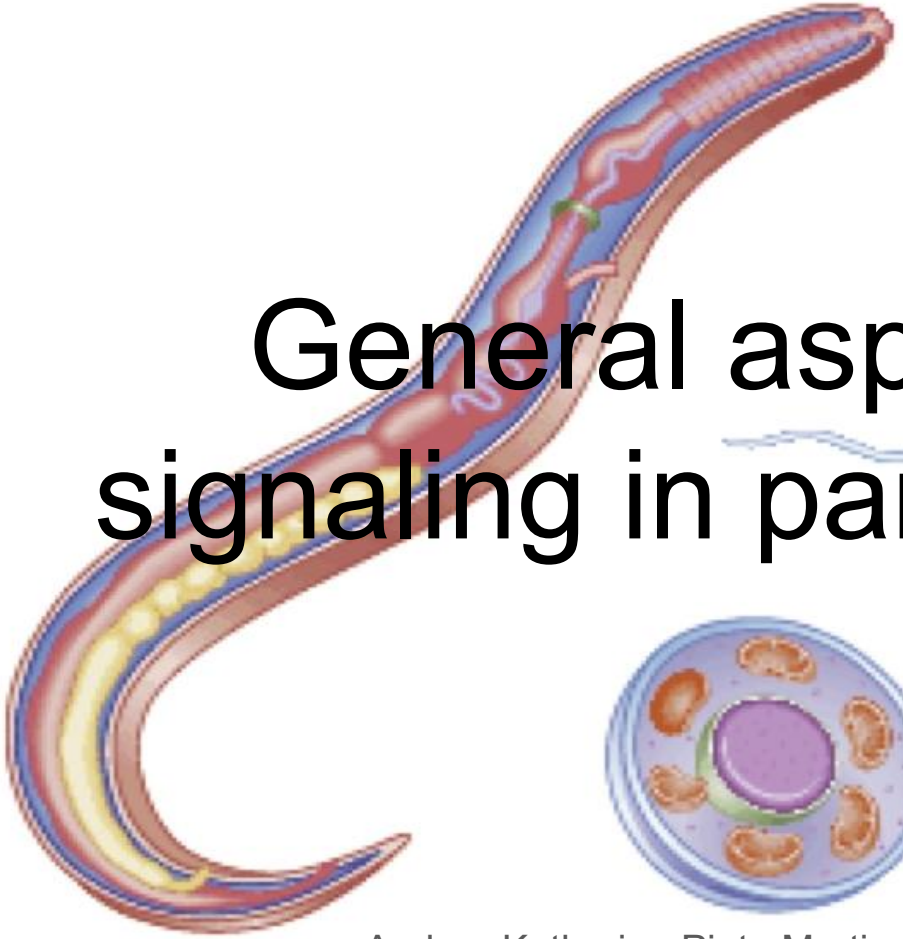
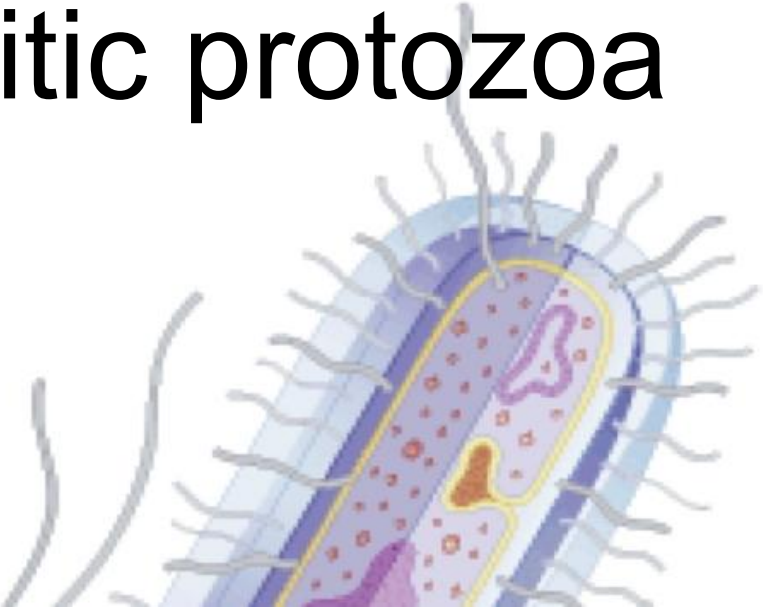
General aspects of Ca^{2+} signaling in parasitic protozoa



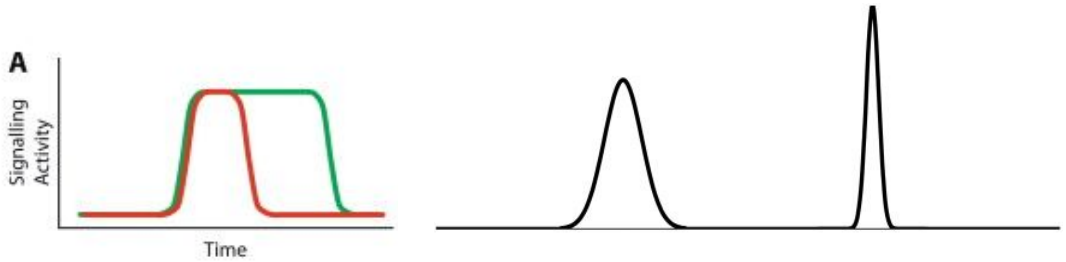


Andrea Katherine Pinto Martinez
andreakpintom@usp.br

O que é um sinal celular?

Quais características deveria ter?

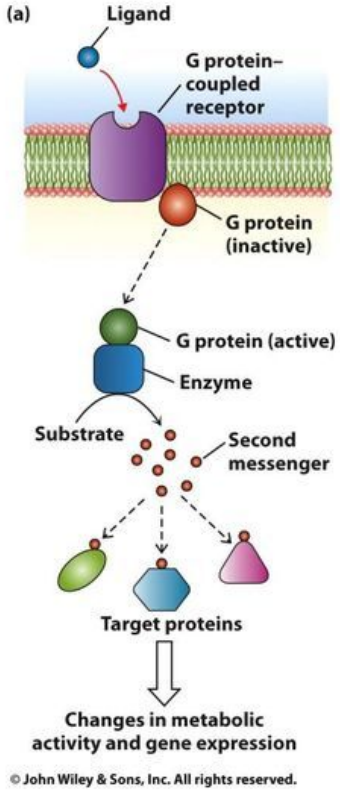
- Sinal físico ou químico → comunica uma mensagem { Duração, Amplitude, localização } resultado
- Se liga a outras moléculas (receptores, segundos mensageiros e efetores) → transdução do sinal
- Adaptação ou modulação (não é persistente)

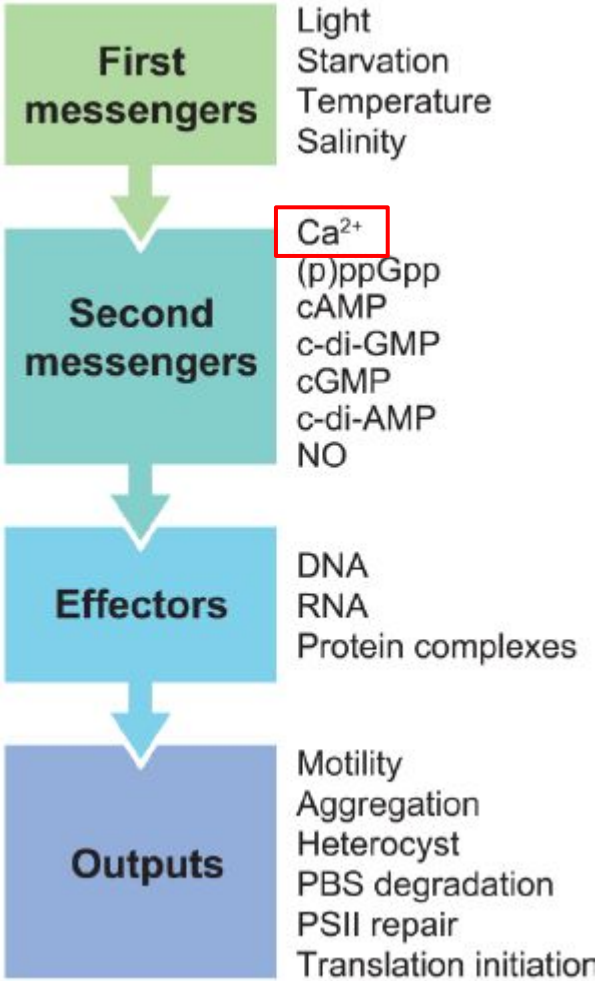


Katharina F. Sonnen and Alexander Aulehla. 2014. *Seminars in Cell & Developmental Biology*

Termos usados na transdução de sinal:

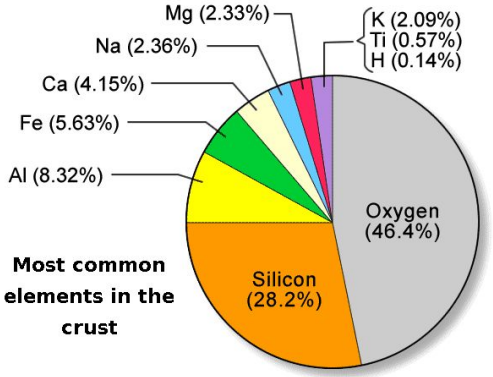
- Ligante (primeiro mensageiro)
- Receptor (transductor)
- Efetor primario
- Segundo mensageiro
- segundo efetor
- moléculas alvo (proteína/DNA)





Marco Agostoni and Montgomery Beronda, 2014.

Porque o Ca^{2+} entre outros íons?

