Cellular and Molecular Biophysics

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UNIVERSITÀ DI TORINO

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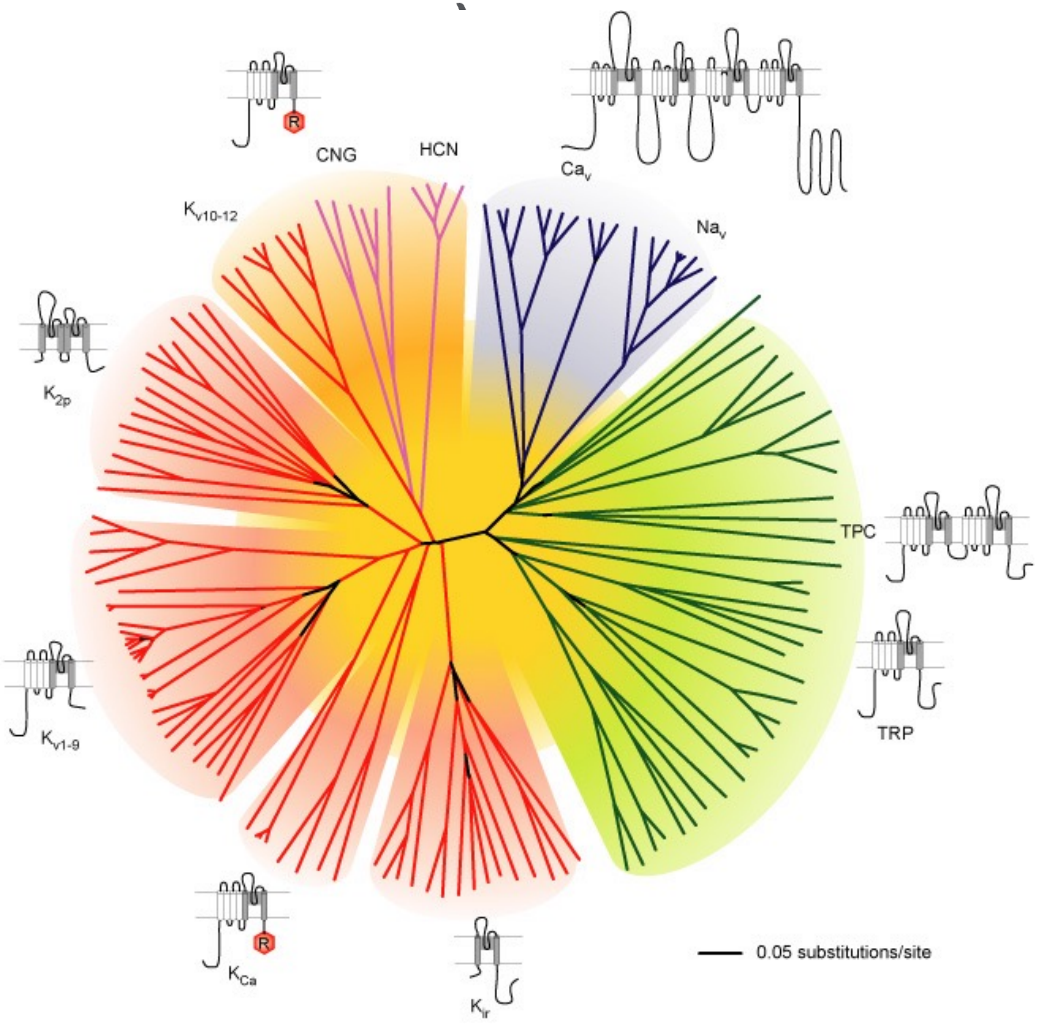
Corso di laurea in LM Biotecnologie Industriali- LM Fisica

Department of
Life Sciences
and Systems Biology

pH sensitive ion channels and role in tumor progression



pH sensitive ion channels and role in tumor progression

- INTRODUCTION TO PH
- ACIDIC TUMOR MICROENVIRONMENT
- PH-SENSITIVE ION CHANNELS
- PH SENSITIVE ION CHANNELS AND ROLE IN TUMOR PROGRESSION

What is pH?

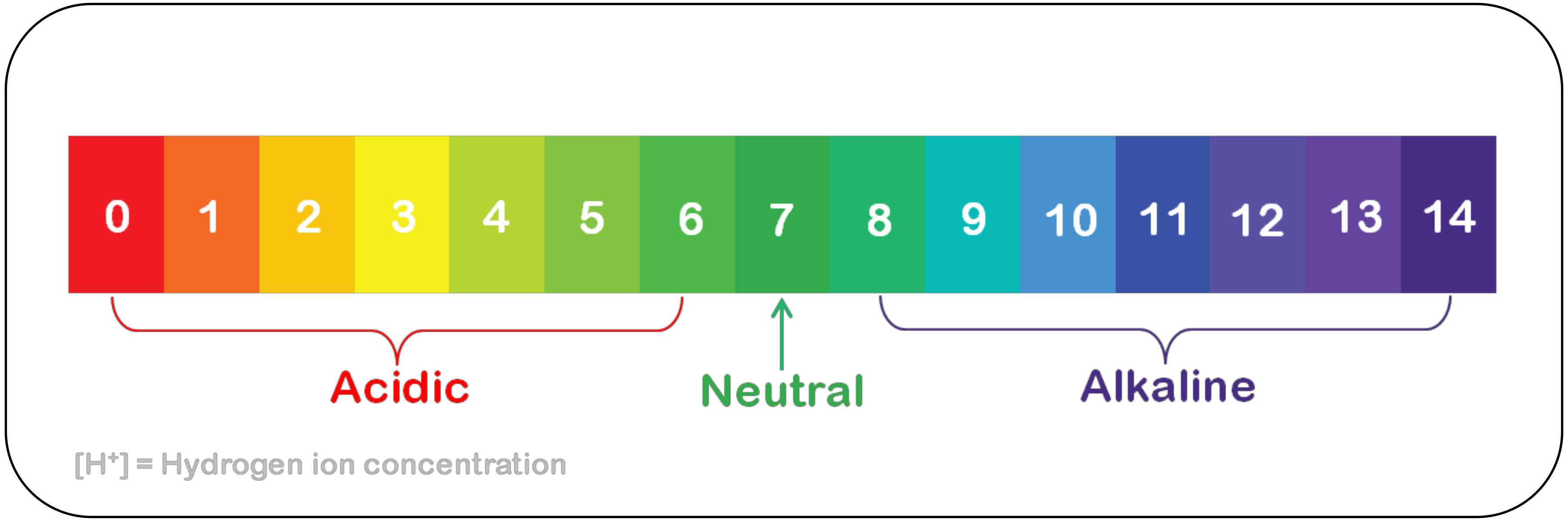
Hydronium ions: $[\mathbf{H_3O}^{+}]$

1) Dissociation product of water: $2\,H_2O \rightleftarrows H_3O^{+} + OH^{-}$

2) Present in very low concentrations (10^{-7} M)

3) High mobility

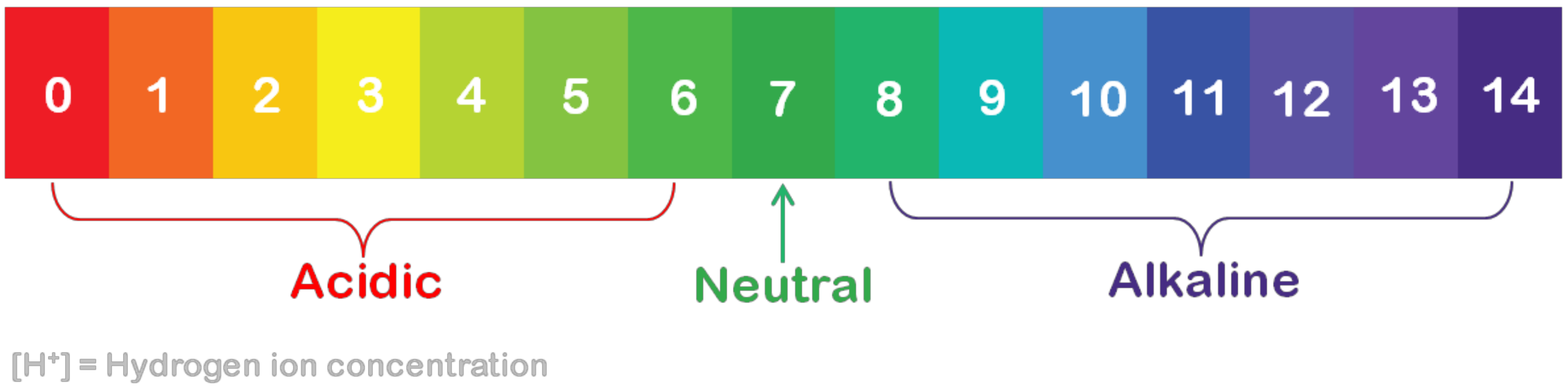
What is pH?



Sörenson (1909):

$$\boxed{pH = -\log [H_3O^+]}$$

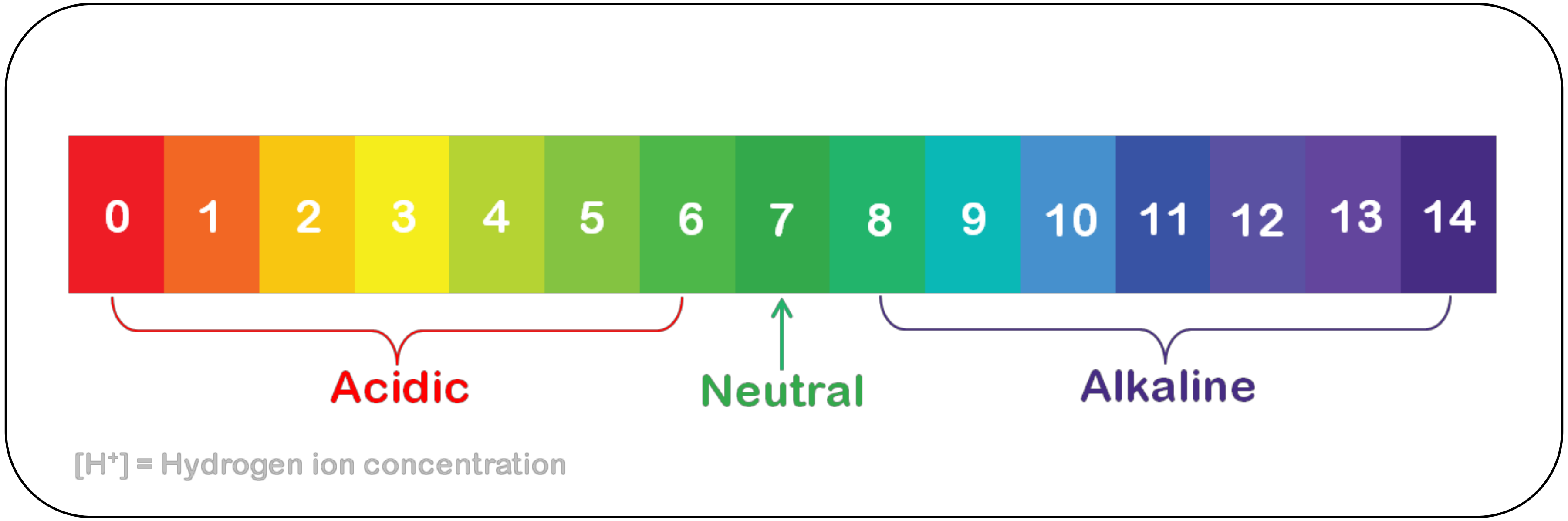
What is pH?



Sörenson (1909):

$$pH = - \log [1 \times 10 - 5]$$

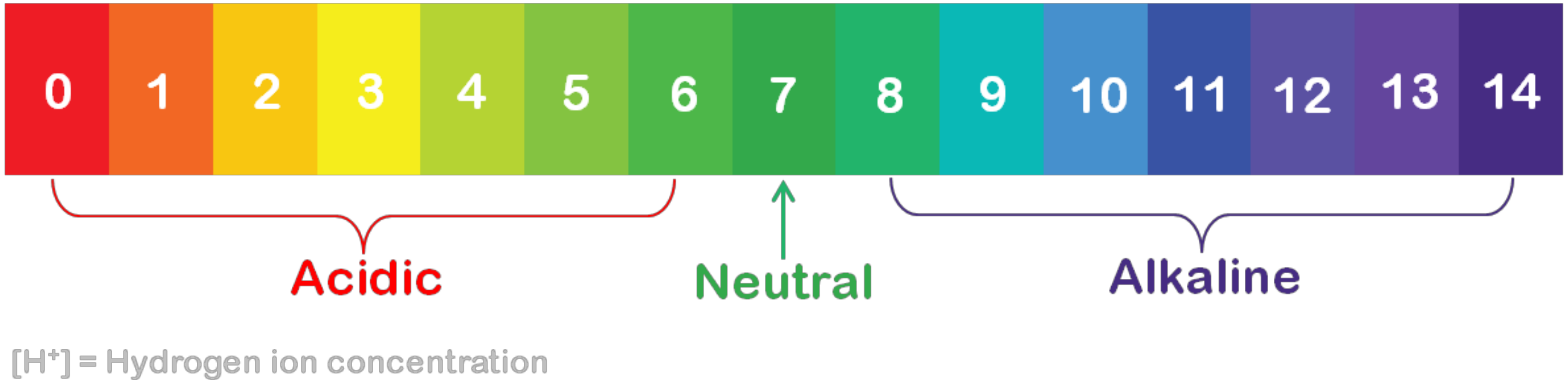
What is pH?



Sörenson (1909):

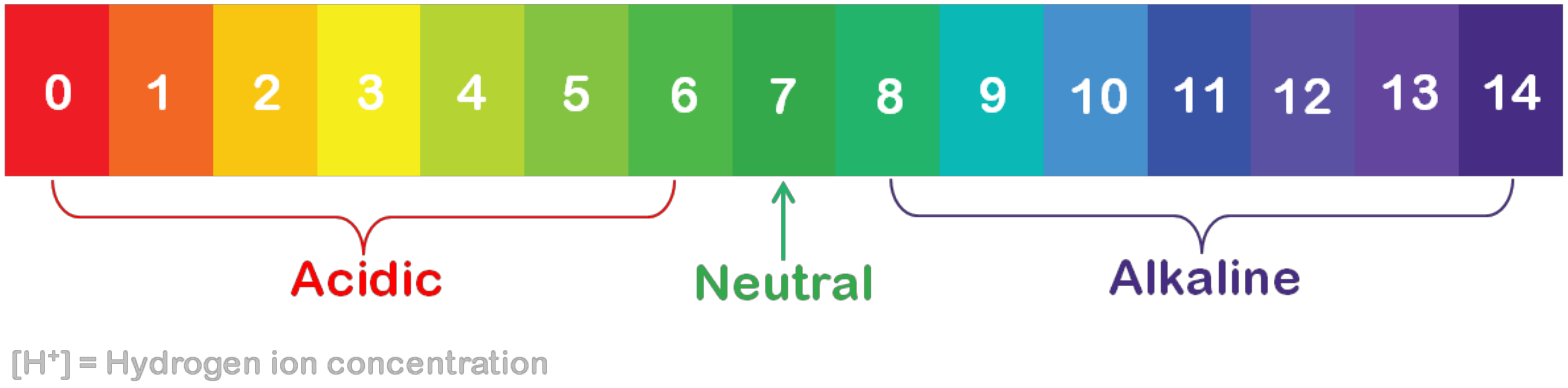
$$\boxed{pH = -(-5.0) = 5.0}$$

What is pH?



