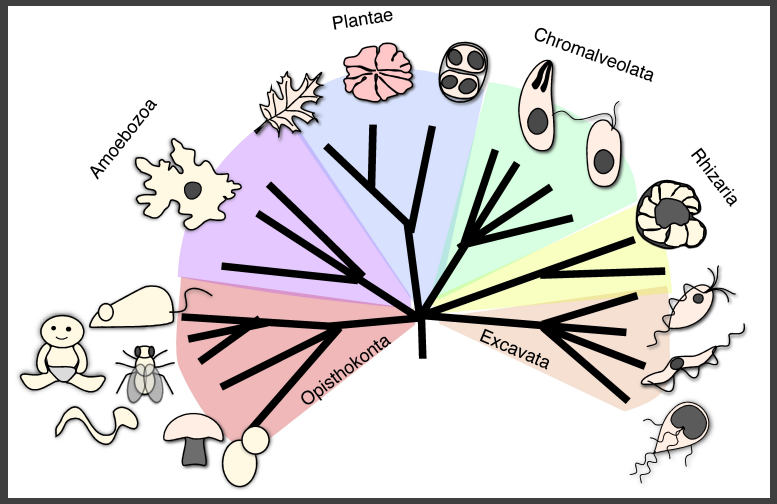
Molecular and Cellular biology Refresher, MeBoP2018

Lilach Sheiner, PhD,

wellcome centre molecular parasitology

Sheiner Lab

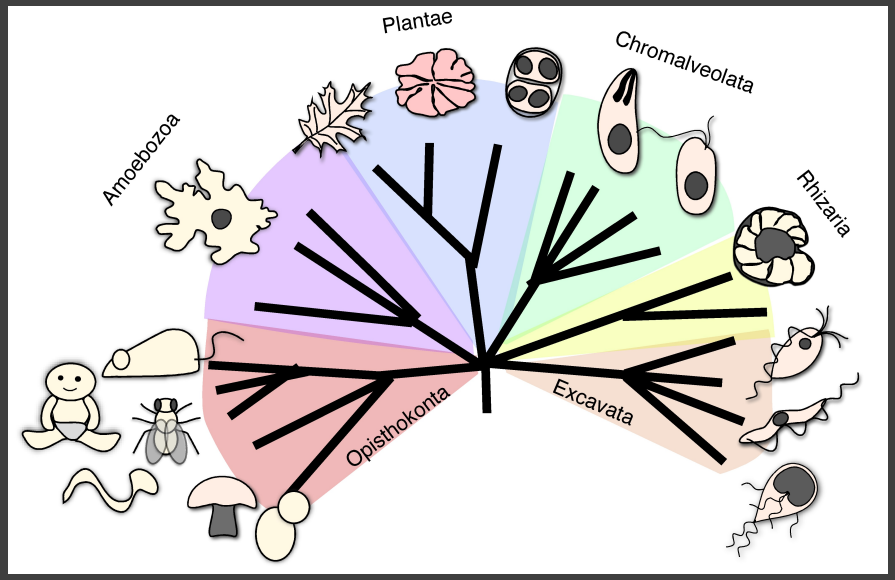


Important outcome of studying basic cell biology of parasites

- Broad understanding of divergent eukaryotic life
- Potential to expose new treatment strategies

Eukaryotic parasites

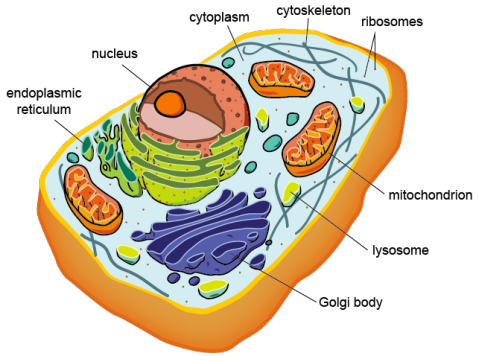
Toxoplasma - David, Lilach, Dominique, Chris, Jon
Hammondia - Jon
Cryptosporidium – Mattie, Alex, Karin
Plasmodium - Kirk, Akhil, Freddy, Jeremy
Teilaria - Phillip

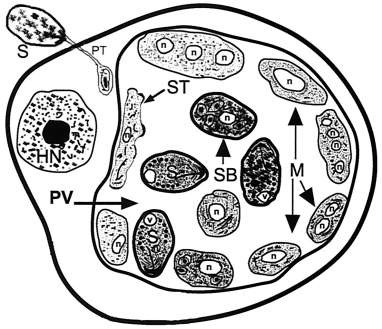


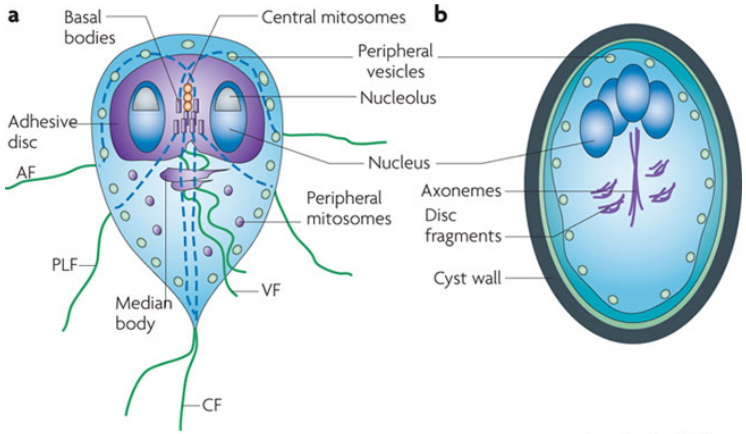
Schistosoma
Poppy, Jim

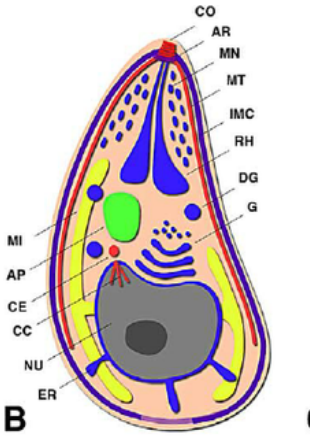
Leishmania – Charle, Ibrahim
Trypanosoma – Richard, Barrie, Shula, Isabel
Giardia – Karin, Alex

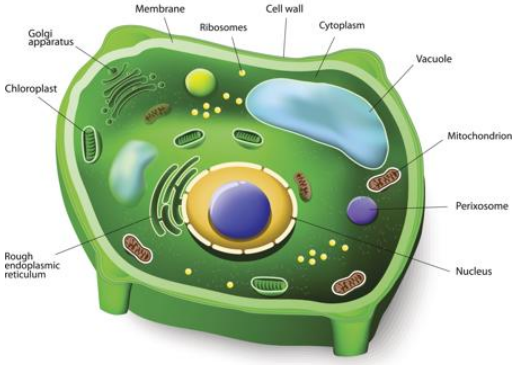
Diversity of intracellular structures in eukaryotes