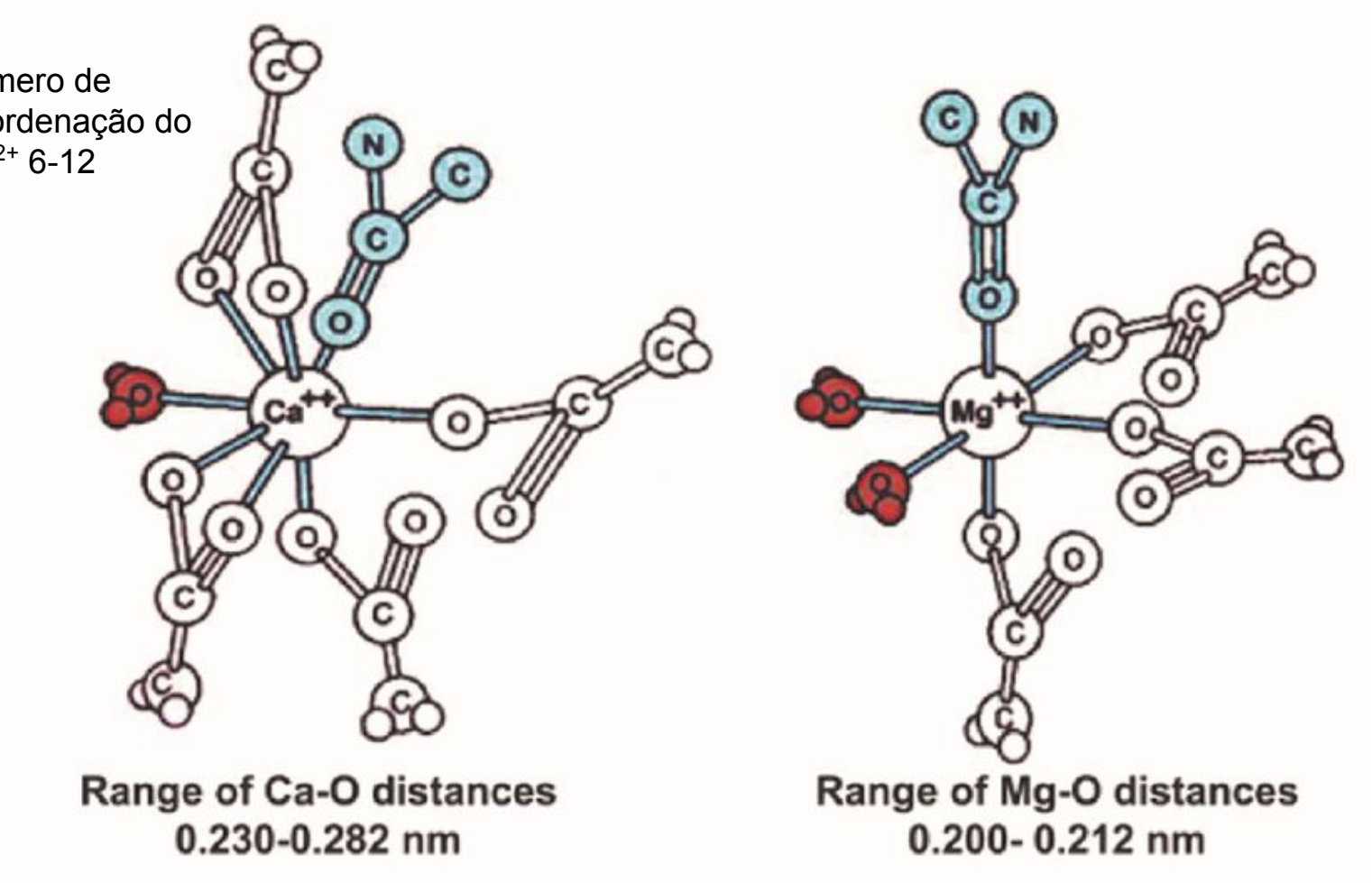


- Quinto elemento e terceiro metal mais abundante na crosta terrestre. → células devem lidar com ↑ $[Ca^{2+}]$ Amplamente disponível
- Baixa solubilidade e forma sais insolúveis especialmente com fosfatos (energy currency) → Demanda regulação ↓ $[Ca^{2+}]i$ (~100 nM)
- Química de coordenação peculiar
  - Carga
  - raio iônico
  - polarizabilidade
  - energía de hidratação
  - raio do íon metálico hidratado

permite → Desenvolvimento de moléculas complexas que ligam Ca^{2+} com sítios de geometria variável

E. Carafoli and J. Krebs, 2016.

número de coordenação do Ca^{2+} 6-12

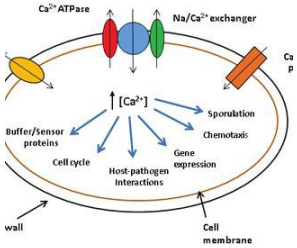


E. Carafoli and J. Krebs, 2016.

Homeostase de cálcio

- Todos os mecanismos usados para regular a concentração intracelular de Ca^{2+}
- Ubicuo

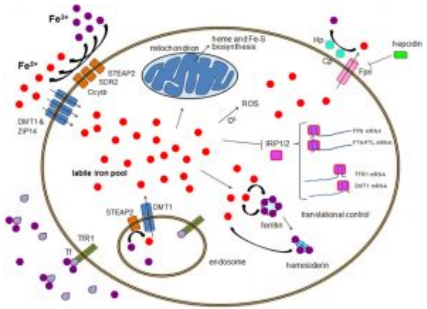
Procariotas



entrada e extrusão
~70 CaBPs

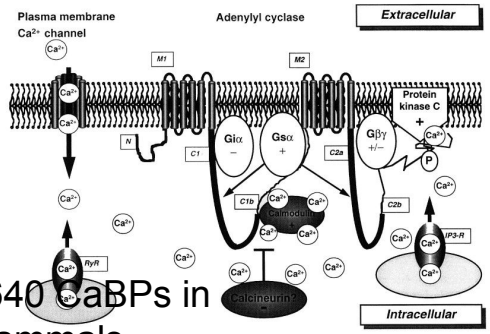
proteínas de união a Ca^{2+}

Eucariotas



compartimentalização

Eucariotas multicelulares



3640 CaBPs in mammals

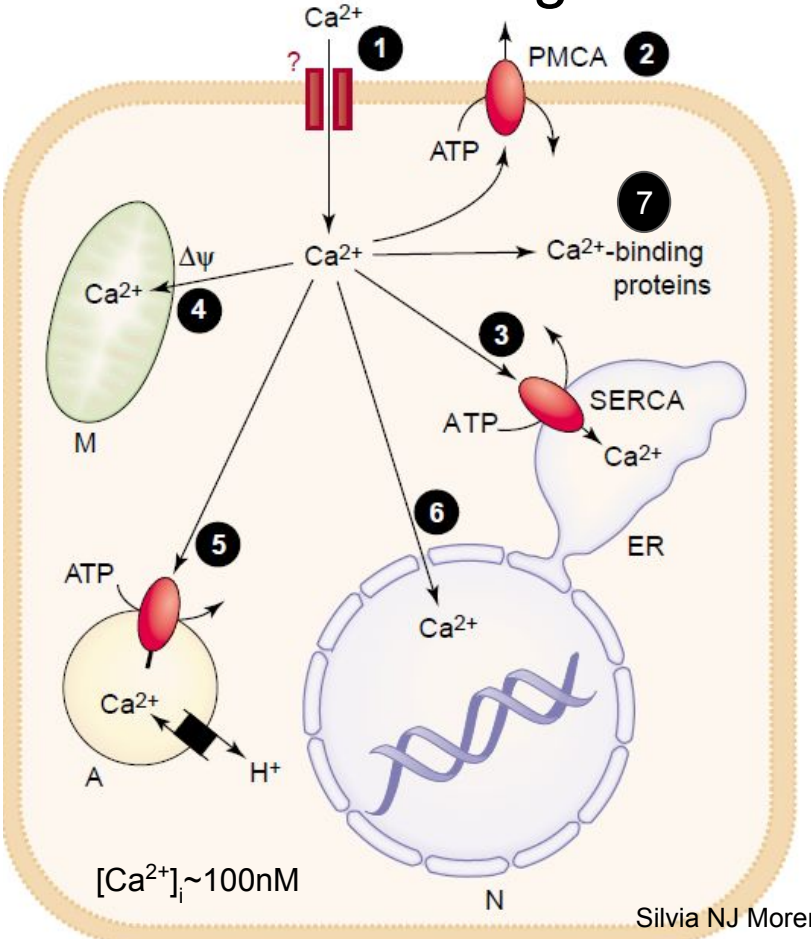
R. Maynard Case et al., 2007; Cai Xi et al., 2014

Protozoarios:

Functions regulated by Ca^{2+}	
Trypanosoma cruzi	Flagellar activity (motility), infectivity, cell proliferation
Trypanosoma brucei	Flagellar activity (motility), infectivity, cell proliferation
Leishmania spp.	Flagellar activity (motility), infectivity, cell proliferation
Plasmodium spp.	protein secretion, motility, cell invasion, cell progression, egress from red blood cells
Toxoplasma gondii	gliding motility, conoid extrusion, microneme secretion, and host cell invasion
Cryptosporidium parvum	Surface binding and invasion, protein secretion, gliding motility, and egress
Entamoeba histolytica	Life cycle development (growth and encystation), cytolytic activity
Giardia lamblia	Excystation and physiopathology
Trichomonas vaginalis	Adherence to mucosal epithelial cells and haemolytic activity

Maldonado Moreno et al., 1994; Yakubu et al., 1994; Docampo and Huang, 2015, Misra et al., 1991; Huang et al., 2013; Douglas A. Pace et al., 2014; Neil McCallum-Deighton and Anthony A.Holder, 1992; Silvia N.J. Moreno et al., 2011; Tooba Sarkhosh et al., 2019; Qiang Zhang et al., 2021; A. Makioka et al., 2001; Alok Bhattacharya et al., 2006; Sushumna Gorowara et al., 1991; David. S. Reiner et al., 2003; Koyama et al., 2009

Mecanismos reguladores de Ca^{2+} em protozoarios:



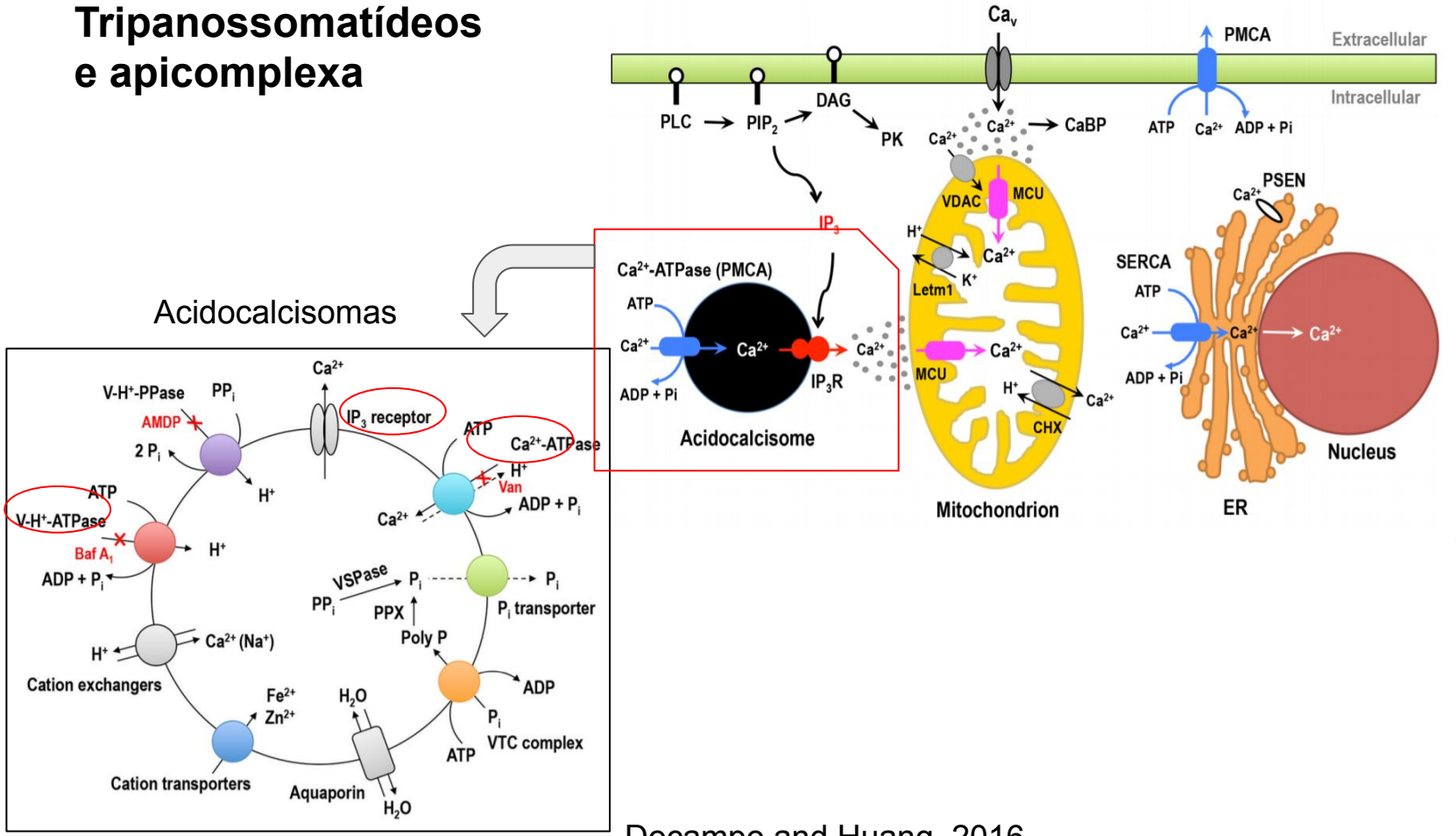
1.Ca^{2+} channel (entrance). **2.** PMCA (extrusão). **3.** SERCA. **4.** Mitocondria: MCU, Na^{+}/Ca^{2+} **5.** Acidocalcisomas: IP3R homologs (in *T. cruzi*), PMCA, H^{+}-ATPase, Na^{+}/H^{+} e Ca^{2+}/H^{+}. **6.** Núcleo

7. Ca^{2+} binding proteins: CaM, CaM-like proteins, calcireticulin, and Ca^{2+} binding proteins present in the flagellum. CDPKs are particularly important in apicomplexa.

