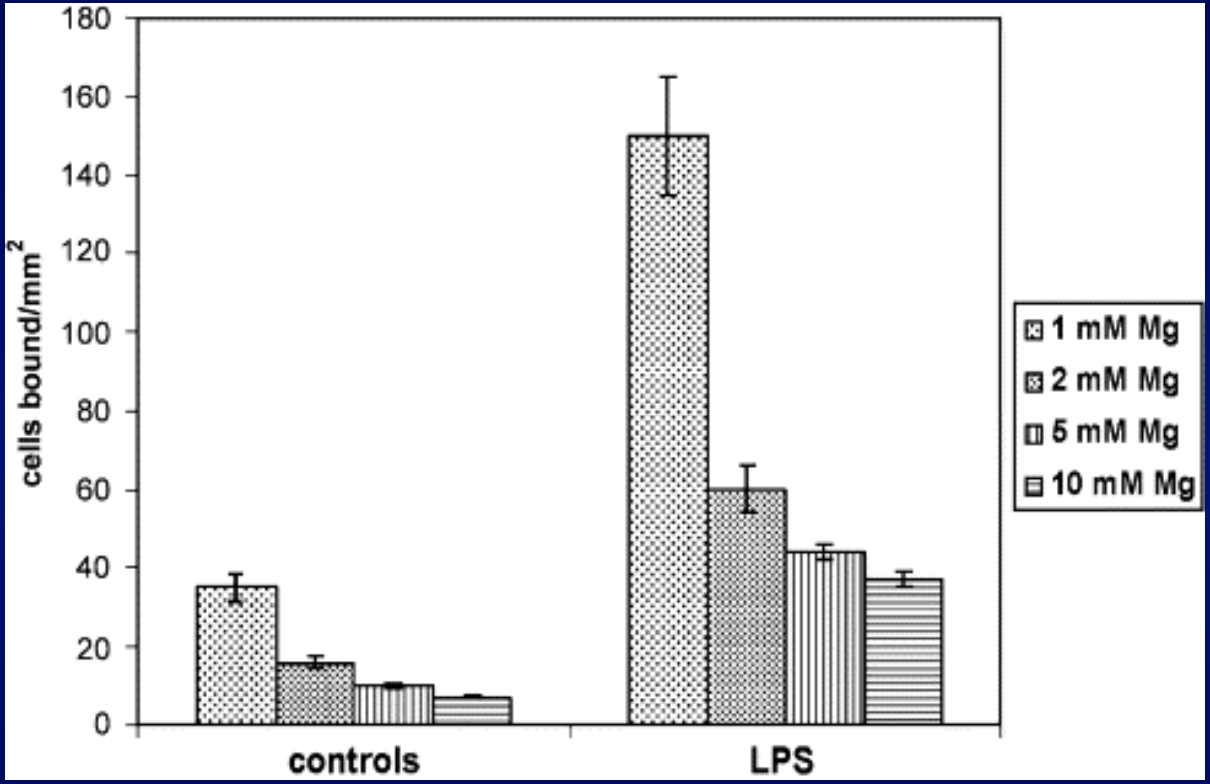
Increasing Mg^{2+} is associated with decreased p21 and p27 and increased cyclin D and CDK4 expression

Mg^{2+} supplementation rescues the growth arrest phenotype of TRPM7 deficient ES cells



Ryazanov. Nature 2010:1109

High concentrations of magnesium modulate vascular endothelial cell behaviour in vitro



Induction of HUVEC/U937 interactions by high magnesium. Confluent HUVEC were cultured in 1.0, 2.0, 5.0 and 10.0 mM Mg for 3 days and exposed or not to LPS (0.5 µg/ml). After 4 h, U937 cells were added. One hour later, the nonadherent **U937 cells were...**

Maier. Biochimica et Biophysica Acta (BBA). 2004

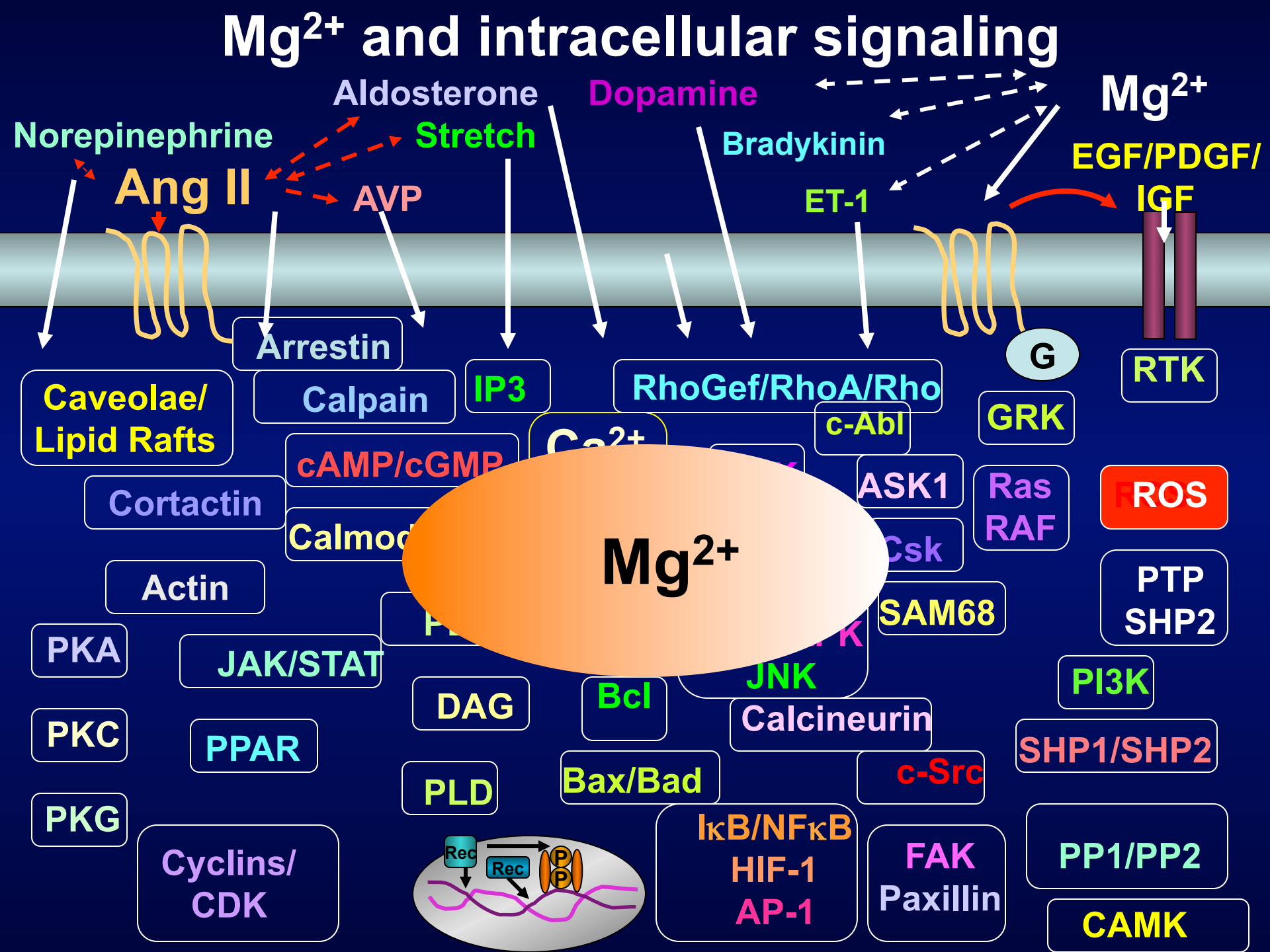
Functions of cellular Mg^{2+}

Mg++-induced endothelial cell **migration**: Substratum selectivity and receptor-involvement
Lapidos. Angiogenesis 2001;4.

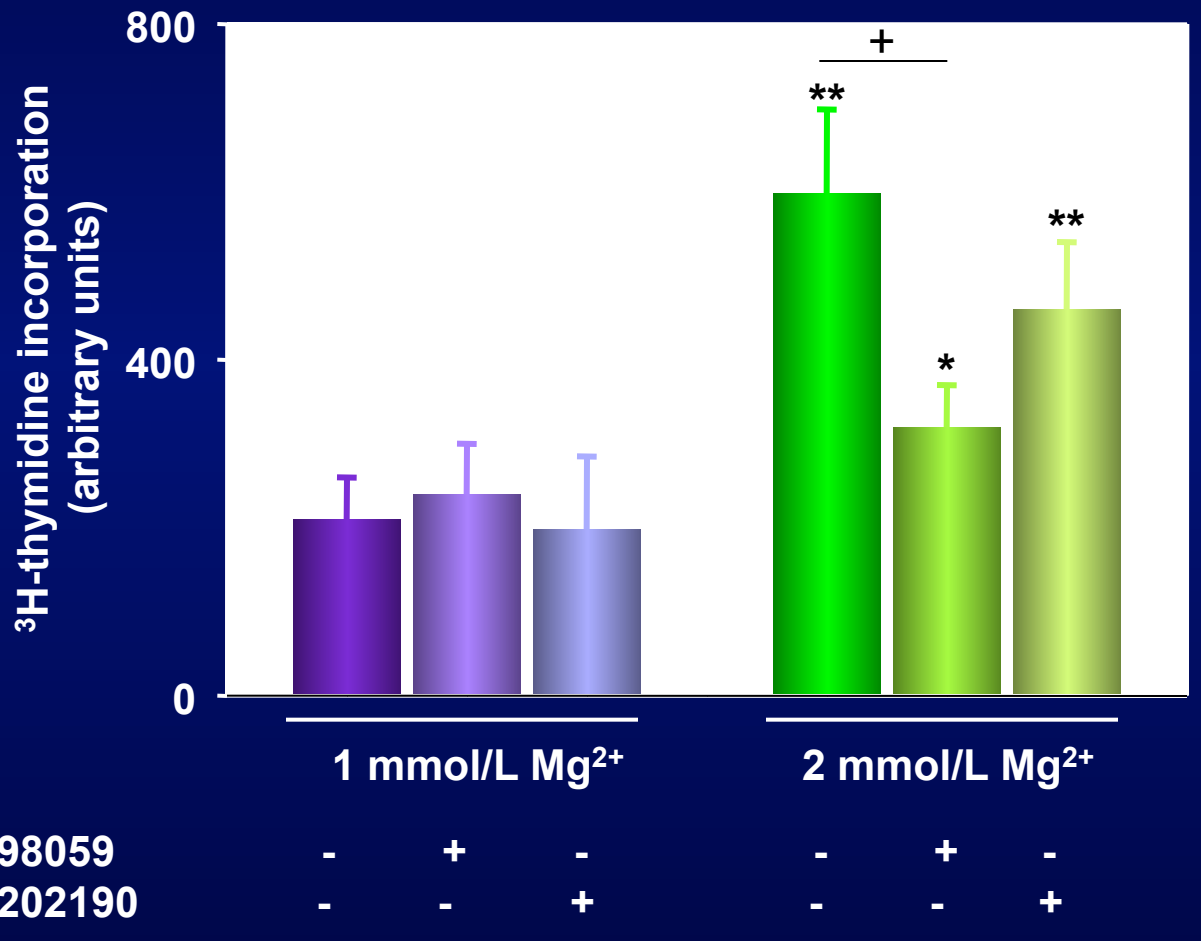
Magnesium decreases **inflammatory** cytokine production.
Sugimoto. J Immunol 2012

Magnesium: The missing element in molecular views of cell **proliferation** control
Rubin. Bioessays. 2005

Vascular smooth muscle cell **differentiation** to an osteogenic phenotype involves TRPM7 modulation by Mg2+
Montezano Hypertension 2011


Mg2+ and intracellular signaling
Aldosterone
Dopamine
Mg2+
Norepinephrine
Stretch
Bradykinin
EGF/PDGF/
IGF
Ang II
AVP
ET-1
Arrestin
G
RTK
Caveolae/
Lipid Rafts
Calpain
IP3
RhoGef/RhoA/Rho
GRK
c-Abl
cAMP/cGMP
ASK1
Ras
RAF
ROS
Cortactin
Csk
Mg2+
Actin
SAM68
PTP
SHP2
PKA
JAK/STAT
JNK
PI3K
DAG
Bcl
Calcineurin
PKC
PPAR
SHP1/SHP2
Bax/Bad
c-Src
PLD
PKG
IκB/NFκB
HIF-1
AP-1
FAK
Paxillin
PP1/PP2
Cyclins/
CDK
Rec
Rec
P
P
CAMK

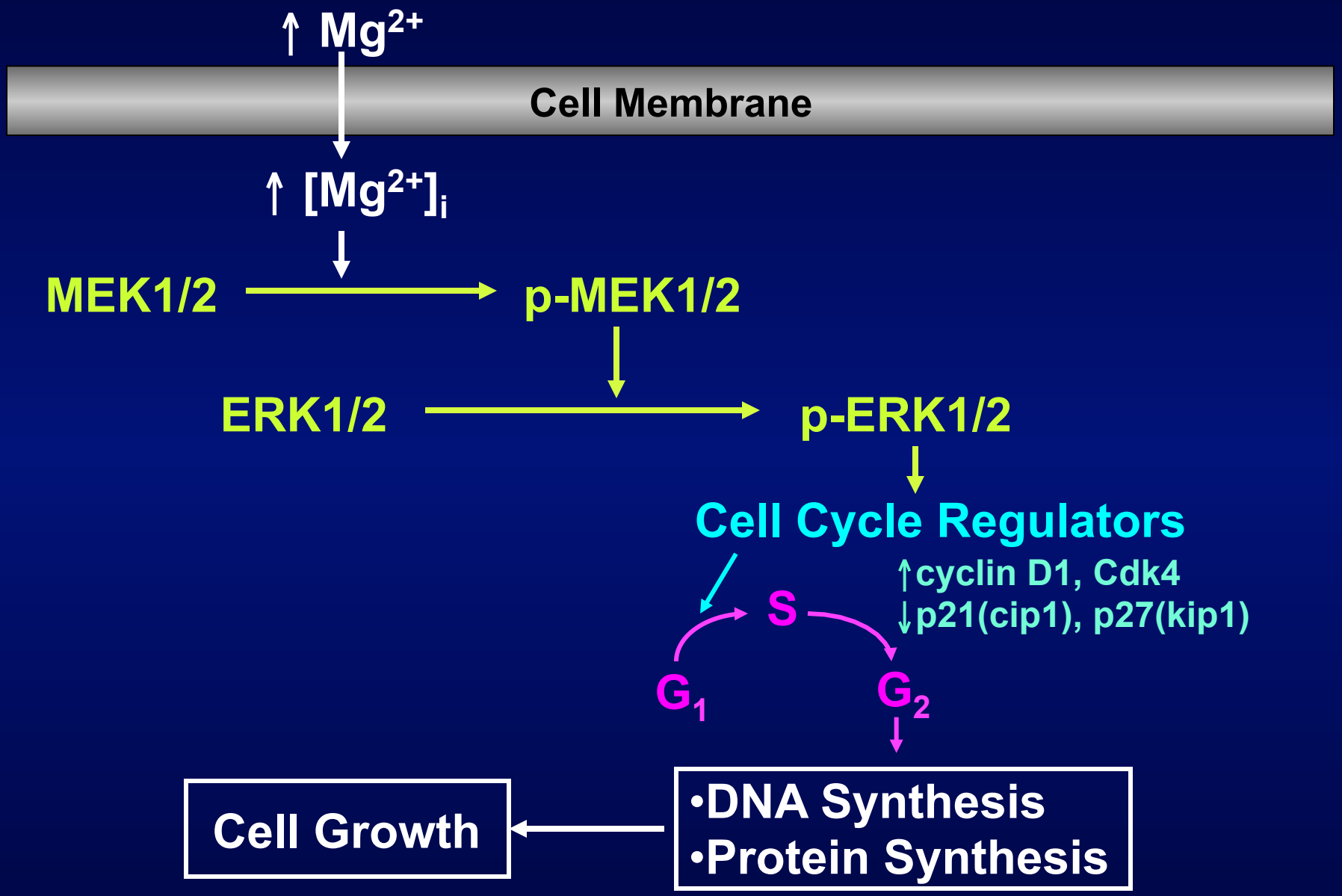
PD98059, MEK inhibitor, but not SB202190, p38MAPK inhibitor, attenuates Mg^{2+}-induced vascular growth