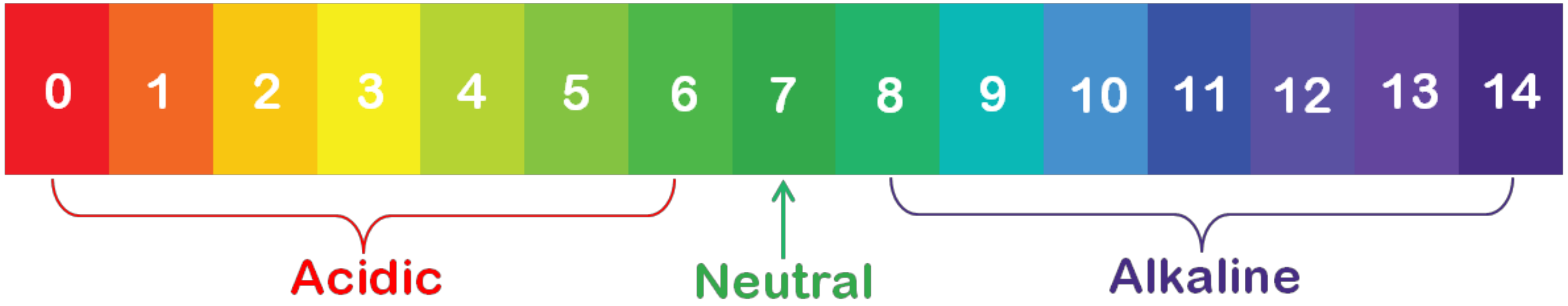
pH $[H_3O^+]\uparrow$

What is pH?



$$pH\downarrow \qquad [H_3O^+]\uparrow$$

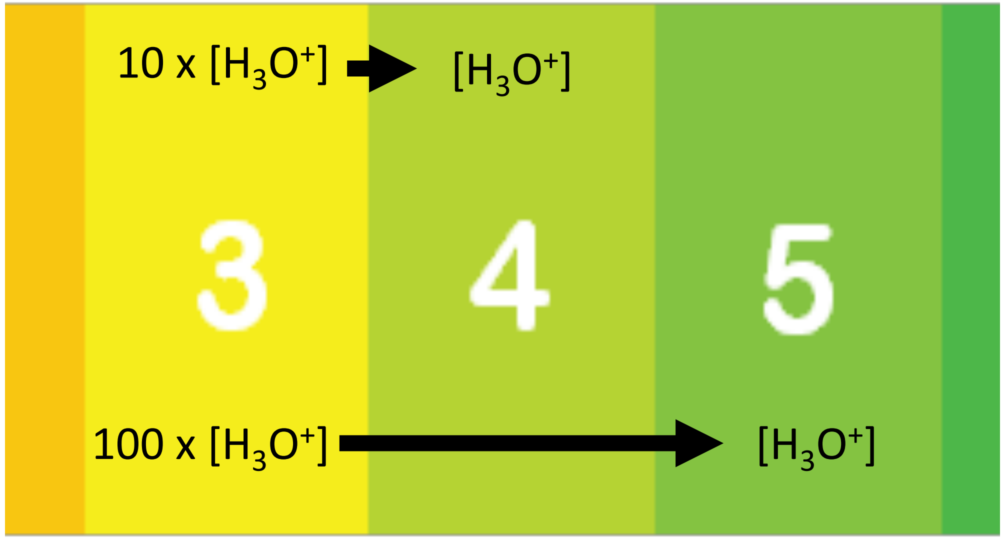
What is pH?



$[H^+]$ = Hydrogen ion concentration

$\pm$ 1 pH unit $\longrightarrow$ $\pm$ 10 x $[H_3O^+]$

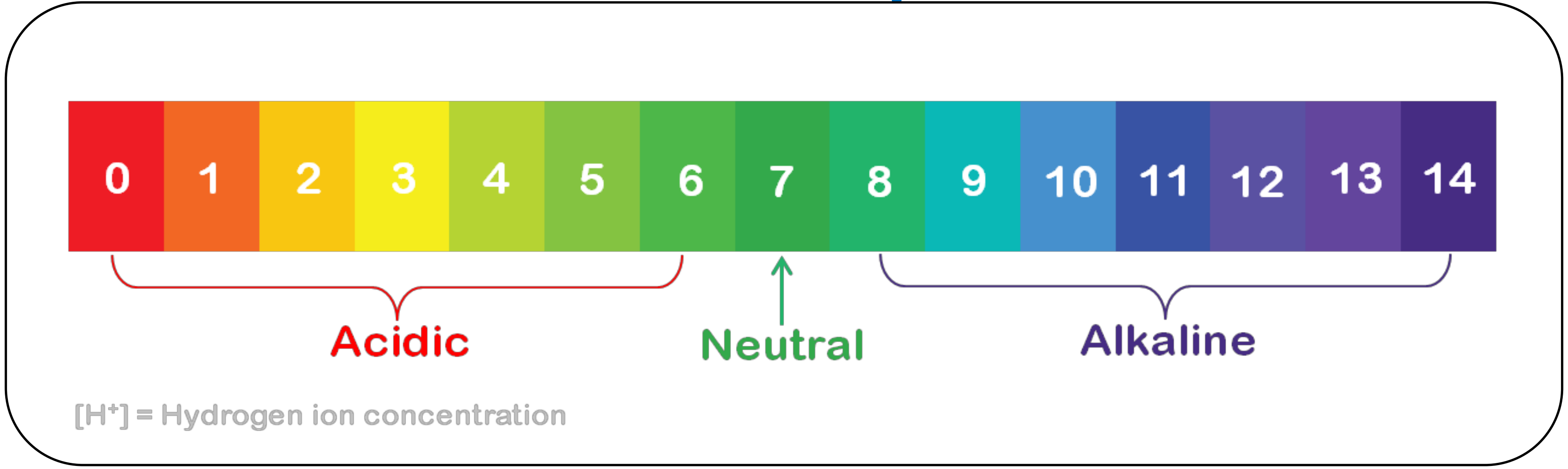
What is pH?



± 1 pH unit

± 10 x [H_3O^+]

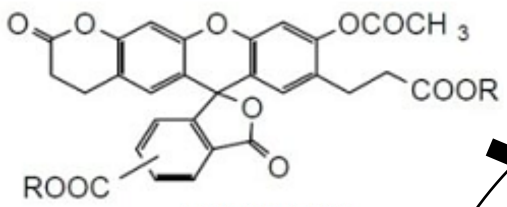
What is pH?



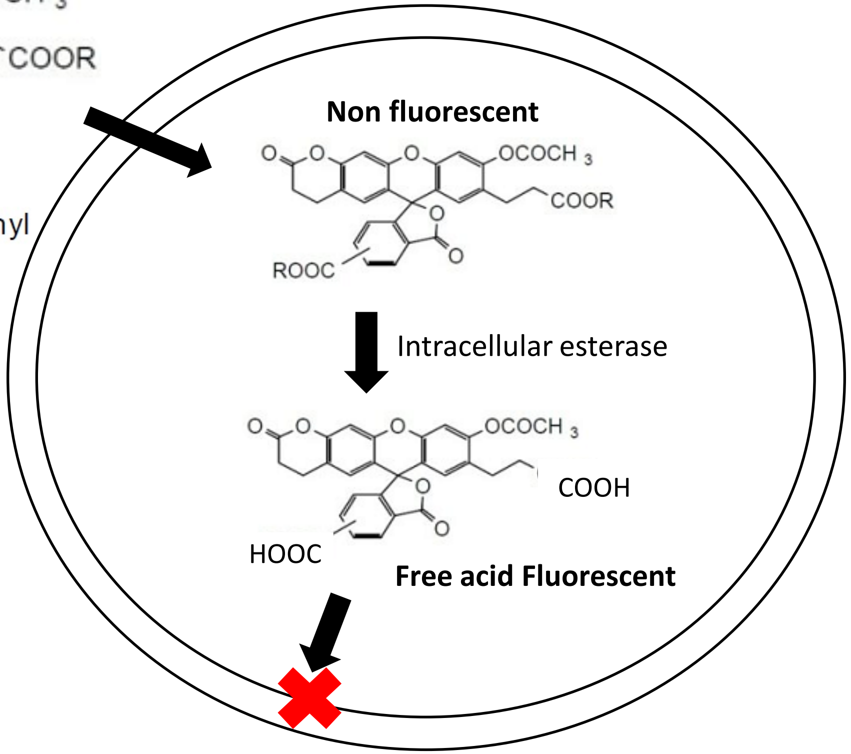
Acidic	$[H_3O^+]$	>	$[OH^-]$	$\longrightarrow$ pH < 7
Basic	$[H_3O^+]$	<	$[OH^-]$	$\longrightarrow$ pH > 7
Neutral	$[H_3O^+]$	=	$[OH^-]$	$\longrightarrow$ pH = 7

Intracellular pH in normal cells: measurement with pH indicators

Non fluorescent



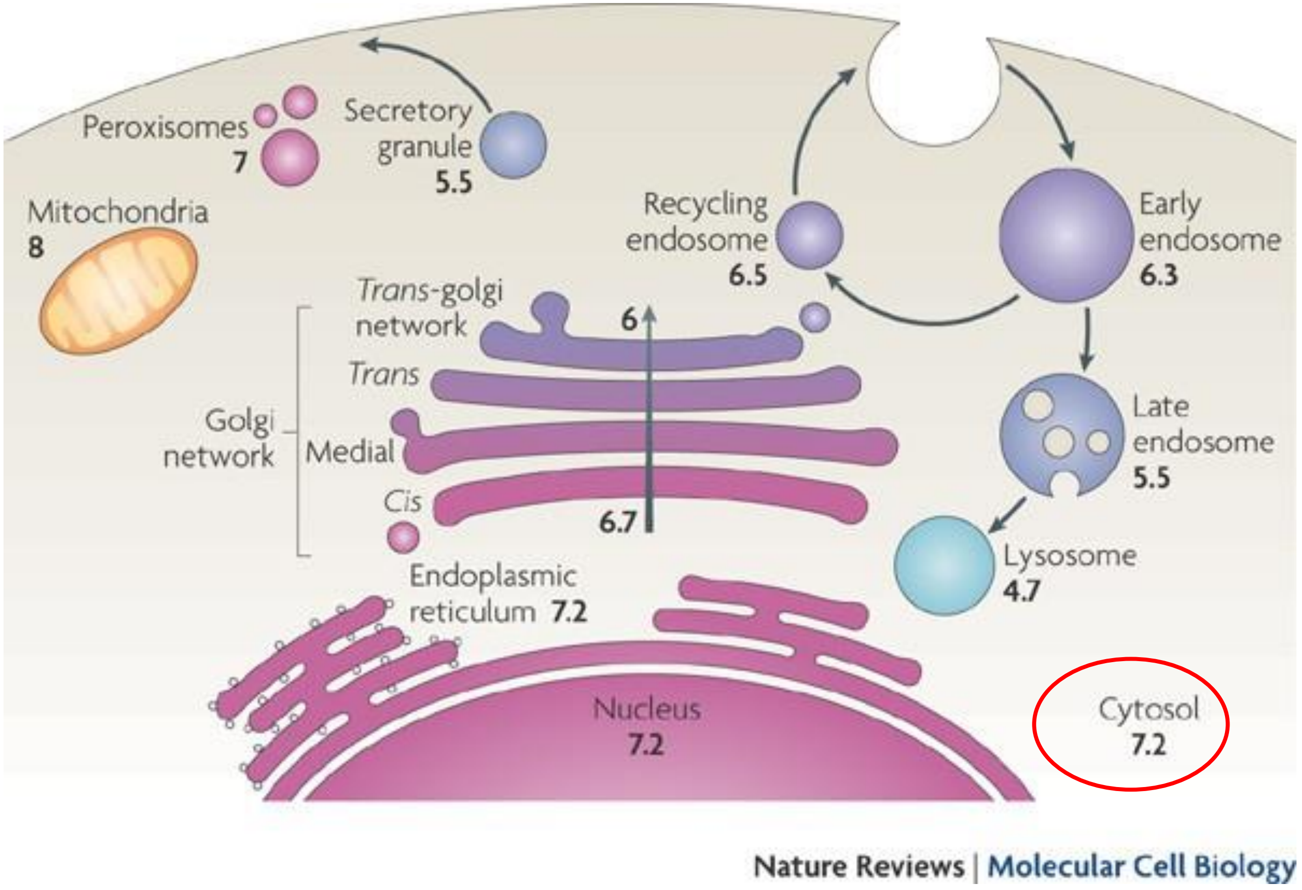
BCECF-AM
R: acetoxymethyl



pH indicators: **BCECF, AM**

- Ratiometric probe (dual-excitation at 490 nm and 440 nm, detection at 535 nm)
- Cell permeant in ester form
- pH range from 6.2 to 9.5

Intracellular pH in normal cells



From Casey J.R. et al., Nat Rev Mol Cell Biol, 2009

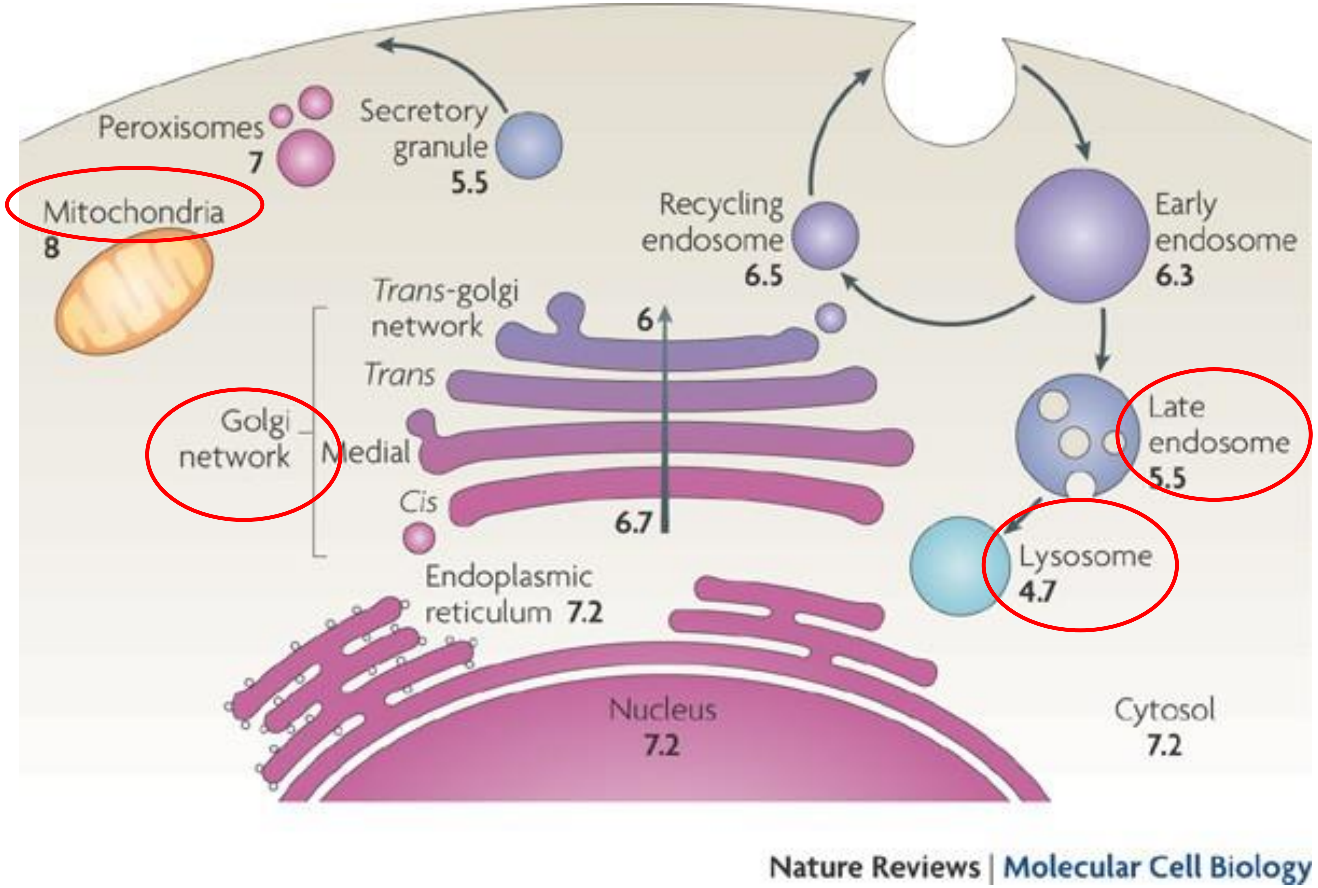
$pH_i = 7.2$

$pH_e = \sim 7.4$

pH indicators: BCECF, AM

- Ratiometric probe (dual-excitation at 490 nm and 440 nm, detection at 535 nm)
- Cell permant
- pH range from 6.2 to 9.5

