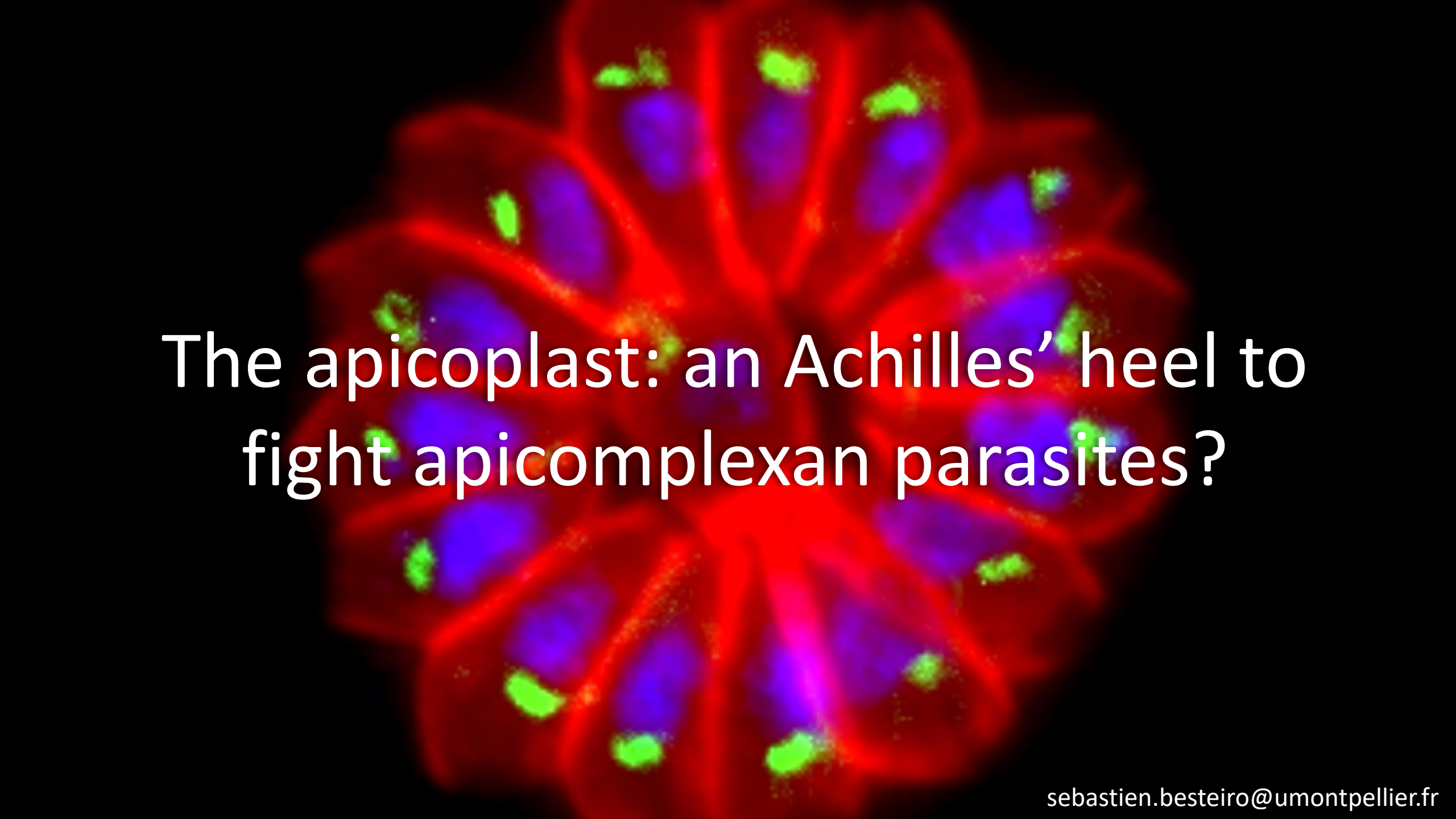


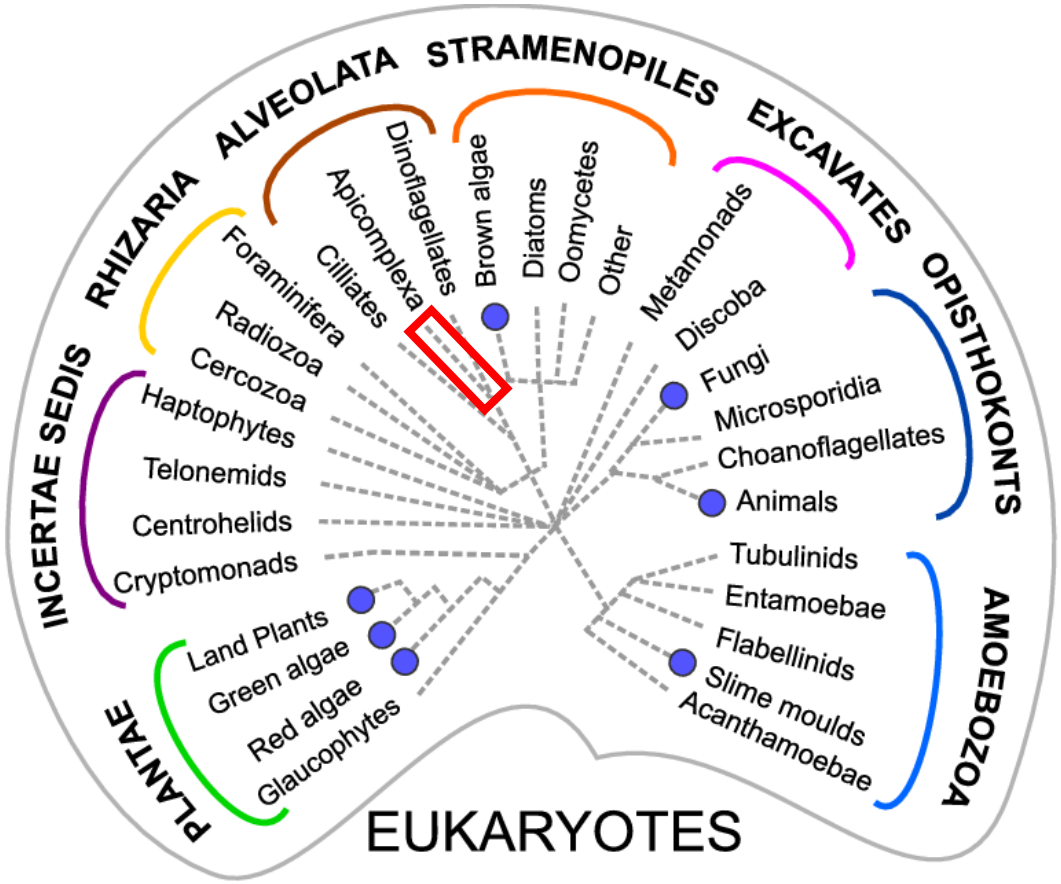
The apicoplast: an Achilles’ heel to fight apicomplexan parasites?