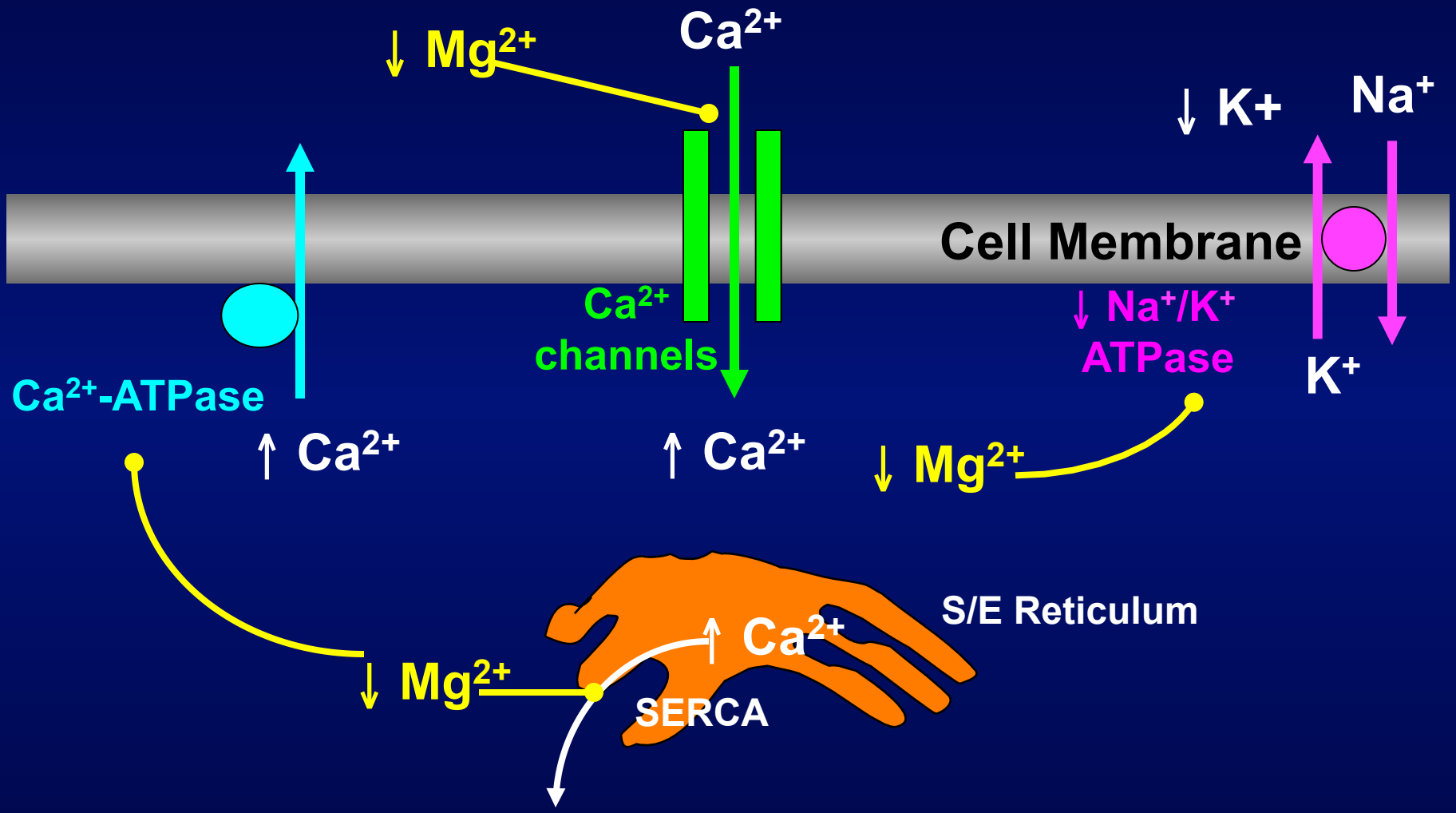
*p<0.05 vs 1 mmol/L
**p<0.01 vs 1 mmol/L
+p<0.01

J Cell Physiol. 2003;197(3):326

Mechanisms Whereby $[Mg^{2+}]_i$ Influences Cell Growth



Mg^{2+} influences Ca^{2+} and K^{+} Homeostasis



- 40% patients with hypomagnesemia have hypokalemia.
- 60% patients with hypokalemia have hypomagnesemia.

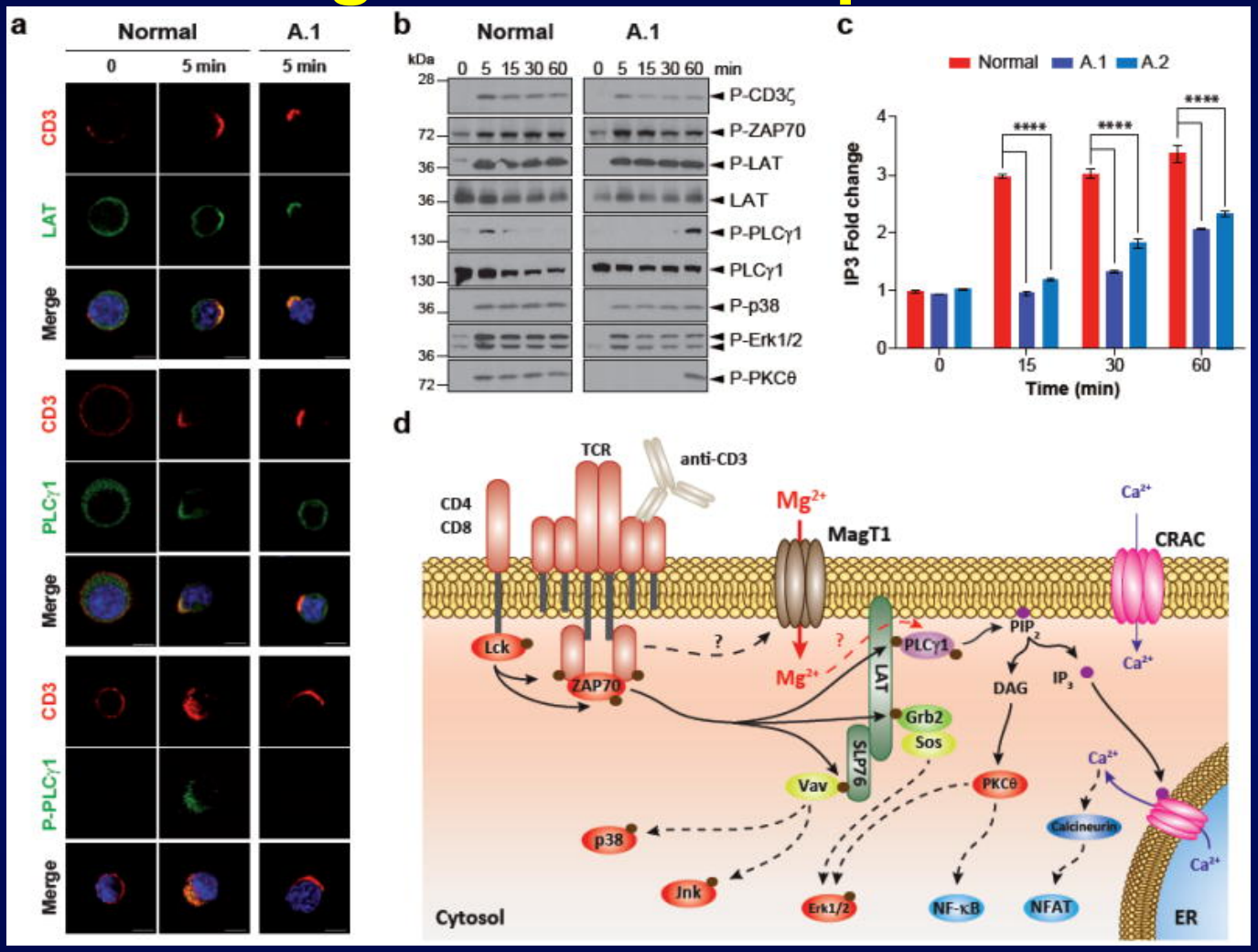
Second messenger role for Mg^{2+} revealed by human T-cell immunodeficiency

Li et al. Nature 2011:475:471–476

Immunology: Magnesium in a signaling role.

Wu. Nature. 2011;475(7357

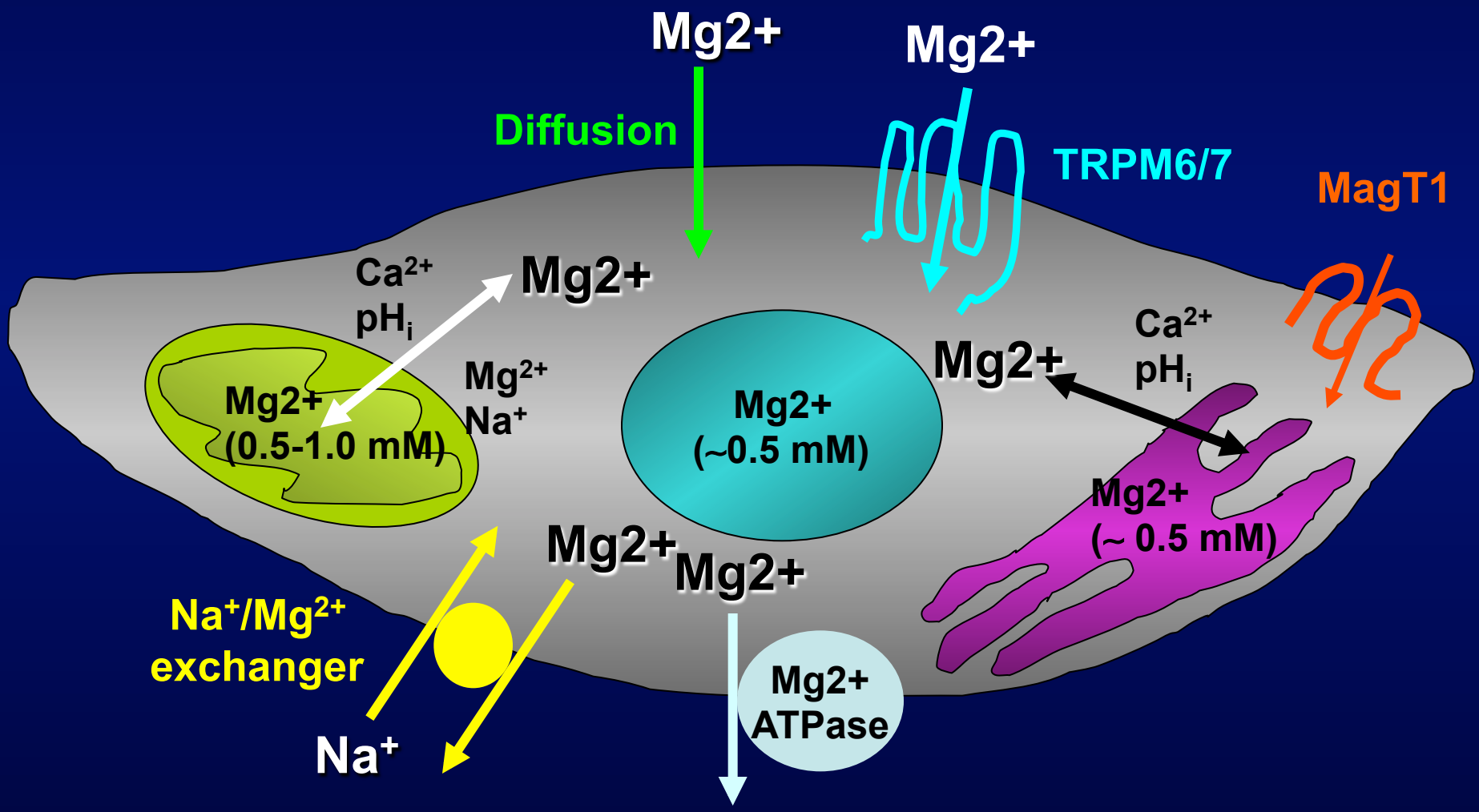
Signaling in T cells from healthy and MagT1-deficient patients



(a,b). Signaling proteins. (c) cellular IP3 level (c) in healthy control and patient T cells stimulated with Acd3

(d). Schematic -MagT1 mediated Mg^{2+} influx participates in TCR signaling

For Mg^{2+} to Influence Cellular Events, Mg^{2+} itself needs to be Regulated: Cellular level



Editorials

Mysteries of Magnesium Homeostasis

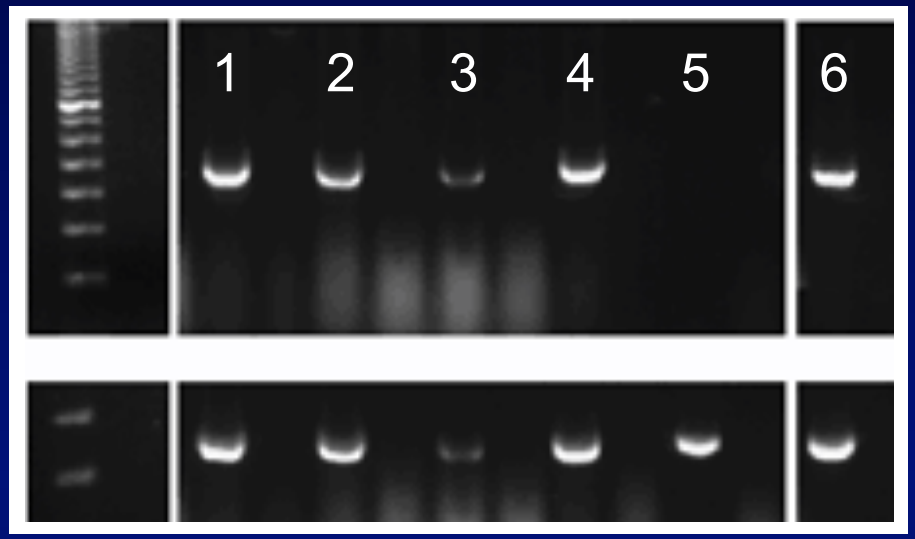
Elizabeth Murphy

Circulation Research. 2000;86:245

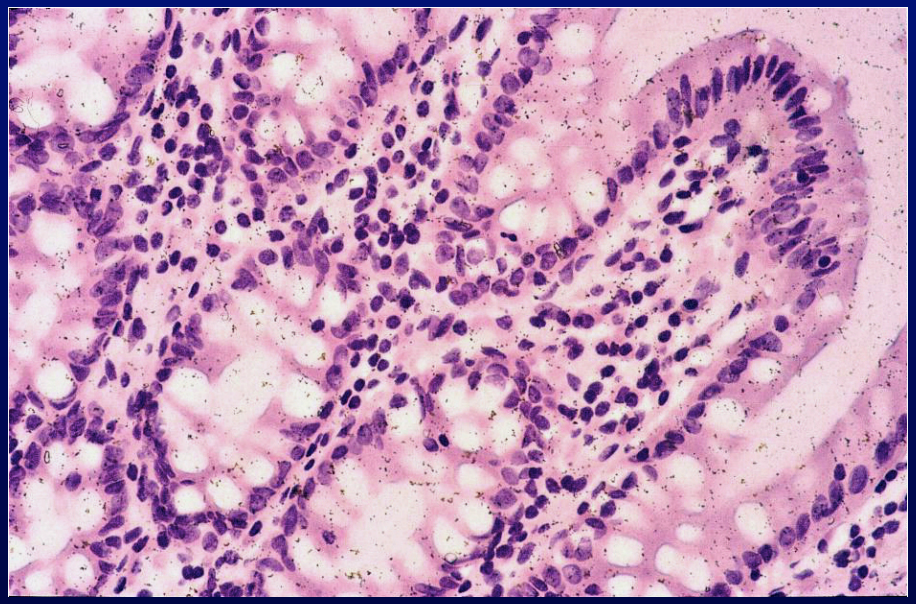
Mg^{2+} transporters in mammalian cells

Transporter	Mutation/disease
TRPM6	Hypomagnesemia+hypocalcemia
TRPM7	
MagT1	X-MEN
SLC41A1	
SLC41A2	
CNNM3	
MRS2	