Intracellular pH in normal cells



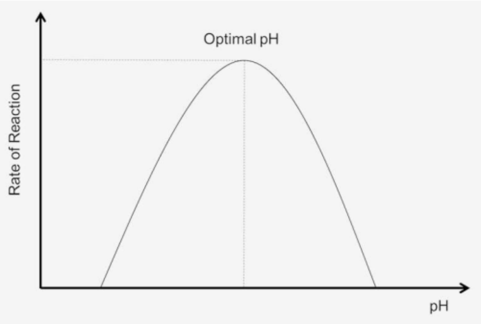
pH_i = 6.8 – 7.2

pH_e = ~ 7.4

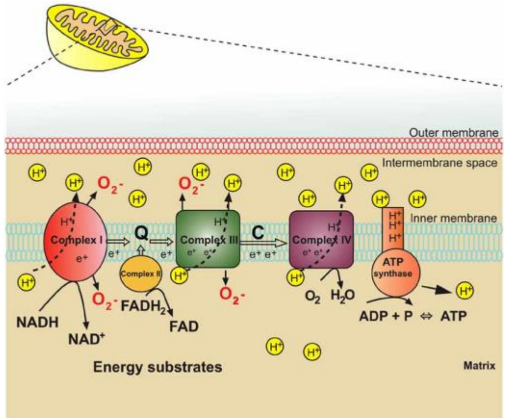
From Casey J.R. et al., Nat Rev Mol Cell Biol, 2009

Intracellular pH: *why is it important?*

Virtually all proteins depend on pH to maintain structure and function (Ex. Enzymes):



The proton-motive force is key to the generation and conversion of cellular energy:



Intracellular pH: *why is it important?*

Every cellular process can be affected by changes in intracellular pH

- **Metabolism**
- **Membrane potential**
- **Cell growth and proliferation**
- **Movement of substances across the membrane**
- **Polymerization of the cytoskeleton, etc**

NEED FOR PH REGULATORY MECHANISMS

Intracellular pH: how is it regulated?

For fast, localized pH_i changes

1. Intrinsic buffer capacity of intracellular weak acids and bases

(phosphate groups, aa's side chains)

2. HCO_3^- buffer capacity:

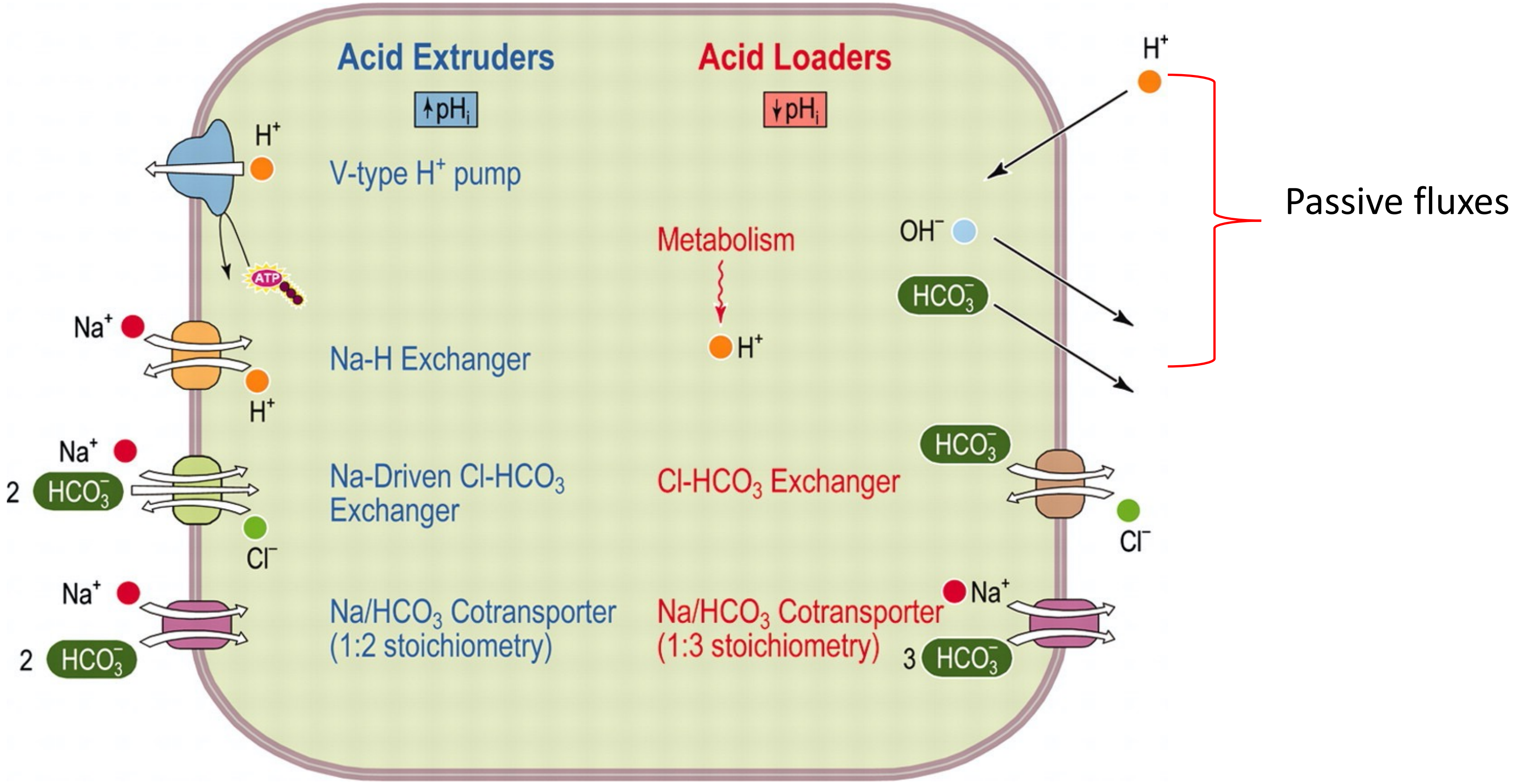
$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

Carbon dioxide — Water — Carbonic acid (Weak acid) — Bicarbonate ion (Weak base)

Carbonic Anhydrase

Intracellular pH: how is it regulated?

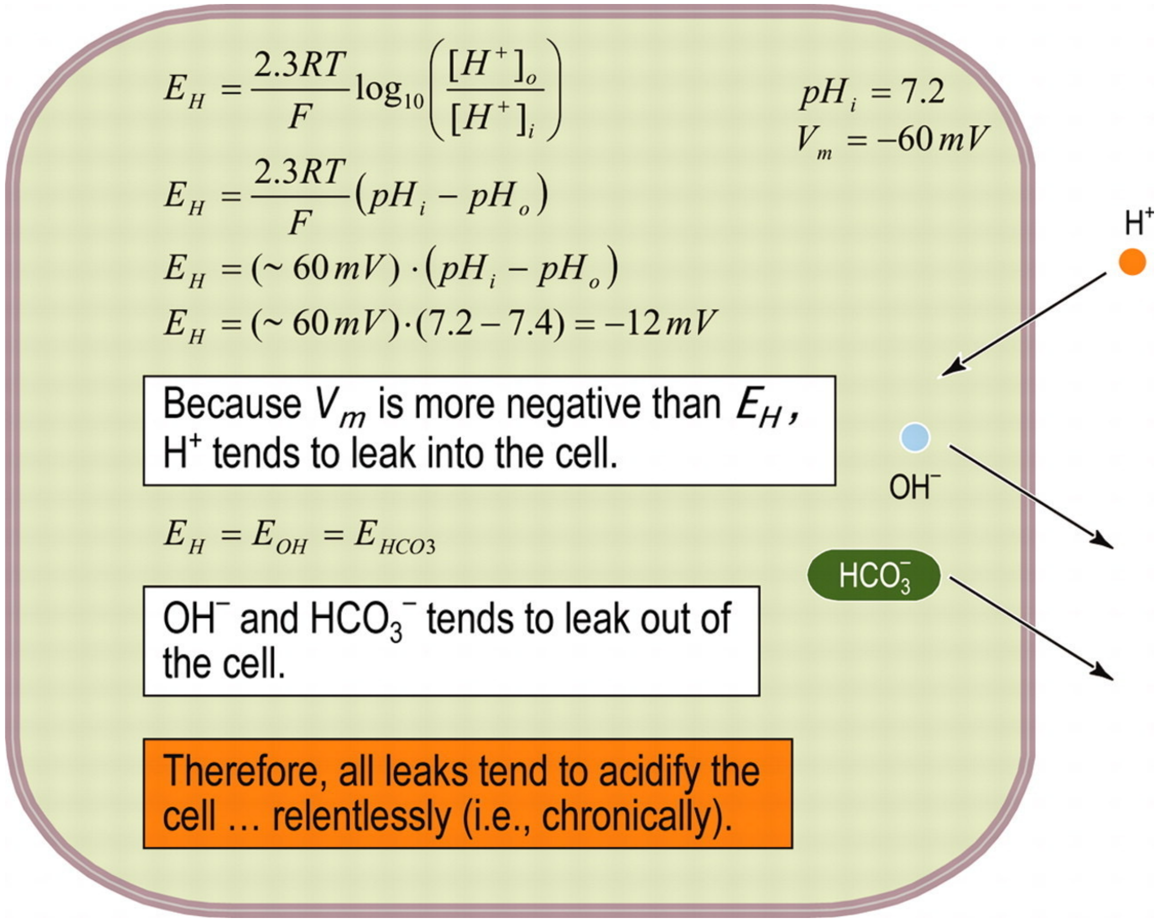
Steady-state pHi depends on the balance between chronic acid extruders and chronic acid loaders. Every extrusion of H+ or intrusion of HCO3- will increase the pH. Eevery intake of H+ or extracellular fluxes of HCO3-, will decrease pH



WF Boron, Advances in Physiology Education, 2004

Intracellular pH: how is it regulated?

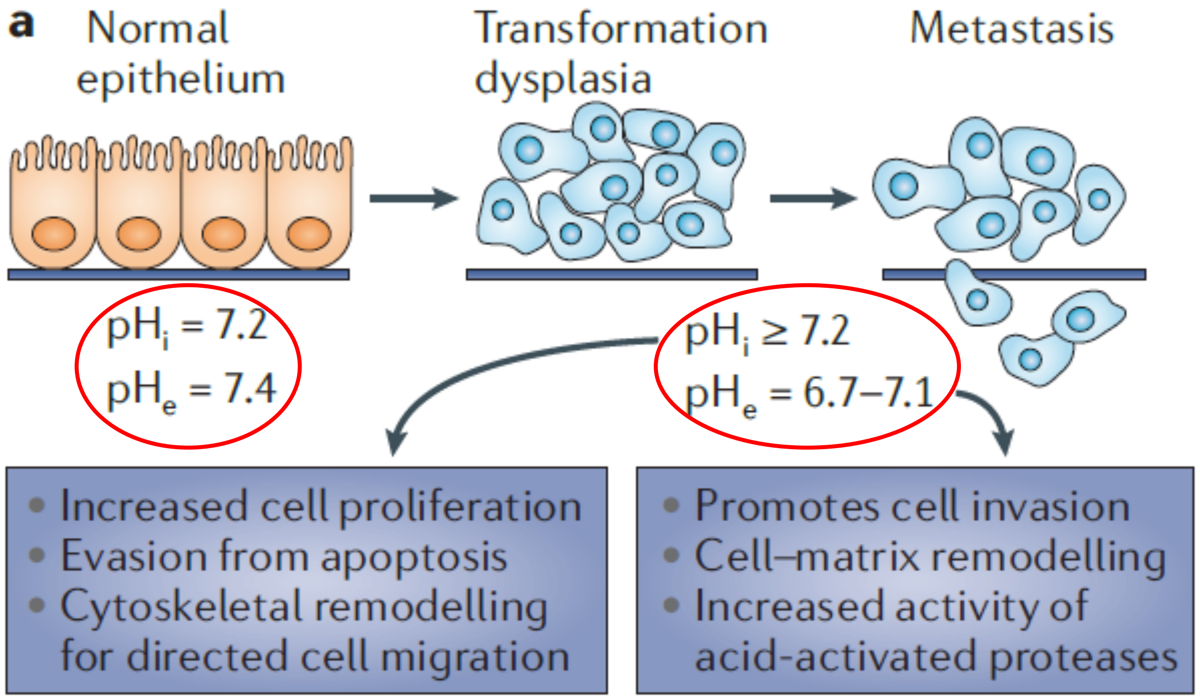
Chronic acid and alkali loads produced by **passive fluxes** of charged weak acids and bases across the plasma membrane. In this example, we assume that the cytosolic pH is 7.2 and that the transmembrane voltage is −60 mV.