sebastien.besteiro@umontpellier.fr

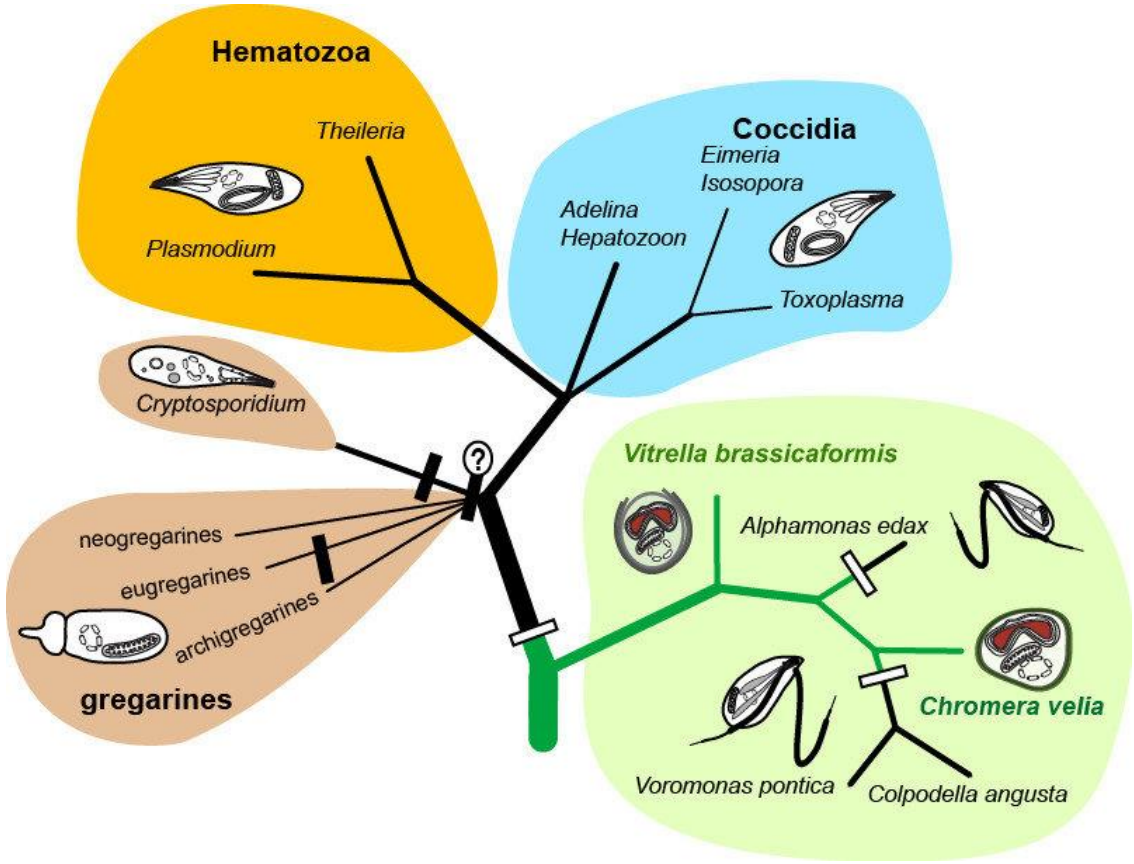
Biology and evolution of Apicomplexa

Apicomplexa is a diverse phylum of protists that includes thousands of **obligate intracellular parasites**

Main parasite groups include **Hematozoa** (blood parasites), **Coccidia** or **Gregarines** (parasites of invertebrates)
Non-parasitic photosynthetic relatives include **Chromerids**



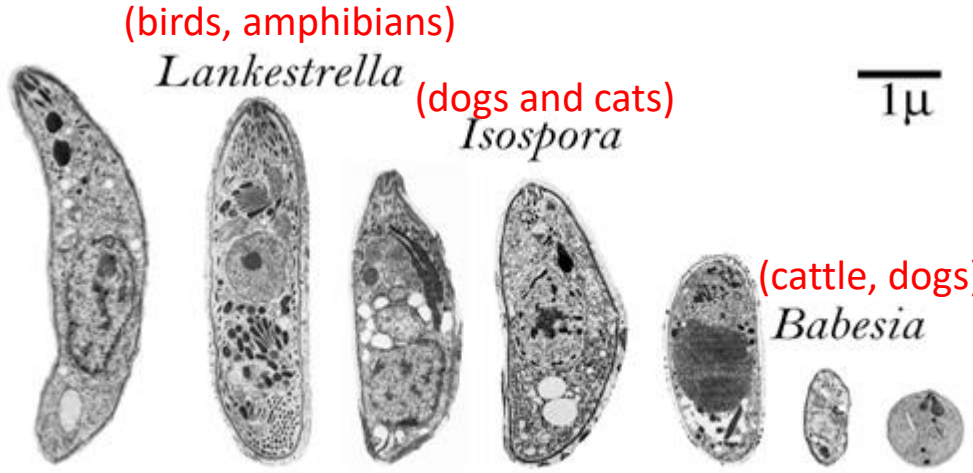
Kazamia et al. Ecol Lett. 2016



Tomčala et al. Biomolecules 2020

Apicomplexa can infect a wide variety of vertebrate hosts

The phylum Apicomplexa encompasses **more than 5000 species**, that can infect a large variety of hosts