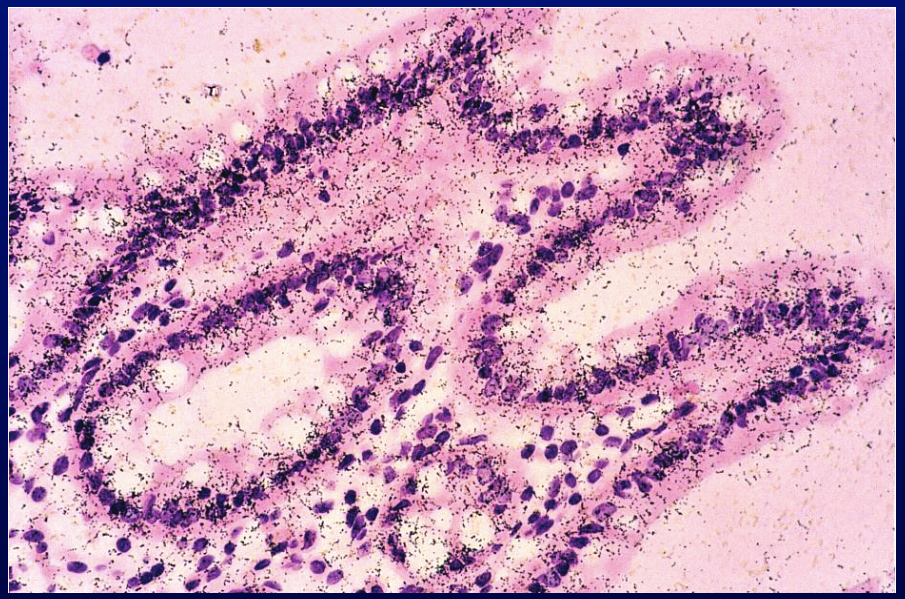
TRPM6 Expression in the Gastrointestinal Tract



1. Duodenum
2. Jejunum
3. Ileum
4. Colon
5. Liver
6. Kidney



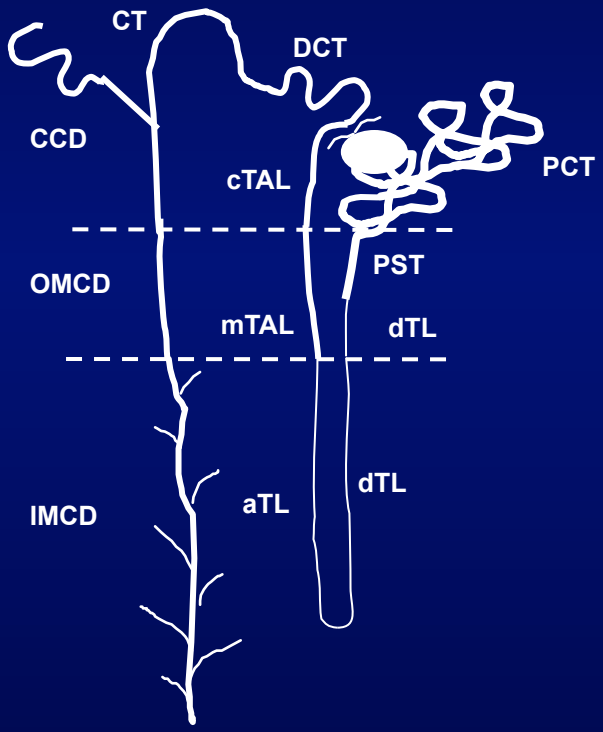
sense

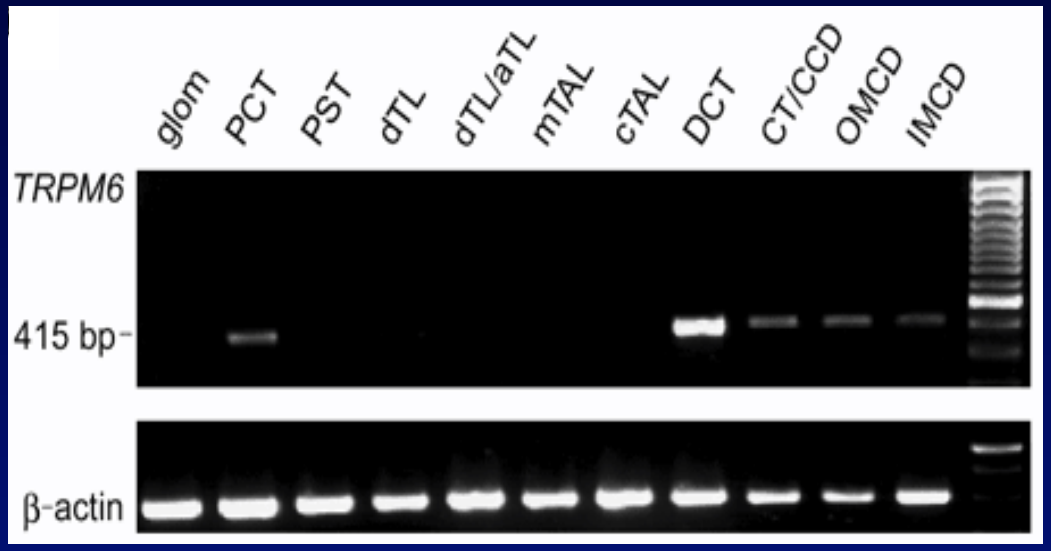


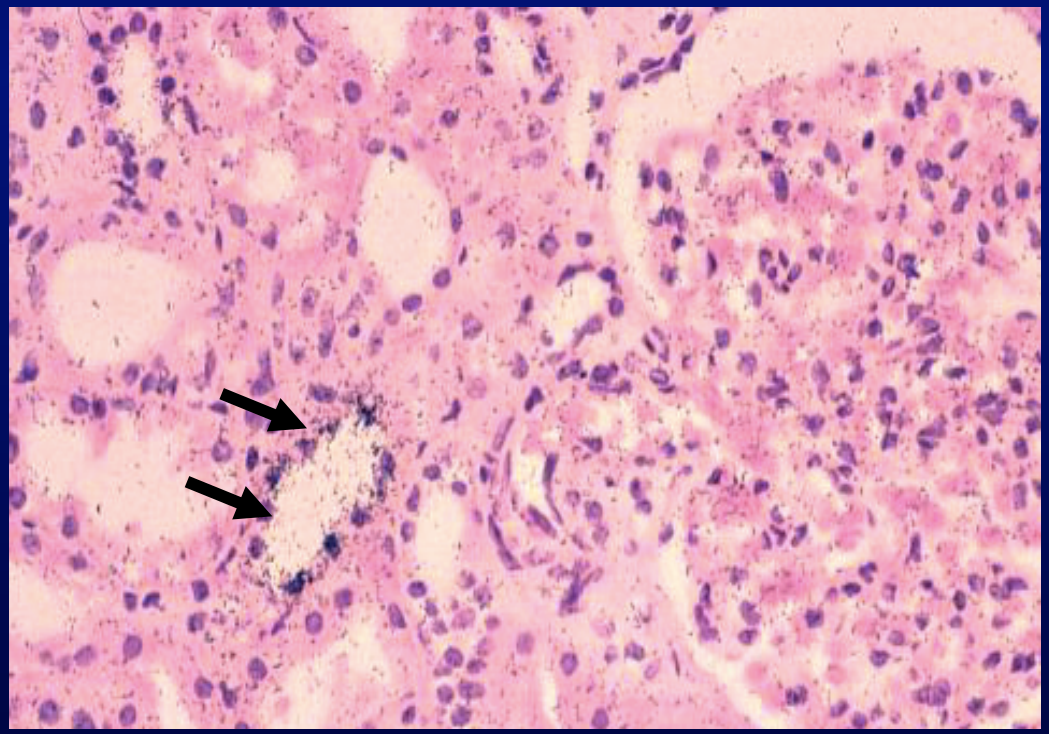
antisense

Schlingmann et al. Nat Genet 2002

TRPM6 expression along the nephron

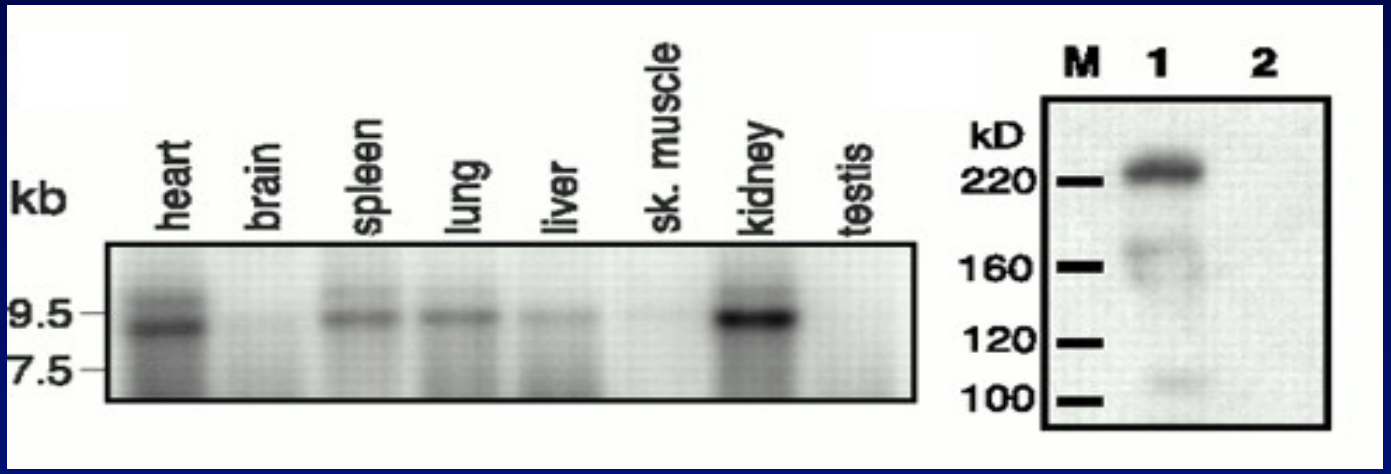






Schlingmann et al. Nat Genet 2002

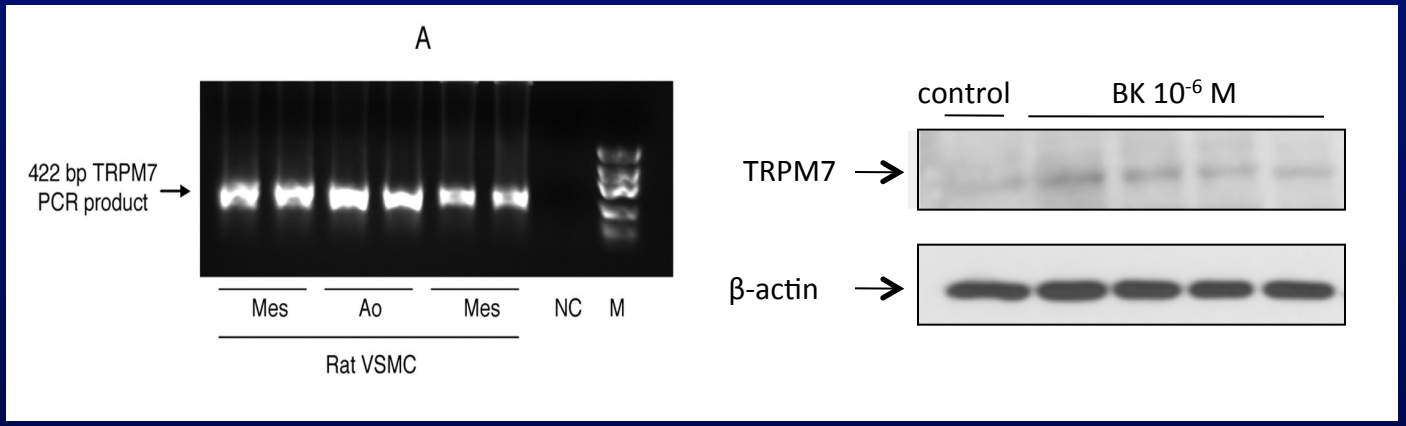
Expression of TRPM7



Northern blot analysis of TRPM7

Western blot of TRPM7

Science 2001;291:1043

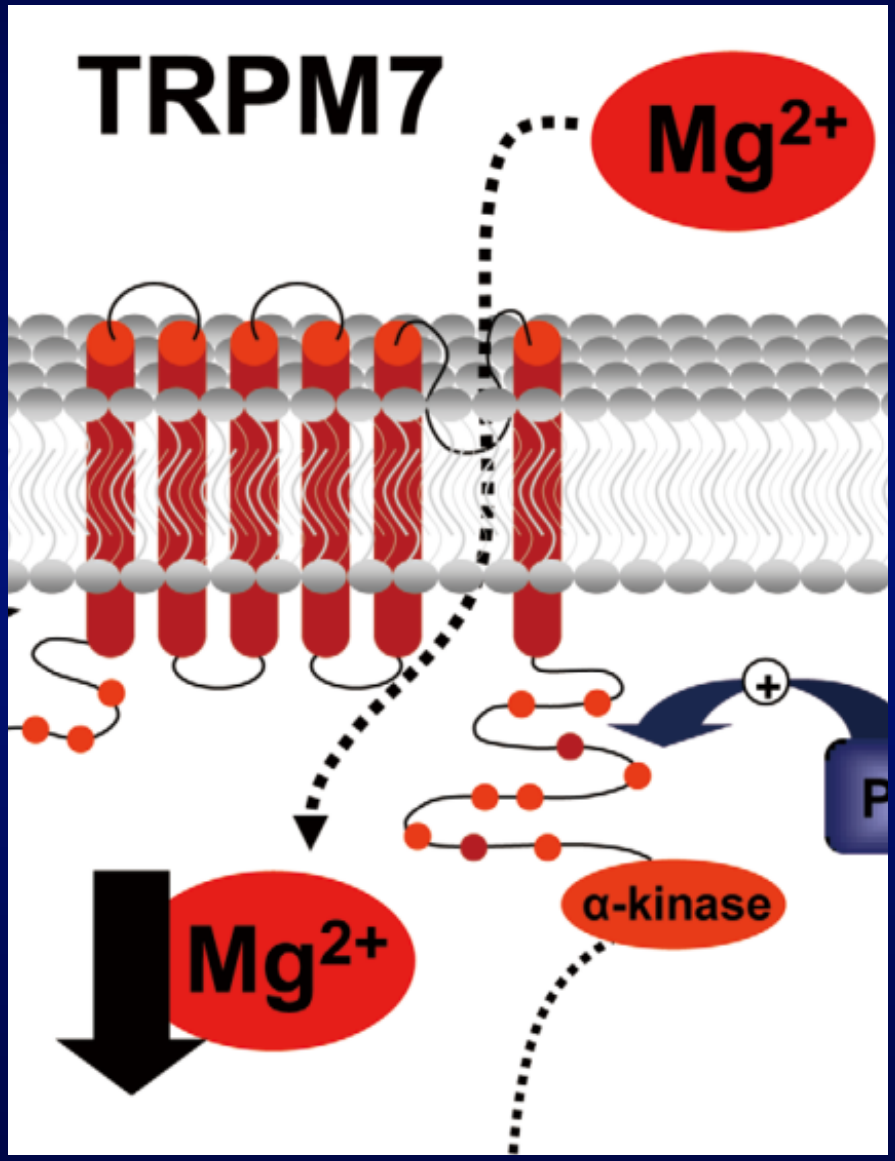


Mouse VSMC

Circ Res 2010

Transmembrane Mg^{2+} Transporter – TRPM6/7

(Ryazanova, Runnels, Nadler, Bindels, Fleig, Gudderman, Chubanov)