WF Boron, Advances in Physiology Education, 2004

pH in cancer cells:

Dysregulated pH as promoter of cancer progression

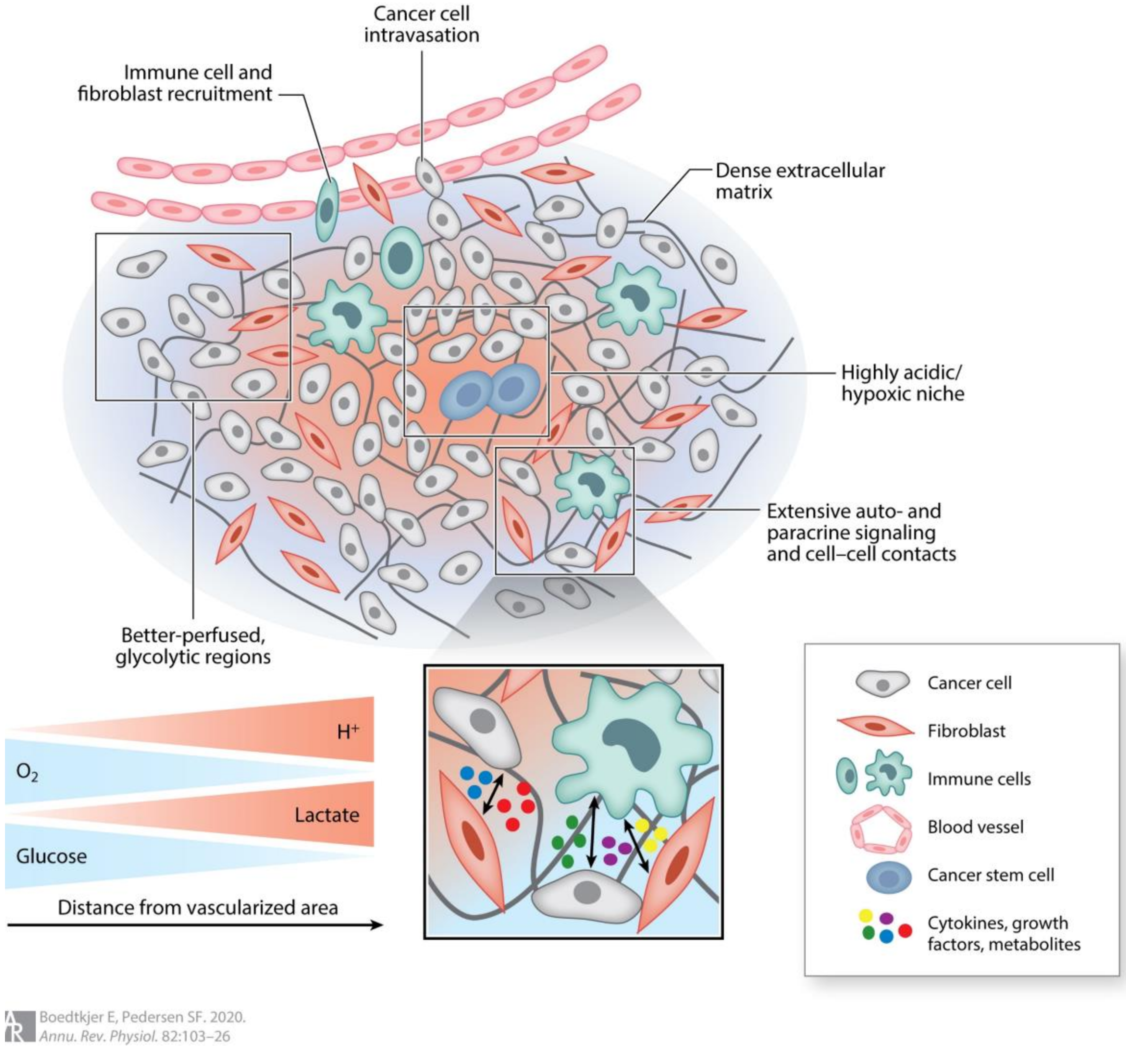


From Bradley A. et al. Nature reviews, 2011

Reversed gradient in cancer cells

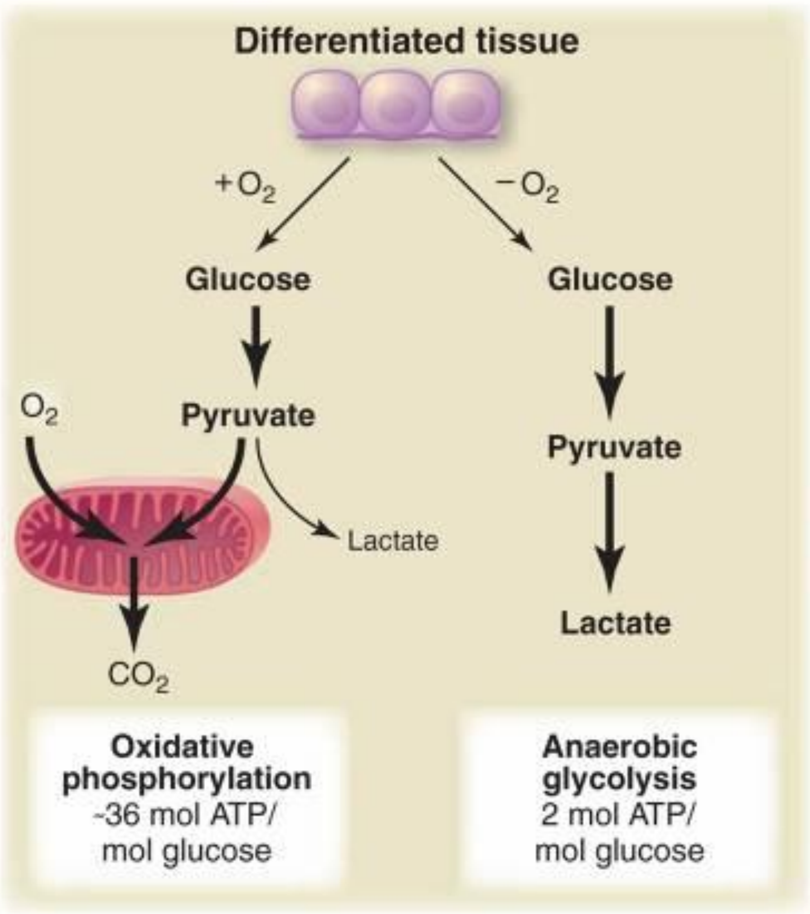
The intracellular pH (pHi) of cancer cells is usually slightly (0.1–0.2 pH units) more alkaline than the extracellular pH (pHe)

Acidic tumor microenvironment



- Uncontrolled proliferation: secretion of acid metabolites
- Warburg effect
- Hypoxia
- deficient blood perfusion and H^+ venting ability
- CO_2 produced from oxygenated tumor areas

Acidic tumor microenvironment: WARBURG EFFECT



From Matthew G. et al., Science, 2009

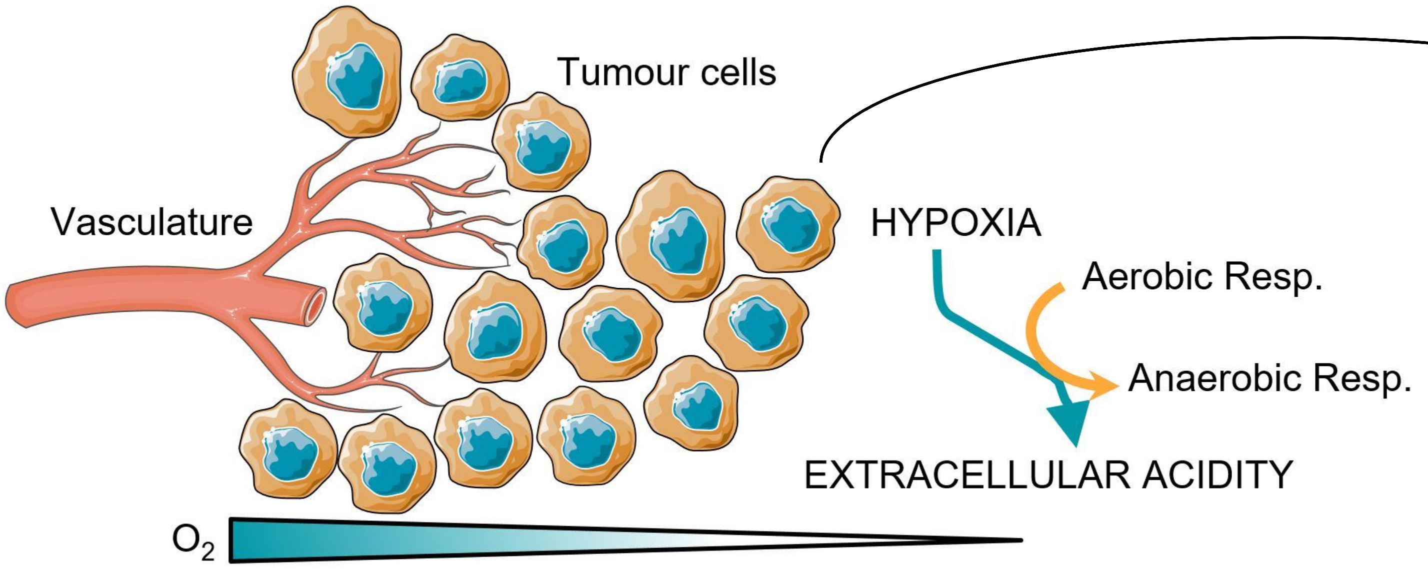
Physiological situation in normal cells: energy in the form of ATP

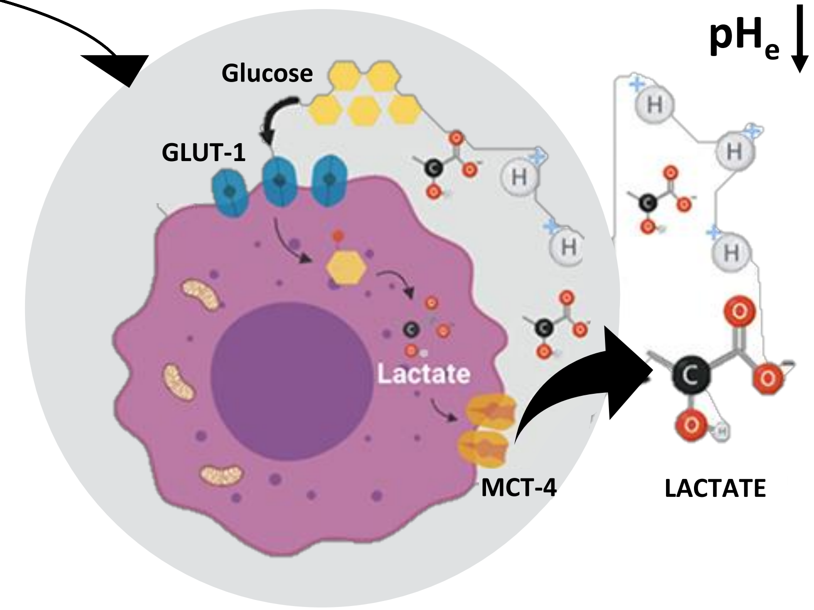
In presence of oxygen, glucose is metabolized to produce pyruvate through the glycolysis pathway. In the mitochondria, the vast majority of pyruvate is oxidized to produce NADH, used for maximizing ATP synthesis trough the oxidative phosphorylation: HIGH ATP YIELD

In case oxygen is lacking, cells can ensure NAD+ (and then glycolysis) by converting pyruvate to lactate via anaerobic glycolysis: LOW ATP YIELD

Acidic tumor microenvironment: WARBURG EFFECT

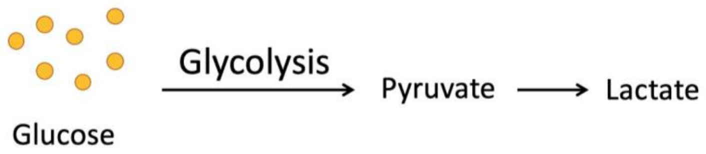
In cancer cells: aerobic glycolysis





Modified from De la Cruz-Lopez K.G. et al., 2019

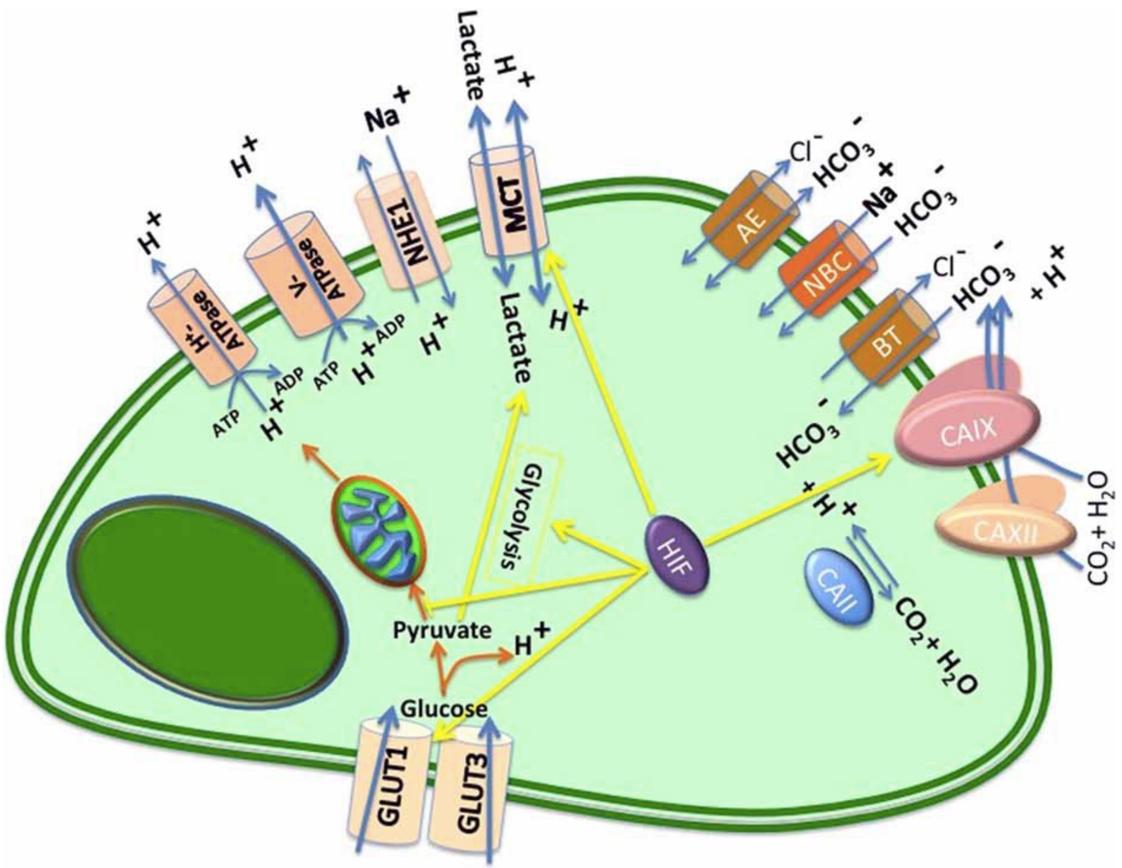
Warburg effect



This effect is also present in normal **proliferative** cells and in cancer cells we can also have some oxidative phosphorylation

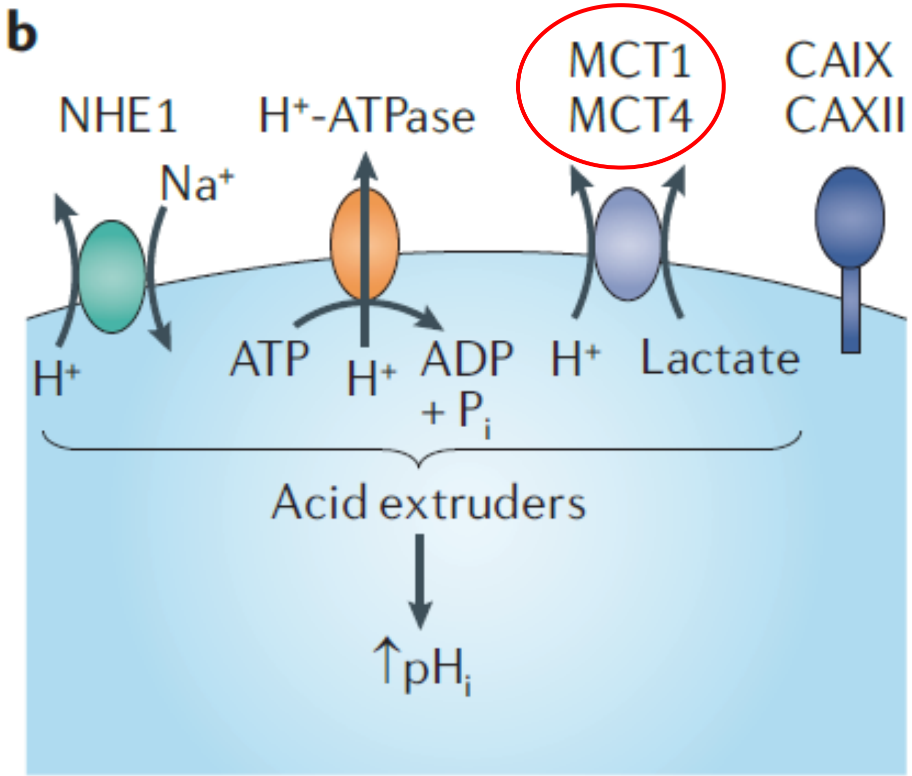
Major pH regulators in a cancer cell.