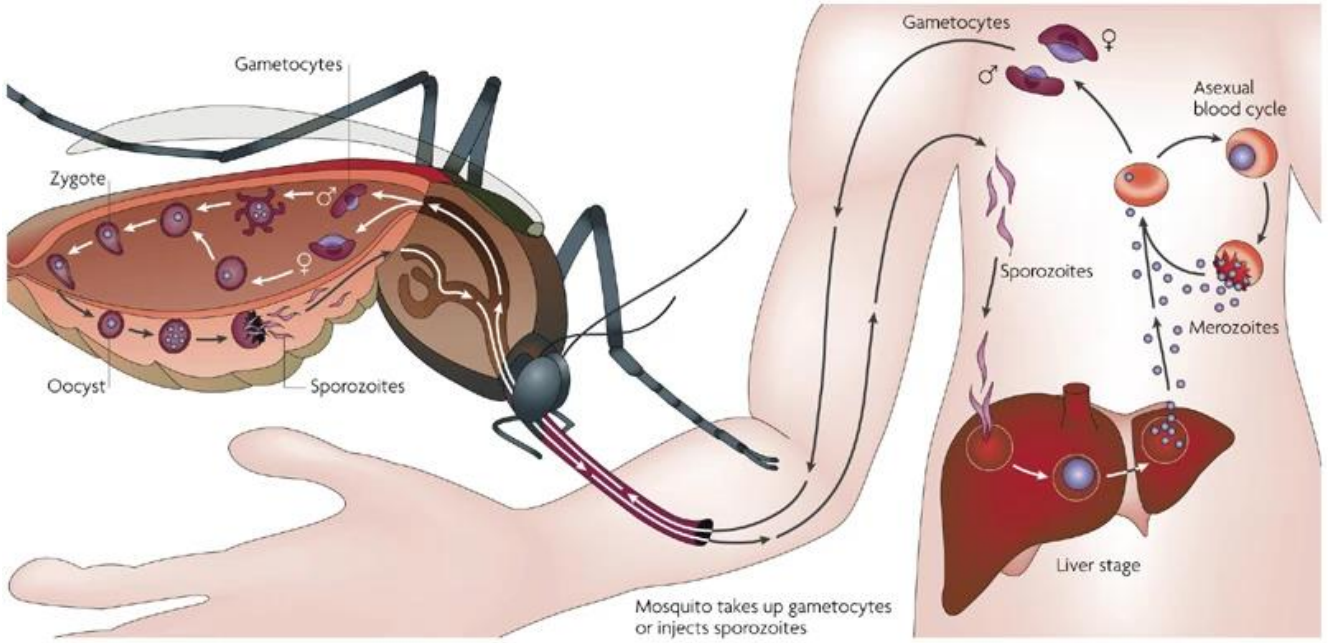
Collectively, apicomplexan parasites constitute a **heavy burden for global health of humans and other animals**, including poultry, cattle, and other livestock

Plasmodium and *Toxoplasma* are important human pathogens

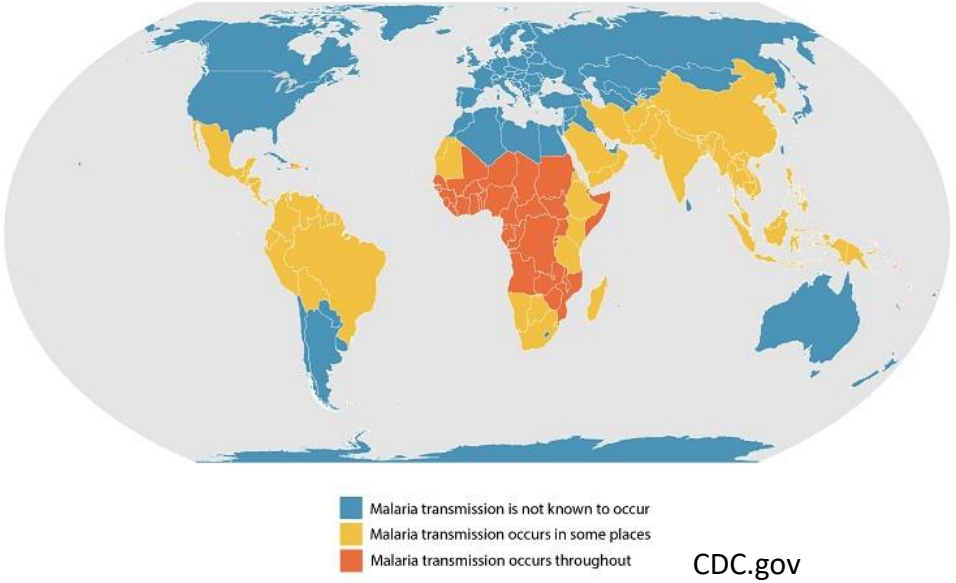
Malaria is a **mosquito-borne** disease caused by several ***Plasmodium*** species

During its life-cycle, the parasite alternates between the mosquito vector and a human host: **sporozoites** are injected from the salivary gland of a mosquito and will develop in the **liver** of the host, before reaching the blood circulation where they initiate a repeated **asexual replication cycle in erythrocytes**, although a small subset of parasites produce **sexual progeny** that can be taken up by a mosquito upon a subsequent bloodmeal to complete the cycle

It kills about **half a million people each year**, essentially in **tropical and subtropical regions** of the world