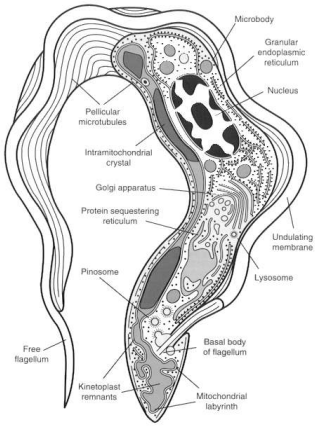


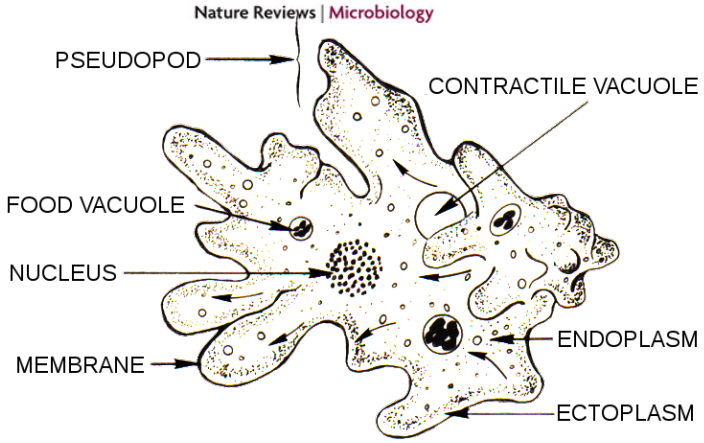












Important outcome of studying basic cell biology of parasites

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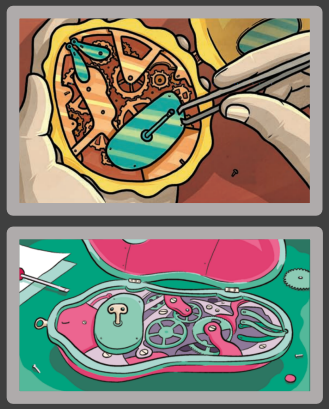
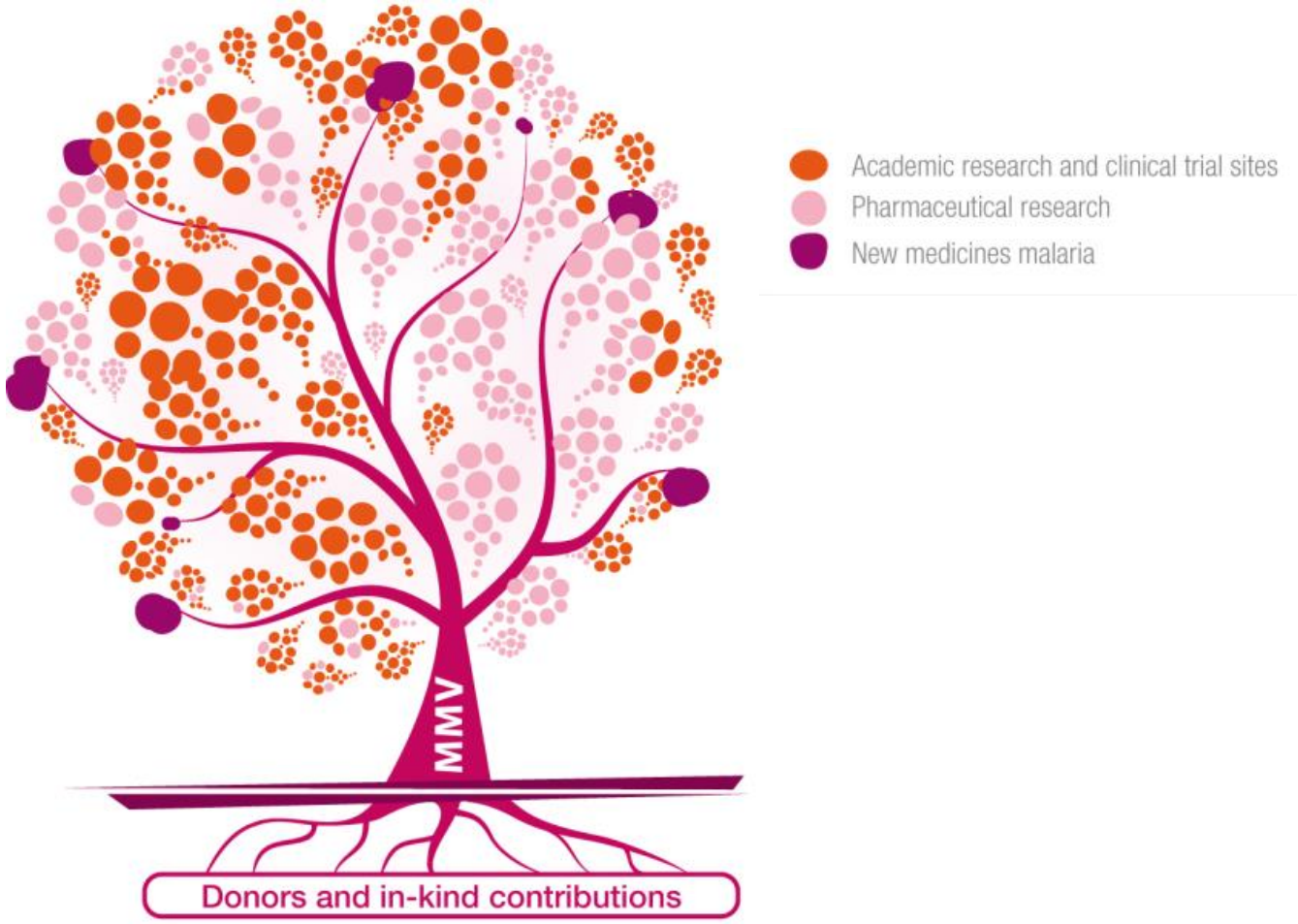


Jeremy Burrows



ExCyte Ltd

INNOVATOR OF THE YEAR

BBSRC FLIP Flexible Interchange Programme

MEDECINS SANS FRONTIERES

Dr Barrie (Bernadette) Rooney CEO

TROZON X

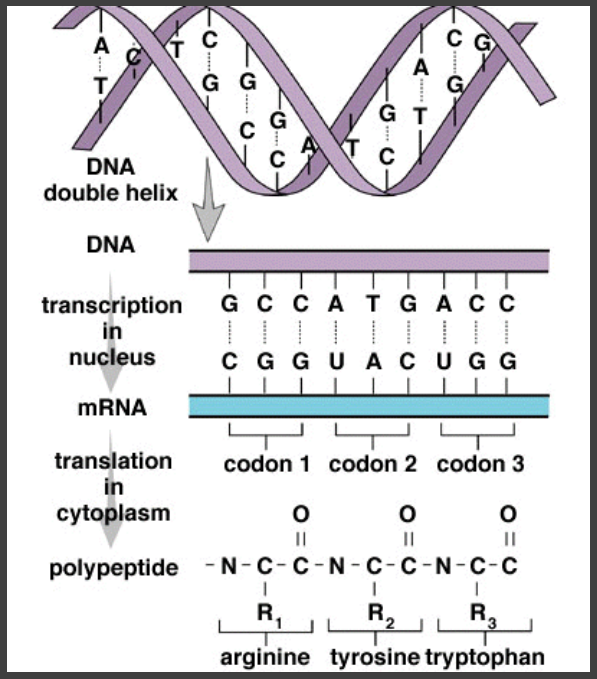
How to make a rapid HAT diagnostic Test

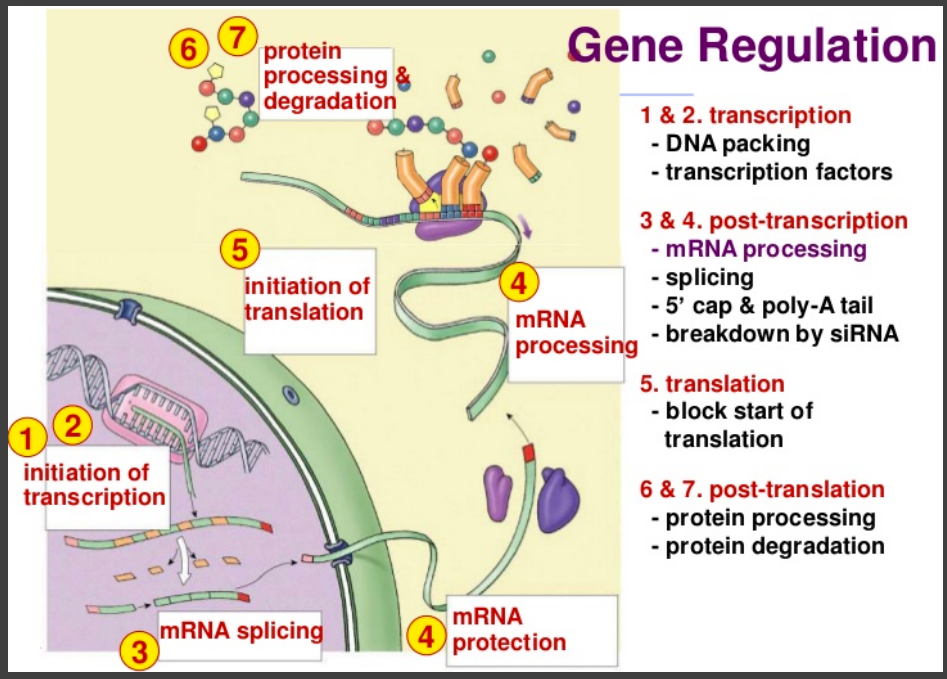
- Use sequence databases
  - proteomics, Mimotrops, other 'omics'
- Select human disease specific antigen sequence
  - non variable surface proteins, repeat proteins
- Express recombinant antigen in surrogate
  eg E.coli, Yeast or Leishmania tarentolae (kinetoplastid)
- Purify His tagged recombinant antigens
- Test antigens against sera from infected people
- Develop prototype
- Field test

Biomarker on Test line

TROZON X

DNA - > RNA -> protein -> function
Controls of function exist at all levels





https://www.slideshare.net/jayswan/chapter-18-gene-regulation

DNA - > RNA -> protein -> function
Controls of function exist at all levels

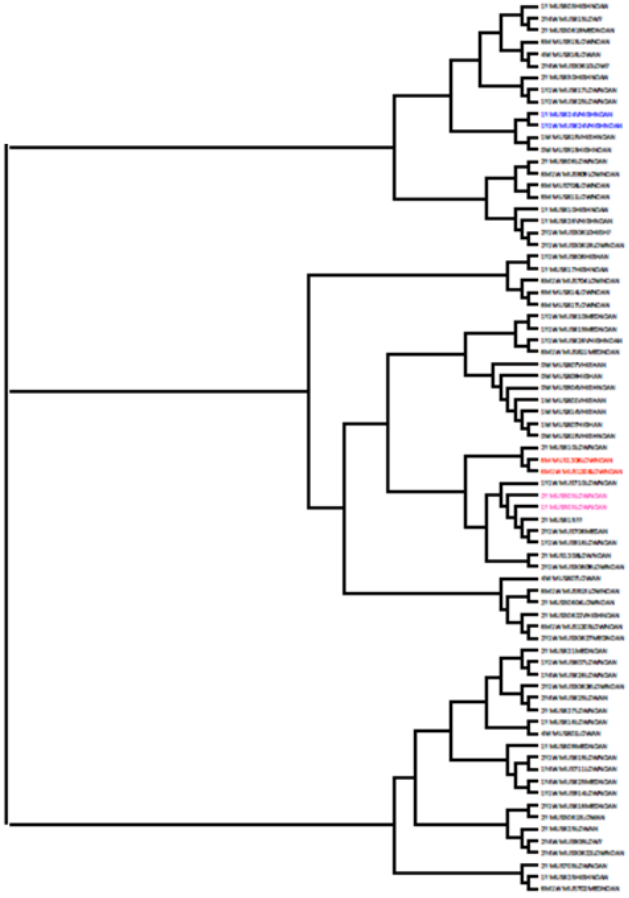
DNA mutations lead to variability in gene functions

Poppy Lamberton

Alex Grinberg

Karin Troell

Uganda - Treatment failures?