After glucose uptake by specific transporters (GLUT1andGLUT3), glucose is converted to pyruvate, generating 2ATP per glucose and proton. Based on Pasteur effect, in the presence of oxygen, pyruvate is oxidized to HCO−3, generating 36 additional ATP per glucose; in the absence of oxygen pyruvate is reduced to lactate, which is exported to extracellular space. However, as Warburg proposed glycolysis is potent in cance rcells. Notably both processes produce protons (H+), which cause acidification of the extracellular space. This figure represents main proteins that regulate intracellular and extracellular pH intumors, including: monocarboxylate transporters(MCTs),which transport lactic acid and other monocarboxylates formed by the glycolytic degradation of glucose; the plasma membrane proton pump vacuolar ATPase (V-ATPase); Na+/H+ exchangers (NHEs); anion exchangers (AEs); carbonic anhydrases (CAII,CAIX,andCAXII); Na+/HCO−3 co-transporters (NBCs), and HCO−3 -transporters (BTs).



Damaghi M, et al., Front in Physiology, 2013

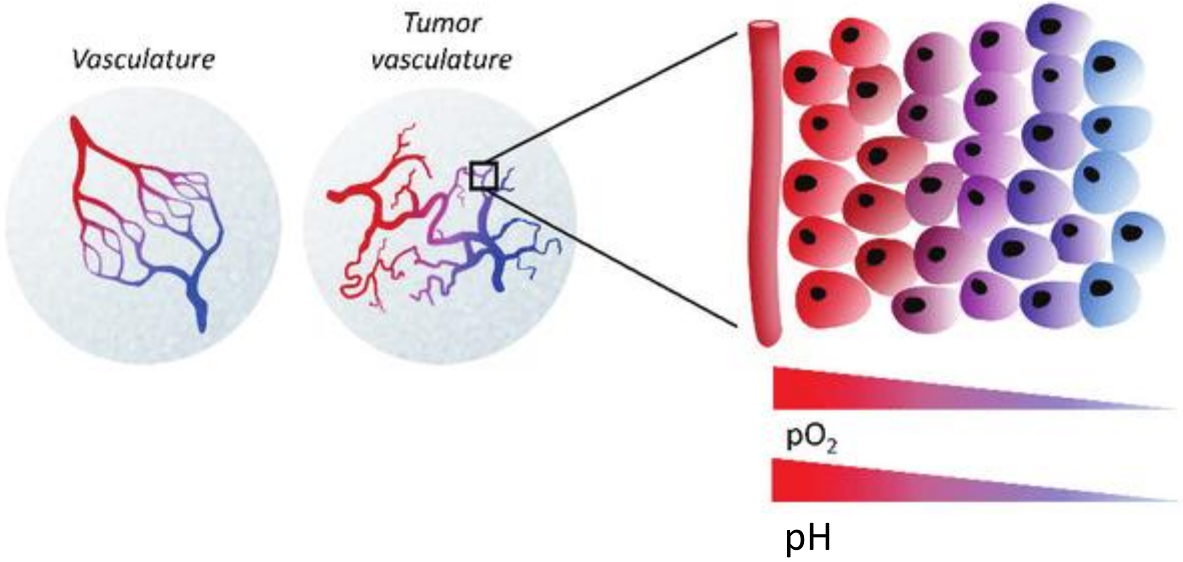
Acidic tumor microenvironment: upregulation of acid extruders



From Bradley A. et al. Nature reviews, 2011

Increase of the expression and/or activity of plasma membrane transporters, particularly **acid extruders**, and carbonic anhydrases (CAs) and monocarboxylate transporters (MCTs), which maintain the higher pHi and lower pHe of tumor cells

Acidic tumor microenvironment: HYPOXIA

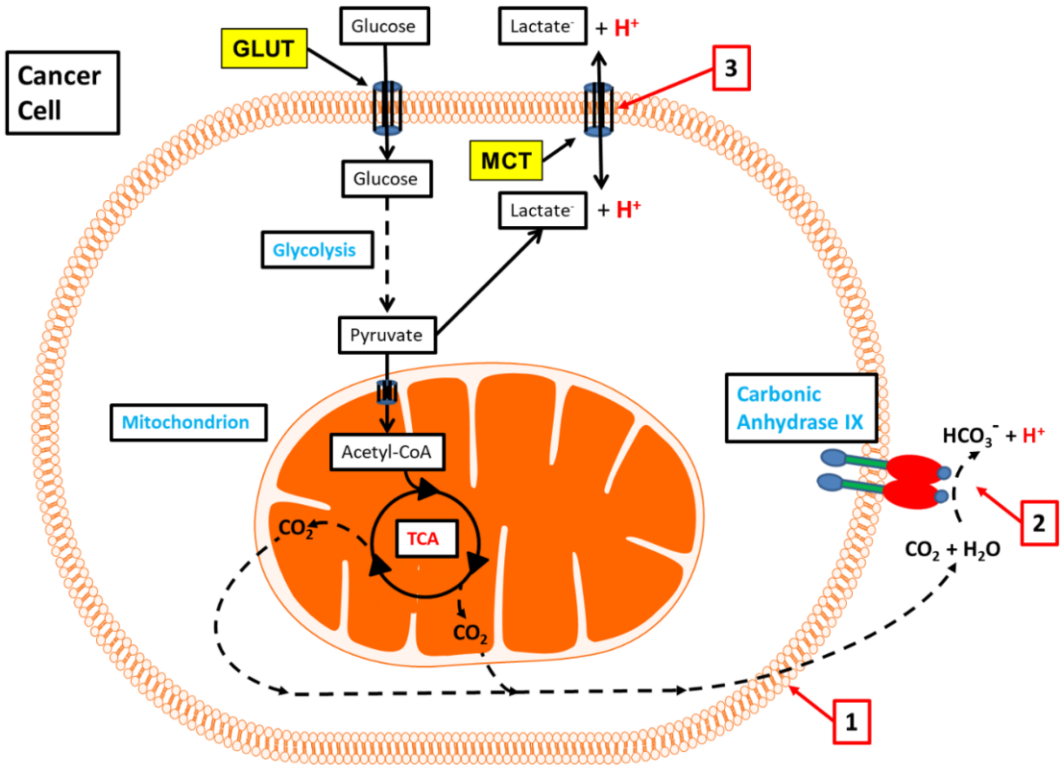


From Ramachandran S.. et al. Genes, 2015

Tumor angiogenesis leads to the formation of disorganized blood vessels impairing the oxygen and nutrient supply to cancer cells. This translates in:

- **localized hypoxic tumor regions**
- **upregulation of HIF-1** (hypoxia inducible factor 1), that promotes the expression of glucose transporters, lactate dehydrogenase, pyruvate dehydrogenase kinase, etc.

Acidic tumor microenvironment: CO_2 PRODUCTION



From Lee S.H and Griffiths J., Cancers, 2020

1. The major by-product of oxidative energy metabolism, CO_2 diffuses across the cell membrane lipid bilayer into the extracellular space, along its concentration gradient;
2. **On the extracellular surface of the cell membrane, Carbonic Anhydrase IX catalyses the hydration of CO_2 to form H^+ and HCO_3^-**
3. In parallel, lactate, exits the cell through the monocarboxylate transporter

GLUT, glucose transporter; MCT, monocarboxylate transporter.

Acidic tumor microenvironment: key word is heterogeneity

1. **Tumor pH_i is alkaline respect to normal cells** → highly acidic extracellular pH influences intracellular pHi, acidifying the intracellular pH. This means that in tumor areas that are poorly oxygenated and highly acidic, localized pHi will be more acidic too. While highly perfused tumor areas will show less acidic pHe. **Tumor pH values are relative, changing in different areas of the tumor cell and tumor microenvironment**

2. **Cancer cells perform aerobic glycolysis** → in highly perfused tumor areas, cancer cells can also produce ATP by oxidative phosphorylation, in minor extend respect to normal cells

3. **Warburg effect is a feature of only cancer cells** → normal proliferating cells accumulate high quantities of ATPs obtained by respiration process. Then it starts the Warburg effect and to prepare for cell division energetically. Once cell cycle starts, the cells start to rely on aerobic glycolysis (Warburg effect) for ATP synthesis and lactic acid production and release, in order to raise the intracellular pH from ~6.8 to ~7.2 as needed by cell division. The cells go back to the normal respiration-based ATP production once the cell division phase ends

Acidic tumor microenvironment: key word is heterogeneity

cancers

MDPI

Review

Spatiotemporal pH Heterogeneity as a Promoter of Cancer Progression and Therapeutic Resistance

David E. Korenchan [1] and Robert R. Flavell [1,2,*]

1 Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA 94143, USA
2 Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, USA
* Correspondence: Robert.Flavell@ucsf.edu; Tel.: +1-415-353-3638

for in-depth study…

Dysregulated pH as promoter of cancer progression

pH-dependent cancer hallmarks

CELL PROLIFERATION: promoted by alkaline pH_i

- **pH_i higher than 7.2** promotes cancer cells to entry to S phase and enter and progress through the G2/M phases
- **Higher pH_i** suppresses the mitotic arrest that is triggered by an activated DNA damage checkpoint

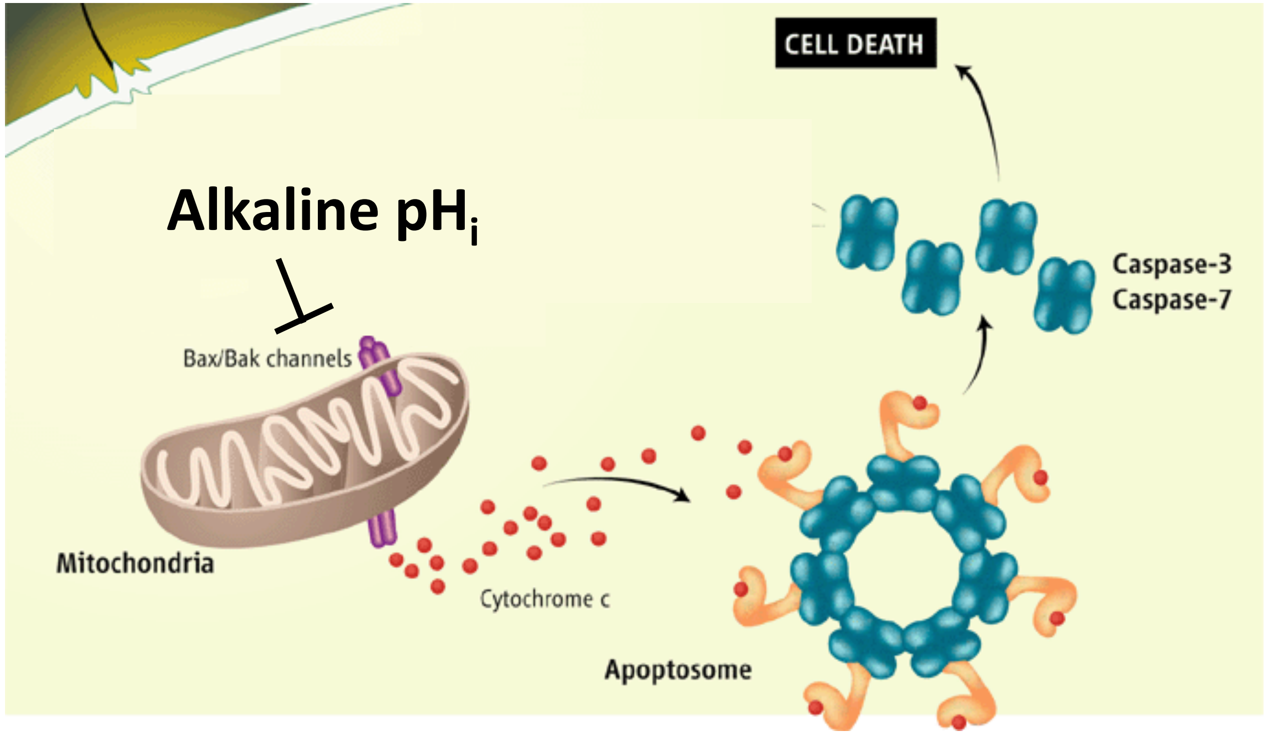
cancer cells high pH_i allows them to **bypass cell cycle checkpoints**, promoting not only proliferation, but also **genetic instability**

Dysregulated pH as promoter of cancer progression

pH-dependent cancer hallmarks

CELL SURVIVAL: promoted by alkaline pH_i

Modified from Colin Adrain and Seamus J. Martin, 2006



- **Acidic intracellular pH** promotes activation of endonucleases
- **Acidic intracellular pH** increases cancer cells' sensitivity to heat or chemotherapy drugs- induced apoptosis
- **Acidic intracellular pH** promotes activation of mitochondrial apoptotic pathway

Dysregulated pH as promoter of cancer progression

pH-dependent cancer hallmarks

METABOLIC ADAPTATION: promoted by alkaline pH_i and acidic pH_e

- **Alkaline pH_i promotes glycolysis**

Lactate dehydrogenase (LDH) optimum pH is 7.5

Phosphofructokinase 1 (PFK1) optimum pH is 7-7.5

- **Acidic pHe and hypoxia promotes glycolysis**

Lactate extrusion alkalinizes pHi

Hypoxia-induced tumor acidosis induces expression of glycolytic enzymes

Dysregulated pH as promoter of cancer progression

pH-dependent cancer hallmarks

CELL INVASION AND MIGRATION: promoted by alkaline pH_i and acidic pH_e

- **Alkaline pH_i promotes directed migration:**
  - ↑ De novo assembly of actin filaments
  - ↑ Activity of CDC42
  - Faster focal adhesion turnover

- **Acidic pHe promotes invasion:**
  - ↑ Formation and maturation of invadopodia
  - ↑ Degradation of ECM

Ion channels as highly sensitive pH-sensors

- Plasma membrane ion channels are optimal pH sensors, as their activity can be modulated by both pHi and pHe
- Plasma membrane ion channels contribute to virtually all basic cellular processes and are also involved in the malignant phenotype of cancer cells

Review

Cell PRESS

Feature Review

Ion channels and the hallmarks of cancer

Natalia Prevarskaya[1], Roman Skryma[1] and Yaroslav Shuba[2]

[1] Inserm, U800, Laboratoire de Physiologie Cellulaire, Equipe labellisée par la Ligue contre le cancer, Villeneuve d'Ascq, F-59650 France; Universite de Lille 1, Villeneuve d'Ascq, F-59650 France
[2] Bogomoletz Institute of Physiology and International Center of Molecular Physiology, NASU, Bogomoletz Str., 4, 01024 Kyiv-24, Ukraine

Physiol Rev 98: 559–621, 2018
Published February 7, 2018; doi:10.1152/physrev.00044.2016

ION CHANNELS IN CANCER: ARE CANCER HALLMARKS ONCOCHANNELOPATHIES?