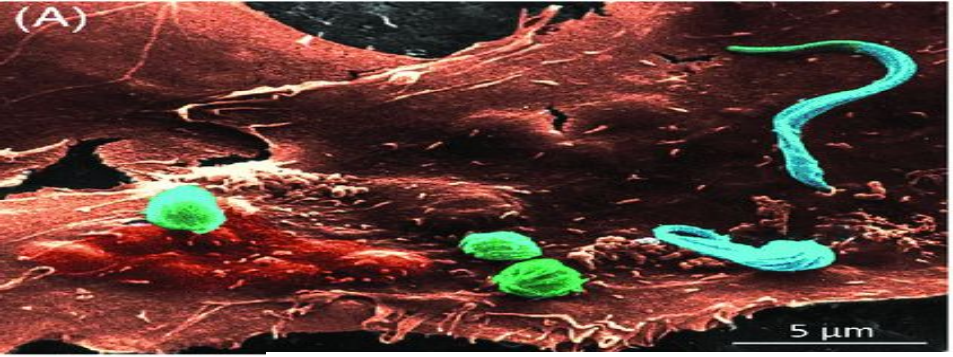
✖ Mitocondria *em Giardia lamblia*, *E. Histolytica* (mitosome/cryptome), e trichomonads (hydrogenosome) a homeostase de Ca^{2+} nesses organelas ainda deve ser estudada.

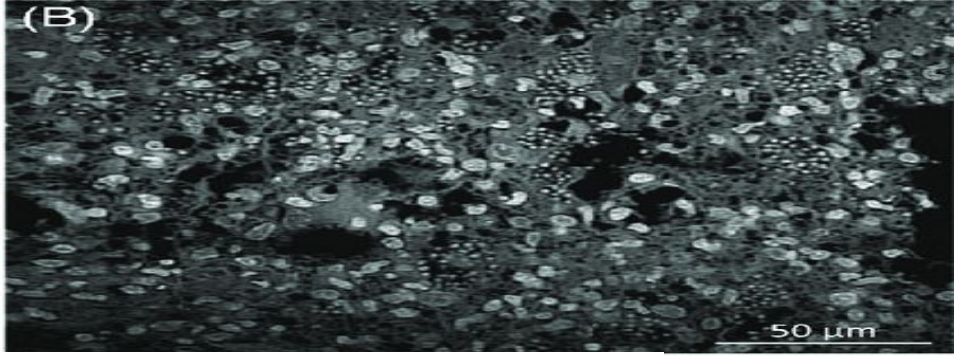
Silvia NJ Moreno and Roberto Docampo. *Current Opinion in Microbiology*. 2003