Child C: 1 year apart

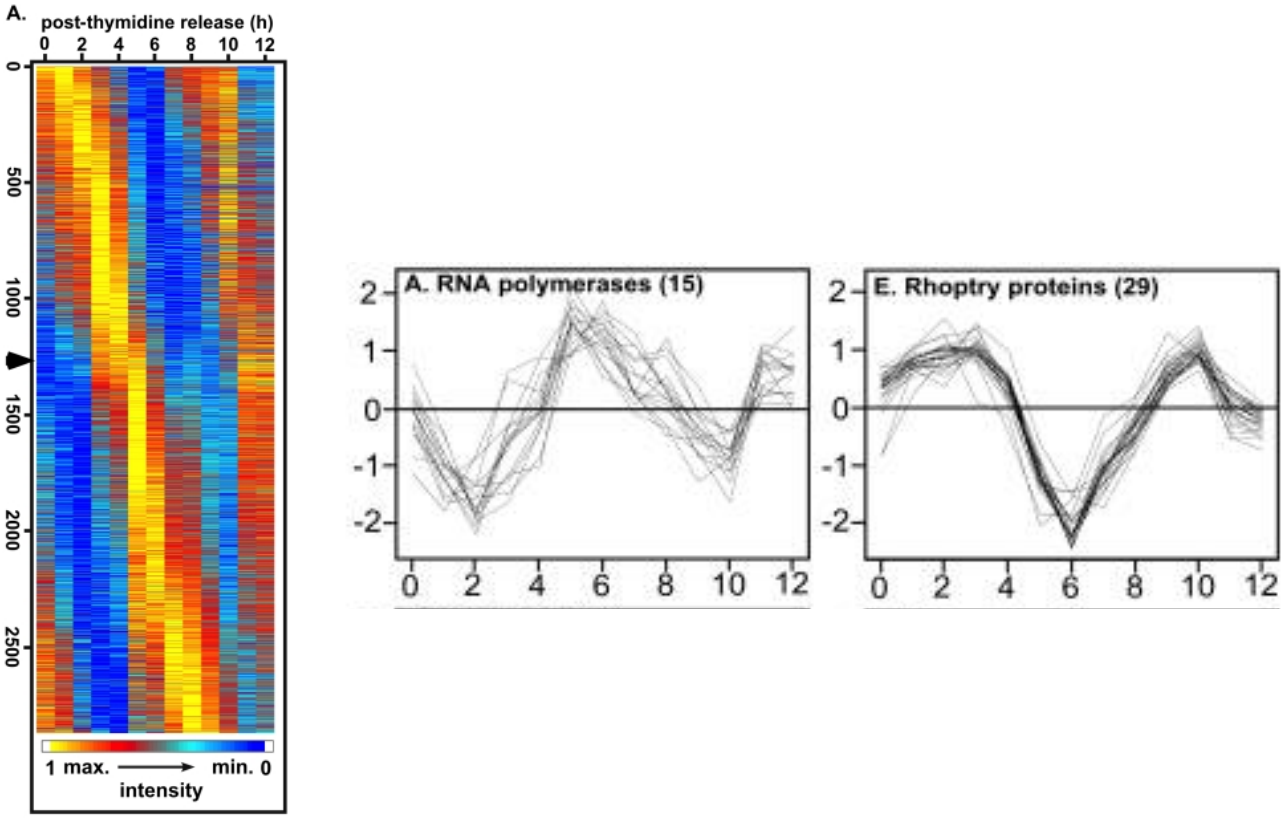
Child D: 1 year apart

Child E: 1 year apart

DNA - > RNA -> protein -> function
Controls of function exist at all levels

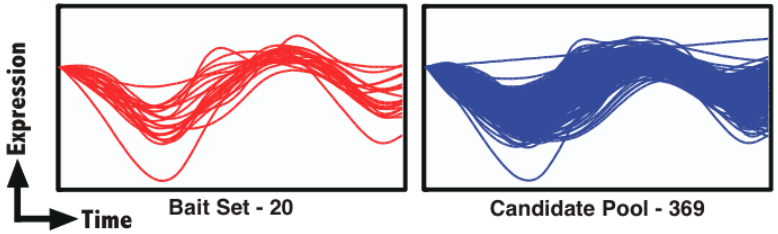
Control of function through transcriptional control => namely control of the production of the mRNA

Identification of new apicoplast proteins



Behnke et al; PLoS one; 2010

Identification of new apicoplast proteins



1/~7000

mRNA expression profiles

1/369

Phyletic distribution

1/57

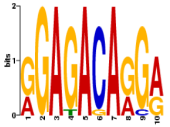
Endogenous tag

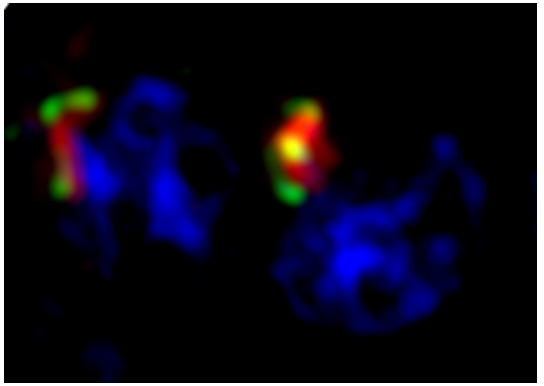
1/30

apicoplast

11

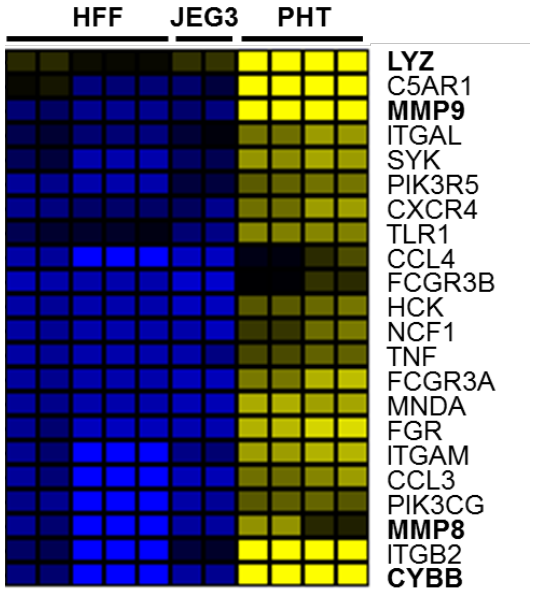
New data for more focused searches

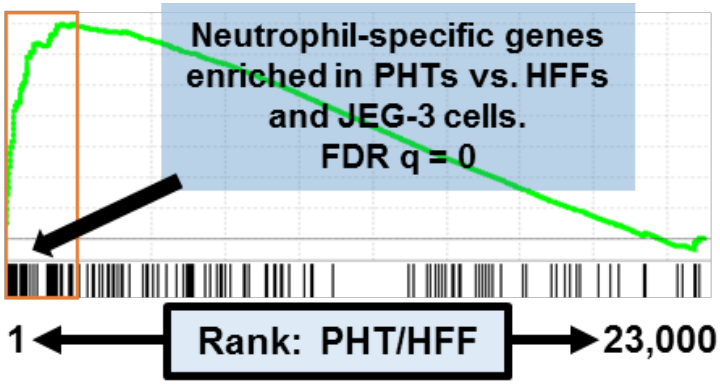




Sheiner et al; PLOS pathogens 2011

Highly significant enrichment for neutrophil-specific genes in the PHT-specific gene set





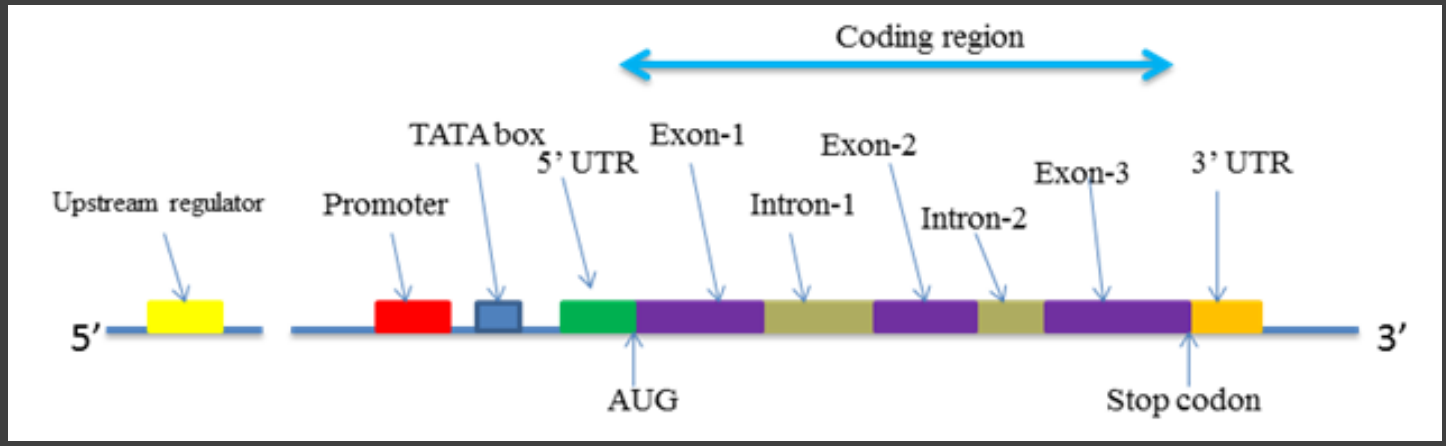
101/161 Neutrophil-specific genes in top 2500 PHT/HFFs