Natalia Prevarskaya, Roman Skryma, and Yaroslav Shuba

INSERM U-1003, Equipe Labellisée par la Ligue Nationale contre le Cancer et LABEX, Université Lille1, Villeneuve d'Ascq, France; Bogomoletz Institute of Physiology and International Center of Molecular Physiology, NASU, Kyiv-24, Ukraine

pH-dependent regulation of ion channels in cancer cells

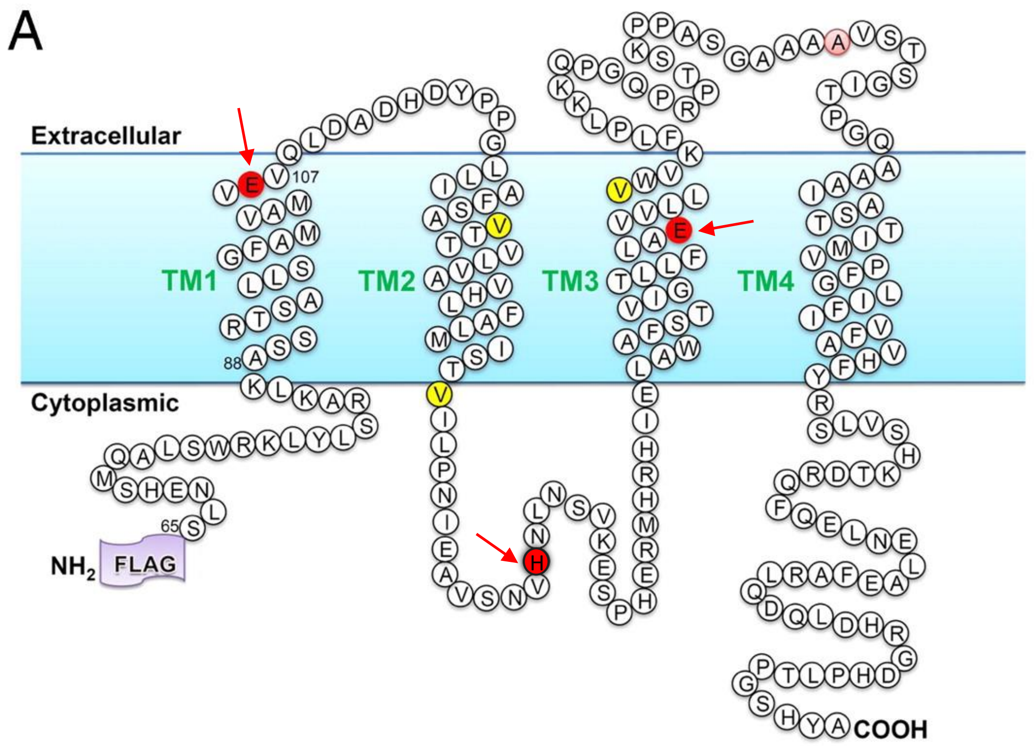
1. Direct Interaction between protons and ion channels

- H^+-binding sites involving titratable side chains with pKa values close to the physiological pH (histidine, arginine, lysine)

Example:

ORAI1 Ca^{2+} permeable channel

- **E106** in TM1 is responsible for pHe sensitivity when Ca^{2+} is the permeant cation
- **E190** located in TM3 is the major sensor of pHe when Na^+ is the charge carrier.
- **H155** located in the intracellular loop is responsible for intracellular pH sensitivity



Modified from Yubin Zhou et al., PNAS, 2010

pH-dependent regulation of ion channels in cancer cells

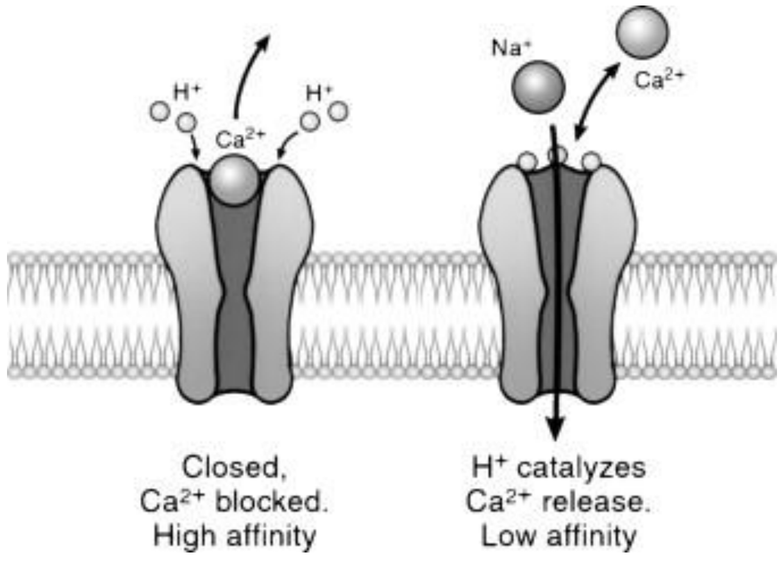
2. Direct Interaction between protons and ion channels

- H^+ competition with other cation-binding sites

Example:

Acid Sensing Ion Channel (ASICs) Na+ permeable channels

- **E106** in TM1 is responsible for pHe sensitivity when Ca^{2+} is the permeant cation
- **E190** located in TM3 is the major sensor of pHe when Na^+ is the charge carrier.
- **H155** located in the intracellular loop is responsible for intracellular pH sensitivity



From Immke D.C and McCleskey E.D, 2003

pH-dependent regulation of ion channels in cancer cells

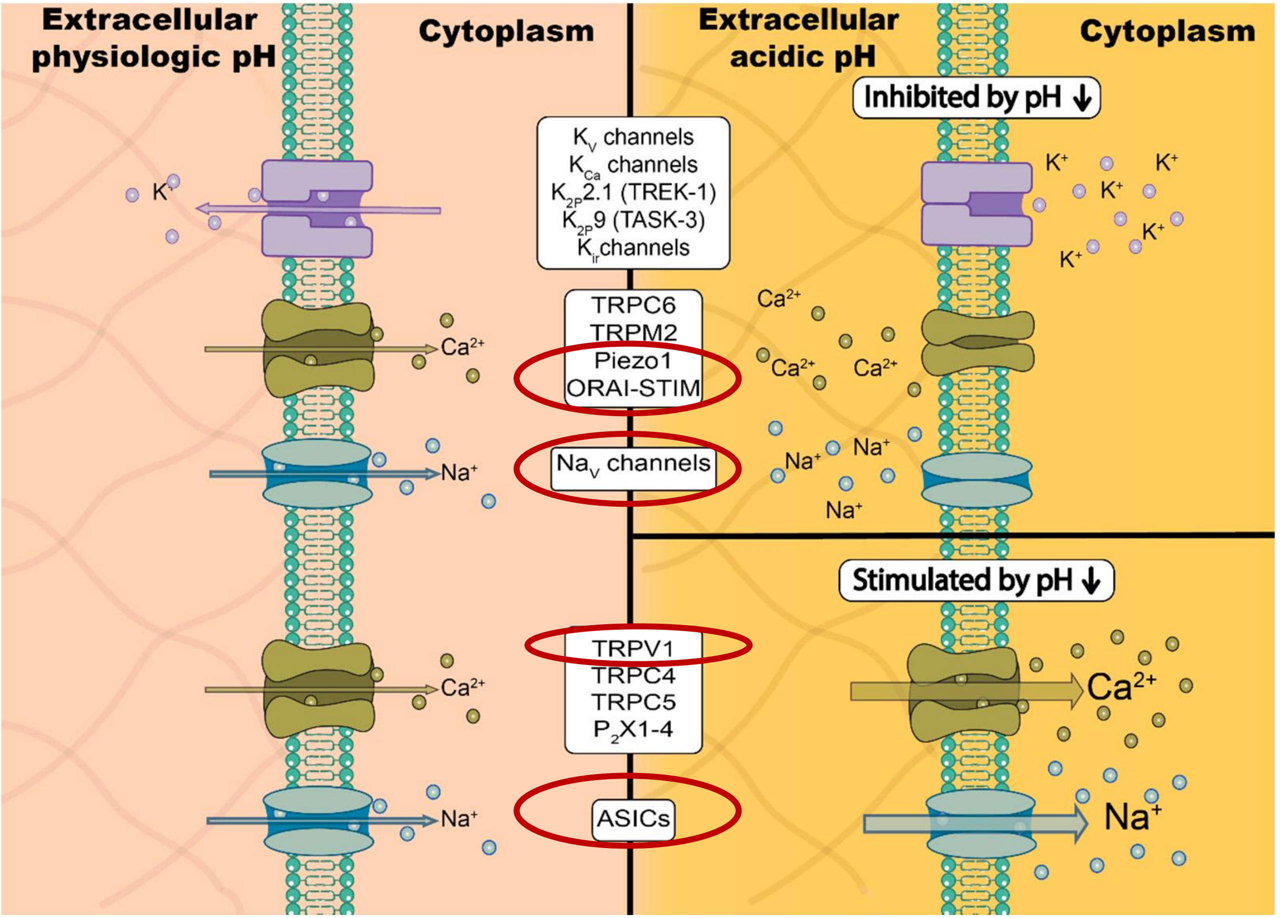
3. Indirect Interaction between protons and ion channels

- Example: Integrins-ion channels interactions

- Several integrins are pH-dependent in cancer and both pHi and pHe can modify integrins “inside-out” and “outside-in” signaling
- K_{Ca}/αvβ3 integrin complex recruits FAK and promotes its phosphorylation, resulting in increased cancer cell proliferation in prostate cancer
- K_V11.1 forms a complex with β1 integrin, increasing colorectal cancer cells’ invasiveness in vitro.

Integrins/ion channels interaction modulated by pH is still not clear

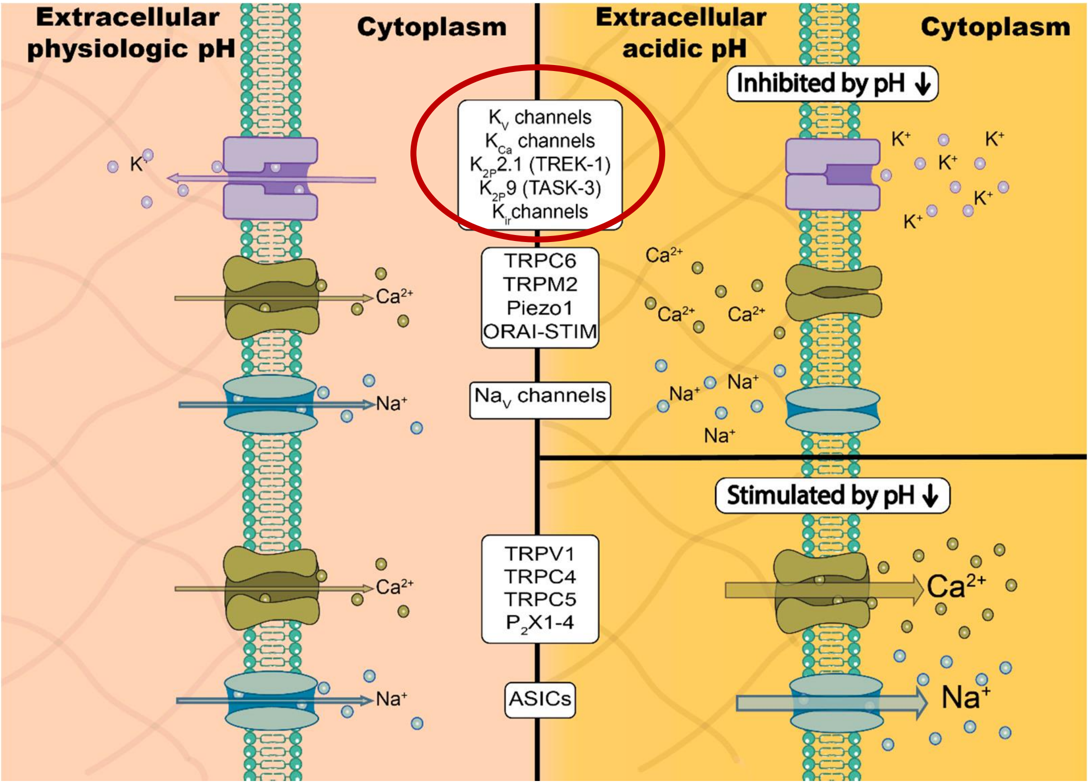
pH-dependent regulation of ion channels in tumor progression



From Petho et al., cancers, 2020

Acidic pHe often inhibits ion channels' activity in cancer cells, depending on ion channel's pH-sensitivity

pH-dependent regulation of potassium channels in tumor progression



From Petho et al., cancers, 2020

- Acidic pHe inhibits K_v (voltage-gated K^+) channels while different K_v channels are differentially regulated by pHi

- Intracellular acidification inhibits K_{ca} channels (K_{ca} 1.1, K_{ca} 2.1-2.3, K_{ca} 3.1) while extracellular pHe doesn't K_{ca} current (K_{ca} 3.1)

There is a lack of existing studies showing the link between the pH dependent activity of K+ channels in cancer cells, so we need to clarify yet the impact of intracellular pHi regulation of K_{ca} channels in the context of tumor progression.

CALCIUM SIGNALING

Ca^{2+} ions

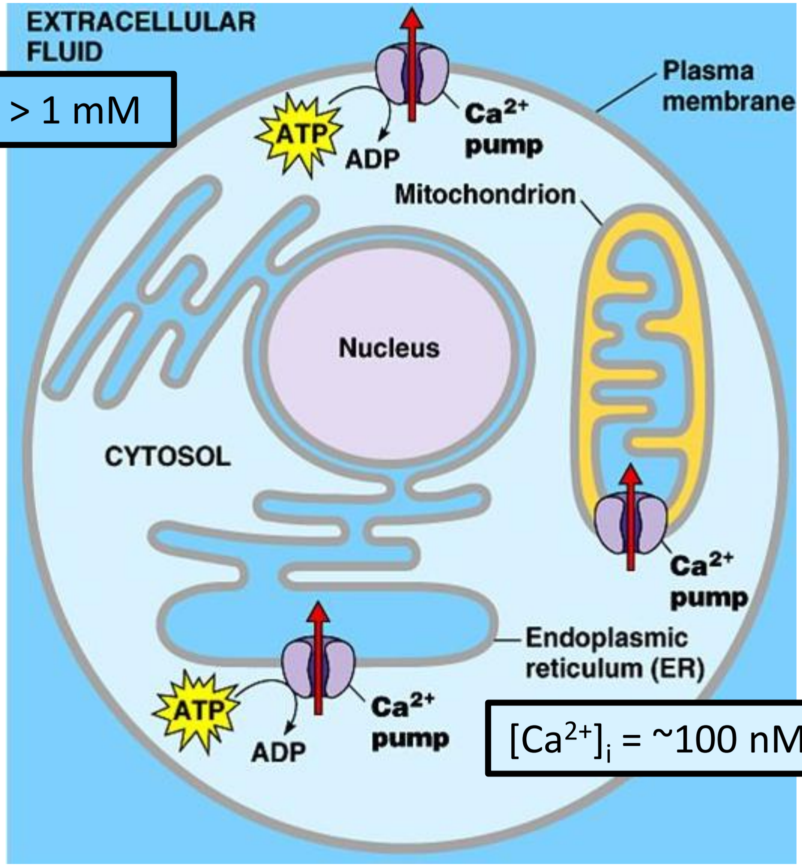
Ubiquitous second messenger that regulates several cellular processes:

- Fertilization
- Secretion
- Gene expression
- Muscle contraction
- Exocytosis
- Neurotransmission
- Cell migration

Etc..