Tripanossomatídeos e apicomplexa

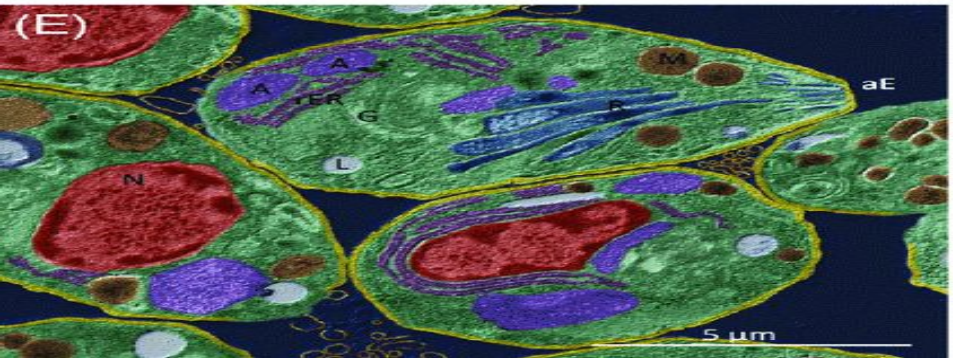


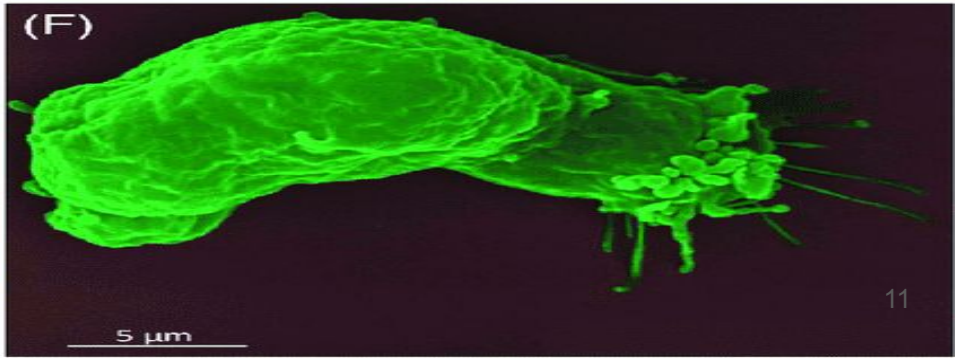
Docampo and Huang, 2016



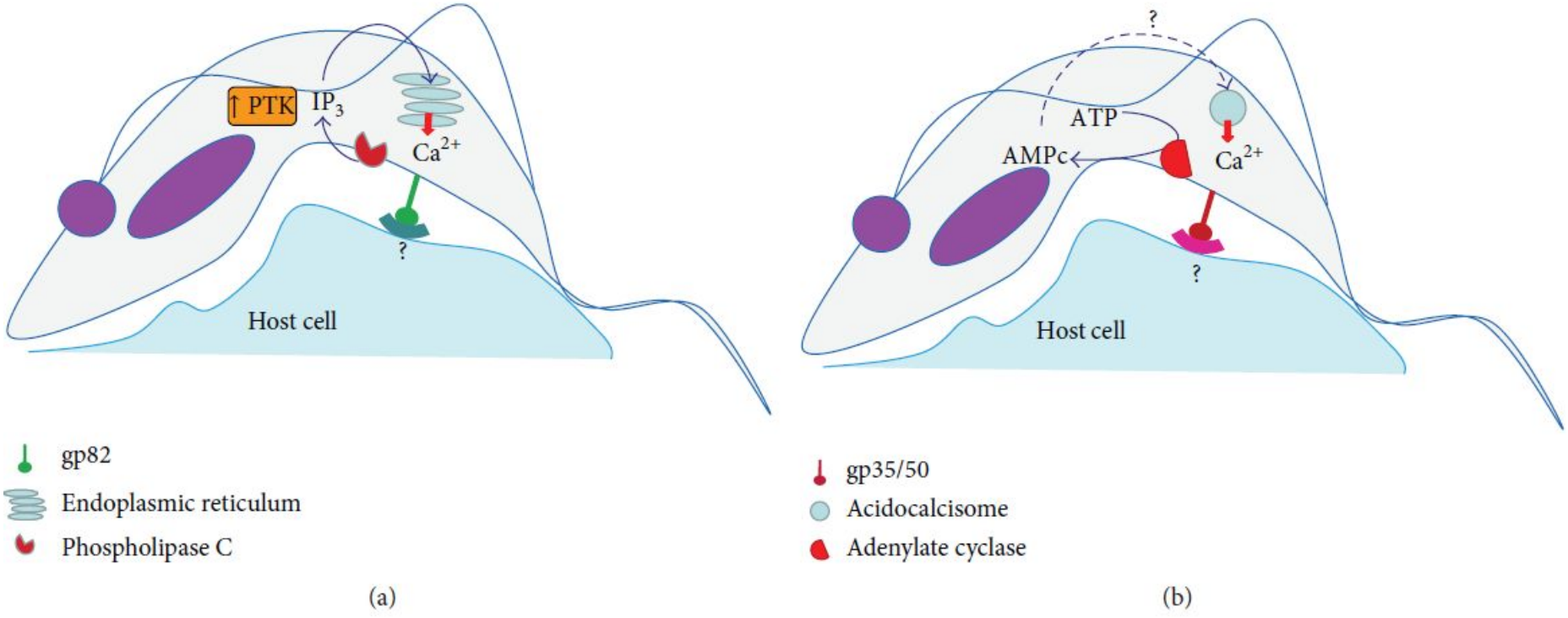


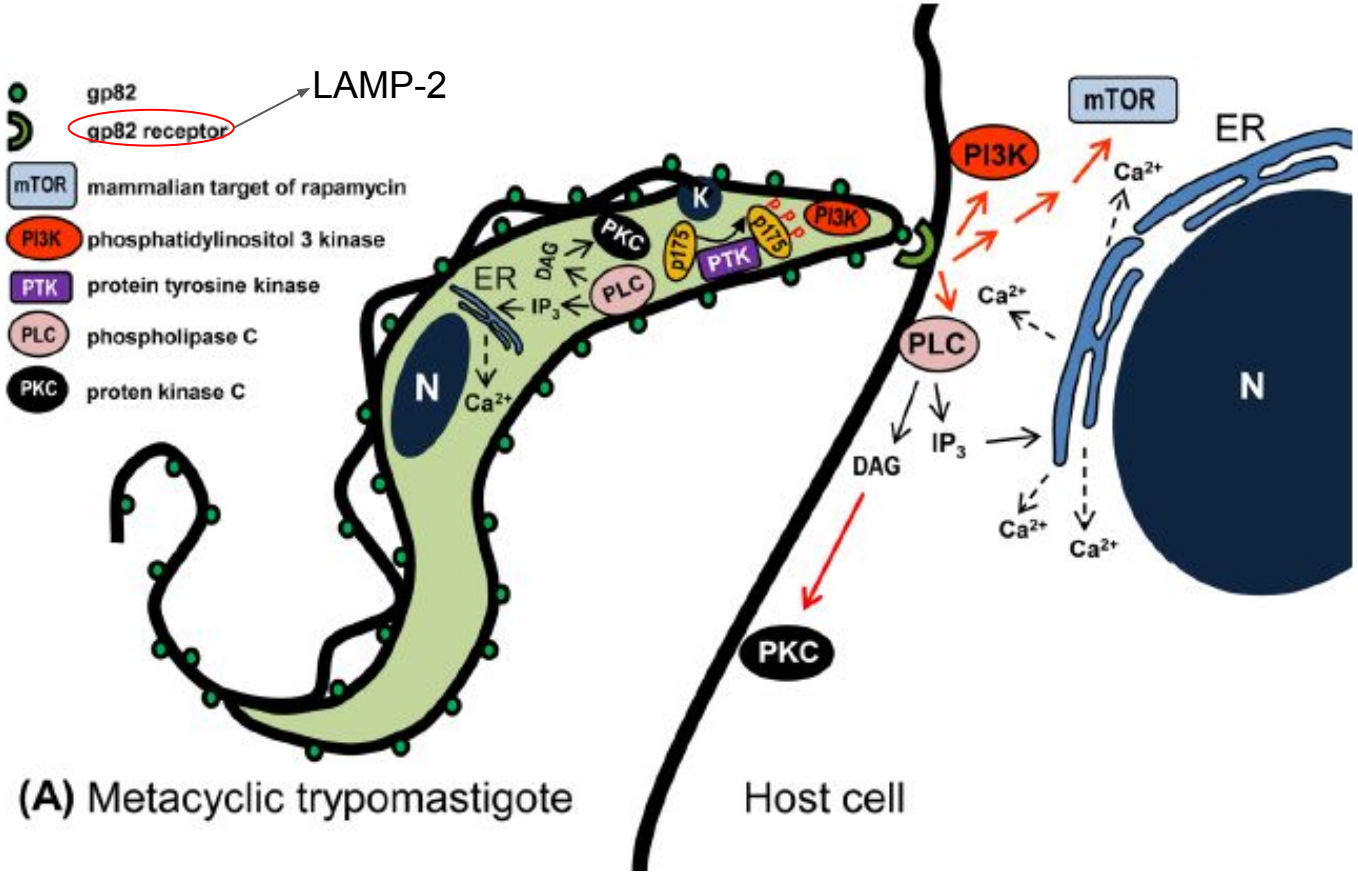
Uma história para o Ca^{2+} em cada parasita



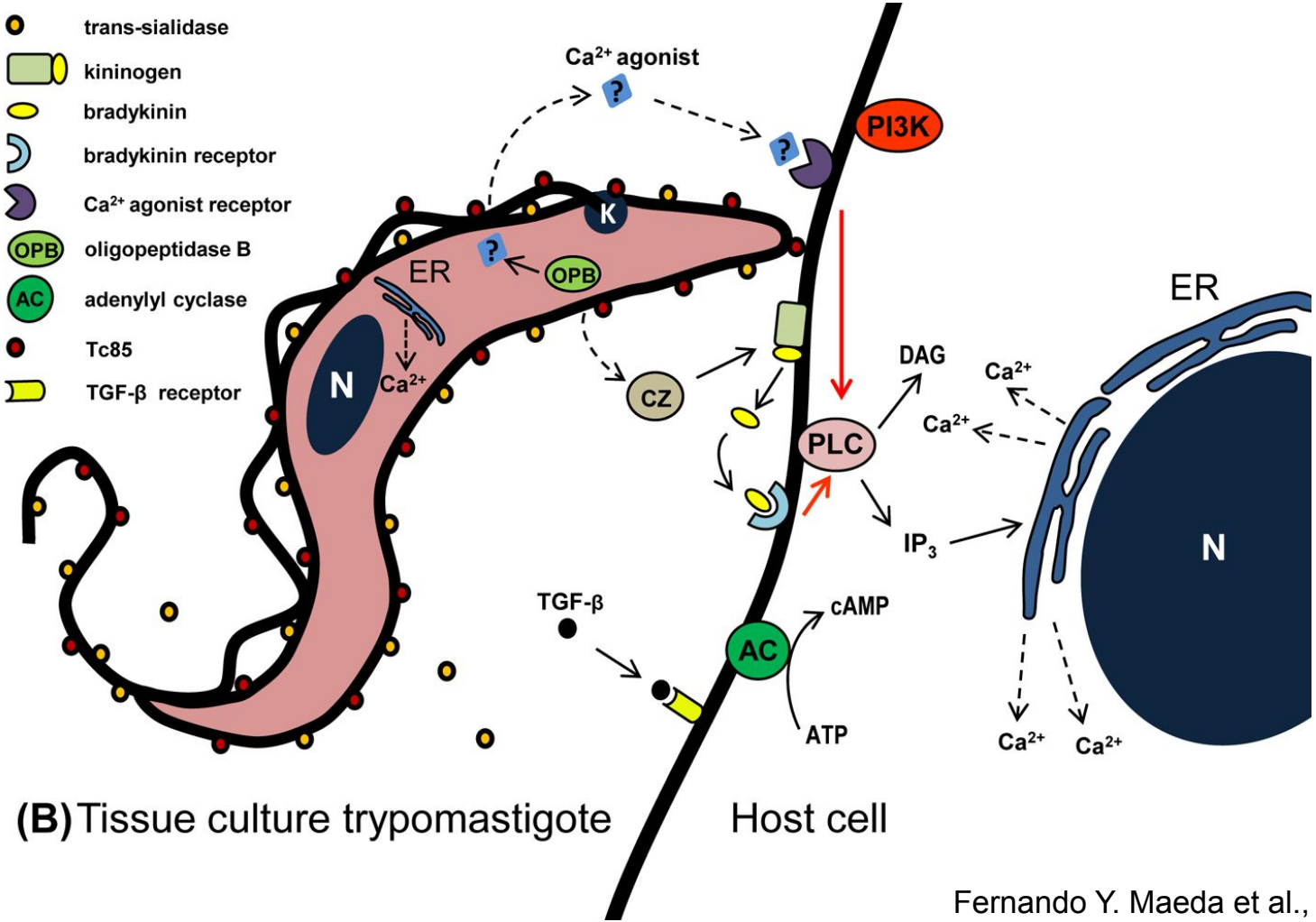


O Ca^{2+} durante a invasão da célula hóspede por *T. cruzi*



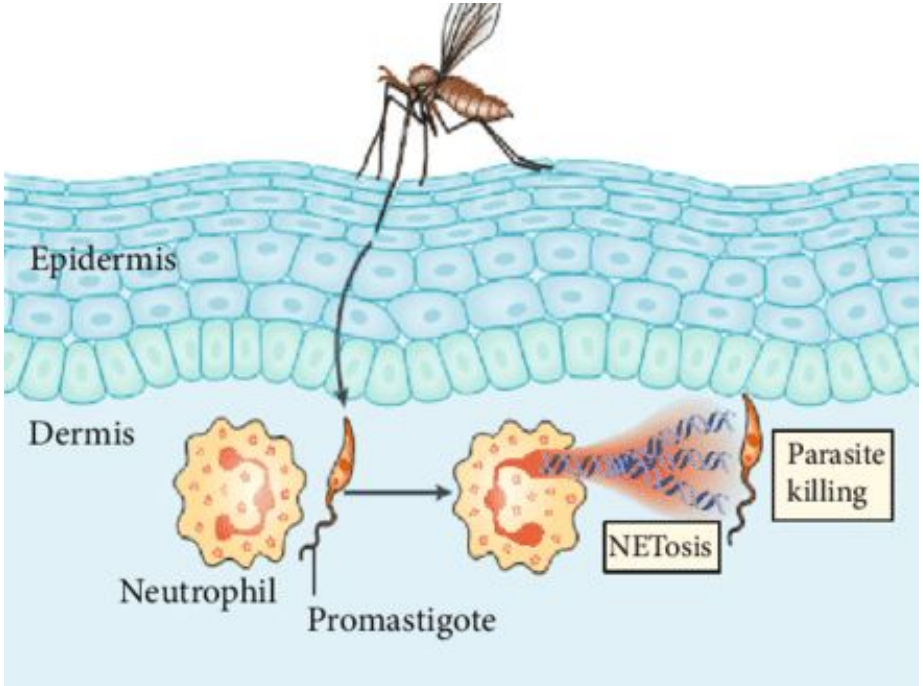


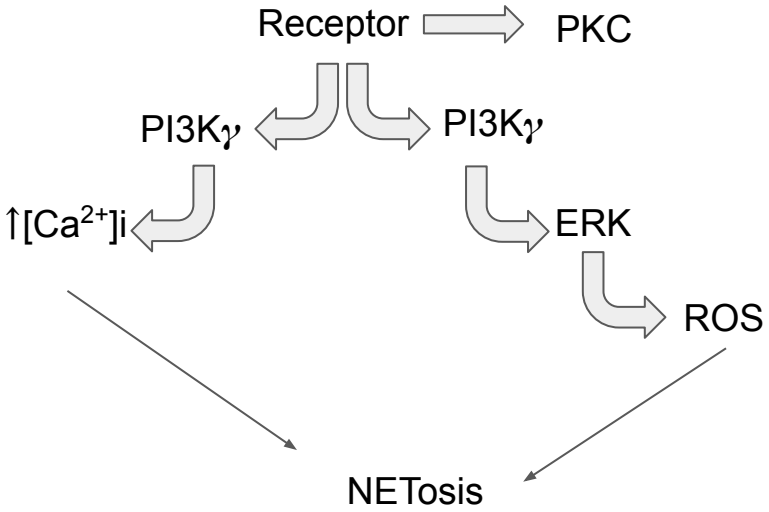
Fernando Y. Maeda et al., 2012; Rodrigues JPF et al., 2019