Su et al. Nature Rev Genet 2007



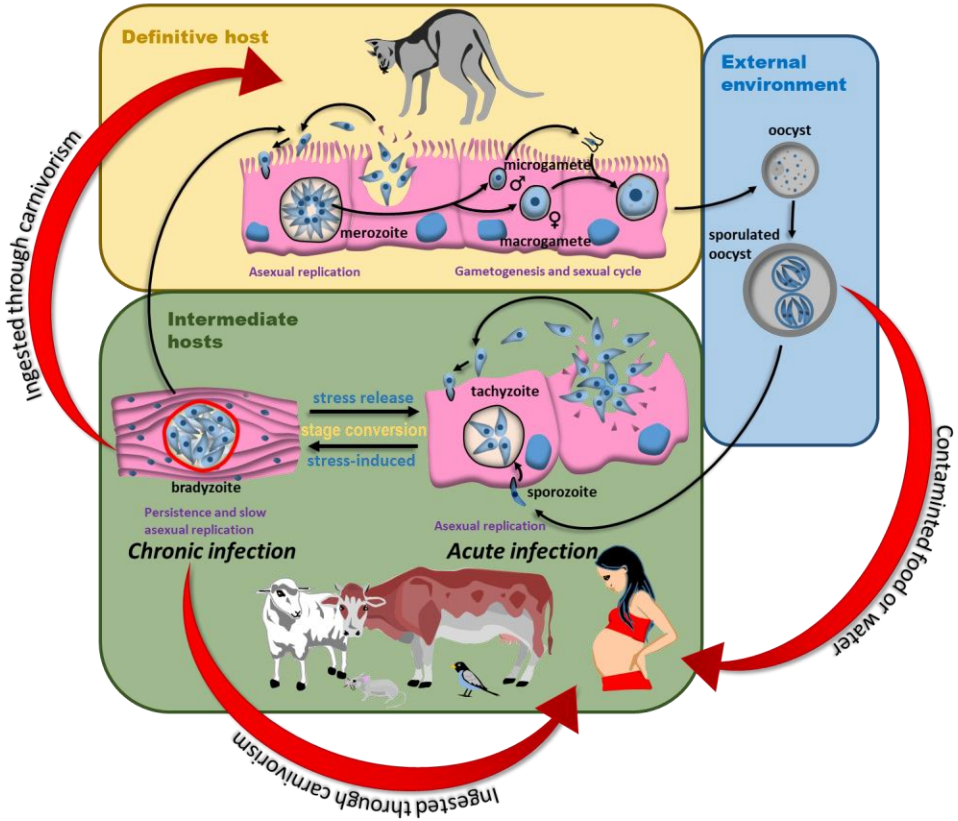
CDC.gov

Plasmodium and *Toxoplasma* are important human pathogens

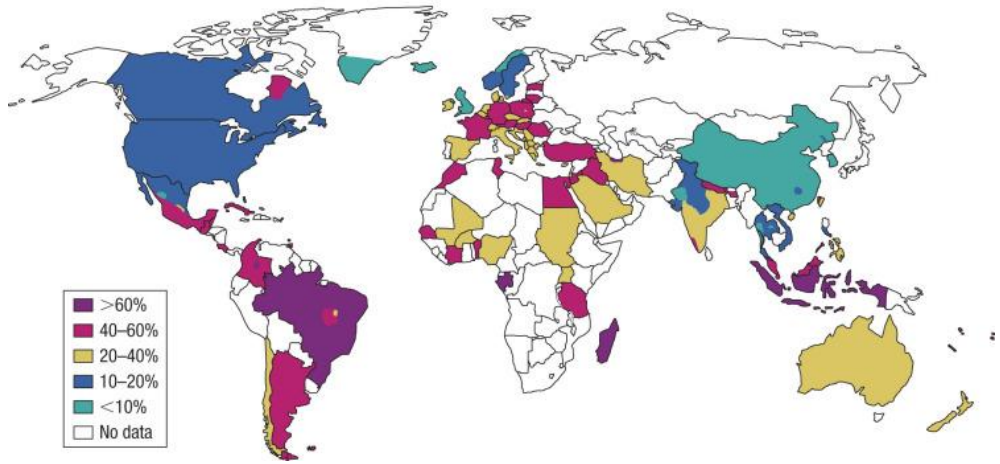
Infection by ***Toxoplasma gondii*** is usually **benign** in healthy individuals, but can have severe outcomes in **immunocompromised individuals and in developing fetuses**

The parasite alternates between different developmental stages including, in the intermediate hosts, **tachyzoites** (responsible for acute toxoplasmosis) and encysted **bradyzoites** (chronic and persistent form)

While prevalence rates vary from region to region, it is estimated that about **one-third of the world's human population is infected** with *T. gondii*



Global status of *Toxoplasma gondii* prevalence



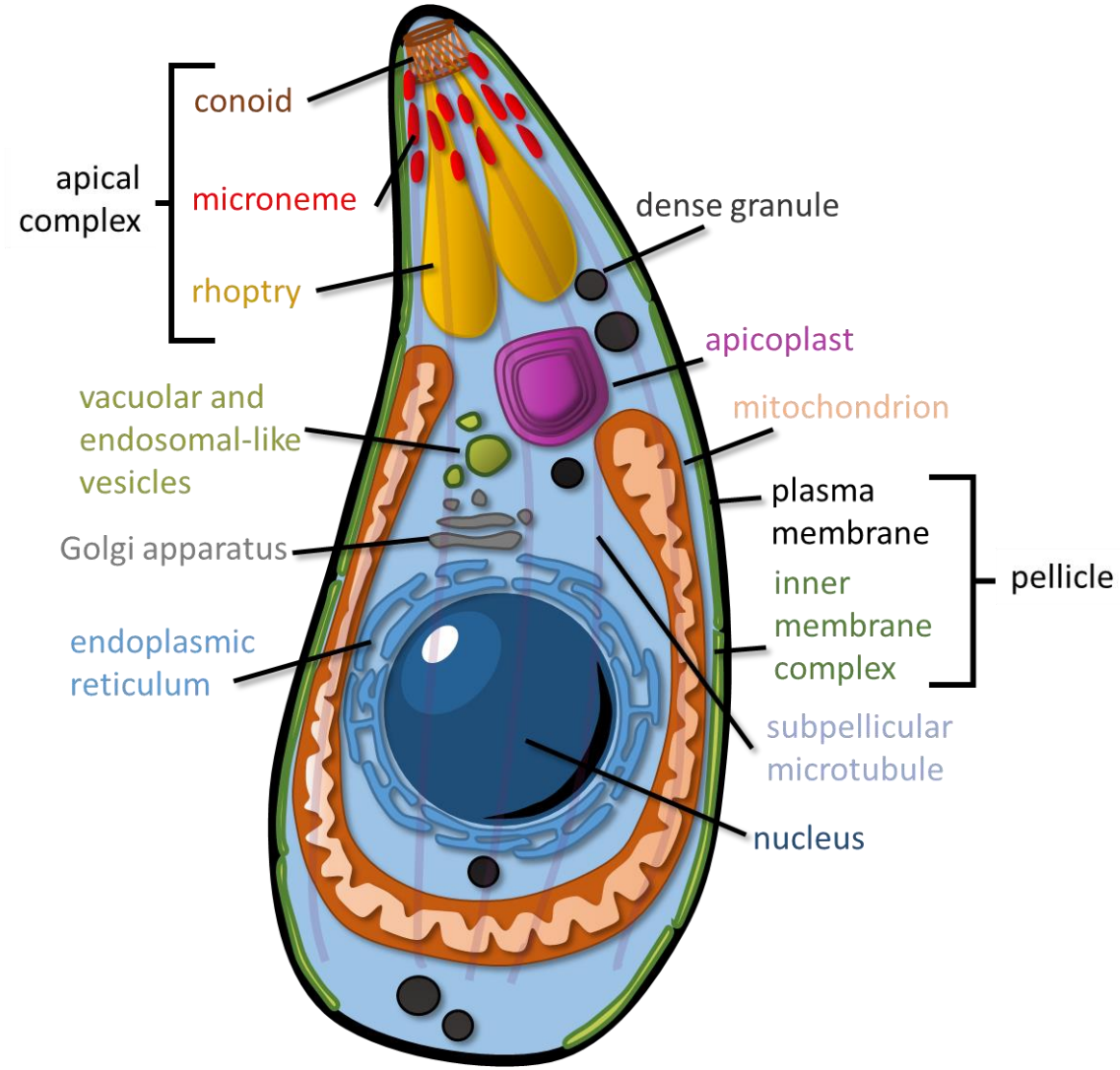
L. Paris, 2020

Apicomplexa contain a number of original organelles

Apicomplexa are eukaryotes and as such contain **typical eukaryotic organelles**, but they also contain some original ones