Fine tune

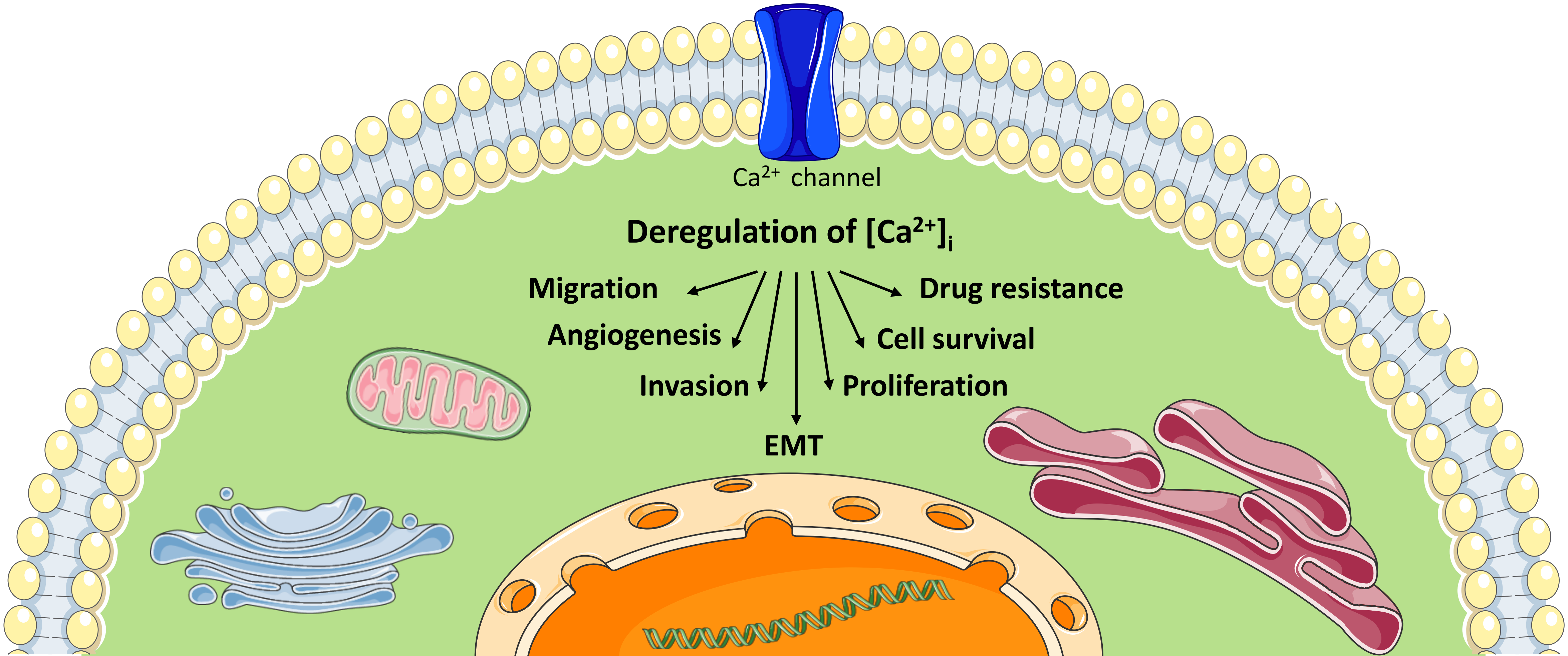
$[Ca^{2+}]_e$ = > 1 mM



$[Ca^{2+}]_i$ = ~100 nM

CALCIUM SIGNALING IN CANCER

Alteration in Ca^{2+} toolkit components' expression and/or function



CALCIUM SIGNALING IN CANCER

Ca^{2+} channels: link between pH and cancer

Deregulation of their expression and/or activity promotes **cancer progression**

Ca^{2+} channels

Several of them are pH sensitive and act as **pH sensors**

1) acidic pH_e and alkaline pH_i support cancer progression, promoting aggressive phenotypes

2) Ca^{2+} -depending signaling is one of major pathways leading to acquisition of aggressive phenotypes (resistance to apoptosis, increased proliferation, increased migration and invasion, etc.)

CALCIUM SIGNALING IN CANCER

Ca^{2+} channels: link between pH and cancer

Ca^{2+} channels

⇅

pHi-pHe

pH and Ca2+ signaling could work in synergy to select the most aggressive cancer cell phenotypes Ca2+ permeable channels as the targets of pH, working "in concert“ for tumor progression

Ca^{2+} channels' regulation by acidic tumor microenvironment

pH-sensitive TRP Channels

TRP family member	Effect of low pHe	Reference
TRPM2	Inactivation	Du J. et al., 2009; Yang W. et al., 2010
TRPM6	Activation	Kozak J.A et al., 2005; Chokshi R et al, 2012
TRPM7	Activation	Jiang et al., 2005; Li M. et al., 2006; Mačianskienė R. et al., 2017
TRPV1	Activation	Tominaga M. et al., 1998; Jordt S.-E. et al., 2000
TRPV4	Activation	Suzuki M. et al., 2003
TRPV6	Inactivation	Cherezova A.L. et al., 2018
TRPC6	Inactivation	Nielsen N. et al., 2017; Iyer S.C. et al., 2015; Wen L. et al., 2016
TRPA1	Activation	De la Roche J. et al., 2013; Hamilton N.B. et al., 2016

pH-dependent regulation of TRP channels in tumor progression

Capsaicin, a component of red peppers, induces expression of androgen receptor via PI3K and MAPK pathways in prostate LNCaP cells

Sophie Malagarie-Cazenave [1], Nuria Olea-Herrero, Diana Vara, Inés Díaz-Laviada

> J Neurochem. 2007 Aug;102(3):977-90. doi: 10.1111/j.1471-4159.2007.04582.x. Epub 2007 Apr 17.

Capsaicin-induced apoptosis of glioma cells is mediated by TRPV1 vanilloid receptor and requires p38 MAPK activation

C Amantini [1], M Mosca, M Nabissi, R Lucciarini, S Caprodossi, A Arcella, F Giangaspero, G Santoni

> Cancer Res. 2009 Feb 1;69(3):905-13. doi: 10.1158/0008-5472.CAN-08-3263. Epub 2009 Jan 20.

Transient receptor potential type vanilloid 1 suppresses skin carcinogenesis

Ann M Bode [1], Yong-Yeon Cho, Duo Zheng, Feng Zhu, Marna E Ericson, Wei-Ya Ma, Ke Yao, Zigang Dong

TRPV1

Transient receptor potential Vanilloid Subfamily 1

- Involved in pain sensation, also elicited by TME
- Activated by acidic pHe: protons directly open the channel or potentiates capsaicin-mediated TRPV1 activation
- Pro- or anti-tumor role depending on cancer cell type and extent of intracellular Ca^{2+} increase

The two proton-dependent effects are not mediated by the same amino acid residues, suggesting that, depending on the stimulus, TRPV1 channels can use different opening states that may convey distinct signals to cells

pH-dependent regulation of TRP channels in tumor progression

› Oncogene. 2012 Jan 12;31(2):200-12. doi: 10.1038/onc.2011.231. Epub 2011 Jun 20.

TRPV4 mediates tumor-derived endothelial cell migration via arachidonic acid-activated actin remodeling

A Fiorio Pla [1], H L Ong, K T Cheng, A Brossa, B Bussolati, T Lockwich, B Paria, L Munaron, I S Ambudkar

Open Access | Published: 13 June 2016

TRPV4 Regulates Breast Cancer Cell Extravasation, Stiffness and Actin Cortex

Wen Hsin Lee, Lee Yee Choong, Naing Naing Mon, SsuYi Lu, Qingsong Lin, Brendan Pang, Benedict Yan, Vedula Sri Ram Krishna, Himanshu Singh, Tuan Zea Tan, Jean Paul Thiery, Chwee Teck Lim, Patrick Boon Ooi Tan, Martin Johansson, Christian Harteneck & Yoon Pin Lim

Scientific Reports **6**, Article number: 27903 (2016) | Cite this article

Pharmacological inhibition of TRPV4 channel suppresses malignant biological behavior of hepatocellular carcinoma *via* modulation of ERK signaling pathway

Yu Fang [a], Guoxing Liu [a], Chengzhi Xie [b], Ke Qian [a], Xiaohua Lei [a], Qiang Liu [a], Gao Liu [a], Zhenyu Cao [a], Jie Fu [a], Huihui Du [a], Sushun Liu [a], Shengfu Huang [a], Jixiong Hu [a], Xundi Xu [a]

TRPV4

Transient receptor potential Vanilloid Subfamily 4

- Permeable to both Ca^{2+} and Na^{+}
- Activated by acidic pHe
- Pro- tumor role in breast cancer, promoting angiogenesis, invasion and metastasis in vivo

Molecular and Cellular Pathobiology

Calcium Promotes Human Gastric Cancer via a Novel Coupling of Calcium-Sensing Receptor and TRPV4 Channel

Rui Xie, Jingyu Xu, Yufeng Xiao, Jilin Wu, Hanxing Wan, Bo Tang, Jingjing Liu, Yahan Fan, Suming Wang, Yuyun Wu, Tobias Xiao Dong, Michael X. Zhu, John M. Carethers, Hui Dong, and Shiming Yang

DOI: 10.1158/0008-5472.CAN-17-0360 Published December 2017 Check for updates

pH-dependent regulation of TRP channels in tumor progression

› Cancer Cell. 2018 Jun 11;33(6):985-1003.e7. doi: 10.1016/j.ccell.2018.05.001. Epub 2018 May 24.

Cancer Cells Co-opt the Neuronal Redox-Sensing Channel TRPA1 to Promote Oxidative-Stress Tolerance

Nobuaki Takahashi [1], Hsing-Yu Chen [1], Isaac S Harris [1], Daniel G Stover [2], Laura M Selfors [1], Roderick T Bronson [3], Thomas Deraedt [4], Karen Cichowski [4], Alana L Welm [5], Yasuo Mori [6], Gordon B Mills [7], Joan S Brugge [8]

› Nat Commun. 2017 Oct 16;8(1):947. doi: 10.1038/s41467-017-00983-w.

TRPA1-FGFR2 binding event is a regulatory oncogenic driver modulated by miRNA-142-3p

Jonathan Berrout [1], Eleni Kyriakopoulou [1], Lavanya Moparthi [2], Alexandra S Hogea [3], Liza Berrout [4], Cristina Ivan [5], Mihaela Lorger [6], John Boyle [7], Chris Peers [7], Stephen Muench [3], Jacobo Elies Gomez [7], Xin Hu [8], Carolyn Hurst [9], Thomas Hall [1], Sujanitha Umamaheswaran [10], Laura Wesley [1], Mihai Gagea [11], Michael Shires [12], Iain Manfield [1], Margaret A Knowles [9], Simon Davies [3], Klaus Suhling [13], Yurema Teijeiro Gonzalez [13], Neil Carragher [14], Kenneth Macleod [14], N Joan Abbott [15], George A Calin [16], Nikita Gamper [3], Peter M Zygmunt [2], Zahra Timsah [17]

TRPA1

Transient receptor potential ankyrin Subfamily 1

- Mechanosensitive channel that also functions as a proton-activated ion channel
- Pro- tumor role and early event in cancer development

› Cancer Prev Res (Phila). 2017 Mar;10(3):177-187. doi: 10.1158/1940-6207.CAPR-16-0257. Epub 2017 Jan 17.

Activation of TRPA1 Channel by Antibacterial Agent Triclosan Induces VEGF Secretion in Human Prostate Cancer Stromal Cells

Sandra Derouiche [1], Pascal Mariot [1], Marine Warnier [1], Eric Vancauwenberghe [1], Gabriel Bidaux [1], Pierre Gosset [2], Brigitte Mauroy [1 3], Jean-Louis Bonnal [1 3], Christian Slomianny [1], Philippe Delcourt [1], Etienne Dewailly [1], Natalia Prevarskaya [1], Morad Roudbaraki [4]

pH-dependent regulation of ASICs channels in tumor progression

> Cell Death Dis. 2017 May 18;8(5):e2806. doi: 10.1038/cddis.2017.189.

ASIC1 and ASIC3 contribute to acidity-induced EMT of pancreatic cancer through activating Ca $^{2+}$/RhoA pathway

Shuai Zhu [1], Hai-Yun Zhou [1 2], Shi-Chang Deng [1 3], Shi-Jiang Deng [1], Chi He [1], Xiang Li [1], Jing-Yuan Chen [1], Yan Jin [1], Zhuang-Li Hu [2], Fang Wang [2], Chun-You Wang [1], Gang Zhao [1]

> Tumour Biol. 2017 Jun;39(6):1010428317705750. doi: 10.1177/1010428317705750.

Acid-sensing ion channels contribute to the effect of extracellular acidosis on proliferation and migration of A549 cells

Yu Wu [1], Bo Gao [1], Qiu-Ju Xiong [2], Yu-Chan Wang [1], Da-Ke Huang [3], Wen-Ning Wu [1 4]

> Oncogene. 2016 Aug 4;35(31):4102-11. doi: 10.1038/onc.2015.477. Epub 2015 Dec 21.