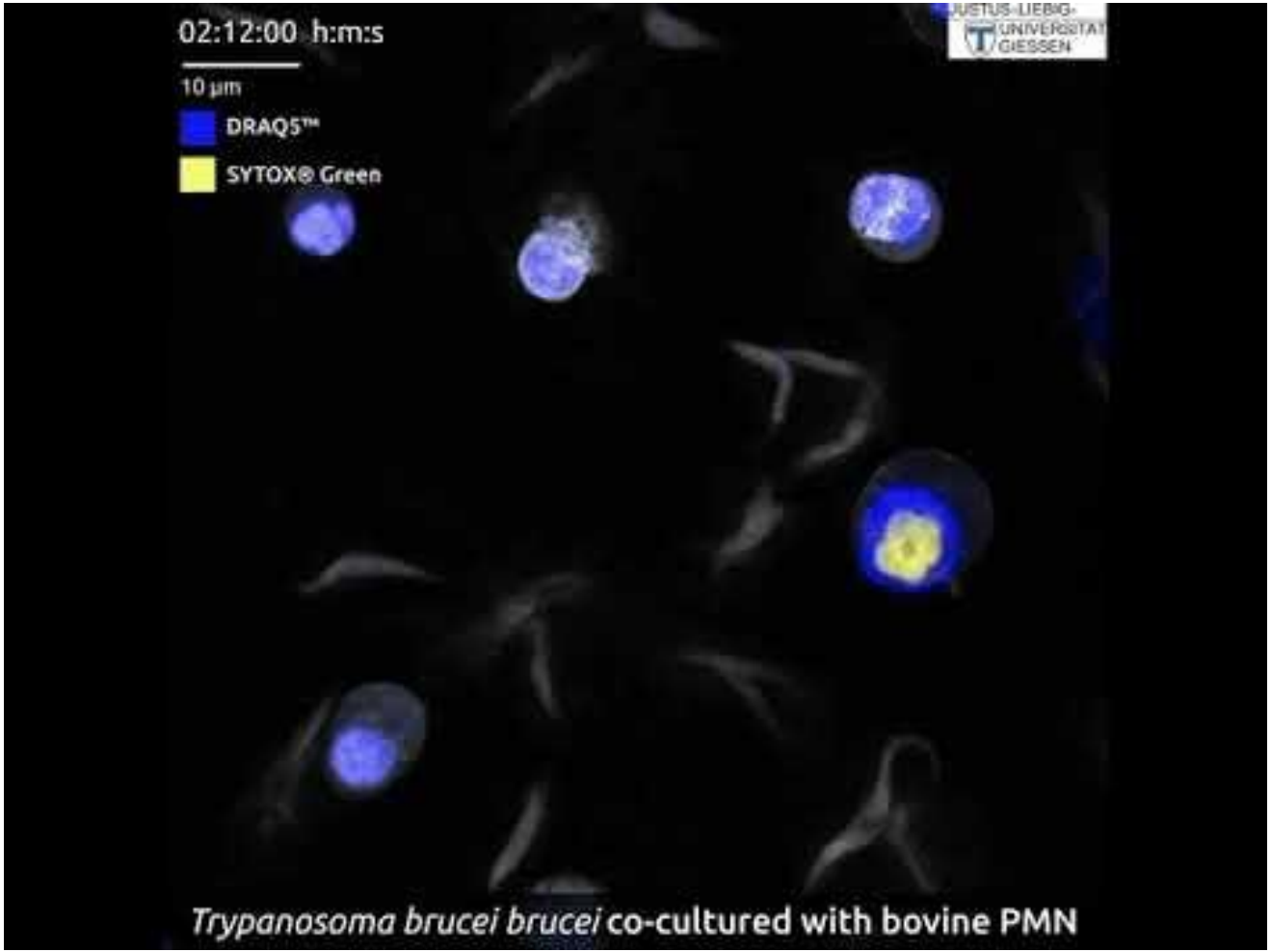
Fernando Y. Maeda et al., 2012

Liberação de NETs inducida por *Leishmania*

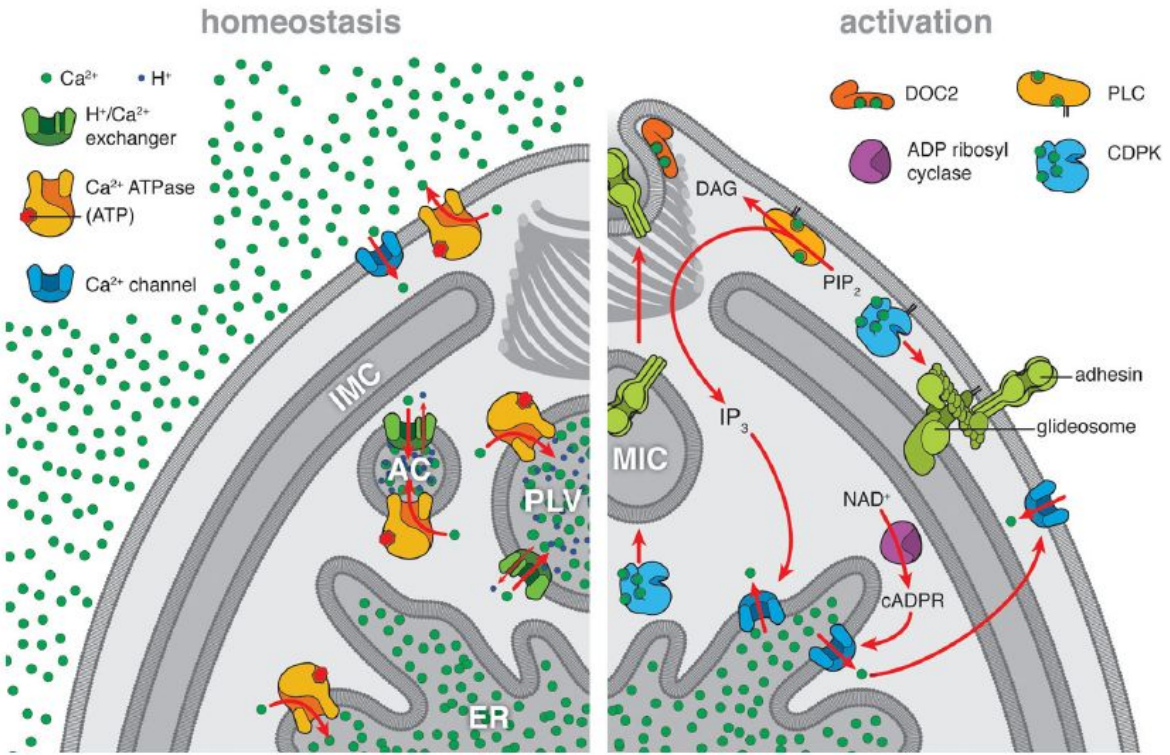


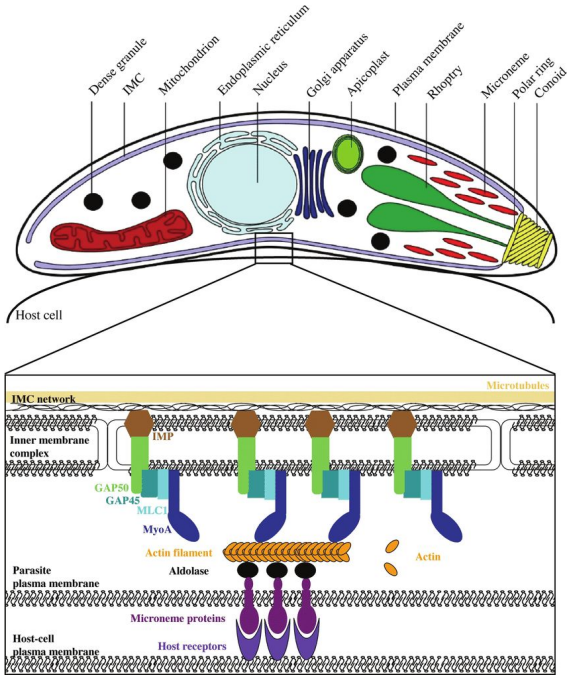


Teshager Dubie and Yasin Mohammed, 2020; Thiago DeSouza-Vieira et al., 2016


02:12:00 h:m:s
10 µm
DRAQ5™
SYTOX® Green
JUSTUS-LIEBIG-
UNIVERSITÄT
GIESSEN
Trypanosoma brucei brucei co-cultured with bovine PMN

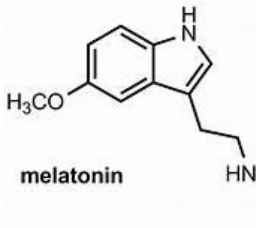
Toxoplasma gondii

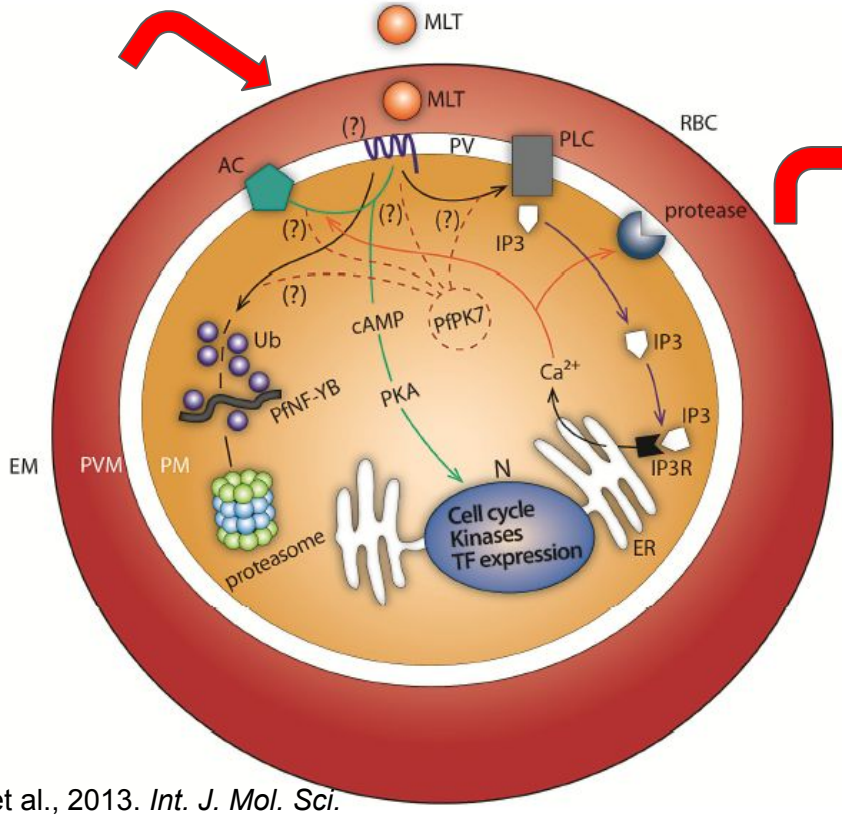


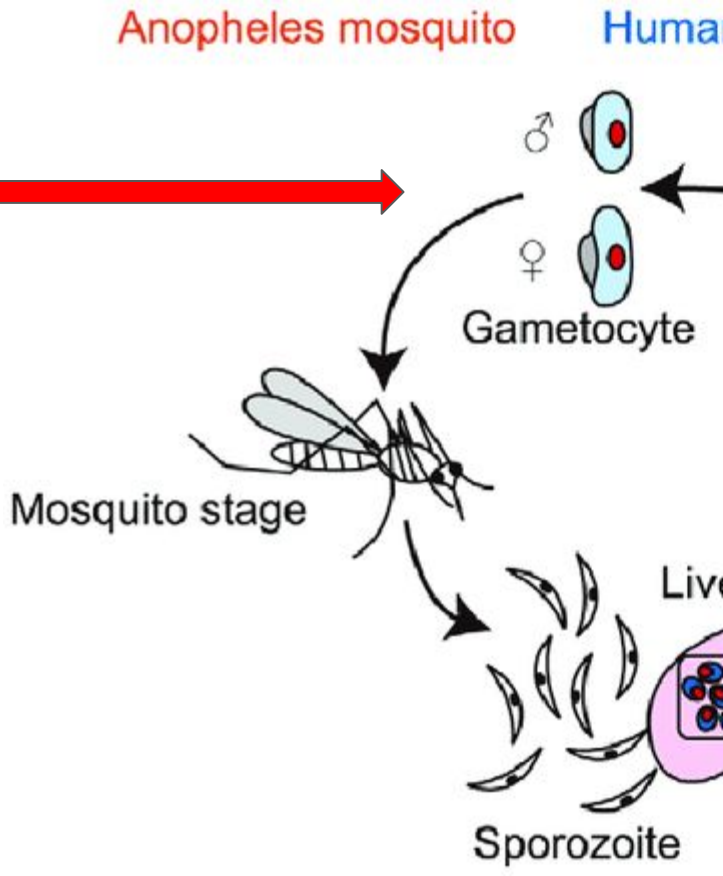


Lourido and Moreno, 2016; FrÉnal K., Foth B.J., Soldati D. (2008)