Interesting hits: MMP9, CYBB (NADPH oxidase), Lysozyme->other neutrophil markers

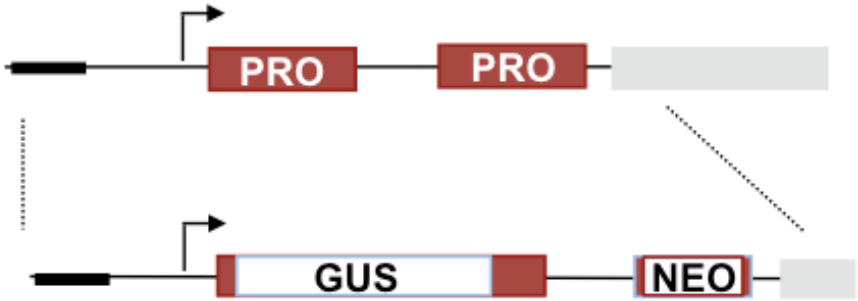
Jon Boyle

DNA - > RNA -> protein -> function
Controls of function exist at all levels

Control of individual gene expression



Using β-glucuronidase (GUS) as a proxy for procyclin expression

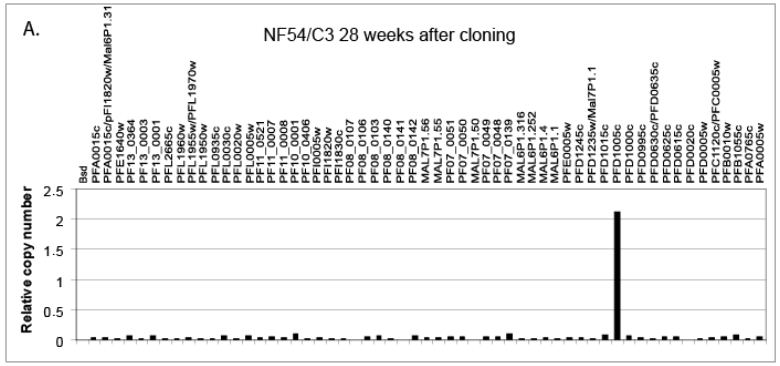


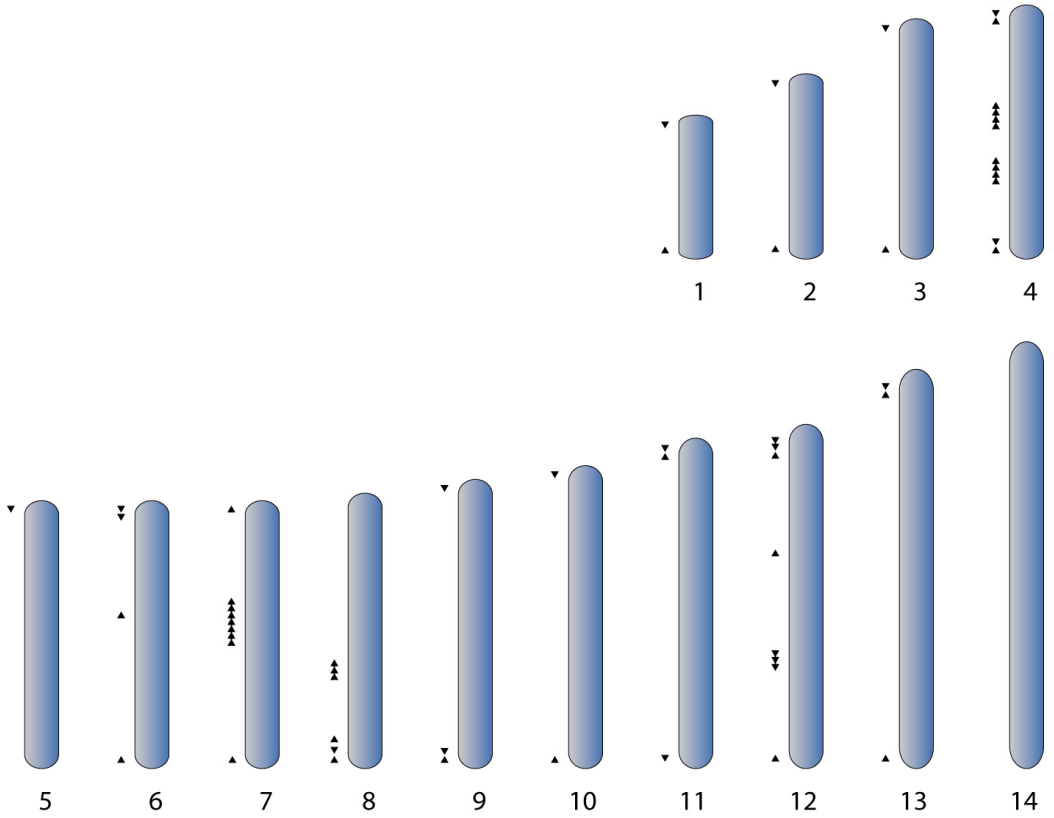
- Replacement of one pair of procyclin genes
- Context and regulatory sequences remain the same
- GUS expression mirrors procyclins in differentiating cells
- Colorimetric /fluorimetric assay suitable for high throughput screens

Sbicego et al (1999) Mol Biochem Parasitol.

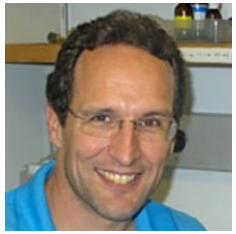
Isabel Roditi

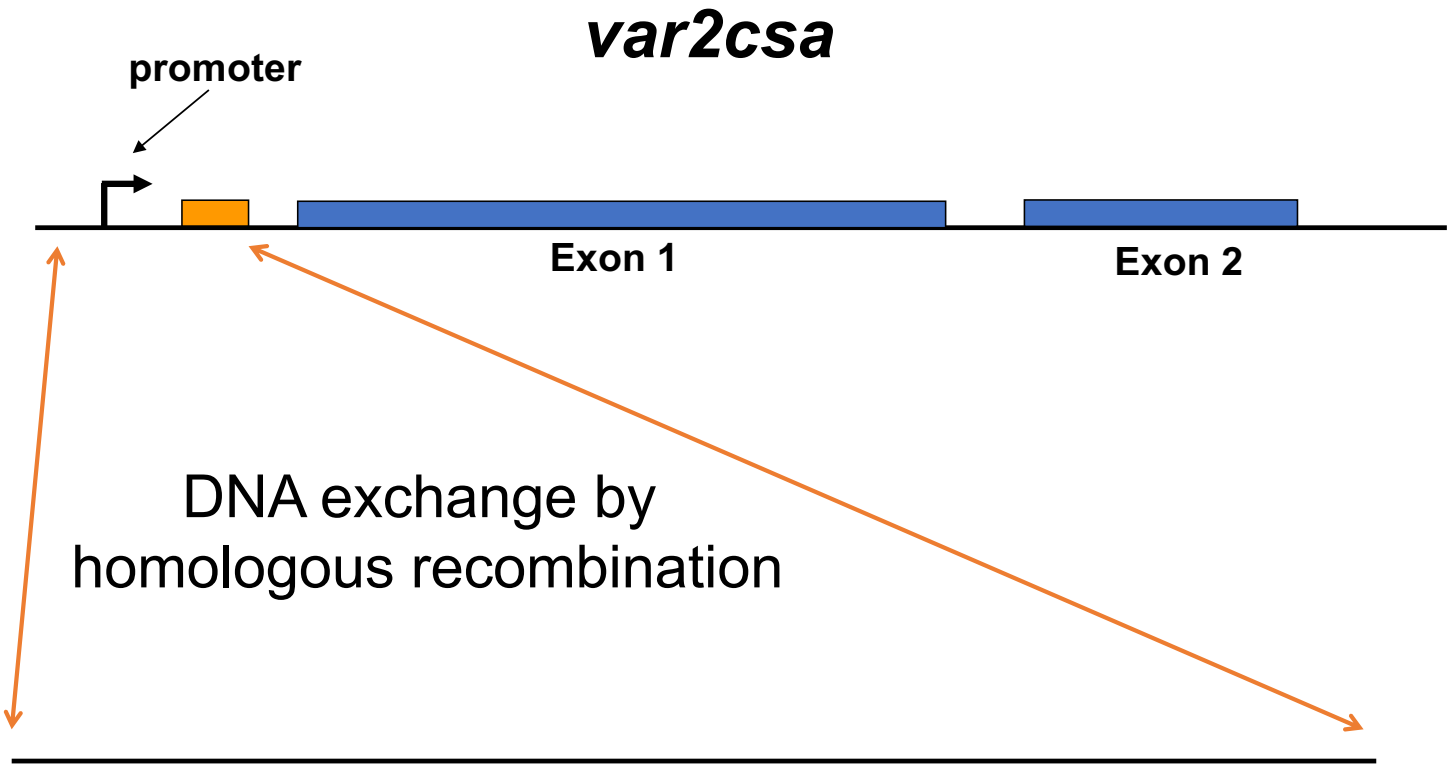
Using qRT-PCR to measure Var gene expression