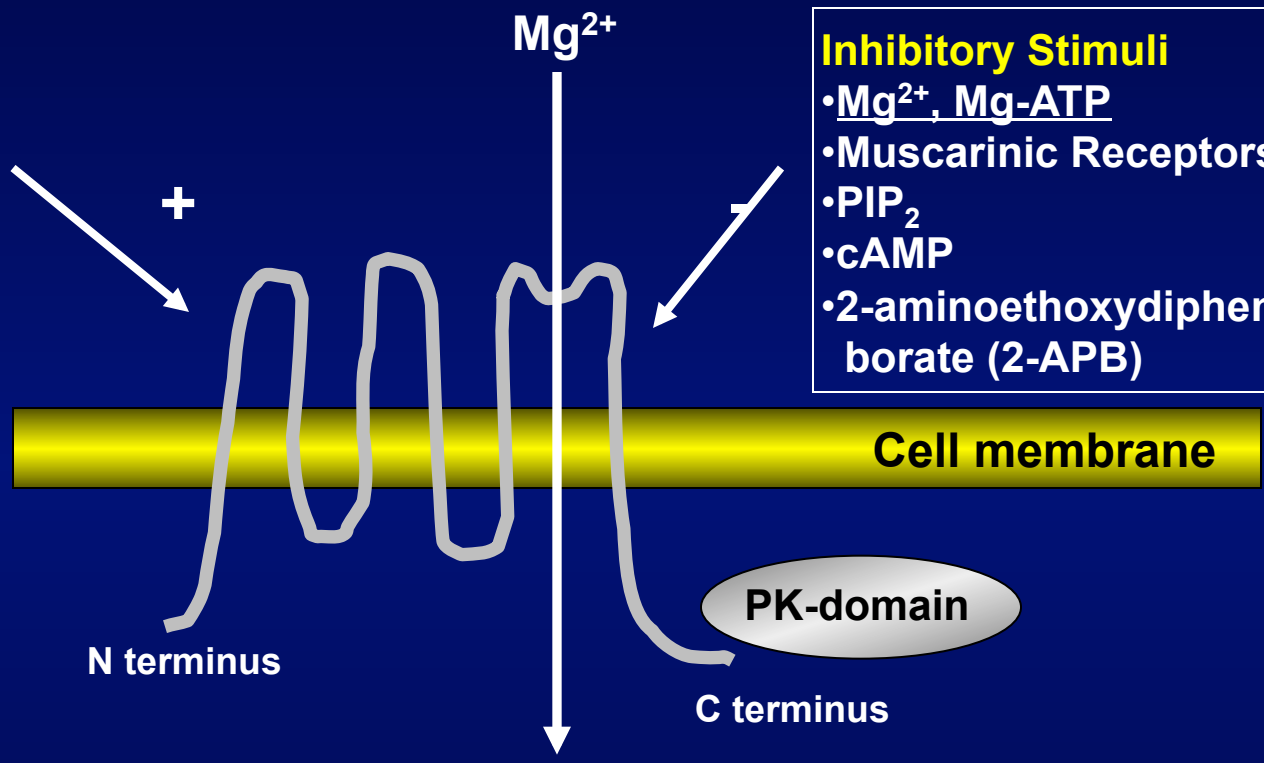
Regulation of TRPM7 (Cell lines)

Stimulatory Stimuli

Receptors
- Bradykinin
- Estrogen
- Muscarinic receptors
- EGFR

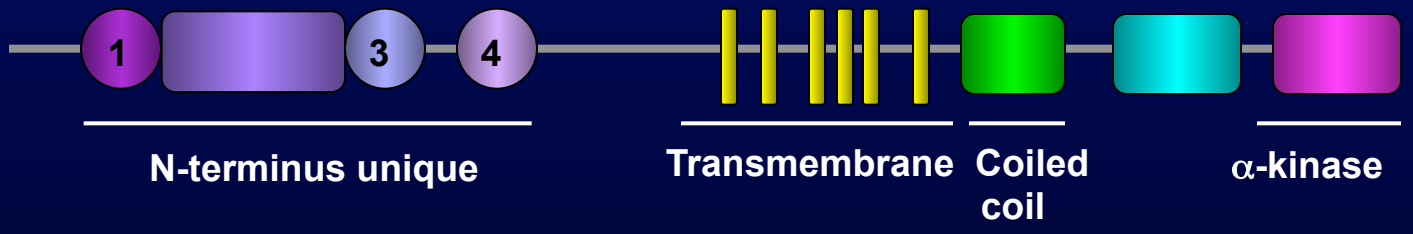
Mechanical factors
- Stretch, flow, pressure

Signaling molecules
- PLC, PKC, PKA
- cAMP
- Phosphatidylinositol bisphosphate (PIP_2)

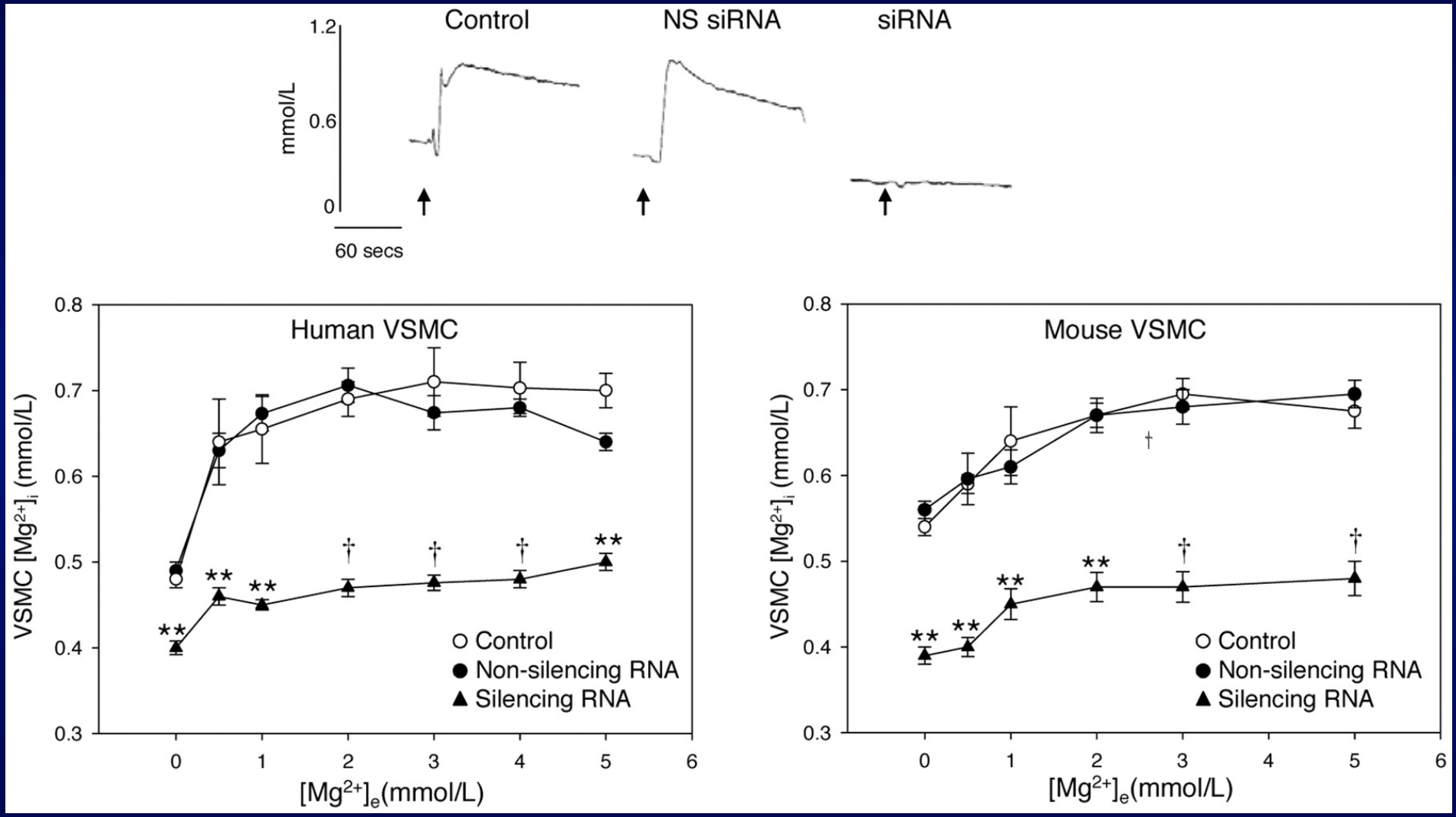


Inhibitory Stimuli
- Mg^{2+}, Mg-ATP
- Muscarinic Receptors
- PIP_2
- cAMP
- 2-aminoethoxydiphenyl borate (2-APB)

Role of kinase domain is unclear



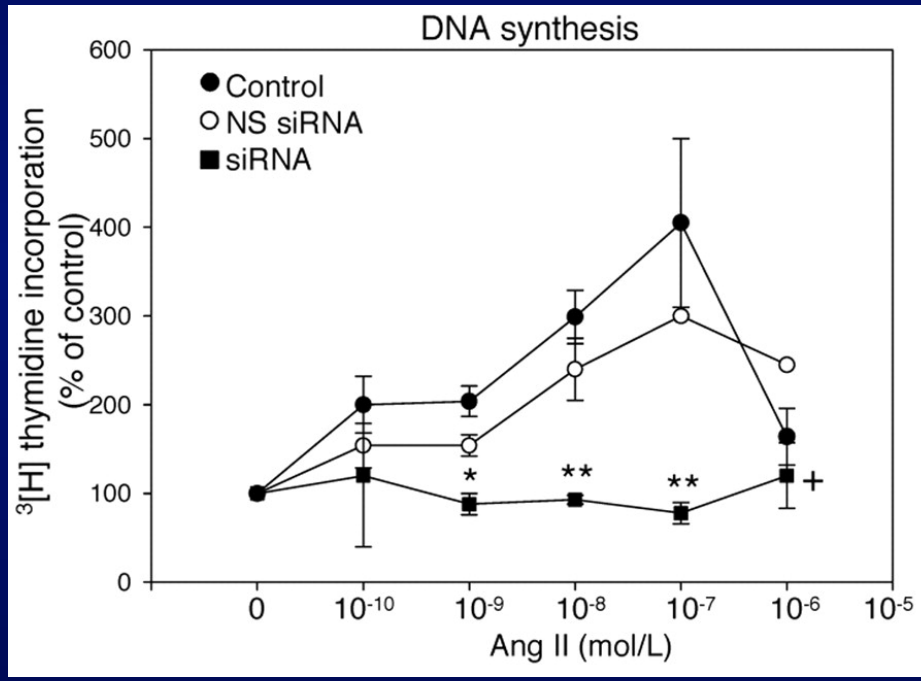
TRPM7 Regulates $[Mg^{2+}]_i$ in VSMCs

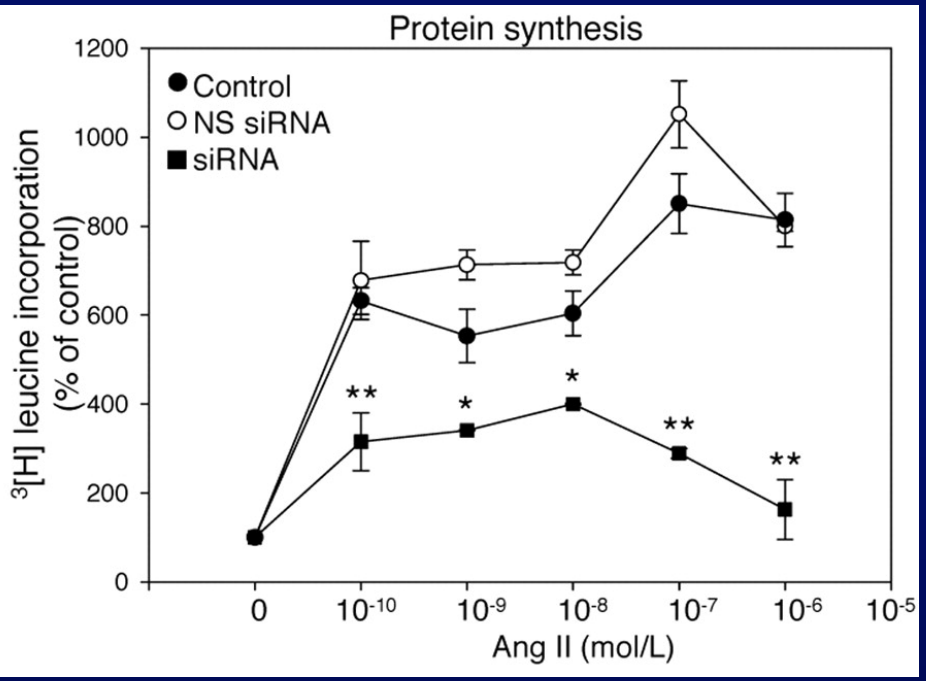


Effects of increasing $[Mg^{2+}]_e$ on $[Mg^{2+}]_i$ in TRPM7-deficient VSMCs.

Touyz. Circ Res. 2005;96(2):207-15

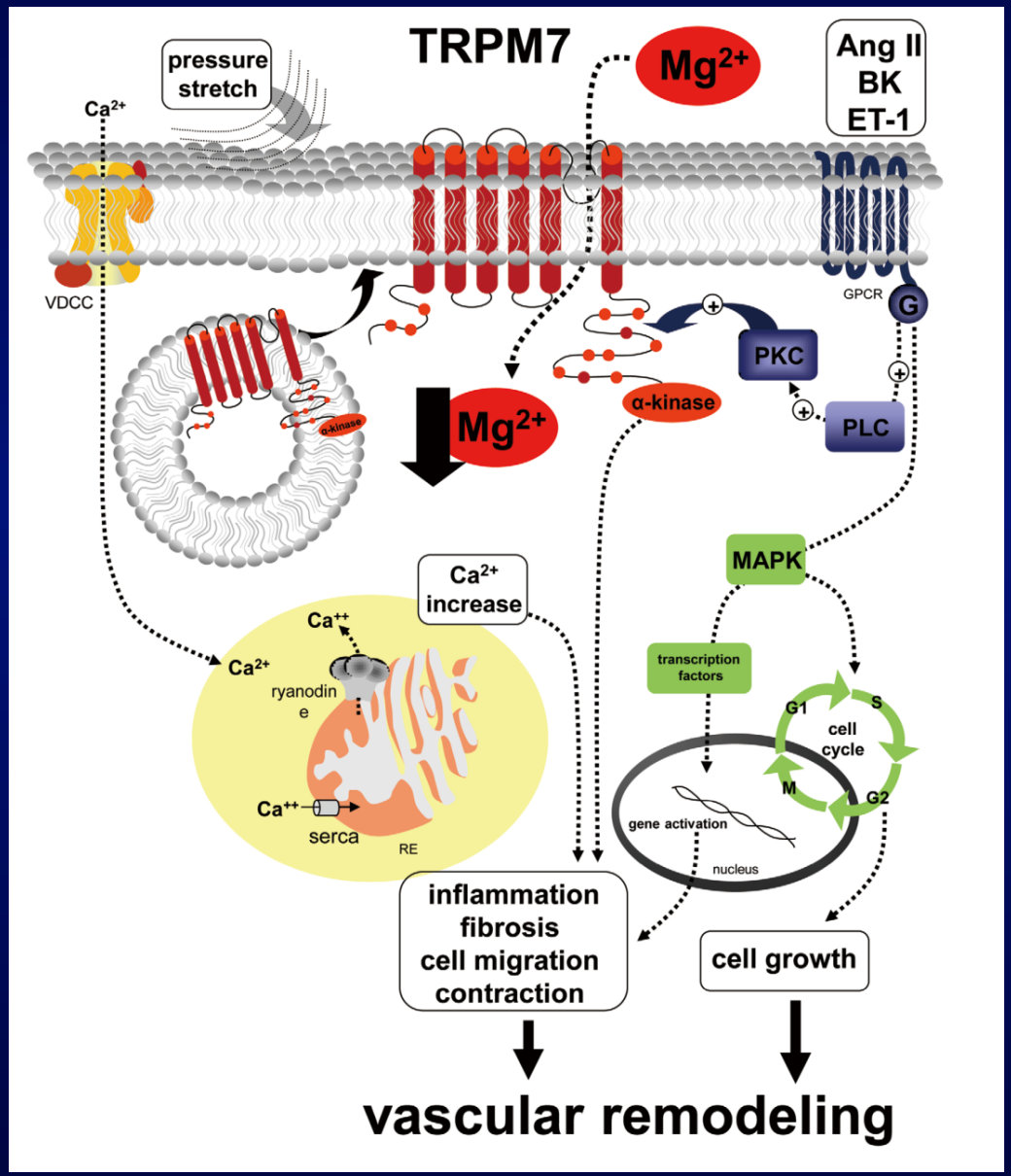
TRPM7 Plays a Critical Role in Ang II-stimulated VSMC Growth