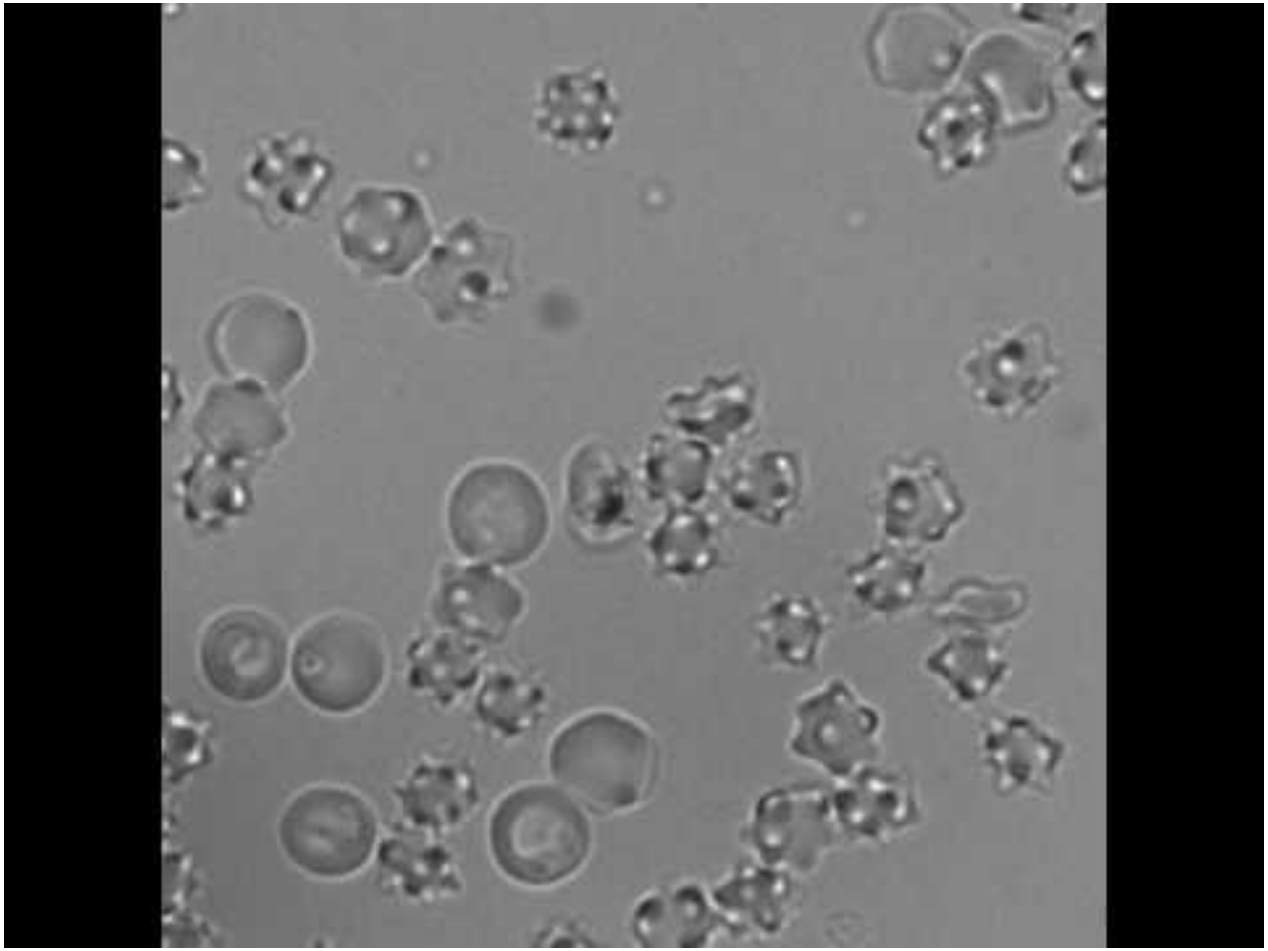
O Ca^{2+} no ritmo circadiano de *Plasmodium sp.*



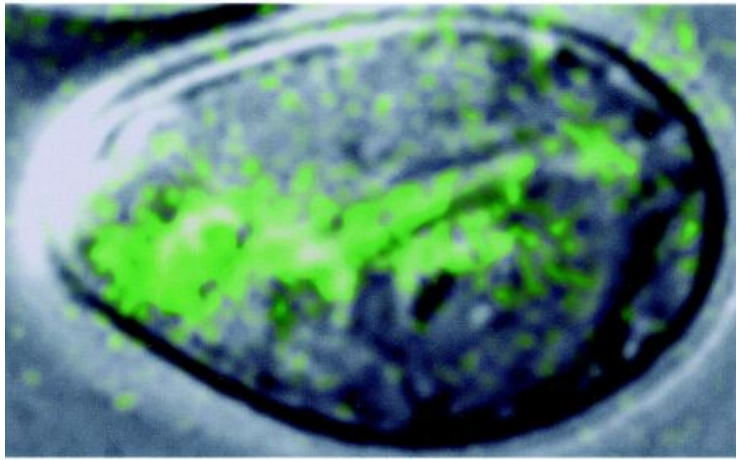




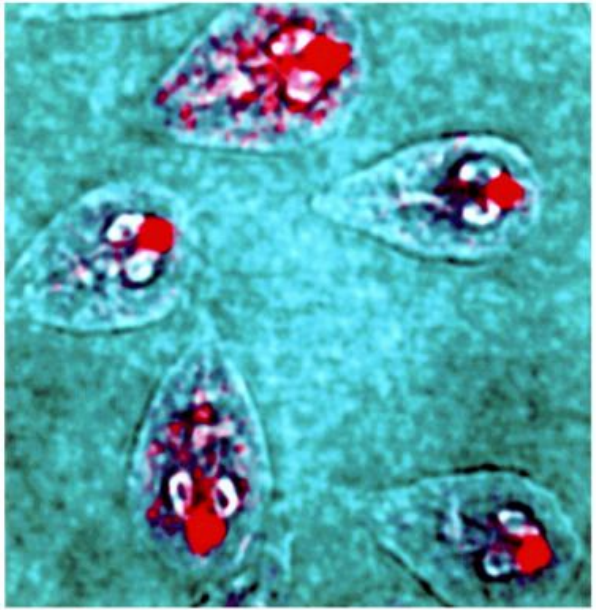
Wânia Rezende Lima et al., 2013. *Int. J. Mol. Sci.*



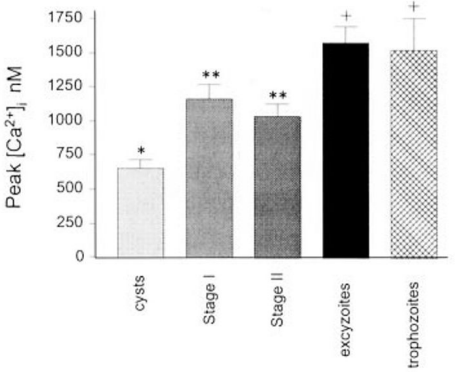
O Ca^{2+} na excistação de *Giardia lamblia*:



Cisto de *G. lamblia* corado com Bodipy-TG

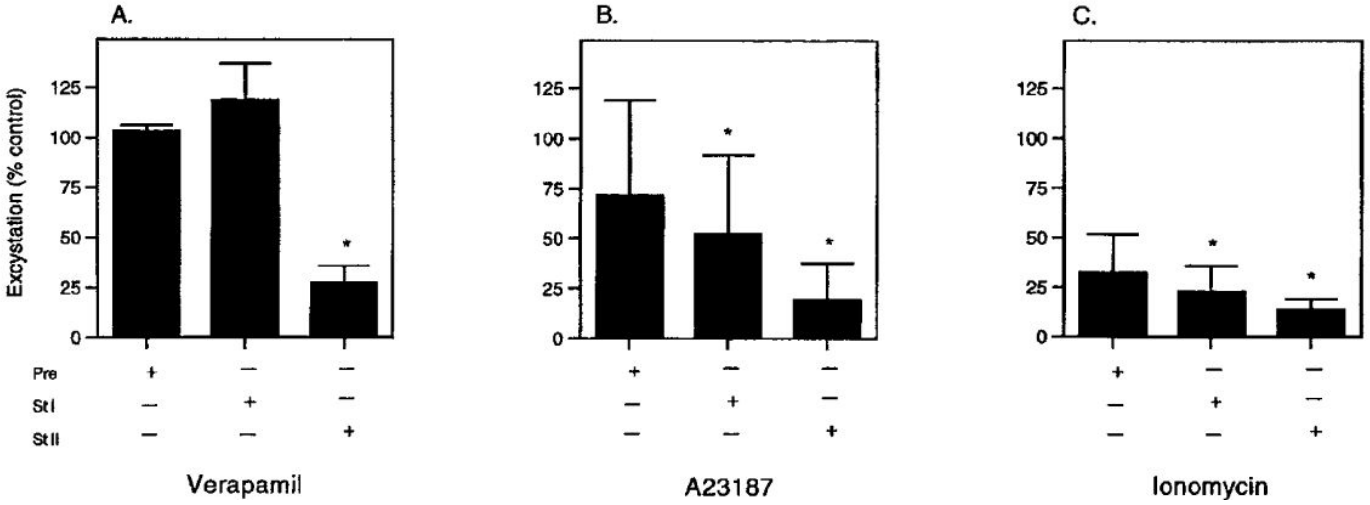


Imunolocalização de gCaM



Compartimentos de Ca^{2+} sensíveis a thapsigargin

David S. Reiner et al., 2003



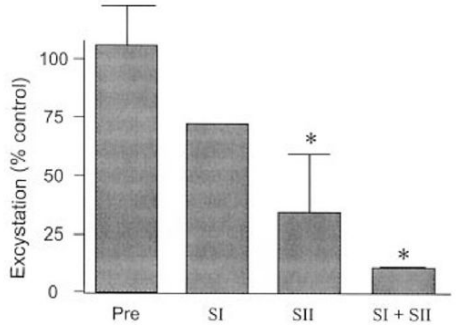


FIG. 3. **Kinetics of excystation inhibition by TG.** 10 μM TG was present only at the stage or stages indicated. *, $p < 0.05$, significant inhibition compared with solvent control.