kirk Deitsch





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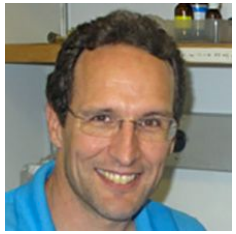
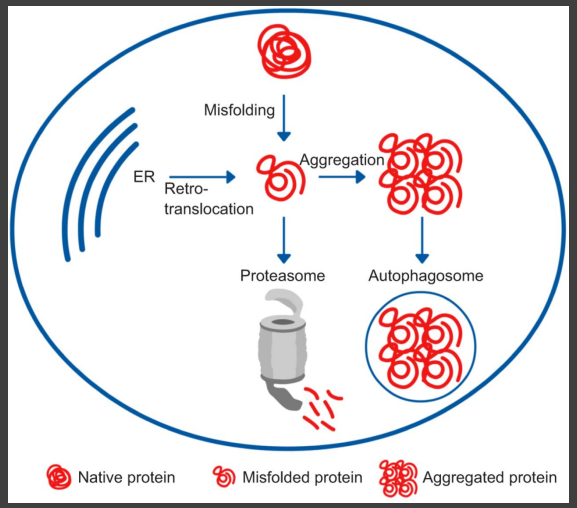
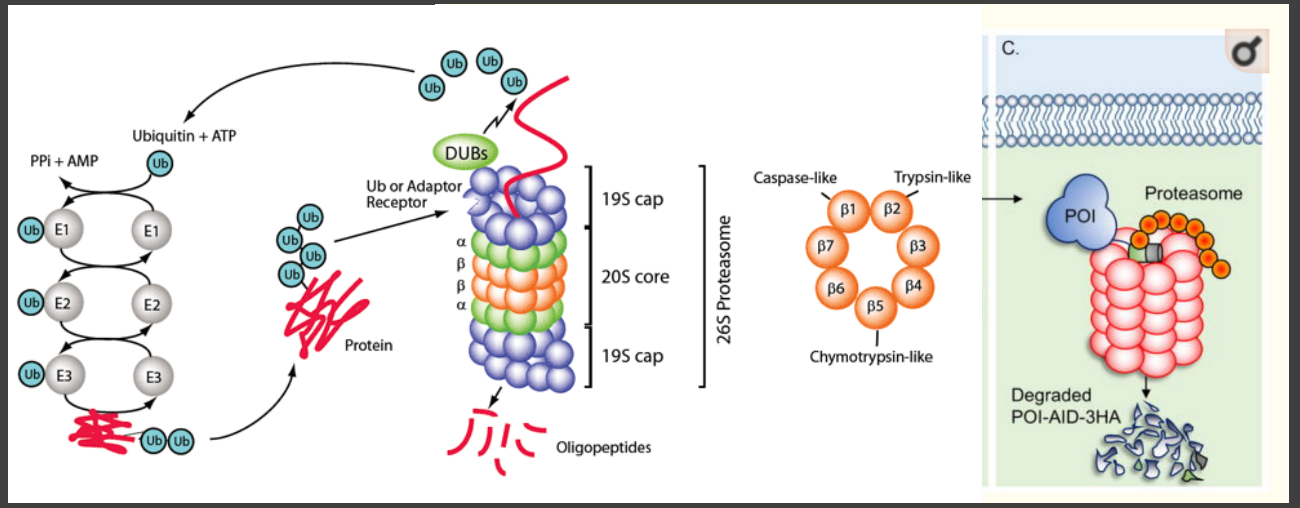


1. Deletion of upstream region
2. Promoter exchange
3. Mutations

DNA - > RNA -> protein -> function
Controls of function exist at all levels

Control of protein expression through protein stability (Mattie auxin)





(Brown et al., Bio Protoc 2018)

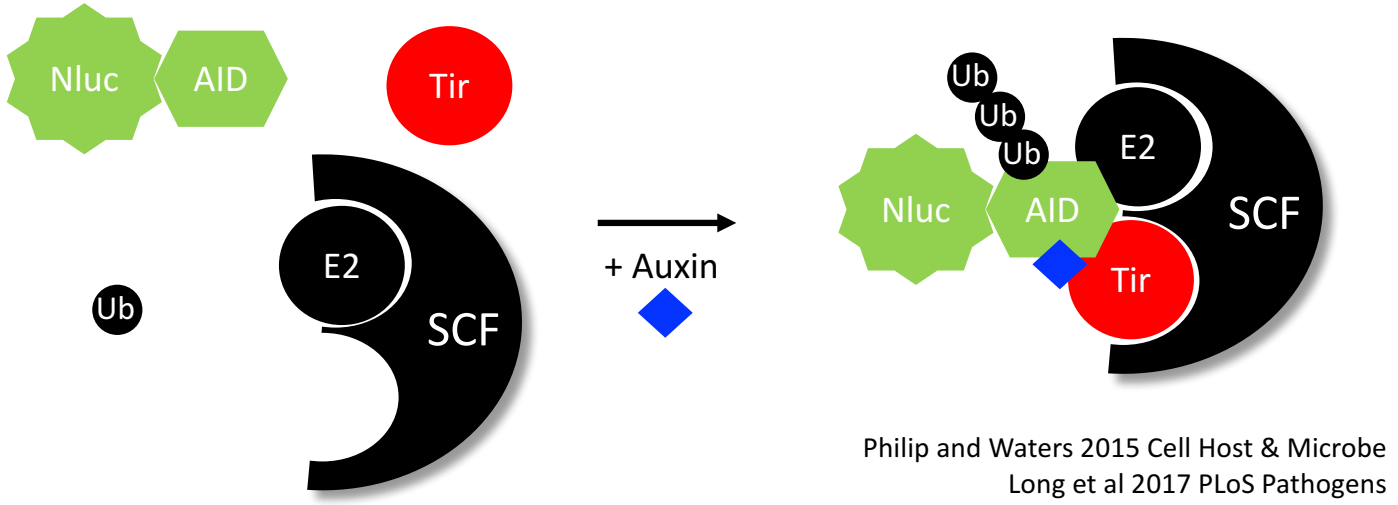
Protein Stability: Auxin degradation system

Tir and **Auxin** system regulates protein stability

- Auxin is a plant hormone, tryptophan derivative.
- Fuse auxin binding domain (AID) to protein of interest (Nluc).
- Express TIR (F-box protein from rice).

Advantages

- Need to be able to tag gene.
- Add auxin only when you want to destabilize protein, not all the time.

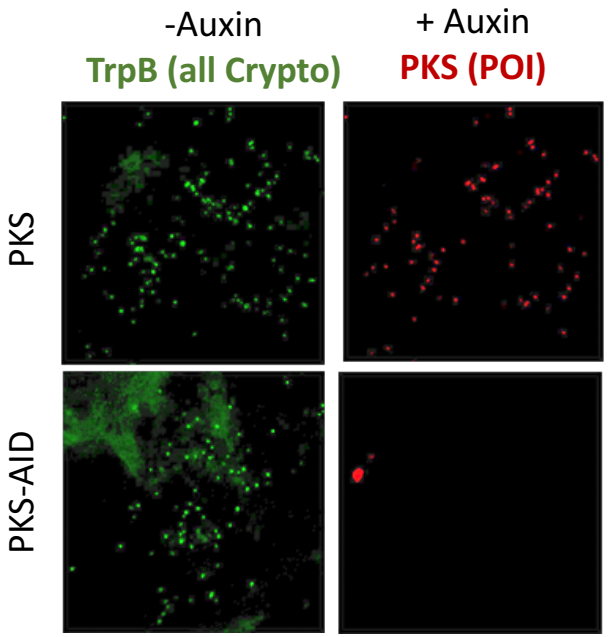


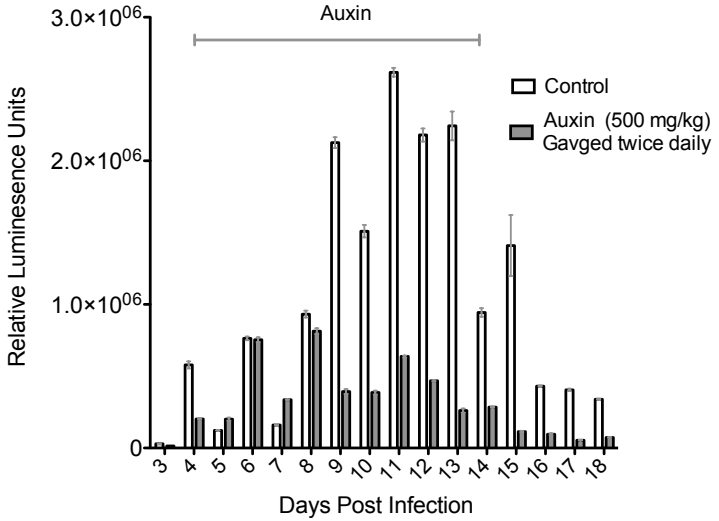
Philip and Waters 2015 Cell Host & Microbe
Long et al 2017 PLoS Pathogens