Regulation of breast tumorigenesis through acid sensors

S C Gupta [1 2], R Singh [1 2], M Asters [1], J Liu [1], X Zhang [3], M R Pabbidi [4], K Watabe [5], Y-Y Mo [1 4]

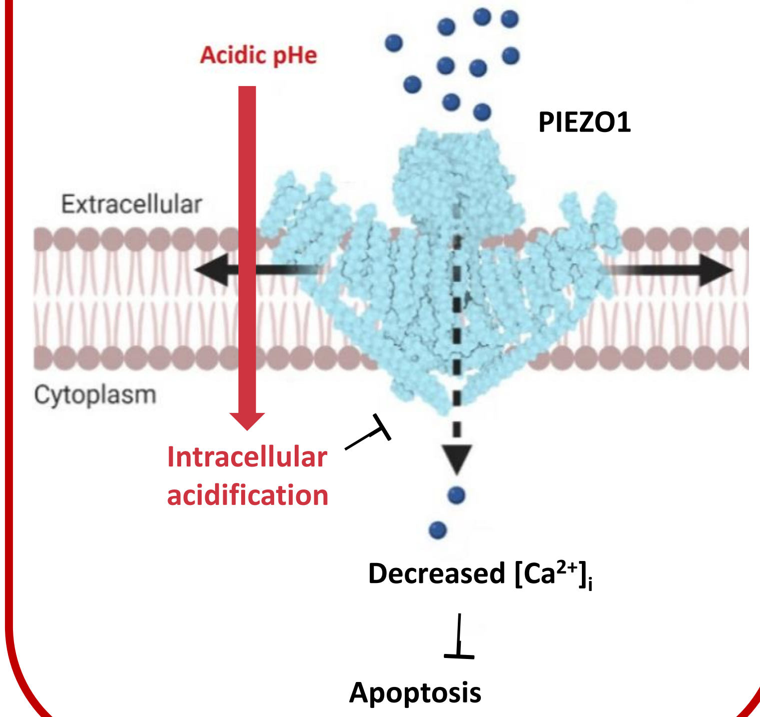
ASICs

Acid-sensing ion channels

- Permeable to Na^+ (ASIC1a and heteromeric ASIC1a/2b channels are also permeable to Ca^{2+})
- Activated by acidic pHe: Competition between protons and Ca^{2+} for binding in the activation site
- They contribute to different hallmarks of cancer, mainly via Ca^{2+} influx

pH-dependent regulation of PIEZO channels in tumor progression

PIEZO1 Channels in PSCs



Modified from Dario De Felice and Alessandro Alaimo, *Cancers*, 2020

PIEZO1 Channels are inhibited by acidic pHe

Inactivation of PIEZO1 might represent a protective mechanism in specific cell types, like in pancreatic stellate cells (PSCs)

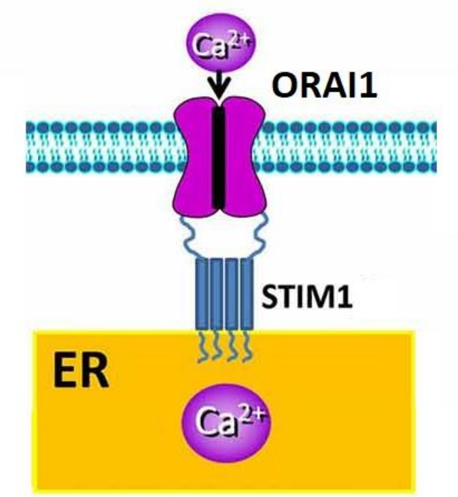
Protonation of Piezo1 Impairs Cell-Matrix Interactions of Pancreatic Stellate Cells

Anna Kuntze[1†], Ole Goetsch[1†], Benedikt Fels[2], Karolina Najder[1], Andreas Unger[1], Marianne Wilhelmi[1], Sarah Sargin[1], Sandra Schimmelpfennig[1], Ilka Neumann[1], Albrecht Schwab[1] and Zoltan Pethő[1*]

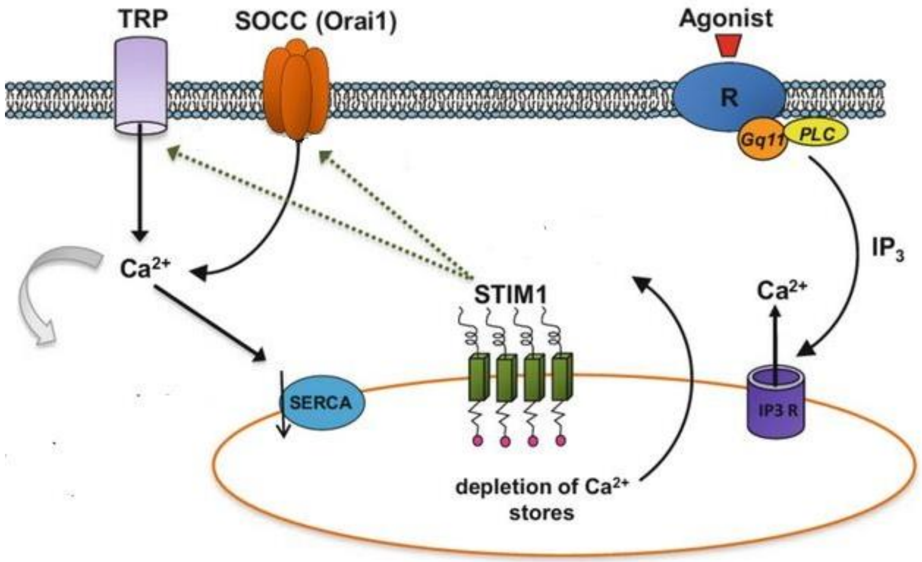
Intracellular pH drop, result of extracellular acidification, inactivates PIEZO1 and Ca^{2+} fluxes are decreased, avoiding apoptosis of PSCs due to calcium overload

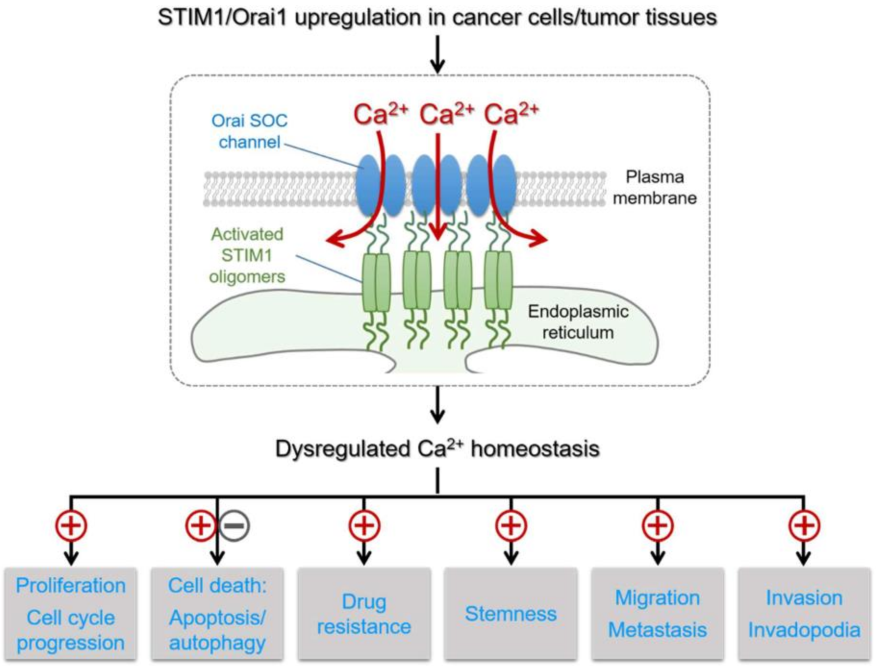
pH-dependent regulation of SOC channels in tumor progression

Store-Operated Calcium Channels



Store-Operated Calcium Entry (SOCE)

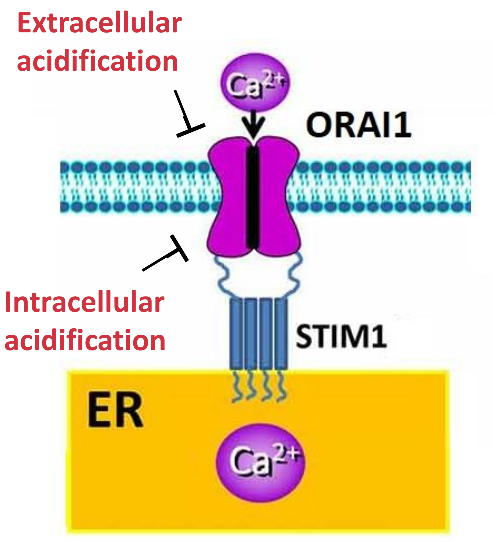




Modified from Secondo A. et al., *Frontiers in Molecular Neuroscience*, 2018

pH-dependent regulation of SOC channels in tumor progression

Store-Operated Calcium Channels



Intracellular and extracellular pH are able to modulate the activity of ORAI channels by affecting its coupling with STIM1 and/or by modifying its gating biophysical properties.

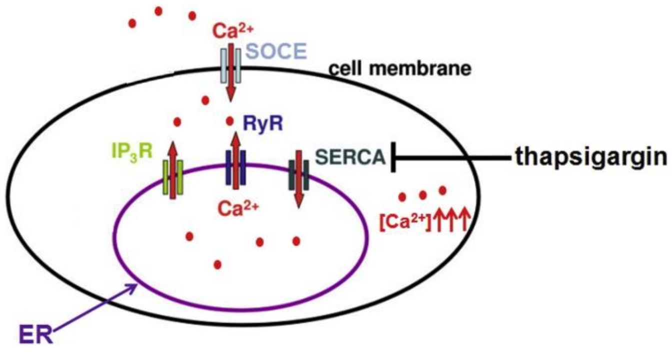
Acidic pH_i and pH_e = **SOCE inhibition**

Alkaline pH_i and pH_e = **SOCE potentiation** (but no activation, it requires store depletion)

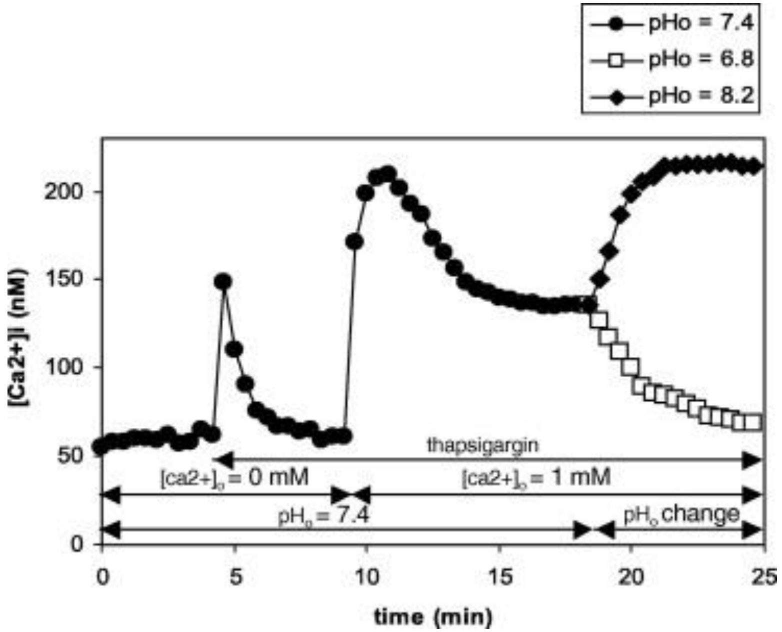
Modified from Secondo A. et al., *Frontiers in Molecular Neuroscience*, 2018

pH-dependent regulation of ORAI1-STIM1 complex in cancer cells

Effect of pH_e on the thapsigargin-mediated Ca^{2+}-entry in ORAI1-STIM1 overexpressing neuroblastoma cells



From Weixia Zhong et al., 2017



Representative experiment of the effect of pH_e on the thapsigargin-mediated Ca^{2+}-entry in SH-SY5Y cells

Biochem Biophys Res Commun. 2005 Nov 18;337(2):571-9. doi: 10.1016/j.bbrc.2005.09.086. Epub 2005 Sep 22.

Store-operated Ca2+-channels are sensitive to changes in extracellular pH

G Laskay [1], K Kálmán, E Van Kerkhove, P Steels, M Ameloot

Acidic pHe suppresses the thapsigargin-mediated Ca^{2+} entry in neuroblastoma cells, while external alkalinisation increased the TG-induced Ca^{2+}-influx

Laskay G. et al., 2005

HYPOXIA-dependent upregulation of ORAI1 and SOCE increase in cancer cells

Orai1 is critical for Notch-driven aggressiveness under hypoxic conditions in triple-negative breast cancers

Xiaoyu Liu [a, b], Teng Wang [c], Yan Wang [d], Zhen Chen [a], Dong Hua [c], Xiaoqiang Yao [b], Xin Ma [a], Peng Zhang [a]

Hypoxia-induced upregulation of Orai1 drives colon cancer invasiveness and angiogenesis

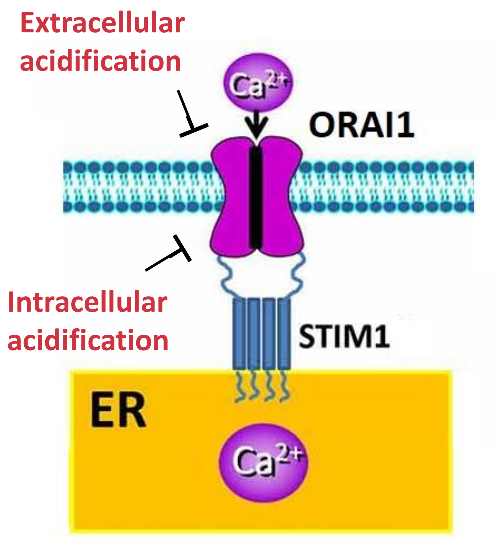
Xiaoyu Liu [1], Xu Wan [2], Hao Kan [2], Yan Wang [3], Fan Yu [2], Lei Feng [2], Jian Jin [2], Peng Zhang [4], Xin Ma [5]

Nicotine enhances store-operated calcium entry by upregulating HIF-1α and SOCC components in non-small cell lung cancer cells

Authors: Yan Wang, Jianxing He, Hua Jiang, Qi Zhang, Haihong Yang, Xiaoming Xu, Chenting Zhang, Chuyi Xu, Jian Wang, Wenju Lu

pH-dependent regulation of SOC channels in tumor progression

Store-Operated Calcium Channels



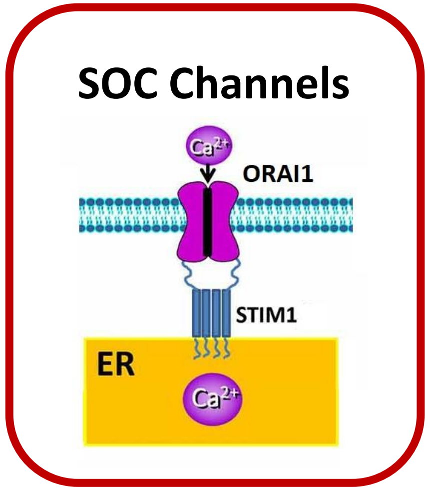
SOCE and acidic tumor microenvironment promote different cancer hallmarks, then its progression

But…

Acidic pH_e = **SOCE inhibition**

Modified from Secondo A. et al., *Frontiers in Molecular Neuroscience*, 2018

pH-dependent regulation of SOC channels in tumor progression



Low pHe mediated SOCE inhibition:

- Ca^{2+} signaling also contributes to tumor suppression by enhancing processes as cell death, senescence and autophagy
- ORAI members assembly to form different combinations of heteromeric Ca^{2+} Release Activated channels (CRACs). The acidic pH of tumor microenvironment may differently regulate heteromeric CRACs.
- Key role of SOCE in immune cell activation: The requirement of Ca^{2+} entry for antitumor immunity might explain the inhibitory effect of acidic tumor microenvironment on SOCE, in order to decrease immune cells' function and protect the tumor

Take home message

- Low pHe and alkaline pHi promote cancer progression, affecting several cancer hallmarks
- Plasma membrane ion channels are optimal pH sensors, as their activity can be modulated by both pHi and pHe
- pH-dependent regulation of ion channels depend on their pH sensitivity, they are often cancer cell type specific
- Tumor acidic pH and Ca^{2+} signaling could work in synergy to select the aggressive cancer cell phenotypes
- Ca^{2+} permeable channels may represent the targets of pHe, representing promising therapeutic targets

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Thank you