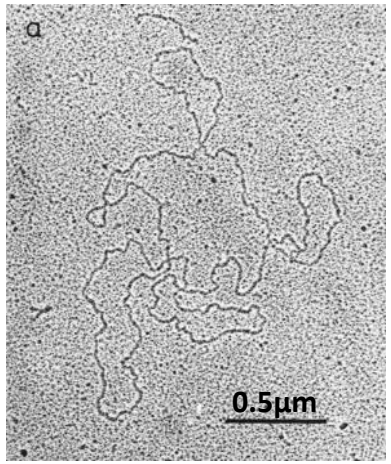
The name of the phylum is derived from an **apical complex** comprising specialised secretory vesicles called rhoptries and micronemes

Most (but not all) Apicomplexa also contain a plastid named **the apicoplast**



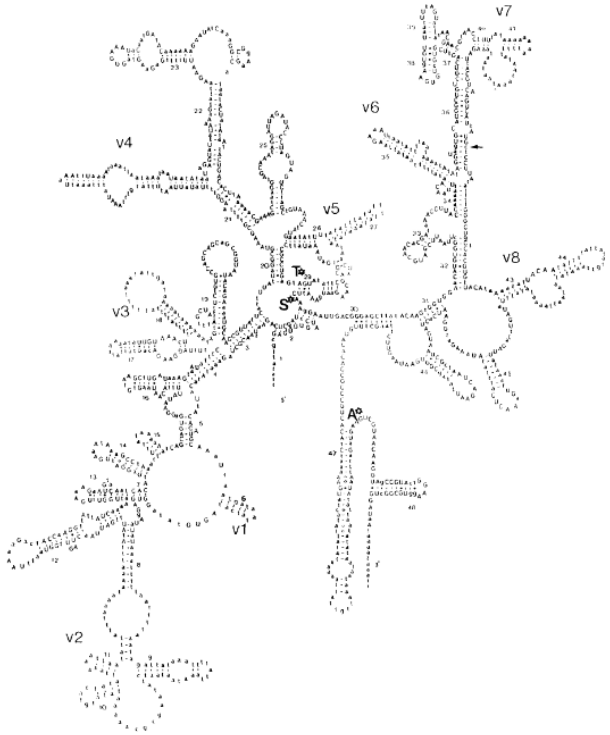
Discovery of the apicoplast

Identification of a **circular, extrachromosomal DNA molecule** in *Plasmodium*



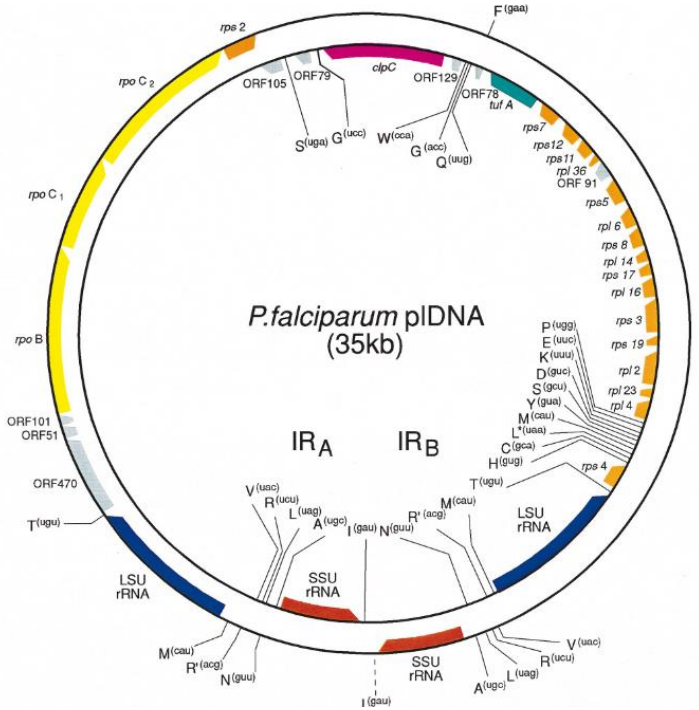
Kilejian A, Biochim Biophys Acta, 1975

rRNA sequences with sequence and inverted repeat arrangement **similar to plastid DNA**



Gardner et al, Mol Biochem Parasitol, 1993

Sequencing of the 35kb molecule, revealing the **plastidic origin** of the genes it encodes

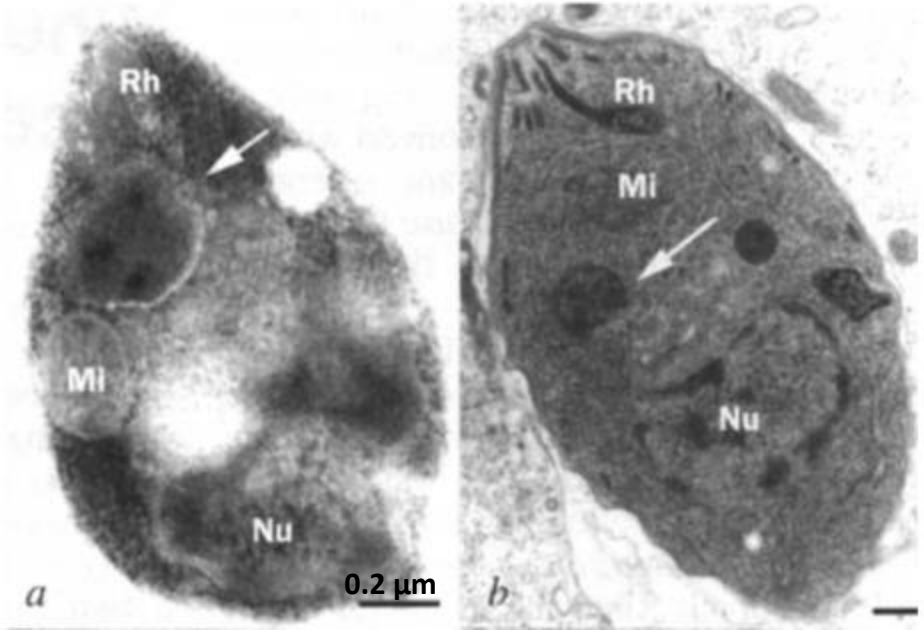


Wilson et al, J. Mol. Biol., 1996

Discovery of the apicoplast

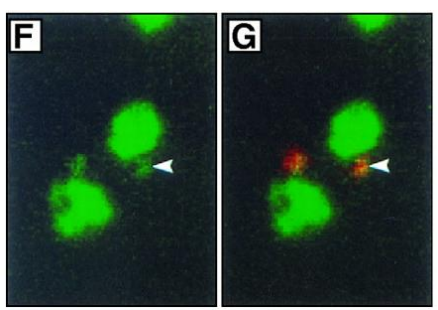
In situ hybridisation with specific probes allowed linking the 35 kb genome with a **four-membrane organelle**