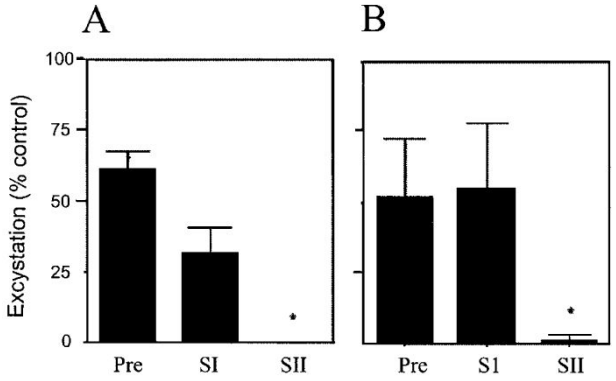
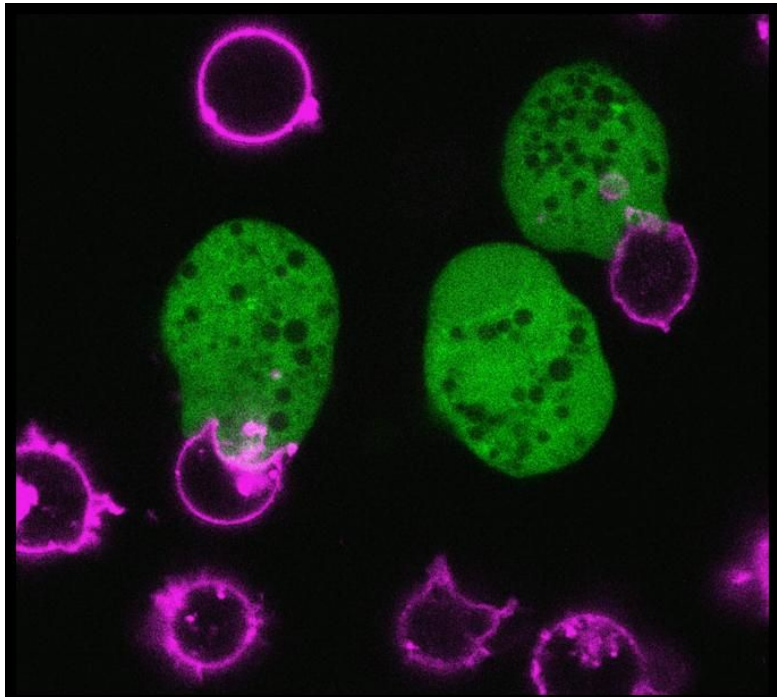


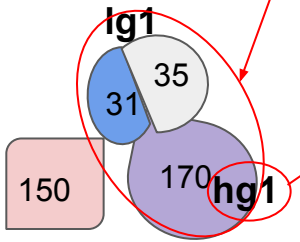
FIG. 5. **Inhibition of excystation by CaM inhibitors.** *A*, trifluoperazine (25 μM); *B*, chlorpromazine (50 μM). *, $p < 0.05$ significant inhibition of excystation compared with solvent control.

David S. Reiner et al., 2003

O Ca^{2+} na patogénesis de Entamoeba histolytica e citólise de células alvo



Reconhecimento: lectina de adherencia
(Gal/GalNAc 260 KDa)



CRD com motivo
DPN (liga Ca^{2+})

hemaglutinação

Citolisis

aumento da $[Ca^{2+}]i$ nas células alvo

Canais de Ca^{2+} inibe morte celular

Katherine Ralston et al., 2014; Mrigya Babuta et al., 2020

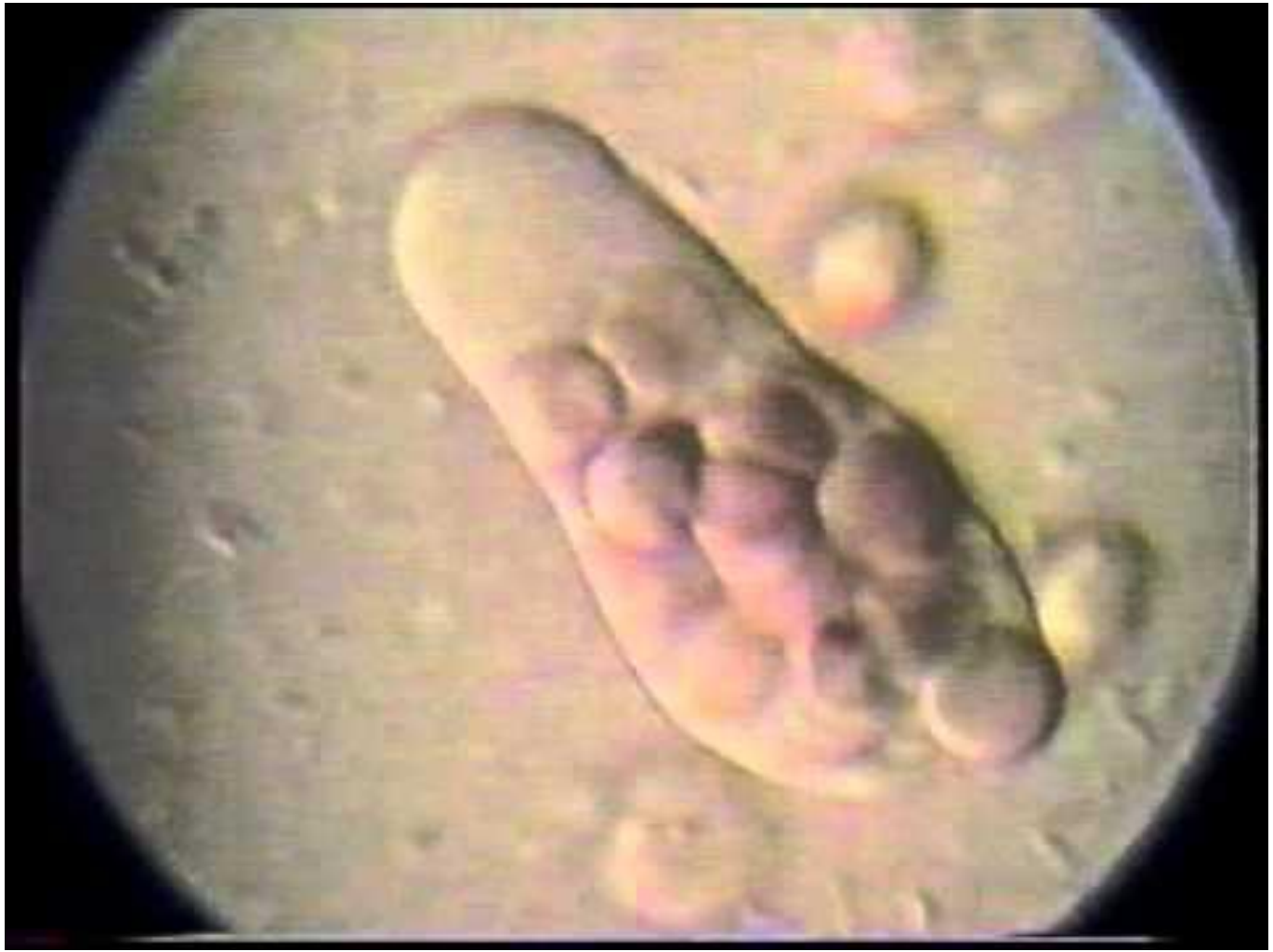


Table 1. List of proteins that interact with calcium and play a role in amebic homeostasis and pathogenesis.

Name of Protein	Function/Role	Reference
EhPMCA	It is present in vacuoles and in cytoplasmic network; however, function is unknown.	[7]
EhSERCA	It is present in vacuoles and in cytoplasmic network. Function is unknown.	[8]
EhSPCA	Putative Ca^{2+}-ATPase that is localized in vacuoles stained with NBD C6-ceramide, a Golgi apparatus marker. Function is unknown.	[10]
EhCCX	CCX that plays a role in programmed cell death and in virulence.	[9]
Ca^{2+}-dependent ATPase/ADPase	They are localized in the inner membrane of cytoplasmic vacuoles that may or may not be phagolysosomes. Function is unknown.	[15,17]
Calpain-like protein	Ca^{2+}-dependent cysteine protease involved in programmed cell death.	[13,14]
Ca^{2+}-dependent thiamine pyrophosphatase	They are localized in the inner membrane of cytoplasmic vacuoles that may or may not be phagolysosomes. Function is unknown.	[16]
Gal/GalNAc	It is involved in the process of invasion because it helps in adhering to the target cells.	[19–22,24,25]
EhCRT	Amebic CRT is involved in the phagocytosis of apoptotic immune cells.	[26,27]
UREBP	It regulates the transcription of amebic genes and inhibits transcription in the presence of Ca^{2+}.	[40,43,44]
EhC2A	It helps in localization of UREBP to the membrane apart from the nucleus.	[44]
EhC2PK	C2PK that is involved in initiation of phagocytosis.	[52,53]
EhCaBP1	Calcium-binding protein 1 that directly regulates erythrophagocytosis and actin dynamics.	[49,50,72]
EhCaBP2	It is 79% identical to EhCaBP1 but neither involved in phagocytosis or pseudopod formation. Function is not known.	[66–68]
EhCaBP3	Calcium-binding protein 3 interacts with the Myosin IB and Arp2/3 complex and plays a role in erythrophagocytosis.	[57,58]
EhCaBP5	Calcium-binding protein 5 is likely to be a light chain of myosin IB that is involved in phagocytosis.	[59]
EhCaBP6	Calcium-binding protein 6, which is involved in cell division and modulates microtubule dynamics.	[69,70]
Grainin1 and 2	EF-hand-motif–containing calcium-binding proteins involved in amebic virulence. It is also speculated they are also involved in vesicle maturation and exocytosis.	[63,64]
EhCaBP7–27	Other calcium-binding proteins encoded in the *E. histolytica* genome. Function is not deciphered yet.	[18]

Mrigya Babuta et al., 2020; Martha Valle-Solis et al., 2018

Conclusões:

- O Ca^{2+} possui uma química de coordenação única que permitiu sua seleção como molécula sinalizadora presente em todas as formas de vida.
- A invasão por *T. cruzi* é um processo de sinalização que envolve múltiplas moléculas e requer a mobilização do Ca^{2+} tanto na célula hóspede como no parasita.
- A mobilização do Ca^{2+} contribui com a produção de NETs nos neutrófilos induzida por *Leishmania sp.*
- A motilidade de *Toxoplasma gondii* requer de sinais mediadas por Ca^{2+} para a ativação do glideosoma e secreção de micronemas.
- A sincronização de *Plasmodium* com o ciclo circadiano é fundamental na adaptação do parasita, depende da sinalização por Ca^{2+} e a ativação de uma série de eventos rio abaixo que modulam o ciclo celular.
- A excistação de *Giardia lamblia* depende de sinais por Ca^{2+} controladas pela entrada do cátion, compartimentalização em reservatórios sensíveis a CaM e CaM.
- Diversos processos celulares na patogênese de *Entamoeba histolytica* dependem de Ca^{2+} (aderência, citolise, facitose, trogocitose) tanto no parasita como na célula alvo.