Mattie Pawlowic

Loss of PKS impairs *in vitro* and *in vivo* growth

- Inserted auxin machinery at Polyketide Synthase (PKS) locus
- Add auxin to destabilize PKS
- Addition of auxin turns off PKS *in vitro*
- Addition of auxin significantly reduces shedding of parasites in feces of infected mice





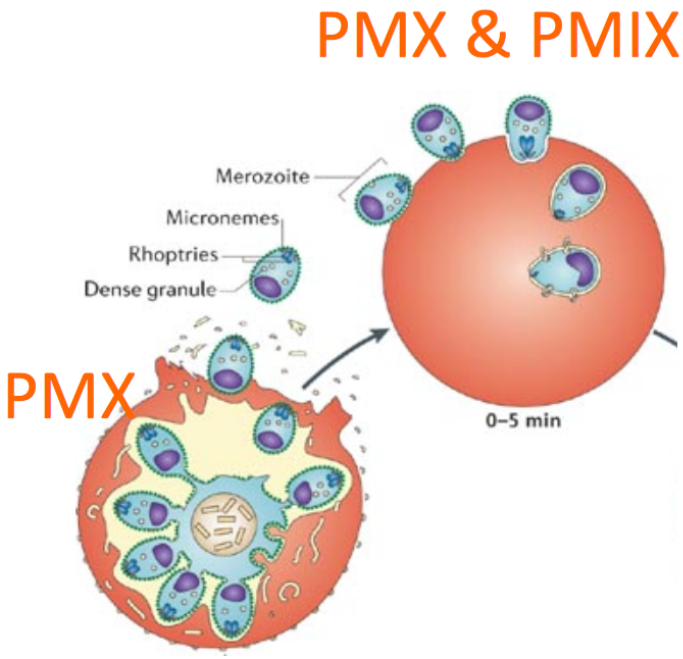
Mattie Pawlowic

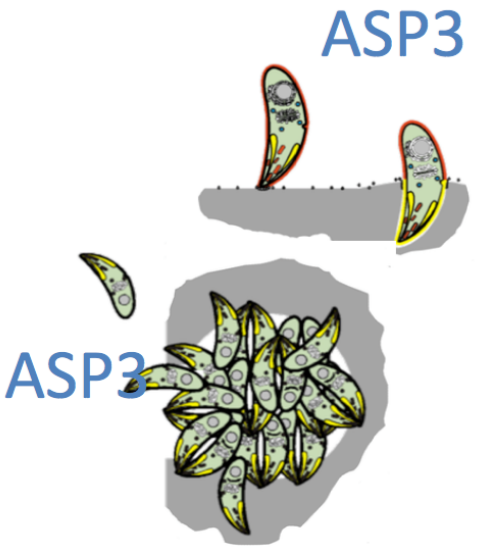
DNA - > RNA -> protein -> function
Controls of function exist at all levels

Control of one protein’s function by another’s function

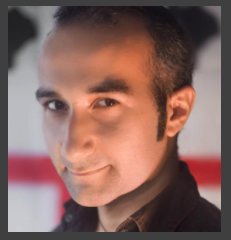
Maturases

Dominique Soldati-Favre

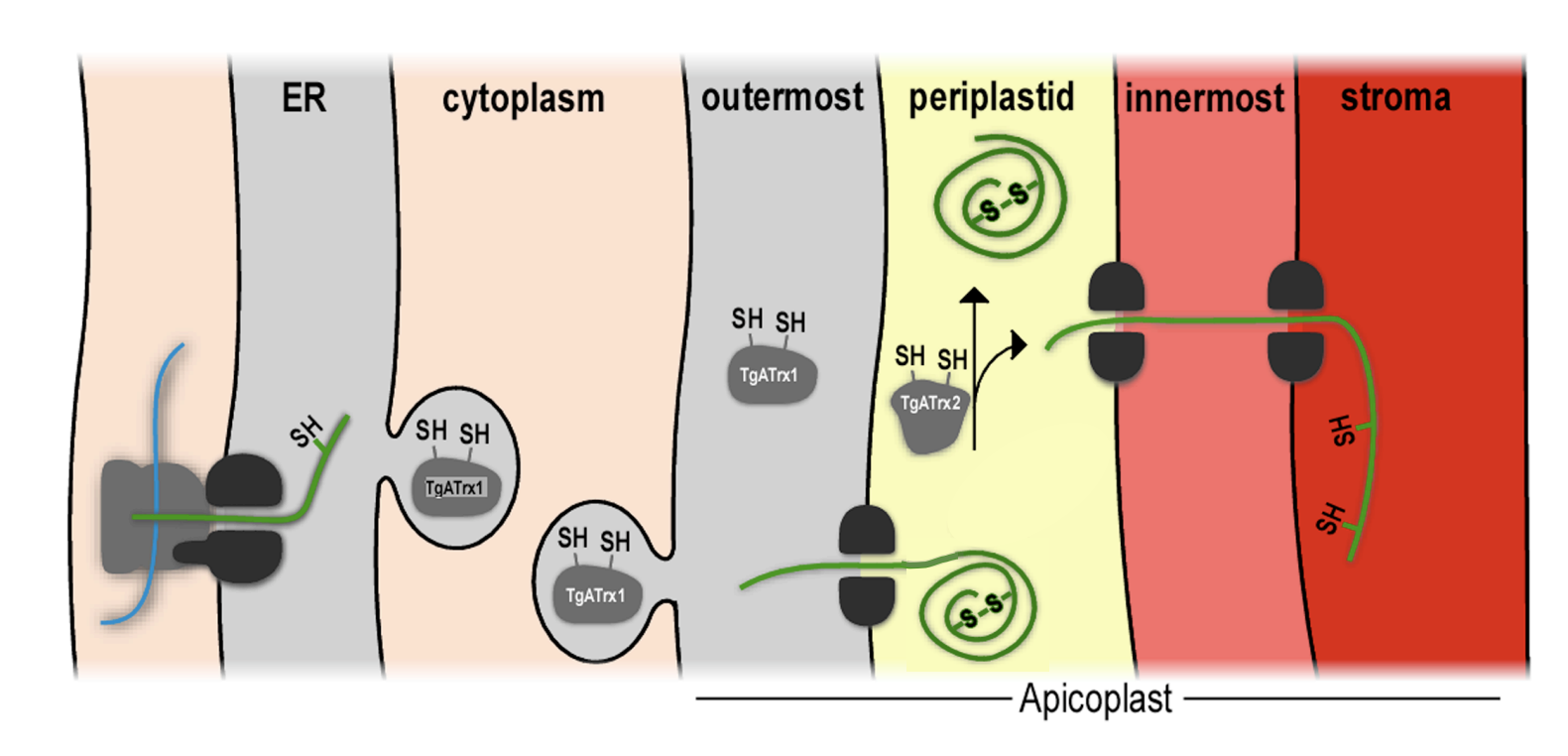




Thioredoxin control the folding of other proteins -> allowing their onward trafficking and correct function