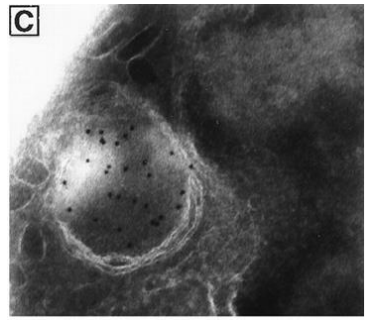
In *Plasmodium*

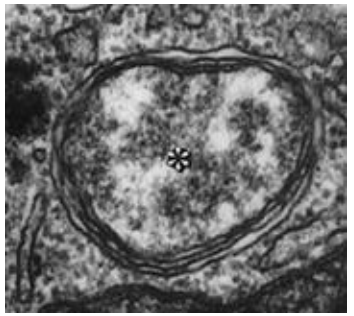


McFadden et al. Nature, 1996

In *Toxoplasma*

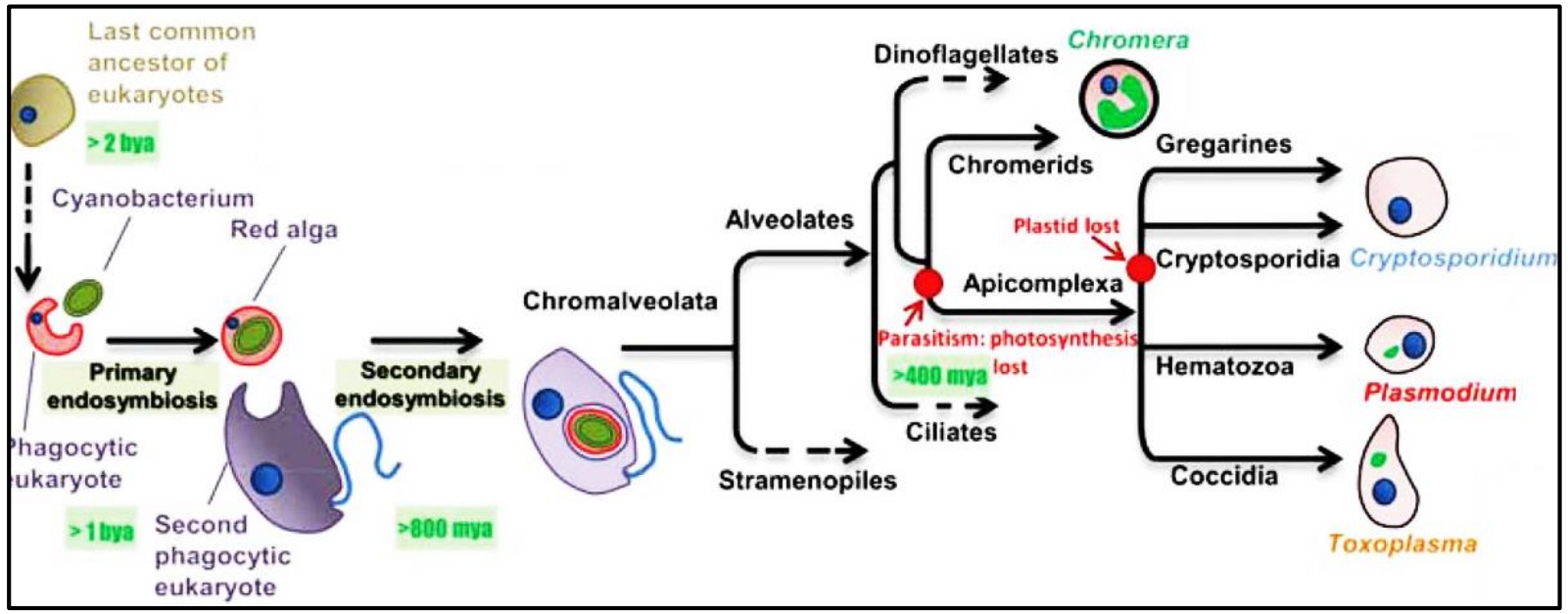






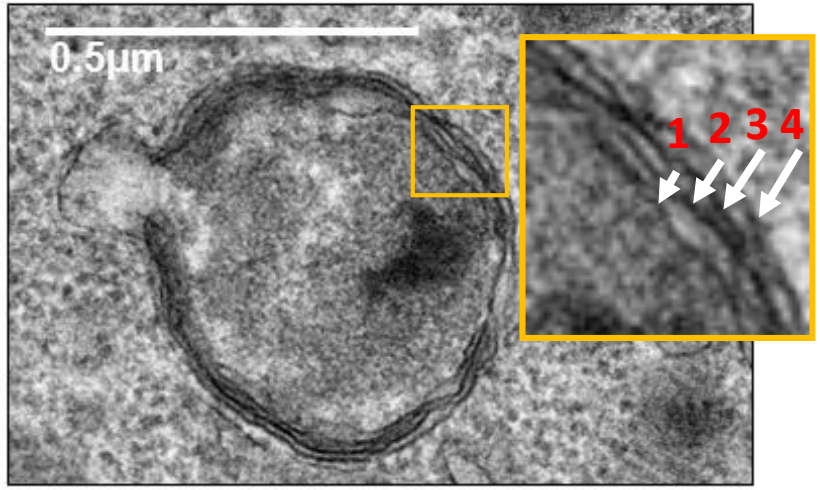
Kholer et al, Science, 1997

Origin of the apicoplast



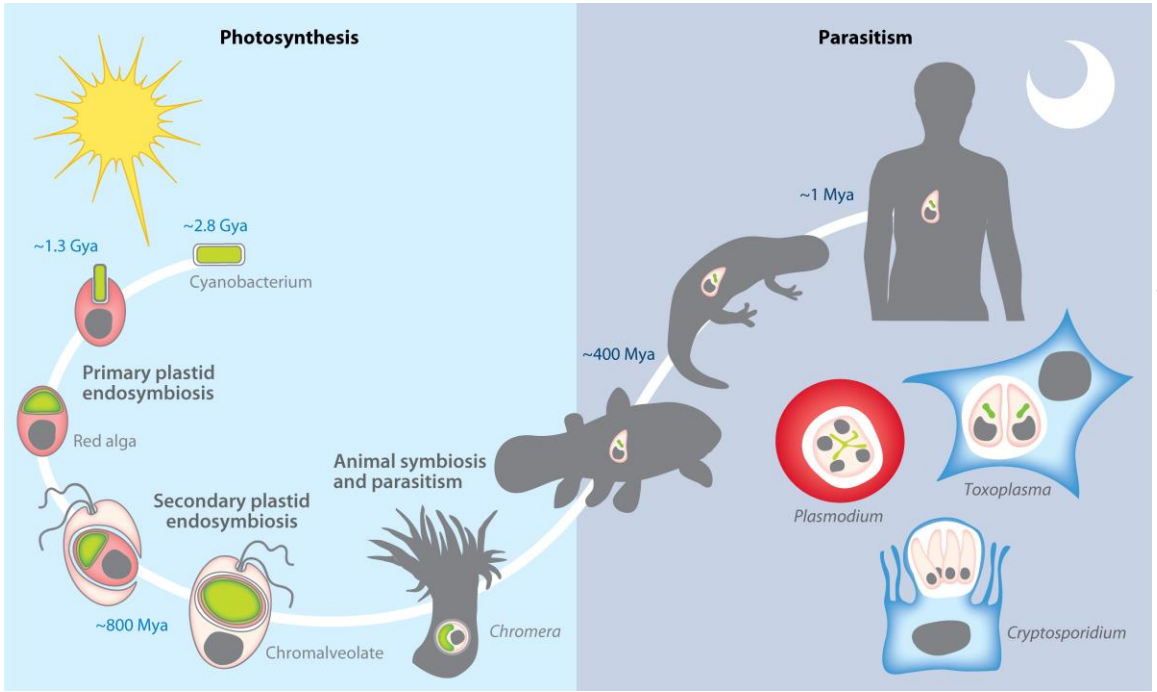
The apicoplast originates from a **secondary endosymbiosis**

It has **lost its photosynthetic capacity**



As a result, it is surrounded by **four membranes**:

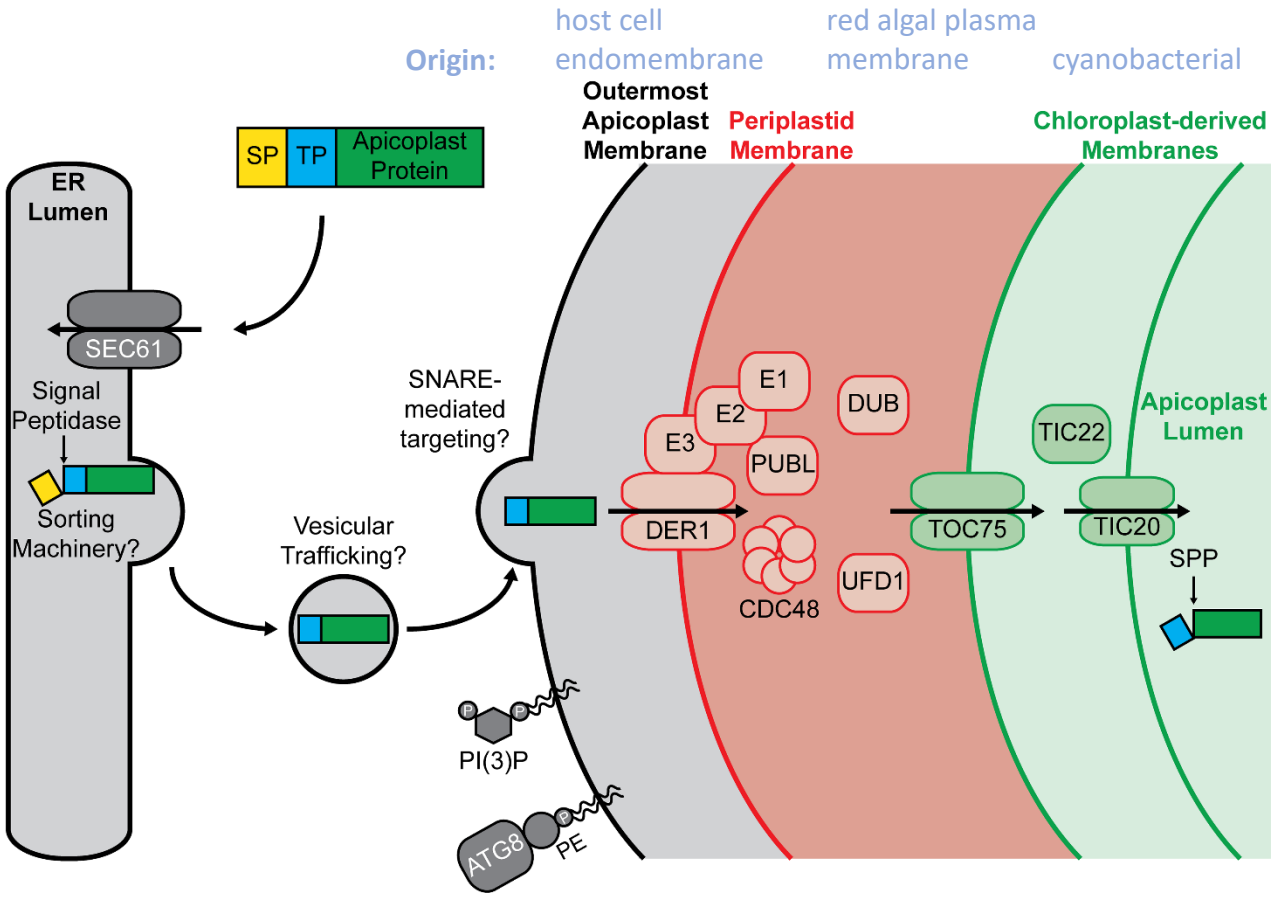
- **4** host cell endomembrane
- **3** red algal plasma membrane
- **1, 2** cyanobacterial, chloroplast-like membranes



van Dooren & Striepen, Ann Rev Microbiol, 2013

Protein targeting to the apicoplast

Although the apicoplast **has its own genome** and machinery for transcription and translation, the vast majority of apicoplast proteins are **nuclear-encoded** and must be imported post-translationally into the organelle



Boucher MJ, Yeh E, PLoS Pathog, 2019

They are usually imported through a **bipartite signal**: the SP mediates import into the ER, after which the TP mediates sorting and trafficking to the apicoplast

Proteins may translocate **directly** from the ER to the apicoplast or traffic through **vesicles**