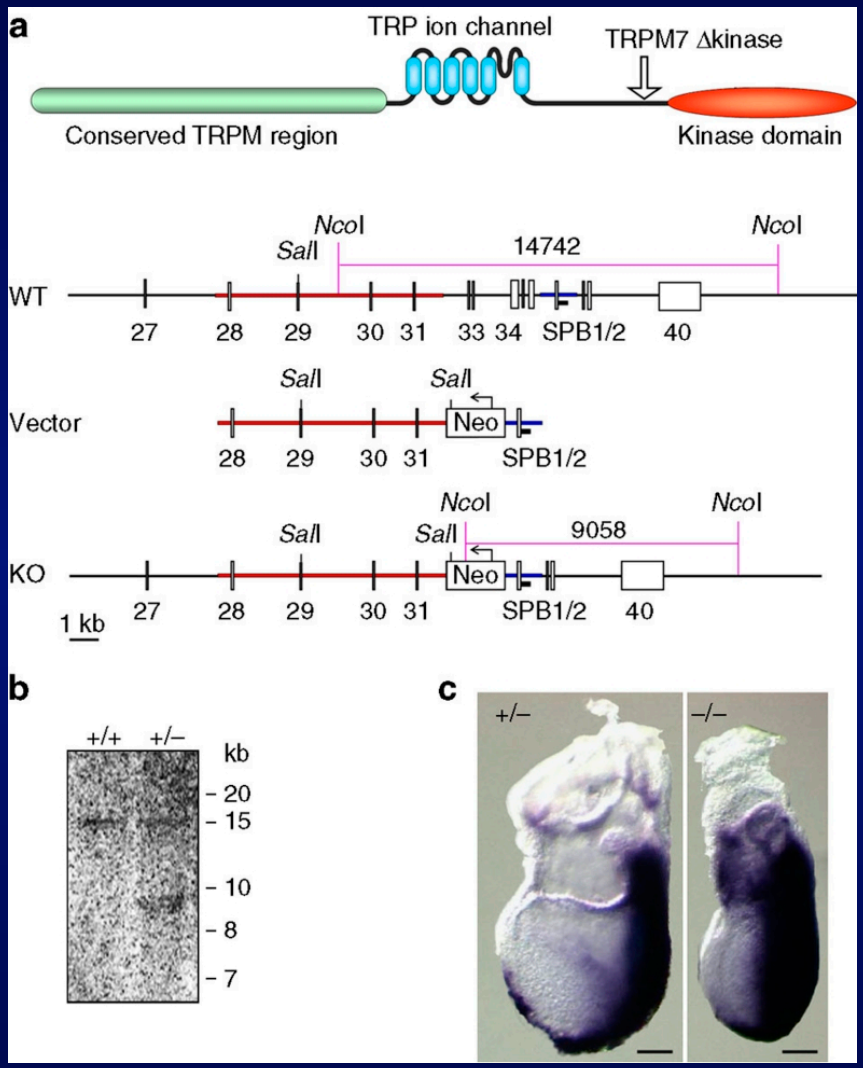


Touyz. Circ Res. 2005;96(2):207-15

TRPM7 and the cardiovascular system



Generation of TRPM7-deficient mice

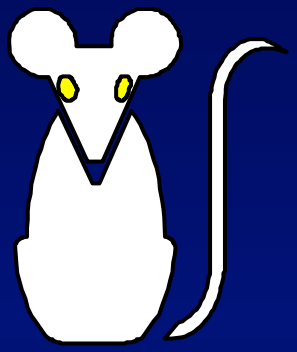


(a) A schematic representation of TRPM7 protein. Arrow indicates position of truncation in TRPM7$^{\Delta kinase}$ mice. (b) Southern blot analysis of genomic DNA from wild-type (+/+) or TRPM7$^{+/\Delta kinase}$ (+/−) mice (c) TRPM7$^{+/\Delta kinase}$ (+/−) and TRPM7$^{\Delta kinase/\Delta kinase}$ (−/−) embryos at 7.5 days Scale bar, 100 μm

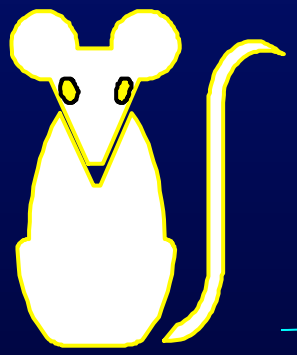
Ryazanov. Nature 2010:1109

Cardiovascular phenotype in TRPM7+/- mice

Control

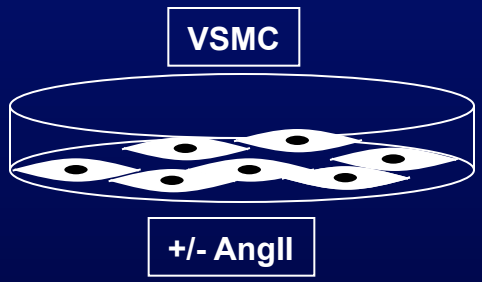


TRPM7$^{+/\Delta kinase}$



Angiotensin II infusion
at 400 ng/kg/min, 2 wks

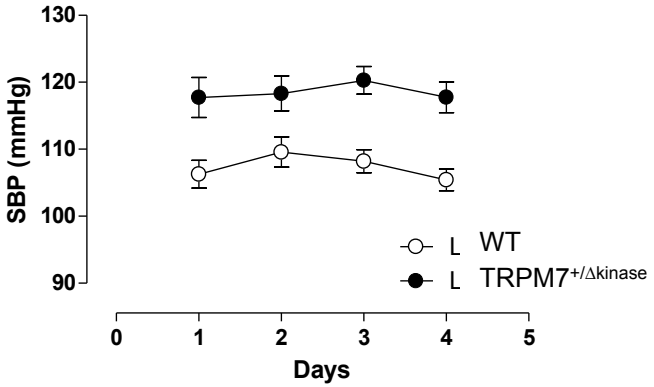
- Blood pressure
- Plasma and urine ions
- Cardiac, vascular and renal injury
- Inflammatory markers
- TRPM7 expression



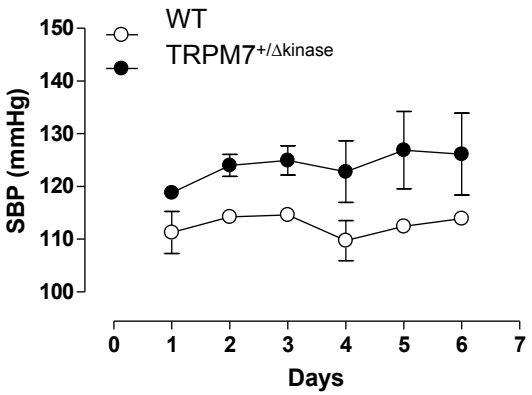
Western Blot
Phospho ERK 1/2
Phospho-JNK
Phospho-p38

Systolic blood pressure

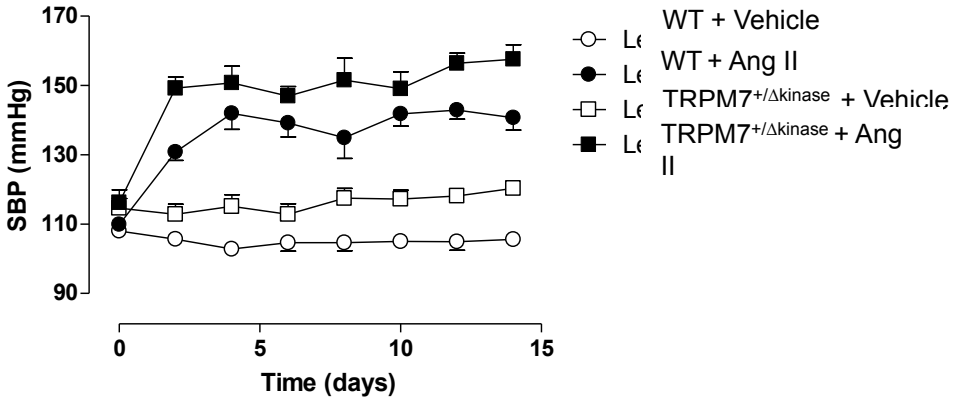
Baseline (Tail-Cuff)



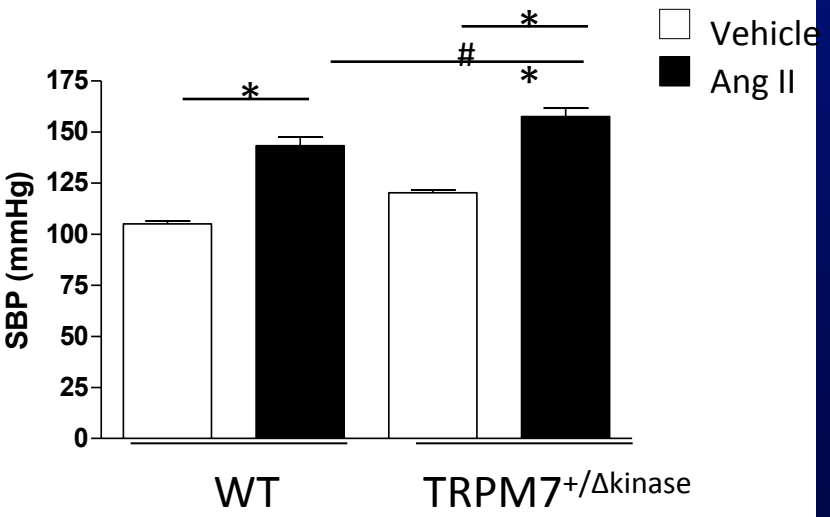
Baseline (Telemetry)



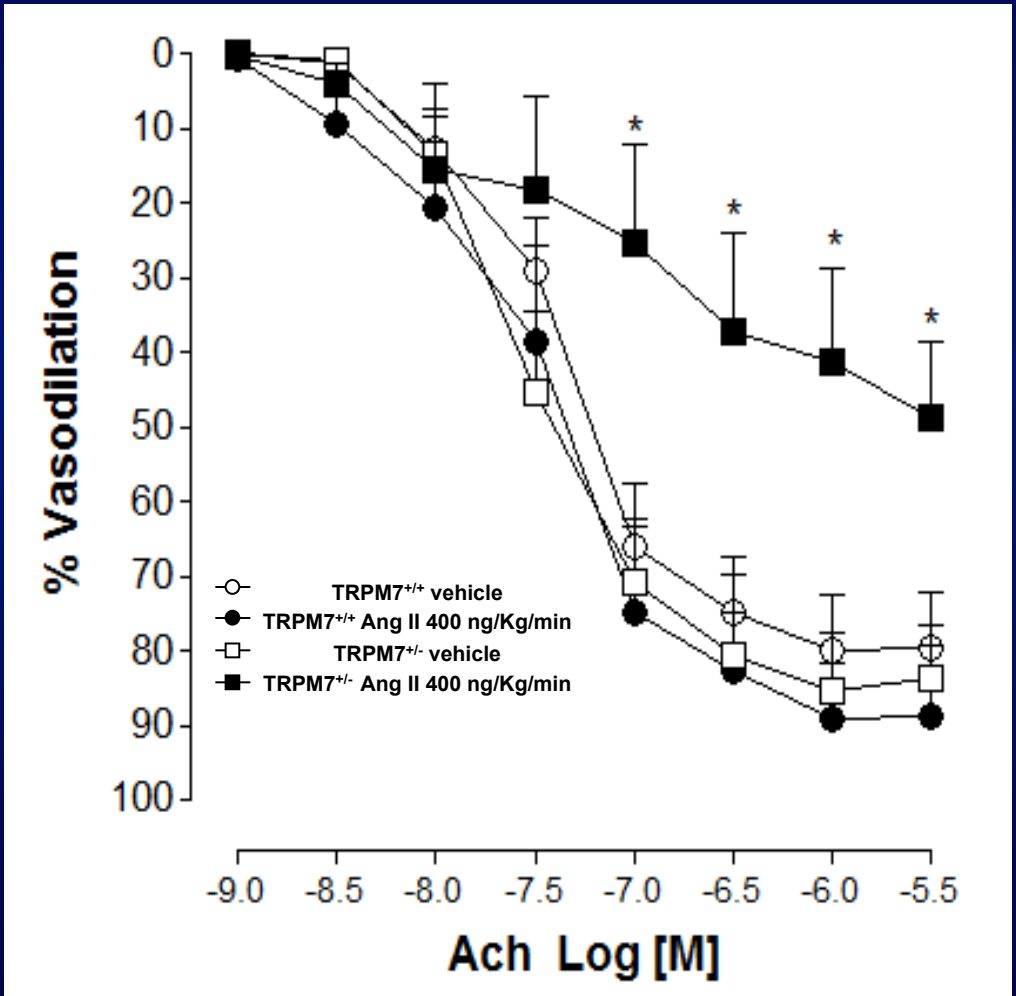
Ang II pump (Tail-Cuff)



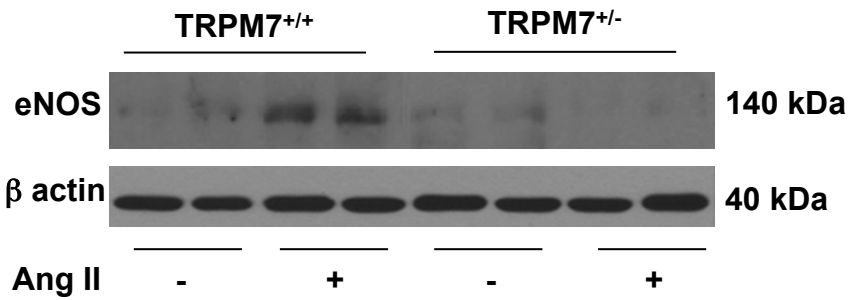
Final SBP (Day 14)

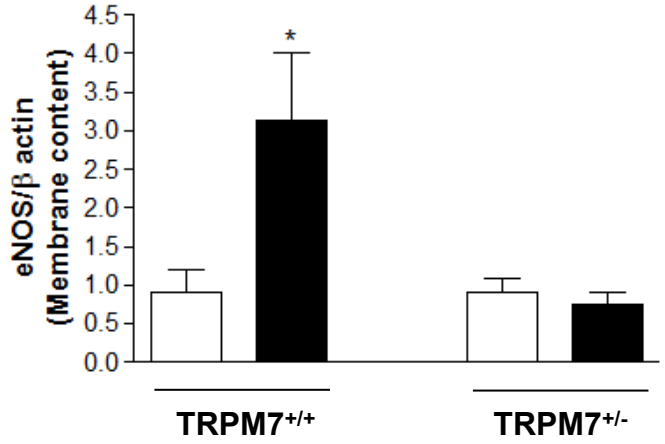


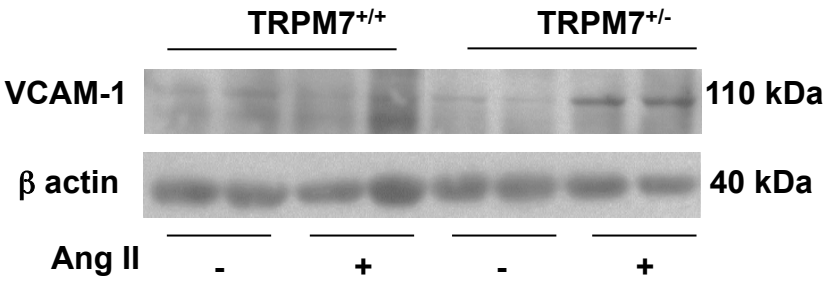
Impaired endothelial function in Ang II-infused TRPM7-deficient mice

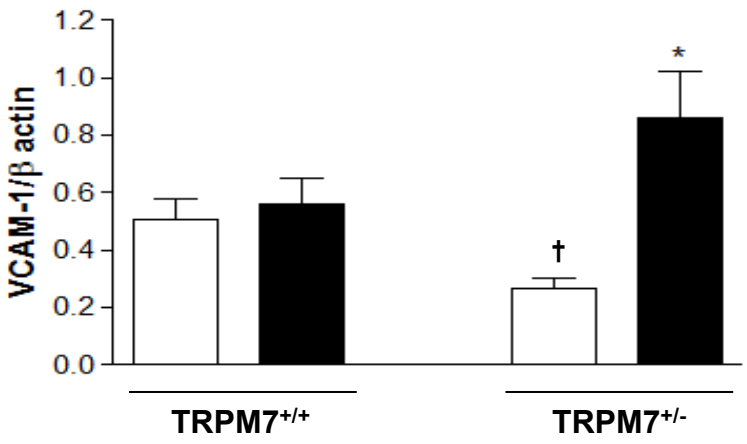


Reduced eNOS (vasodilation) and increased VCAM-1 (inflammation) in vessels from TRPM7$^{+/-}$ mice