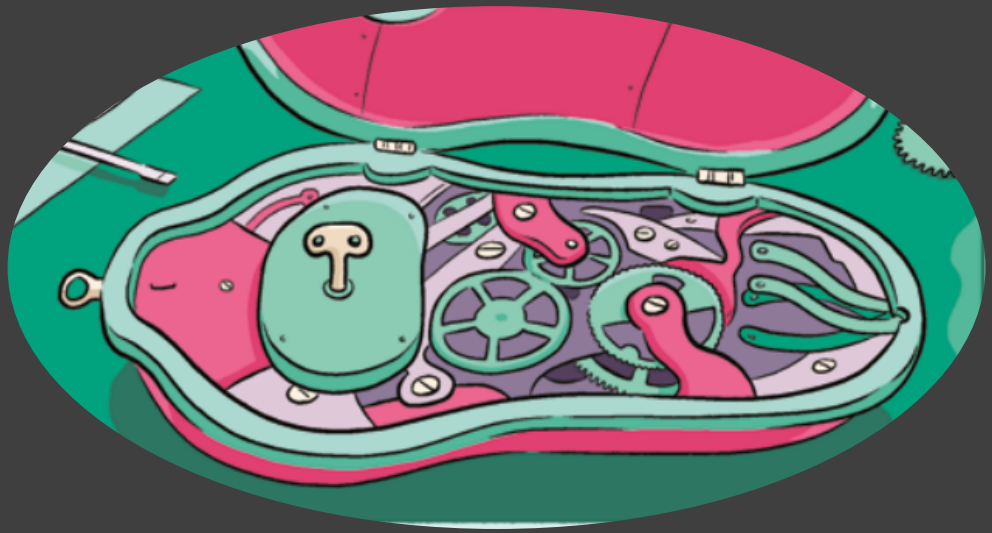


Marco Biddau



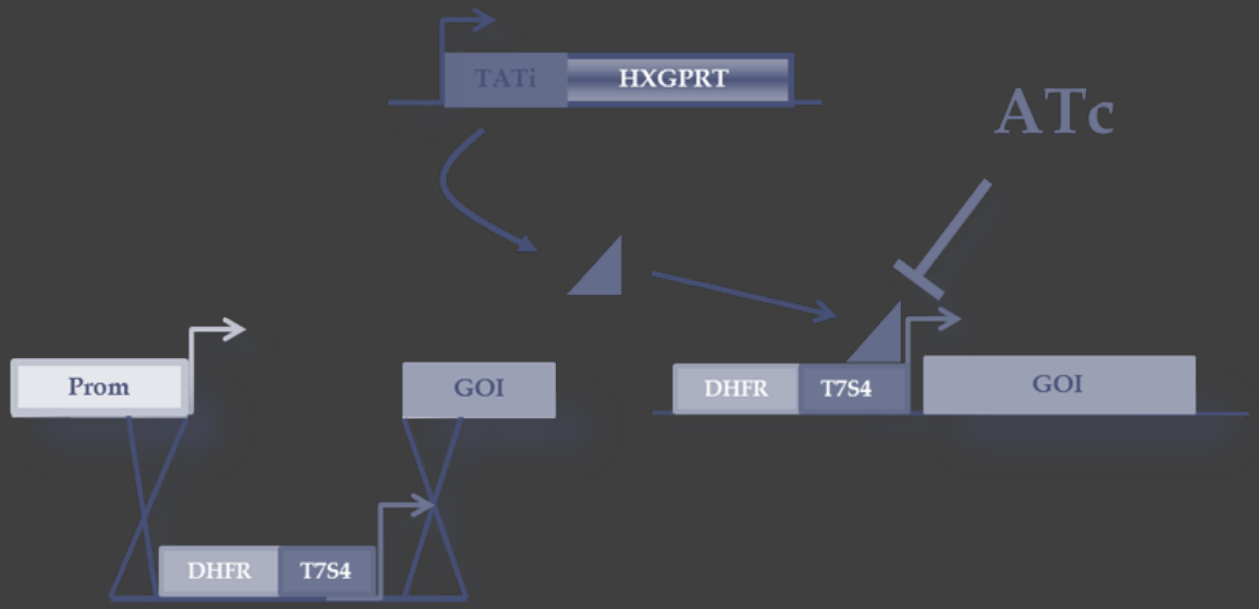
Biddau et al 2018 PLOS path

Cellular and molecular tools used to study parasites

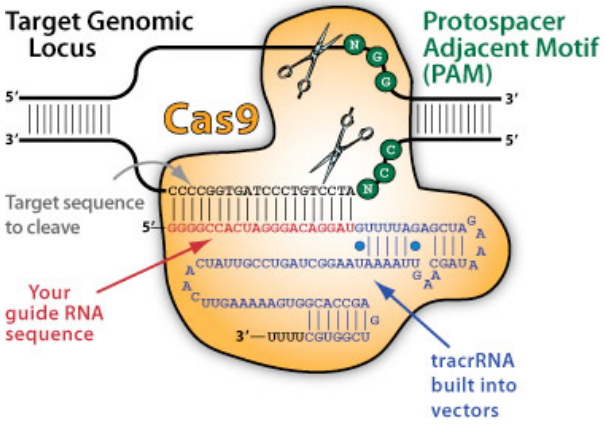


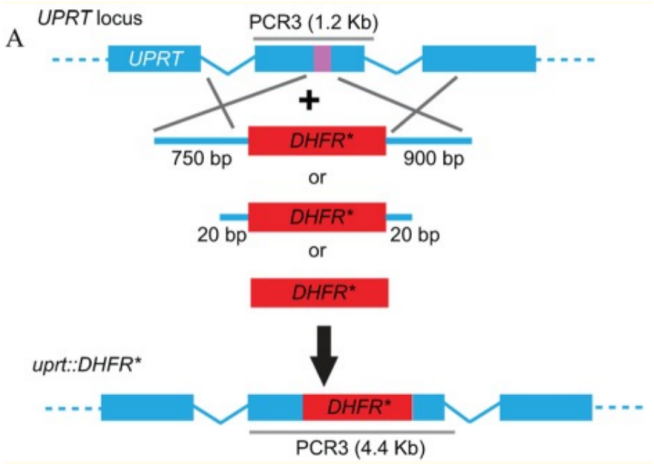
A Tet regulated promoter

Dominique Soldati-Favre

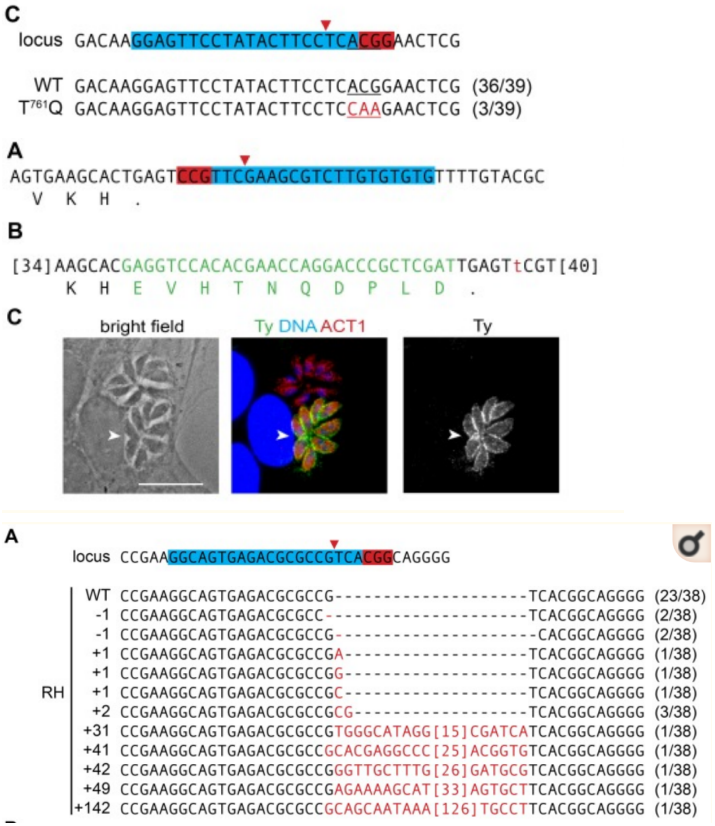


CRISPR/Cas9





Shen et al; 2014 MBio



Sidik et al; 2014 PLOS one

CRISPR tools are available for many parasites

CRISPR-Cas9-mediated single-gene and gene family disruption in Trypanosoma cruzi.
Peng D, Kurup SP, Yao PY, Minning TA, Tarleton RL.
MBio. 2014 Dec 30;6(1):e02097-14. doi: 10.1128/mBio.02097-14.
PMID: 25550322 **Free PMC Article**
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Efficient genome engineering of **Toxoplasma** gondii using **CRISPR**/Cas9.
Sidik SM, Hackett CG, Tran F, Westwood NJ, Lourido S.
PLoS One. 2014 Jun 27;9(6):e100450. doi: 10.1371/journal.pone.0100450. eCollection 2014.
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R Soc Open Sci. 2017 May 3;4(5):170095. doi: 10.1098/rsos.170095. eCollection 2017 May.
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Nat Protoc. 2018 Jan;13(1):307-323. doi: 10.1038/nprot.2017.131. Epub 2018 Jan 11.
PMID: 29323662
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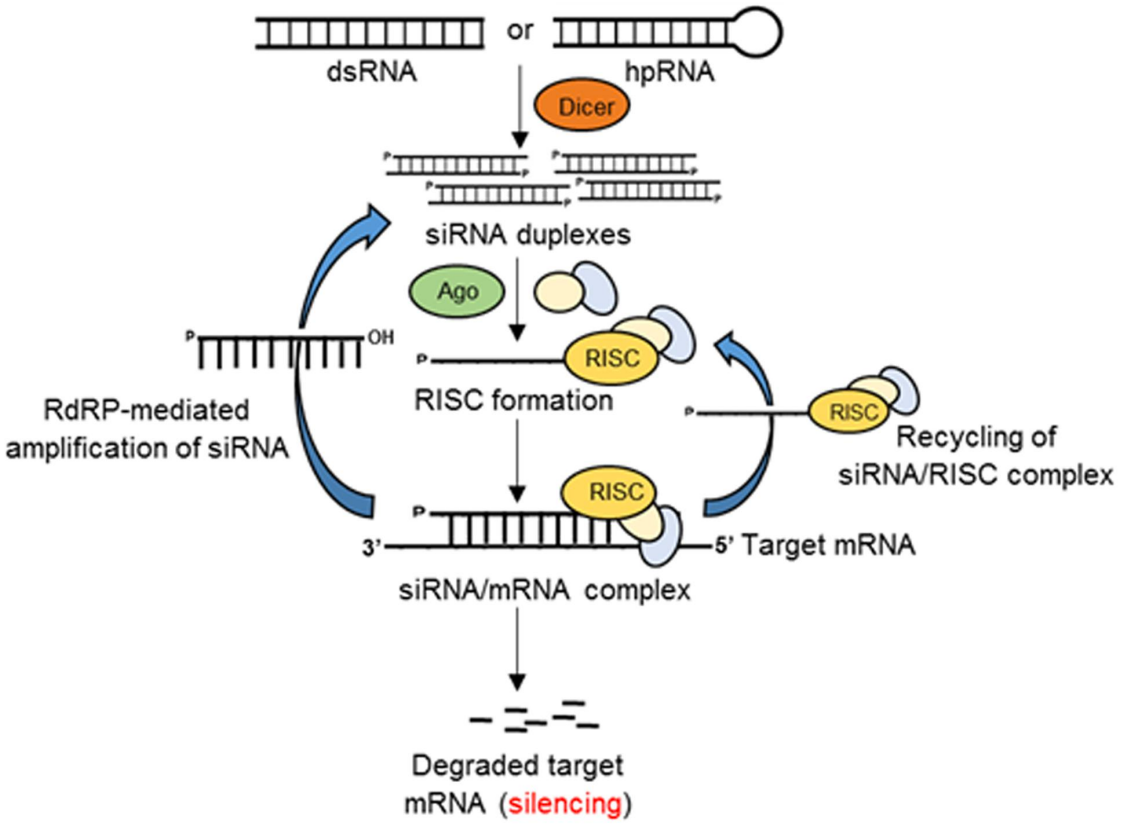
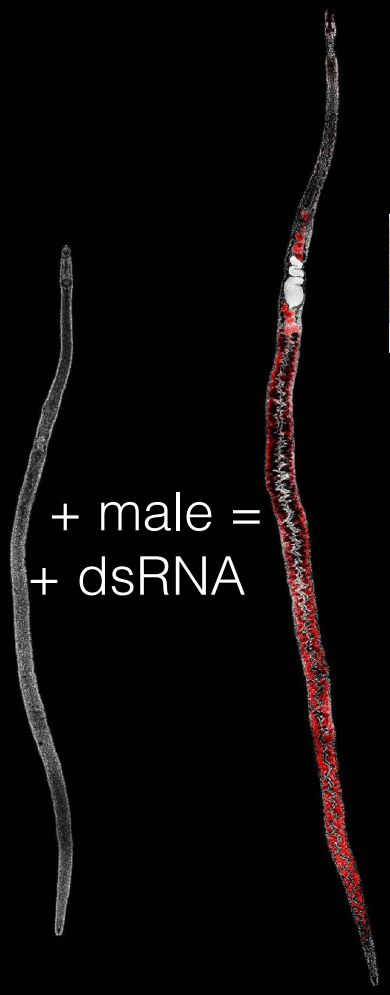
RNA interference (RNAi)