Bibliografia

- Carafoli E, Krebs J. Why Calcium? How Calcium Became the Best Communicator. J Biol Chem. 2016 Sep 30;291(40):20849-20857. doi: 10.1074/jbc.R116.735894. Epub 2016 Jul 26. PMID: 27462077; PMCID: PMC5076498.
- Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol. 2000 Oct;1(1):11-21. doi: 10.1038/35036035. PMID: 11413485.
- Moreno SN, Docampo R. Calcium regulation in protozoan parasites. Curr Opin Microbiol. 2003 Aug;6(4):359-64. doi: 10.1016/s1369-5274(03)00091-2. PMID: 12941405.
- Gupta Y, Goicoechea S, Pearce CM, Mathur R, Romero JG, Kwofie SK, Weyenberg MC, Daravath B, Sharma N, Poonam, Akala HM, Kanzok SM, Durvasula R, Rathi B, Kempaiah P. The emerging paradigm of calcium homeostasis as a new therapeutic target for protozoan parasites. Med Res Rev. 2021 Apr 13. doi: 10.1002/med.21804. Epub ahead of print. PMID: 33851452.
- Maeda FY, Cortez C, Yoshida N. Cell signaling during Trypanosoma cruzi invasion. Front Immunol. 2012 Nov 28;3:361. doi: 10.3389/fimmu.2012.00361. PMID: 23230440; PMCID: PMC3515895.
- Burleigh BA, Andrews NW. Signaling and host cell invasion by Trypanosoma cruzi. Curr Opin Microbiol. 1998 Aug;1(4):461-5. doi: 10.1016/s1369-5274(98)80066-0. PMID: 10066513.
- Benaim G, Paniz-Mondolfi AE, Sordillo EM, Martinez-Sotillo N. Disruption of Intracellular Calcium Homeostasis as a Therapeutic Target Against *Trypanosoma cruzi*. Front Cell Infect Microbiol. 2020 Feb 14;10:46. doi: 10.3389/fcimb.2020.00046. PMID: 32133302; PMCID: PMC7040492.
- Chiurillo MA, Lander N, Vercesi AE, Docampo R. IP3 receptor-mediated Ca2+ release from acidocalcisomes regulates mitochondrial bioenergetics and prevents autophagy in Trypanosoma cruzi. Cell Calcium. 2020 Dec;92:102284. doi: 10.1016/j.ceca.2020.102284. Epub 2020 Sep 2. PMID: 32947181.
- Babuta M, Bhattacharya S, Bhattacharya A. Entamoeba histolytica and pathogenesis: A calcium connection. PLoS Pathog. 2020 May 7;16(5):e1008214. doi: 10.1371/journal.ppat.1008214. PMID: 32379809; PMCID: PMC7205191.
- Scarpelli PH, Pecenin MF, Garcia CRS. Intracellular Ca2+ Signaling in Protozoan Parasites: An Overview with a Focus on Mitochondria. Int J Mol Sci. 2021 Jan 5;22(1):469. doi: 10.3390/ijms22010469. PMID: 33466510; PMCID: PMC7796463.
- DeSouza-Vieira T, Guimarães-Costa A, Rochael NC, Lira MN, Nascimento MT, Lima-Gomez PS, Mariante RM, Persechini PM, Saraiva EM. Neutrophil extracellular traps release induced by Leishmania: role of PI3Kγ, ERK, PI3Kσ, PKC, and [Ca2+]. J Leukoc Biol. 2016 Oct;100(4):801-810. doi: 10.1189/jlb.4A0615-261RR. Epub 2016 May 6. PMID: 27154356; PMCID: PMC5014744.
- Hann J, Bueb JL, Tolle F, Bréchard S. Calcium signaling and regulation of neutrophil functions: Still a long way to go. J Leukoc Biol. 2020 Feb;107(2):285-297. doi: 10.1002/JLB.3RU0719-241R. Epub 2019 Dec 16. PMID: 31841231.
- FrÉnal K., Foth B.J., Soldati D. (2008) Myosin Class XIV And Other Myosins In Protists. In: Myosins. Proteins and Cell Regulation, vol 7. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6519-4_15

Obrigada
Gracias
Thank you

Table 1

Ca^{2+} ATPases that have been cloned and sequenced in parasitic protozoa.

Protozoa	Name	Accession number	Type	Expressed	Function confirmed*
Trypanosoma cruzi	Tca1	U70620	PMCA	Yes	Yes
	TcSCA	AF093566	SERCA	Yes	Yes
Trypanosoma brucei	Tba1	M73769	SERCA	Yes	Yes
	TbA1	AY065988	PMCA	Yes	Yes†
	TbA2	AY065989	PMCA	Yes	Yes†
Leishmania mexicana amazonensis	Lmaa1	U70540	SERCA	Yes	Yes
Plasmodium falciparum	PfATPase6	X71765	SERCA	No	No
	PfATP4	AF203980	New subclass?	Yes	Yes
Toxoplasma gondii	TgA1	AF151372	PMCA	Yes	Yes
Cryptosporidium parvum	CpATPase1	U65981	New subclass?	Yes	No
Entamoeba histolytica	Pmca‡	U20321	PMCA	Yes	Yes
Trichomonas vaginalis	TVCA1	U65066	SERCA	No	No
	TVCA(2-4)‡	AF145282 AF145283 AF145279	Unknown	No	No

*Function confirmed by expression in the same parasite or in a heterologous system and correlation found between expression of the enzyme and Ca^{2+} transport.

†Luo S., Uyemura SA., Moreno SNJ. and Docampo R., unpublished results.

‡Partial sequence in GenBank.

A single amino acid residue can determine the sensitivity of SERCAs to artemisinins

Anne-Catrin Uhlemann[1], Angus Cameron[2], Ursula Eckstein-Ludwig[1], Jorge Fischbarg[3], Pavel Iserovich[3], Felipe A Zuniga[3], Malcolm East[4], Anthony Lee[4], Leo Brady[2], Richard K Haynes[5] & Sanjeev Krishna[1]

Artemisinins are the most important class of antimalarial drugs. They specifically inhibit PfATP6, a SERCA-type ATPase of *Plasmodium falciparum*. Here we show that a single amino acid in transmembrane segment 3 of SERCAs can determine susceptibility to artemisinin. An L263E replacement of a malarial by a mammalian residue abolishes inhibition by artemisinins. Introducing residues found in other *Plasmodium* spp. also modulates artemisinin sensitivity, suggesting that artemisinins interact with the thapsigargin-binding cleft of susceptible SERCAs.

Calflagin Inhibition Prolongs Host Survival and Suppresses Parasitemia in *Trypanosoma brucei* Infection[▽]

Brian T. Emmer,[1] Melvin D. Daniels,[1]† Joann M. Taylor,[1,2]
Conrad L. Epting,[2]* and David M. Engman[1]*

*Departments of Pathology and Microbiology-Immunology[1] and Department of Pediatrics,[2]
Northwestern University, Chicago, Illinois*

Received 12 April 2010/Accepted 13 April 2010

African trypanosomes express a family of dually acylated, EF-hand calcium-binding proteins called the calflagins. These proteins associate with lipid raft microdomains in the flagellar membrane, where they putatively function as calcium signaling proteins. Here we show that these proteins bind calcium with high affinity and that their expression is regulated during the life cycle stage of the parasite, with protein levels approximately 10-fold higher in the mammalian bloodstream form than in the insect vector procyclic stage. We also demonstrate a role for the calflagins in mammalian infection, as inhibition of the entire calflagin family by RNA interference dramatically increased host survival and attenuated parasitemia in a mouse model of sleeping sickness. In contrast to infection with parental wild-type parasites, which demonstrated an unremitting parasitemia and death within 6 to 10 days, infection with calflagin-depleted parasites demonstrated prolonged survival associated with a sudden decrease in parasitemia at approximately 8 days postinfection. Subsequent relapsing and remitting waves of parasitemia thereafter were associated with alternate expression of the variant surface glycoprotein, suggesting that initial clearance was antigen specific. Interestingly, despite the notable *in vivo* phenotype and flagellar localization of the calflagins, *in vitro* analysis of the calflagin-deficient parasites demonstrated normal proliferation, flagellar motility, and morphology. Further analysis of the kinetics of surface antibody clearance also did not demonstrate a deficit in the calflagin-deficient parasites; thus, the molecular basis for the altered course of infection is independent of an effect on parasite cell cycle progression, motility, or degradation of surface-bound antibodies.

Molecular Microbiology (2006) **61**(6), 1523–1532

doi:10.1111/j.1365-2958.2006.05344.x
First published online 23 August 2006

Comparative proteomic analysis of two Entamoeba histolytica strains with different virulence phenotypes identifies peroxiredoxin as an important component of amoebic virulence

Paul H. Davis,[1,2] Xiaochun Zhang,[1] Jianhua Guo,[1] R. Reid Townsend[1] and Samuel L. Stanley, Jr[1,2*]

[1]Department of Medicine, Washington University School of Medicine, St Louis, MO, USA.

[2]Department of Molecular Microbiology, Washington University School of Medicine, St Louis, MO, USA.

phenotypic differences, and identify peroxiredoxin as an important component of virulence in amoebic colitis.

Introduction

Increased expression of grainin proteins associated with decreased virulence

Table 2

Ca^{2+}-binding proteins in parasitic protozoa.

Protozoa	Name	Accession number	Expected or demonstrated function
Trypanosoma cruzi	CaM*	M98551	Cell signaling
	FcaBP	D87512	Unknown
	Calreticulin	AF107115	Ca^{2+}-store/quality control of glycoprotein folding/potent immunogen
Leishmania major	CaM	AL445944	Cell signaling
Leishmania donovani	Calreticulin	U49191	Ca^{2+} store
Trypanosoma brucei	CaM	X56511	Cell signaling
	Calflagin	U06644	Unknown
Trypanosoma brucei gambiense	CaM	K02944	Cell signaling
Plasmodium falciparum	CaM	AE014850	Cell signaling
Toxoplasma gondii	CaM	Y08373	Cell signaling
Cryptosporidium parvum	CaM	AQ842812†	Cell signaling
Entamoeba histolytica	EhCaBP	M84155	Unknown/cytosolic
	Grainin 1	AF085196	Unknown/in granules
	Grainin 2	AF082530	Unknown/in granules
Giardia lamblia	CaM	AF359239	Cell signaling
Trichomonas vaginalis	CaM	U38786	Cell signaling

*CaM, calmodulin.
†Partial sequence.

Cell Calcium 92 (2020) 102284

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

IP_3 receptor-mediated Ca^{2+} release from acidocalcisomes regulates mitochondrial bioenergetics and prevents autophagy in *Trypanosoma cruzi*

Miguel A. Chiurillo [a,b,1,*], Noelia Lander [a,b,1], Anibal E. Vercesi [b], Roberto Docampo [a,*]

[a] Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA, 30602, USA
[b] Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, 13083, Brazil

PLC (PIP2)---> IP3 + DAG

Functions regulated by Ca^{2+} in protozoan parasites:

- Flagellar activity and infectivity in *T. cruzi* (Maldonado Moreno et al., 1994; Yakubu et al., 1994) and *Leishmania spp.* (Docampo and Huang, 2015, Misra et al., 1991)
- Cell proliferation in *T. cruzi* (Docampo and Huang, 2015) and *T. brucei* (Huang et al., 2013)
- Virulence traits, such as gliding motility, conoid extrusion, microneme secretion, and host cell invasion in *Toxoplasma gondi*i (Douglas A. Pace et al., 2014).
- Erythrocyte invasion by *Plasmodium falciparum* and parasite cell motility (NeilMcCallum-Deighton and Anthony A.Holder, 1992; Silvia N.J. Moreno et al., 2011)
- Surface binding and invasion, protein secretion, gliding motility, and egress of *Cryptosporidium parvum* (Tooba Sarkhosh et al., 2019; Qiang Zhang et al., 2021)
- Life cycle development (growth and encystation), cytolytic activity in *Entamoeba histolytica* (A. Makioka et al., 2001; Alok Bhattacharya et al., 2006).
- Excystation and physiopathology in *Giardia lamblia* (Sushumna Gorowara et al., 1991; David. S. Reiner et al., 2003)