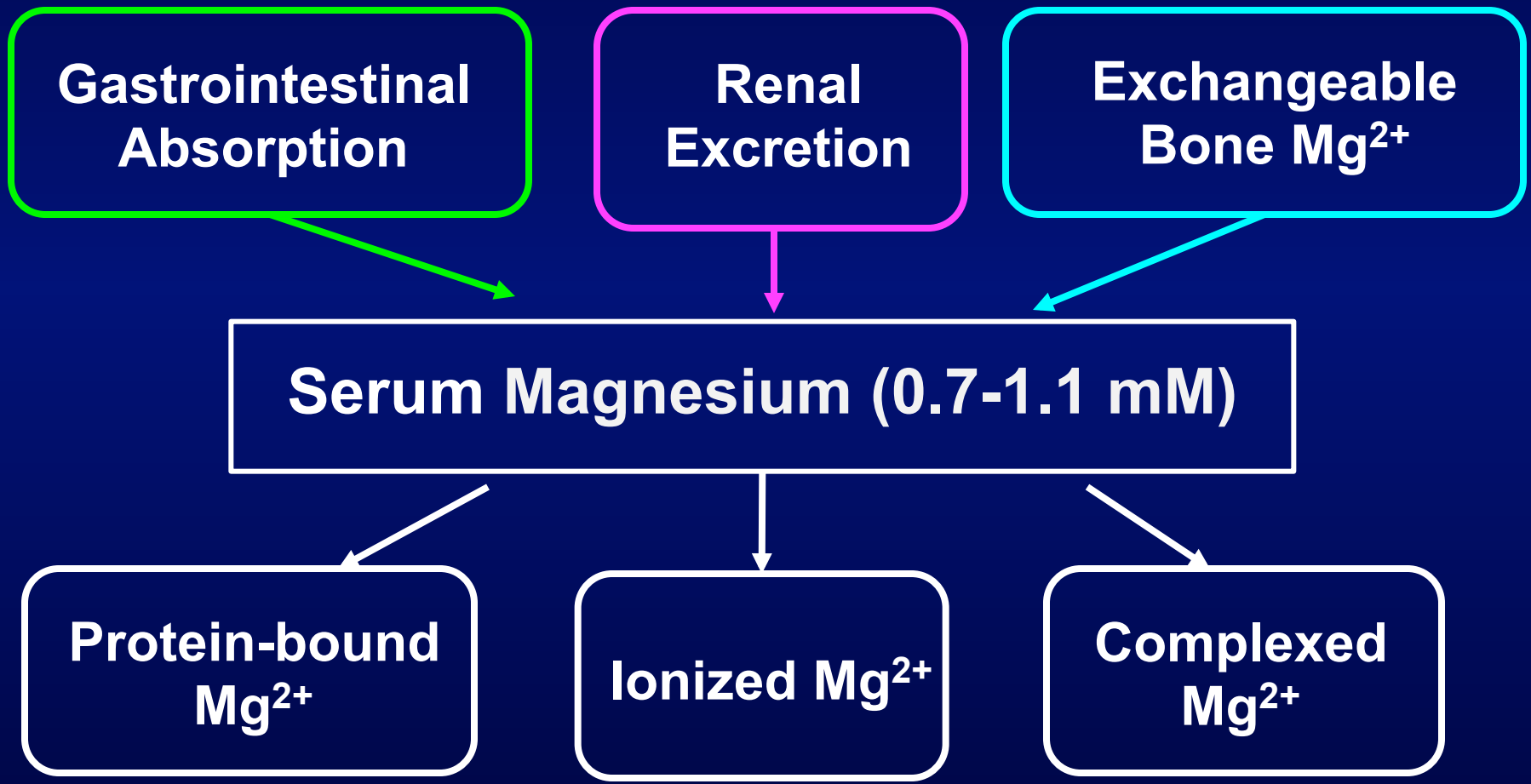




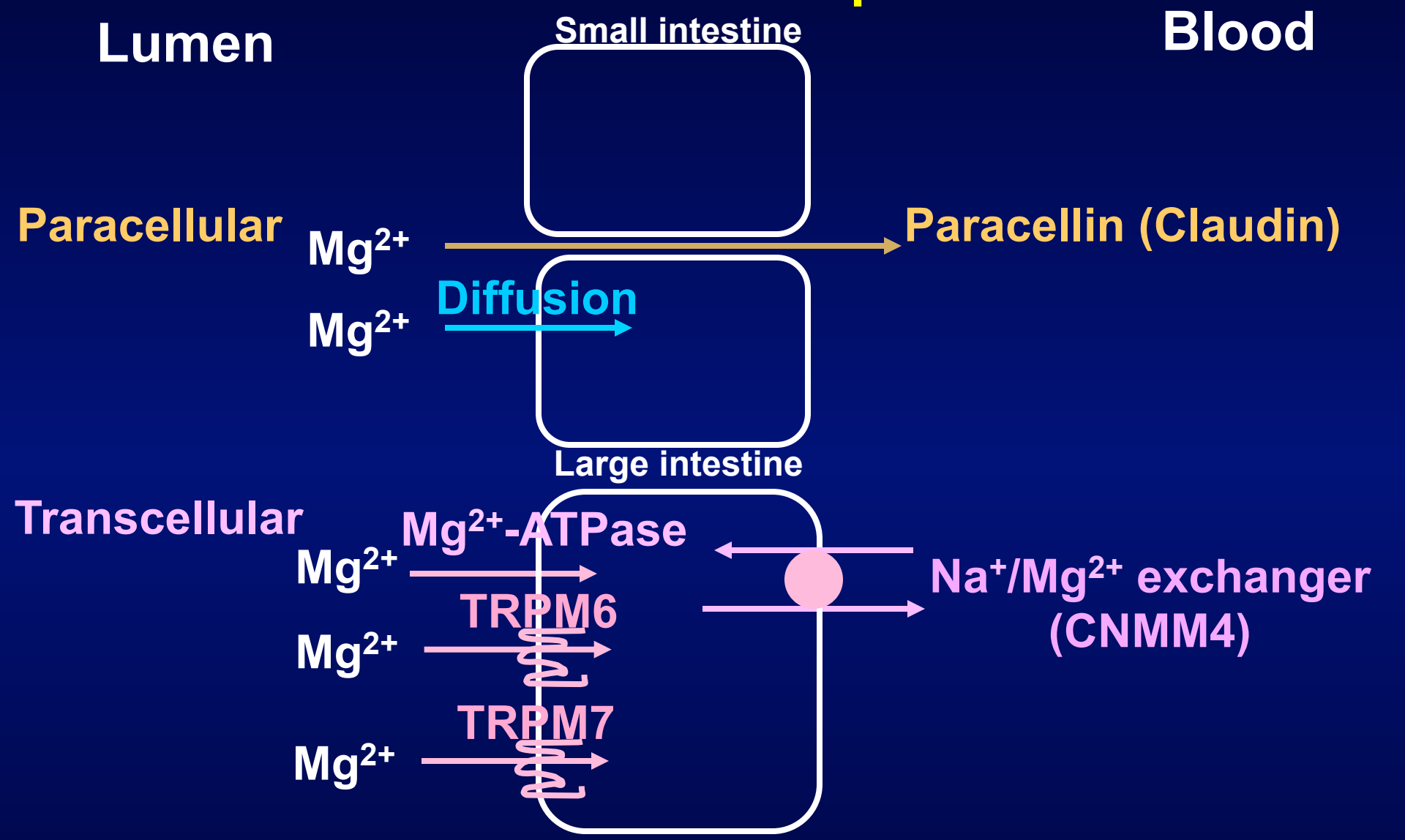
Insights from TRPM7-deficient mice

- Cell survival and viability
- Cardiac development
- Cardiac rhythmicity
- Vasodilation
- Renal function
- Blood pressure regulation

Magnesium Homeostasis in man



Intestinal Absorption



- Mg^{2+}-rich food: vegetables, nuts, cocoa.
- Phytate, fibre, alcohol inhibit Mg^{2+} absorption.
- Daily Mg^{2+} intake = ~ 300 mg/day.

Renal Magnesium Handling

- Kidney/Nephron - main regulator of Mg^{2+} homeostasis.

  - 80% filtered through glomerulus

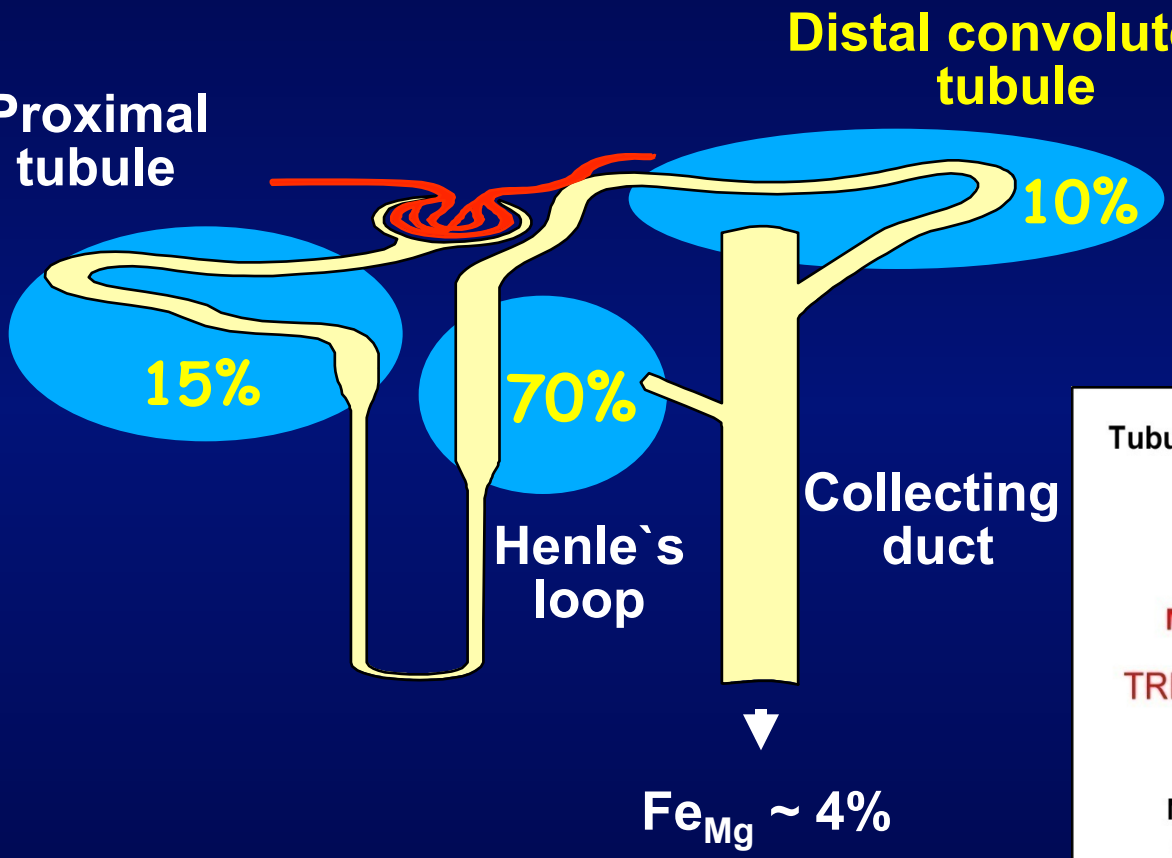
  - 15-20% reabsorbed in proximal tubule

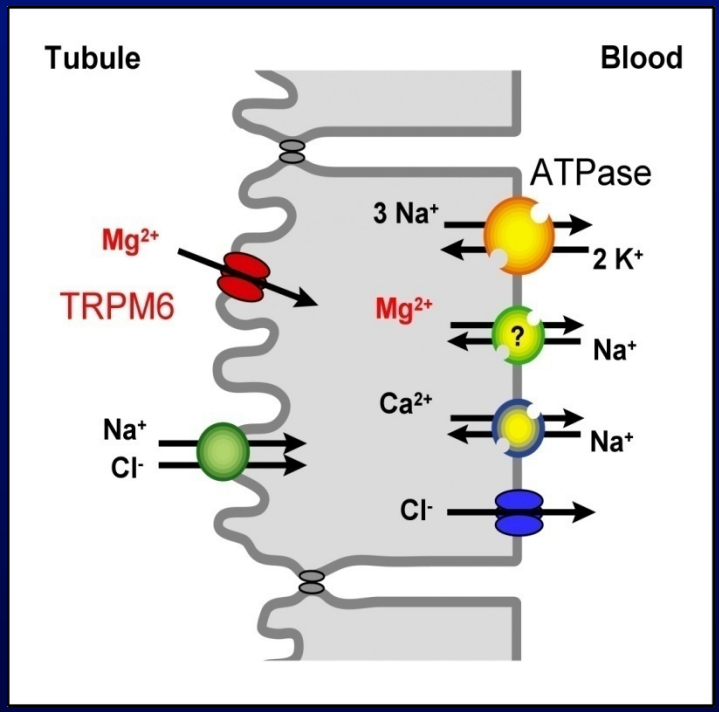
  - 65-75% reabsorbed in **TAL**

  - 5-10% reabsorbed in **distal convoluted tubule**.

- Drugs (diuretics) and hormones (PTH, insulin, aldosterone) influence Mg^{2+} excretion

Magnesium Reabsorption Along the Nephron





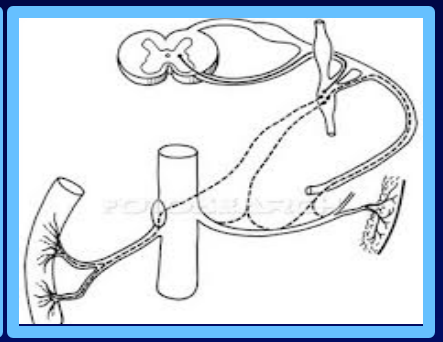
Schlingmann et al.

Magnesium Metabolism – Bone

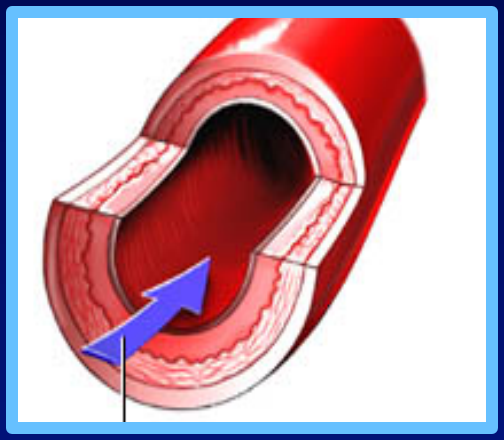
- Major Mg^{2+} reservoir is bone
- ↓ serum Mg^{2+} stimulates Mg^{2+} release from bone.
- 30% bone Mg^{2+} is exchangeable.
- Bone = buffer regulating serum Mg^{2+}.