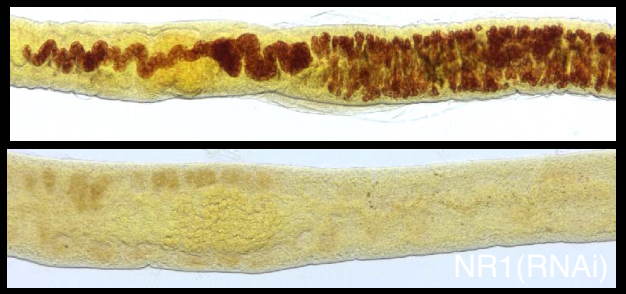
Defining regulators of schistosome reproduction



+ male =
+ dsRNA

Jim Collins

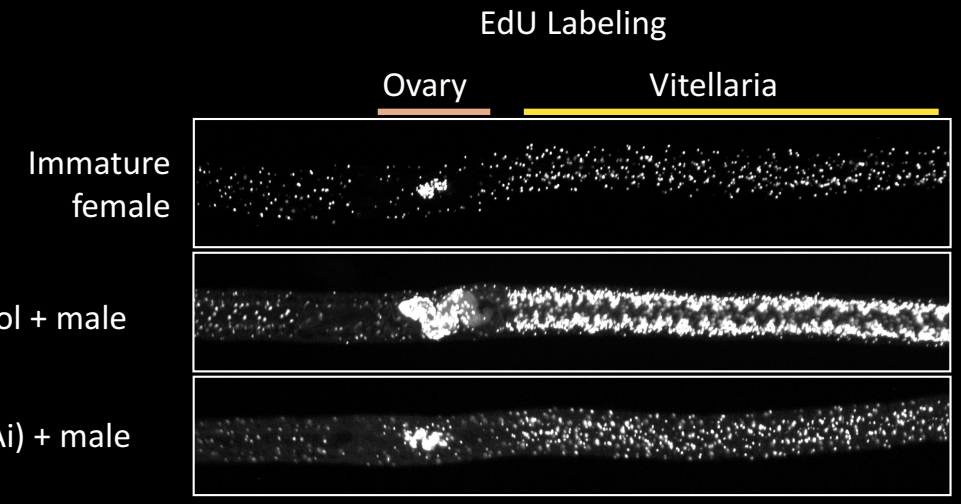
RNAi Screen



19 eggs/pair/day

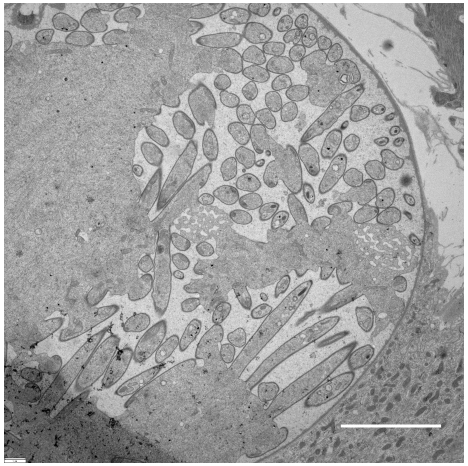
2 eggs/pair/day

Immature female + male = ⬆cell proliferation

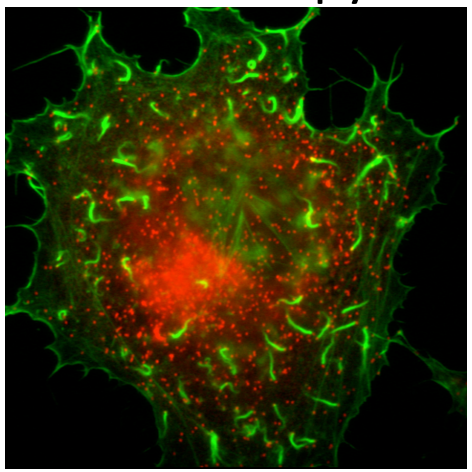


Microscopy techniques

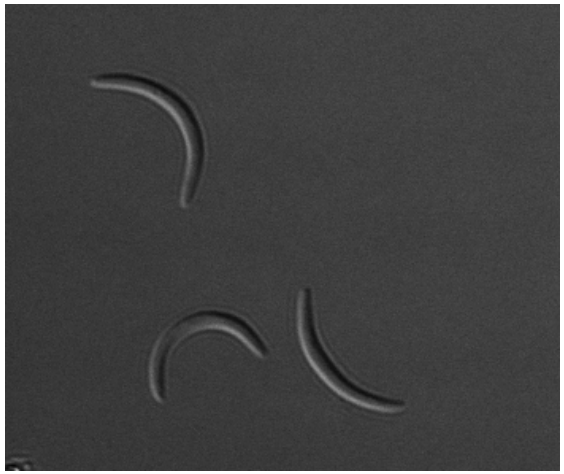
Electron microscopy



Fluorescence microscopy



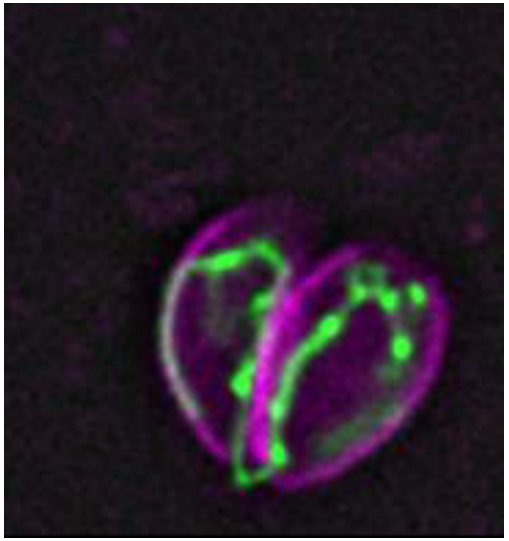
Video (time-lapse) microscopy

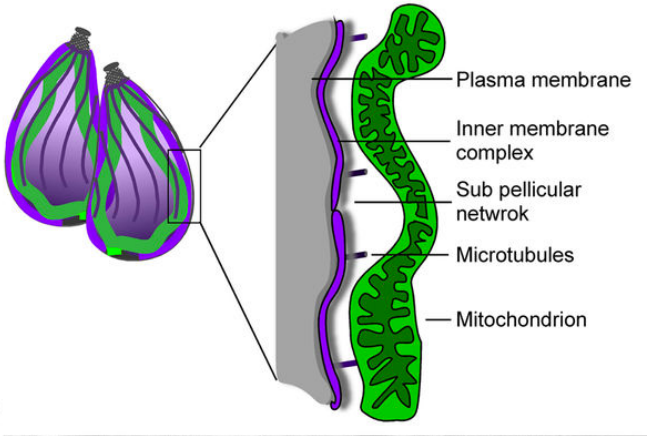


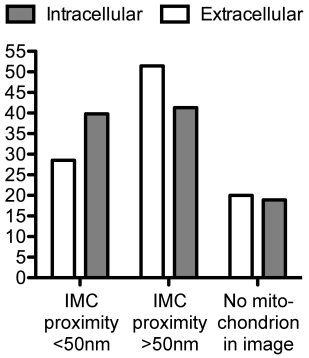
Freddy Frischknecht

www.sporozoite.org

Mitochondrial morphology changes coincide with reduced mito/IMC juxtaposition







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