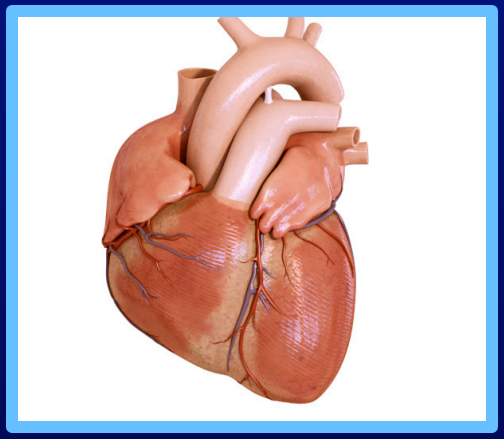
Mg^{2+} and organ function

Central and peripheral nervous system

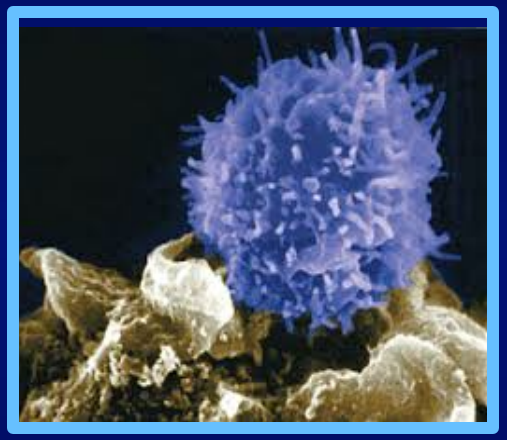


Cardiovascular system





Immune system



Musculoskeletal system

Causes of Mg^{2+} deficiency

1. Decreased dietary Mg^{2+} intake
2. Gastrointestinal malabsorption
3. Increased gastrointestinal loss
   - Diarrhoea
   - Vomiting
   - Laxative abuse

} Gastrointestinal

4. Increased renal loss
5. Congenital or acquired tubular defects
6. Drug-induced

} Renal

7. Endocrine causes
   - Hyperaldosteronism
   - Hyperparathyroidism
   - Hyperthyroidism
   - SIAD
   - Diabetes
8. Other causes
   - Alcoholism
   - Excessuive sweating
   - Severe burns

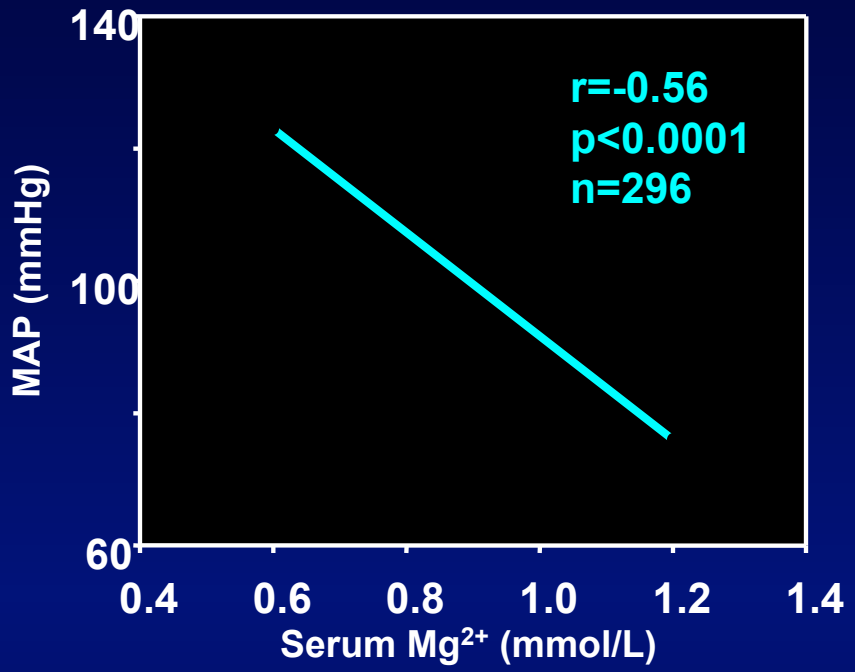
Magnesium:Drug Interactions

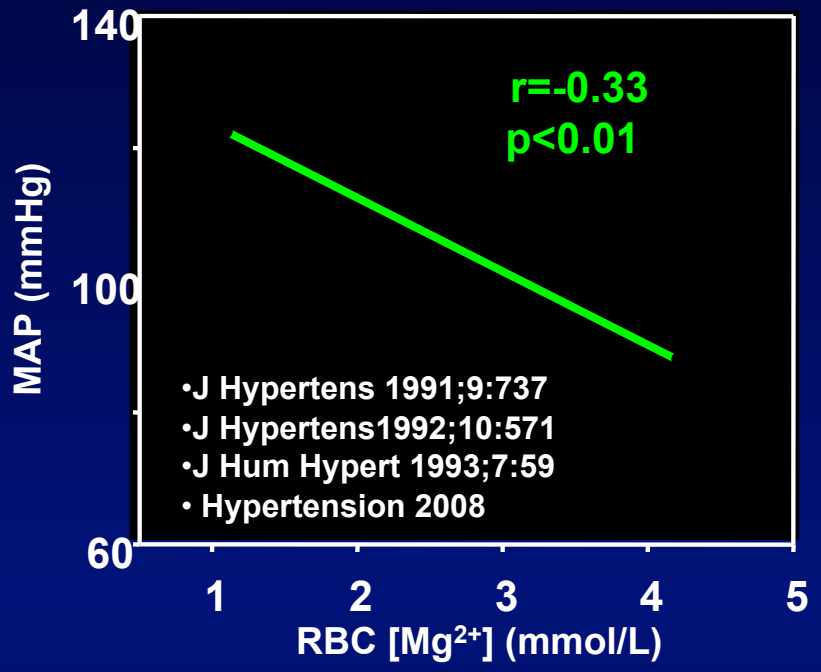
Drug	Interaction
• Diuretics	↑ renal Mg^{2+} loss
• Immunosupressants	Cyclosporine, tacrolimus ⬇ TRPM6
• Antibiotics • Tetracycline	Mg^{2+} binds tet in gut and ↓ absorption.
• Mg^{2+}-containing laxatives/antacids	Chronic use leads to hypermagnesemia
• Tyrosine kinase inhibitors EGF, VEGF, c-Src inhibitors (anti-cancer drugs)	TRPM6/7

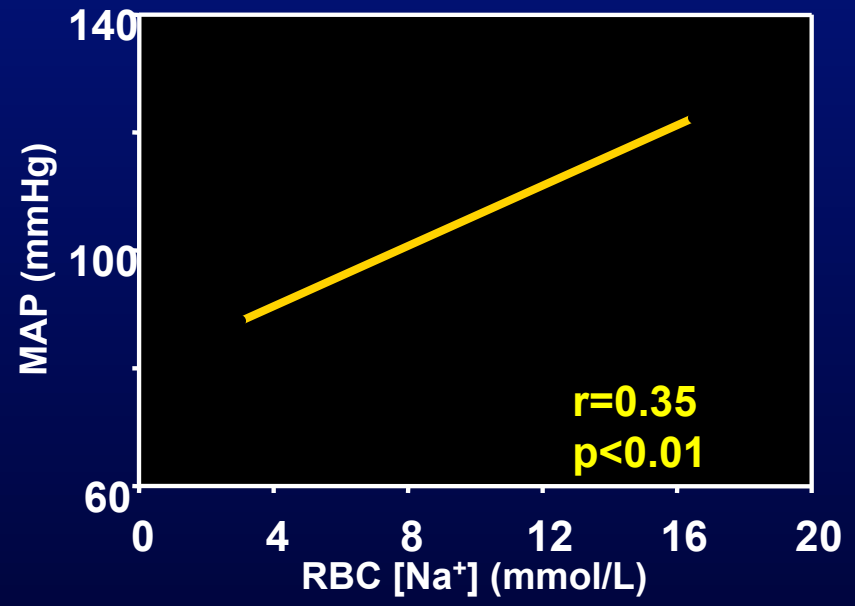
Clinical Conditions and Hypomagnesemia

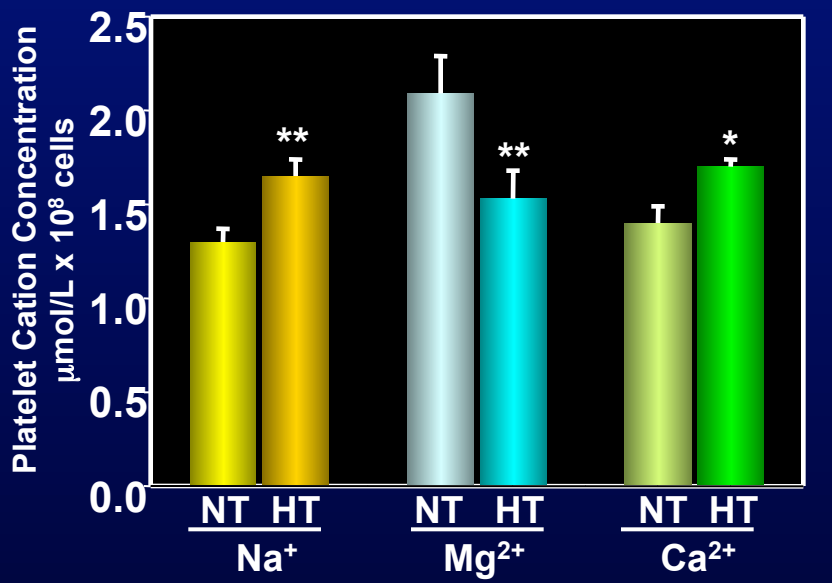
- Chronic diseases: *Hypertension,* diabetes, metabolic syndrome
- Stroke
- *Cardiac disease* (IHD, arrhythmias)
- *Pre-eclampsia* and eclampsia
- Neurodegenerative disorders
- Cancer
- Drug-induced: Cetuximab

Hypertension. Relationship Between BP and Cations



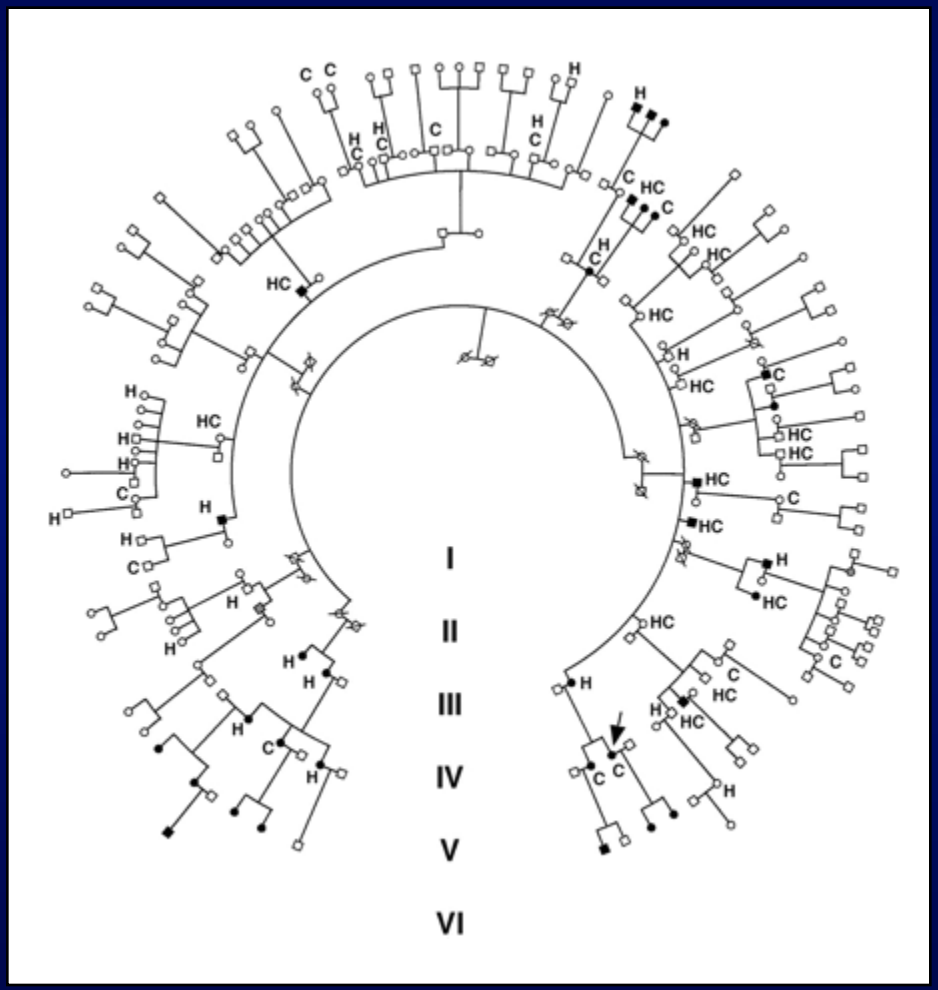






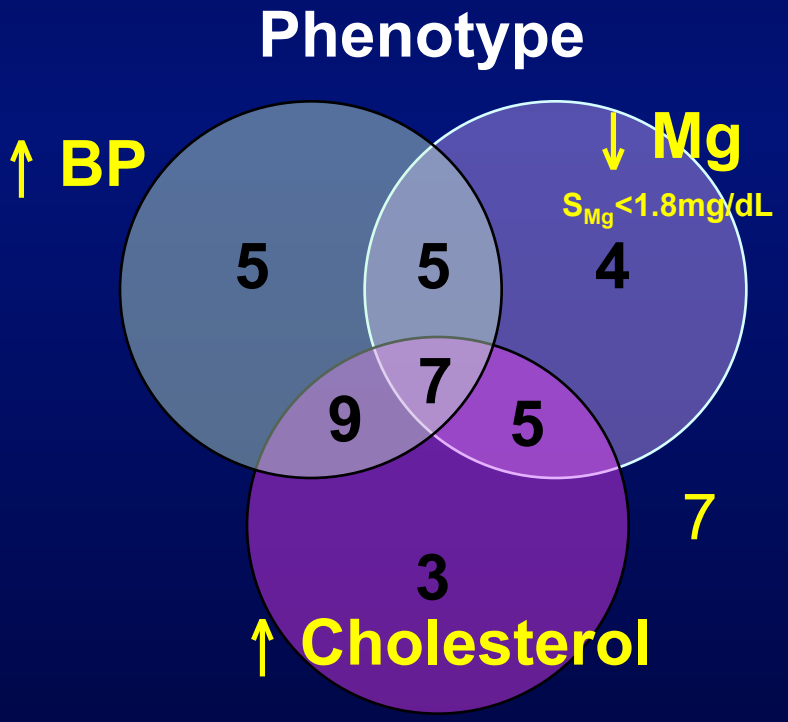
Metabolic Hypomagnesemia

Kindred 129



- transmission of phenotype exclusively by affected females

→ Mitochondrial inheritance



Wilson et al. *Science* 2004

Maternal offspring (n=45)

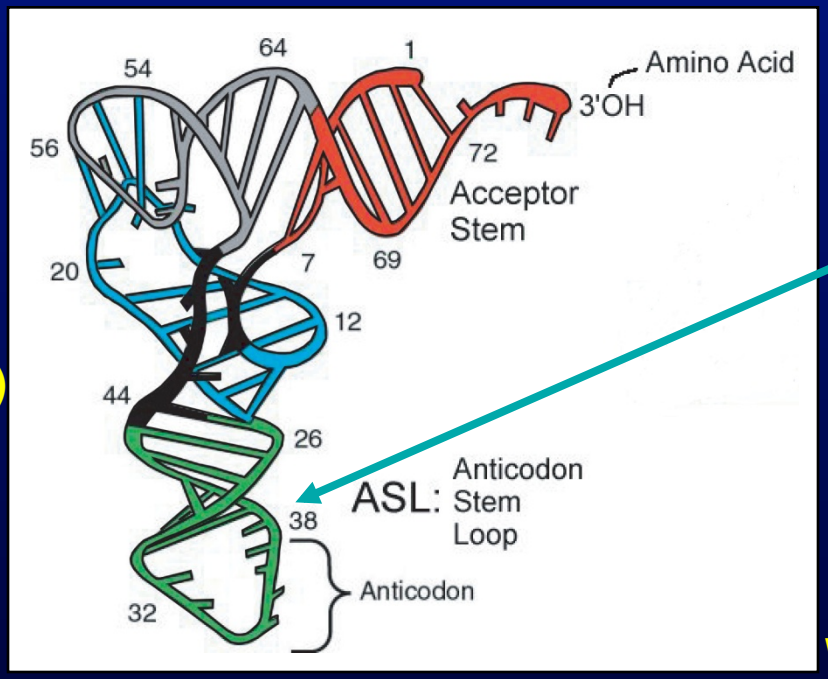
Metabolic Hypomagnesemia is Caused by a Defect in a Mitochondrial tRNA

mitoch. DNA

Homoplasmic mutation substituting cytidine for uridine

t4291c

Isoleucine-tRNA (MTTI)



Wilson et al. *Science* 2004

Mg^{2+} and clinical hypertension: Conflicting data

- Lack of association between serum Mg^{2+} and risks of HT and CVD. Framingham study. *(Khan. Am Heart J. 2010;160)*
- Hypomagnesemia is one of the strongest predictors of gain in LVM over 5 years. *(Reffelmann. Atherosclerosis. 2010. 213).*
- Most clinical studies fail to demonstrate BP-lowering effects of Mg^{2+}.
- Intravenous $MgSO_4$ vs. inhaled NO for moderate, persistent pulmonary hypertension of the newborn. *(Raimondi J Trop Pediatr. 2008;54)*
- *BP lowering in mild HT with Mg- salt replacement (Sarkkinen, Nut J 2011l10)*
- *BP lowering in HT (Kisters)*

Subgroup of Patients who may Benefit from Mg^{2+} Supplementation

- African Americans
- Elderly
- Insulin resistance/metablic syndrome
- Patients on diuretics
- Hypomagnesemic patients
- Patients resistant to therapy
- Severe or malignant hypertension
- Pre-eclampsia.

Magnesium and Pre-eclampsia/Eclampsia

- Mg^{2+} improves endothelial function in pre-eclampsia: ↑prostacyclins, EDRF and ↓platelet activation.
- Mg^{2+} infusion ↓ BP, ↑ renal blood flow and reduces peripheral resistance.

Magpie Trial

10,141 women with pre-eclampsia in 175 hospitals, in 33 countries, showed Mg^{2+} sulphate decreased BP and significantly reduced risk of eclampsia.

(Lancet 2002;359:1877-1890).

Stroke

- Health professional Follow-Up Study: inverse association between Mg^{2+} intake and stroke.

- Mg^{2+} is neuroprotective:
  - blockade of NMDA receptors
  - enhanced cerebral blood flow
  - inhibition of Ca^{2+} influx.

Stroke Trials

- **Intravenous Magnesium Efficacy in Stroke trial (IMAGES) (Lancet 2004;363)**

  Results: Mg^{2+} given within 12 h of acute stroke does not reduce chances of death or disability significantly, although it may be of benefit in lacunar strokes.

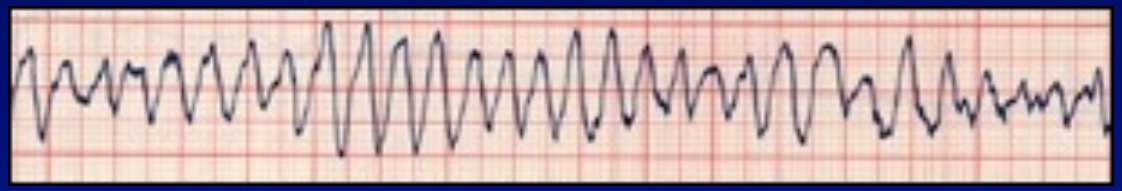
- **Intravenous MgSO4 for aneurysmal subarachnoid hemorrhage (IMASH) trial.**

  (Wong. Stroke 2010;41)

  Results: No clinical benefit.

Magnesium and Ventricular Arrhythmias

- **Torsades de Pointes.** Ventricular arrhythmia associated with prolonged QT syndrome



- Mg^{2+} is the treatment of choice (AHA Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiac Care).
- Recommended dose: 2 g $MgSO_4$ (8 mmol)/ 10 mins, repeated if necessary.

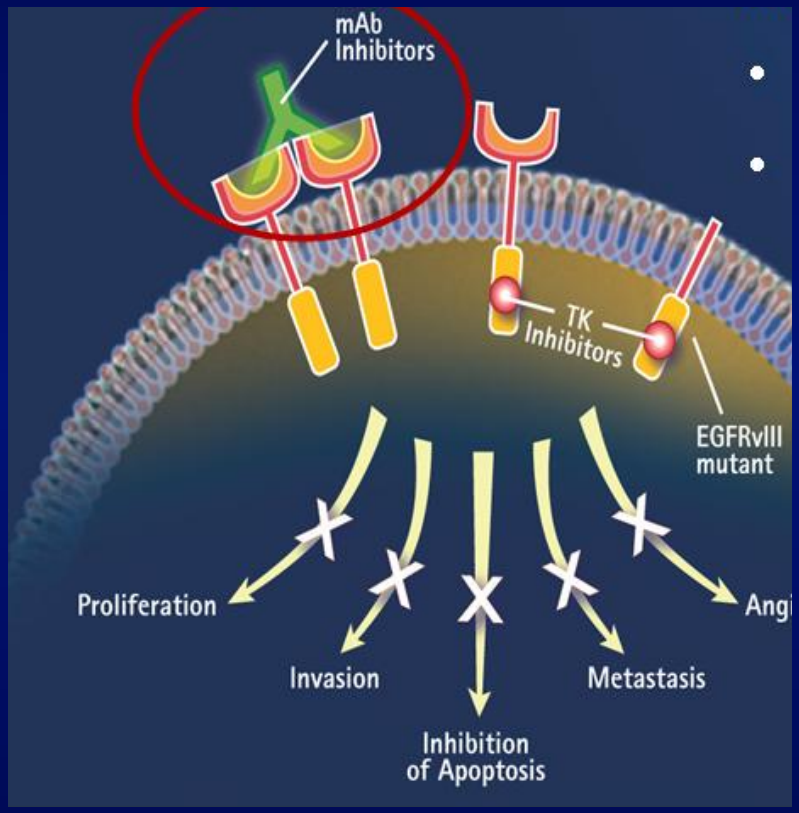
Clinical trials and magnesium

• Preeclampsia	MAGPIE*
• Asthma	MAGNETIC
• Stroke	FAST-MAG, IMAGES
• MI	MAGIC, LIMIT-2, ISIS-2
• Subarrachnoid hemorrhage	IMASH, MASH II

*Mg^{2+} showed benefit

Cetuximab

- Chimeric monoclonal antibody selective for EGFR.
- FDA approved for metastatic colorectal cancer.
- Adverse events:
- rash, diarrhoea, fatigue, neutropenia, hypertension, severe hypomagnesemia
- Meta-analysis: hypomagnesemia in 97% patients.

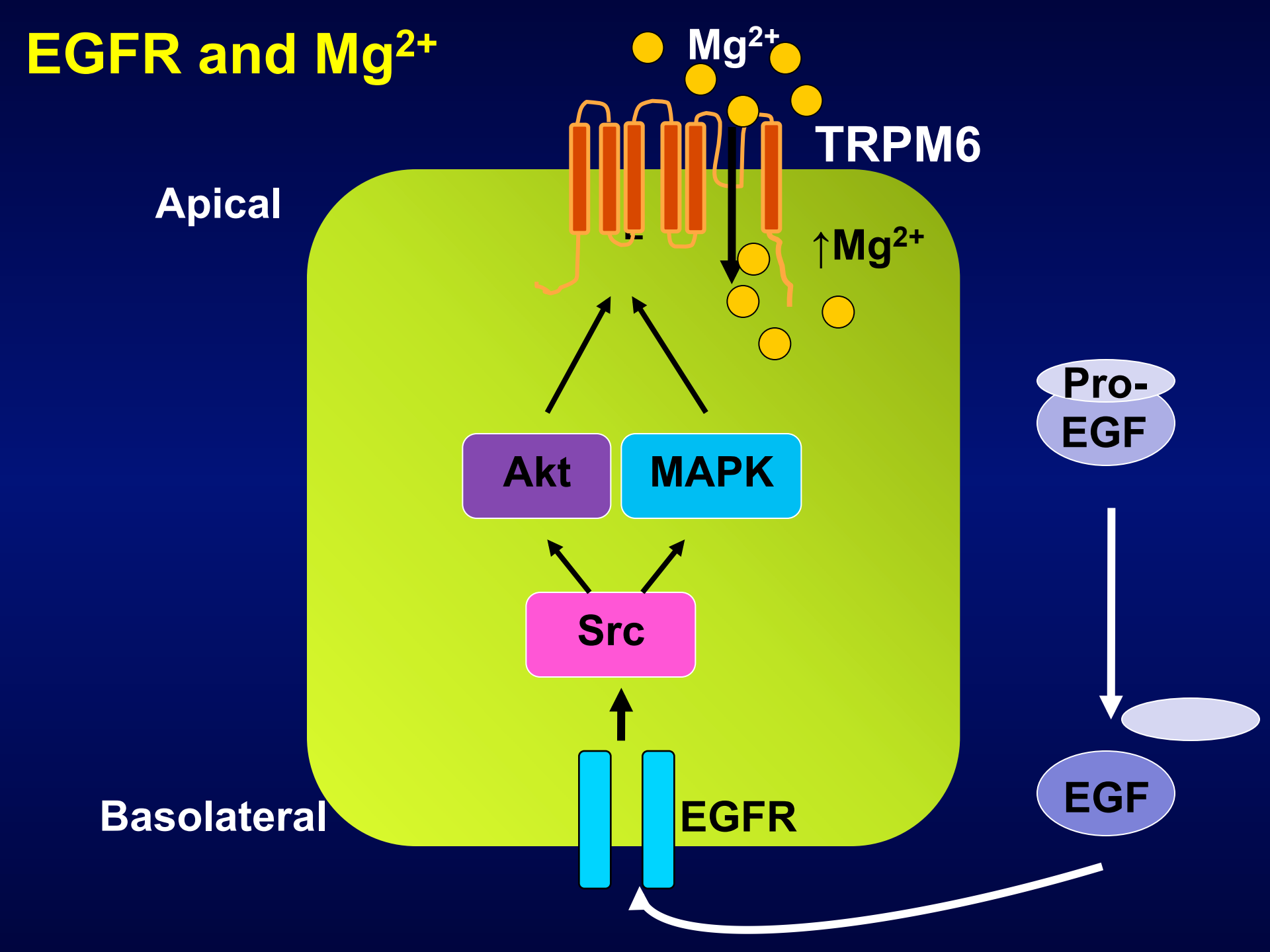


Cetuximab-induced hypomagnesemia

- Due to renal Mg^{2+} wasting
- Class effect – all EGFR monoclonal Abs .
- Normalization when cetuximab stopped.
- Rx – daily IV Mg^{2+} (weekly Rx ineffective).
- Monitor serum Mg^{2+}
- Early hypomagnesemia = surrogate marker of cetuximab efficacy. (Vincenzi. Ann Oncol 2011;22)

Molecular Mechanisms

- Isolated autosomal recessive renal hypomagnesemia due to **EGF gene mutation**. (Groenestege 2007)
- Mutation causes impaired basolateral sorting of pro-EGF and reduced activation of EGFR (~ EGFR inhibition by cetuximab).
- So what is the relationship between EGFR, cetuximab and Mg^{2+}?.


EGFR and Mg2+
Mg2+
TRPM6
Apical
↑Mg2+
Pro-
EGF
Akt
MAPK
Src
EGF
Basolateral
EGFR

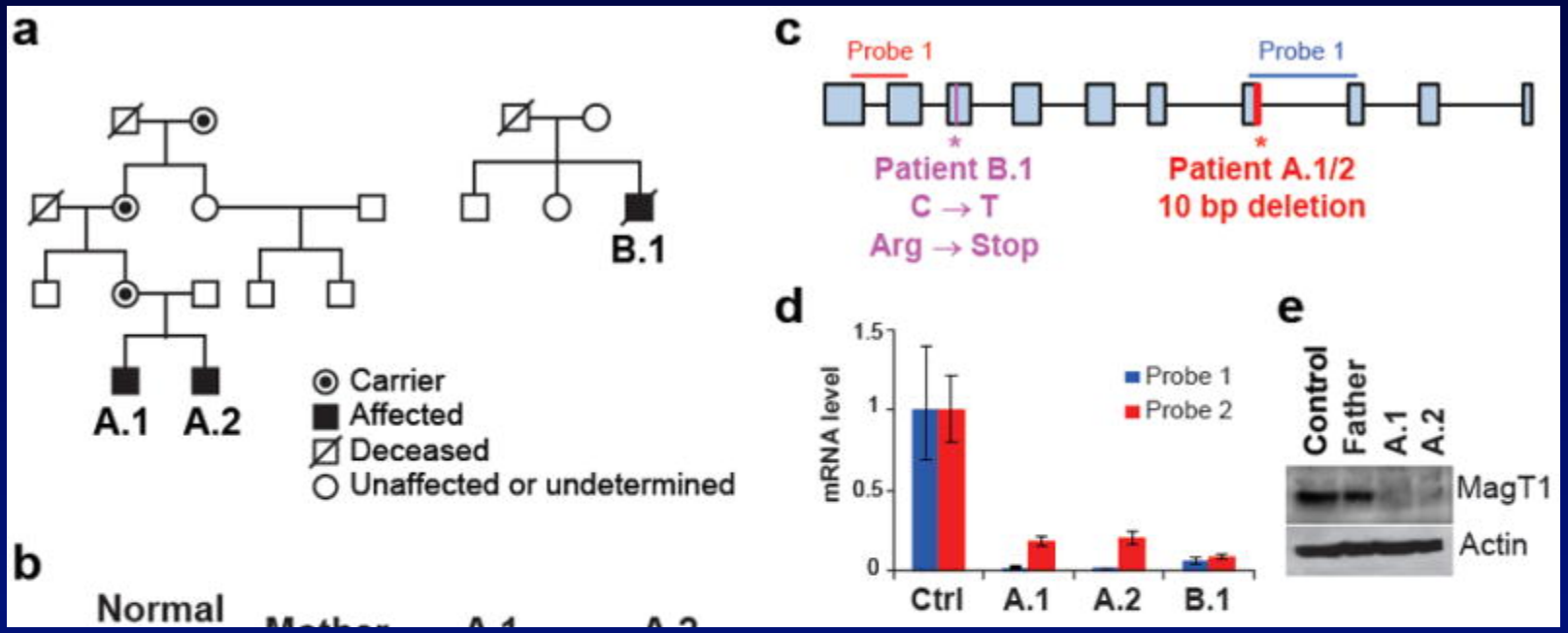
TRPM6 and Cetuximab

- EGFR inhibition by cetuximab leads to decreased activation of TRPM6.
- ↓TRPM6 activation leads to ↓ Mg^{2+} reabsorption and consequent hypomagnesemia.
- Other tyrosine kinase inhibitors????

MagT1

- Membrane protein with 5 transmembrane domains
- Contains a N-linked glycosylation site
- N-terminal region contains 4 cAMP-dependent protein kinase phosphorylation sites.
- Gene located on X chromosome
- Regulated by extracellular Mg^{2+}
- Selective Mg^{2+} transporter

  – Goytain and Quamme. BMC Genomics. 2005:6:48



- **Mutations in MAGT1, in a novel X-linked human immunodeficiency characterized by CD4 lymphopenia, viral infections and defective T-lymphocyte activation.**
- **Transient Mg^{2+} influx is induced by antigen receptor stimulation in normal T cells and by growth factor stimulation in non-lymphoid cells.**
- **MAGT1 deficiency abrogates the Mg^{2+} influx, leading to impaired responses to antigen receptor engagement.**

Clinical Assessment of Mg^{2+} Status

Clinical challenges

- Mg^{2+} is an intracellular cation
- No lab test tracks total body Mg^{2+} levels.
- Changes in serum Mg^{2+} do not reflect intracellular levels.

Clinical assessment

- Serum Mg (total vs ionized) (photometry, atomic absorption spectroscopy).
- Metabolic studies: Mg^{2+} loading
- Probes, fluorescence markers - research

Manifestations of Magnesium Deficiency

Cardiac Manifestations
- atrial fibrillation
- ventricular arrhthmias
- Torsades de pointes
- Hypersensitivity to cardiac glycosides

Neurological Manifestations
- convulsions
- nystagmus
- athetoid movements
- apathy
- delirium, coma

Neuromuscular Manifestations
- positive Chvostek's sign
- positive Trousseau's sign
- tetany, muscle cramps
- muscle fasciculations and tremor
- muscle weakness

Electrolyte disturbances
- hypokalemia, hypocalcemia

Immunodeficiency

Conclusions

- Magnesium plays a key role in regulating physiological processes.
- Body magnesium is regulated by gut, bone, kidneys.
- Cellular Mg^{2+} is tightly regulated
- TRPM6/7 and MagT1 are major transcellular Mg^{2+} transporters.
- Assessment of Mg^{2+} in the clinic is challenging

Conclusions

- Hypomagnesemia - underdiagnosed
- Renal Mg^{2+} wasting disease due to mutations in TRPM6, paracellin 1, MagT1, NCC, Na^{+}/K^{+} ATPase.
- Hypermagnesemia is caused by laxative and antacid overuse, especially in patients with renal failure.
- Mg^{2+} not recommended in the standard treatment of hypertension, IHD, stroke, diabetes.
- Conditions in which Mg^{2+} is recommended as Rx:

  - Torsades de Pointes; Eclampsia.

- Cetuximab is associated with hypomagnesemia
- To date large clinical trials of Mg^{2+} have been negative.

Moving forward in Mg^{2+} research

- Better understanding of basic mechanisms of cell biology and signaling of Mg2+.
- Elucidate mechanisms of Mg2+ regulation.
- Therapeutic targeting of Mg2+ transporters and regulators.
- Better tools to assess Mg2+ in basic and clinical research.

- Exciting time for Mg2+ research

Magnesium in Translational Medicine
Smolenice Castle, May 2014

USA
UK
Germany
Italy
France
Japan
Slovakia
Finland
Romania
Spain
Netherlands

Mg^{2+}