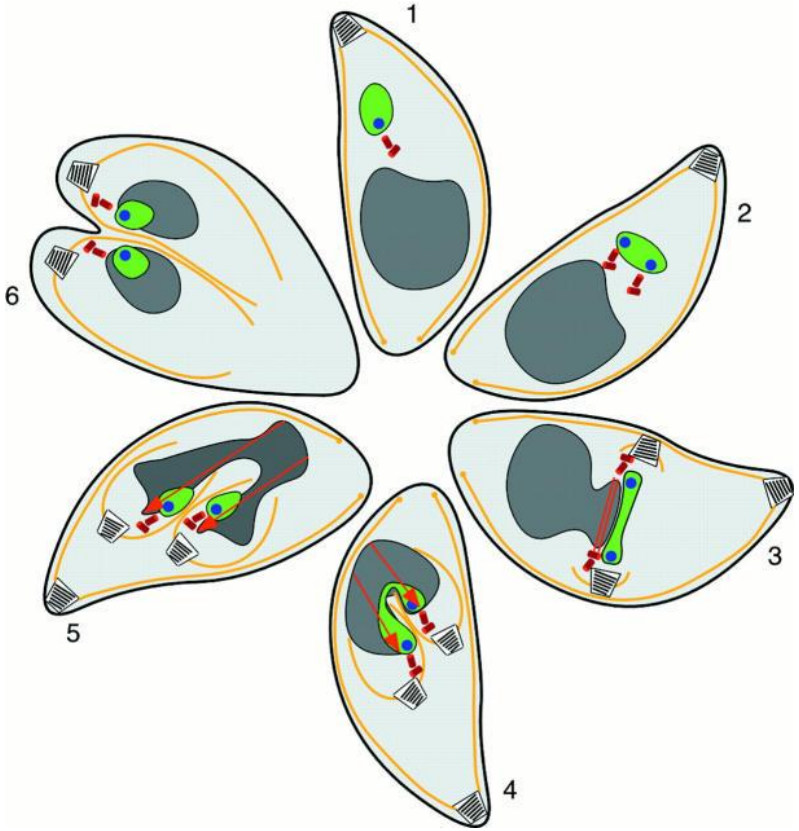
To get proteins through the periplastid membrane, Apicomplexa use the **ERAD system**, that is usually involved in retrotranslocating misfolded proteins from the ER to the cytoplasm

Then classical chloroplast-like **TOC/TIC translocons** ensure import into the apicoplast lumen

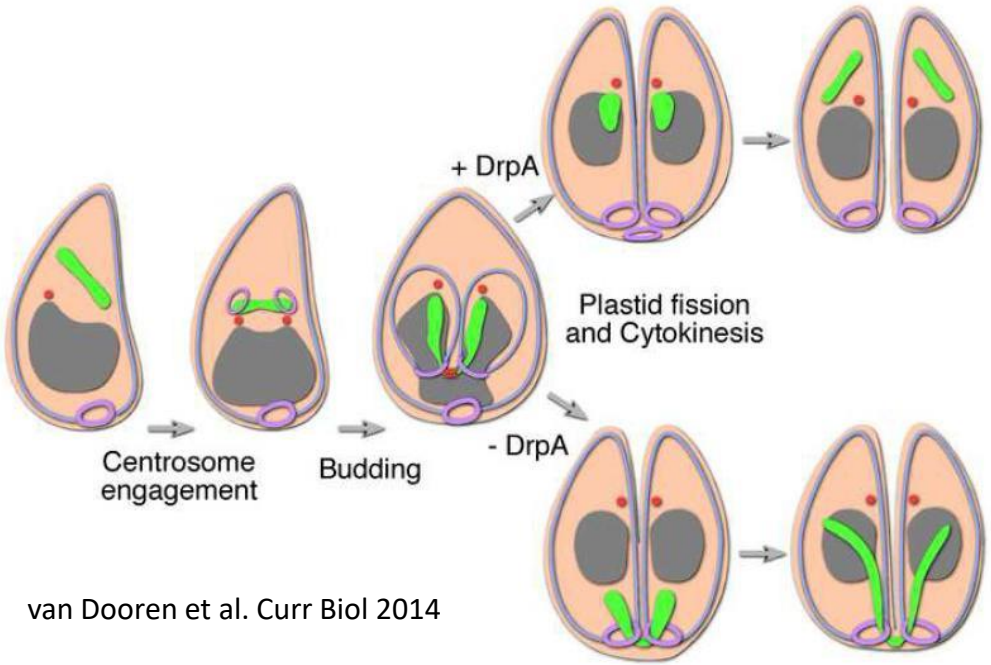
Apicoplast replication

Toxoplasma tachyzoites divide by a process called **endodyogeny**, by which two daughter cells develop inside a mother cell

During this process, the apicoplast is duplicated and inherited by daughter cells in a coordinated manner, **driven by its association with the centrosome**



Striepen et al. J Cell Biol, 2000



van Dooren et al. Curr Biol 2014

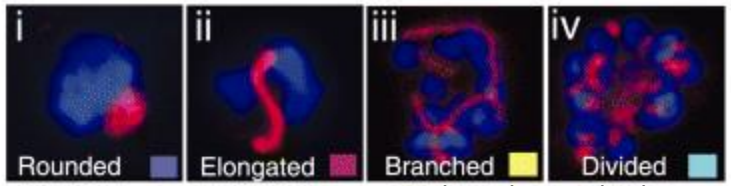
Fission is mediated by the DrpA dynamin, which is different from dynamins involved in plastid fission in plants and algae

Apicoplast replication

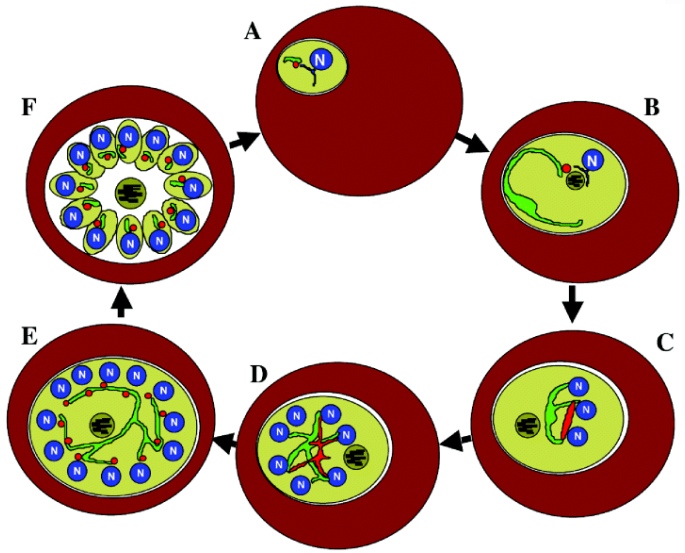
Asexual blood and liver stages of *Plasmodium* parasites divide by a process termed **schizogony** where parasites undergo repeated rounds of mitosis and nuclear division, producing a **multinucleate cell** that then undergoes daughter budding and cytokinesis in a single event to produce multiple (thousands in the case of liver-stage schizonts) parasites

During schizogony, apicoplasts, start of as small, rounded organelles, and undergo **extensive elongation and branching before coordinated fission** and inheritance in newly-formed parasites

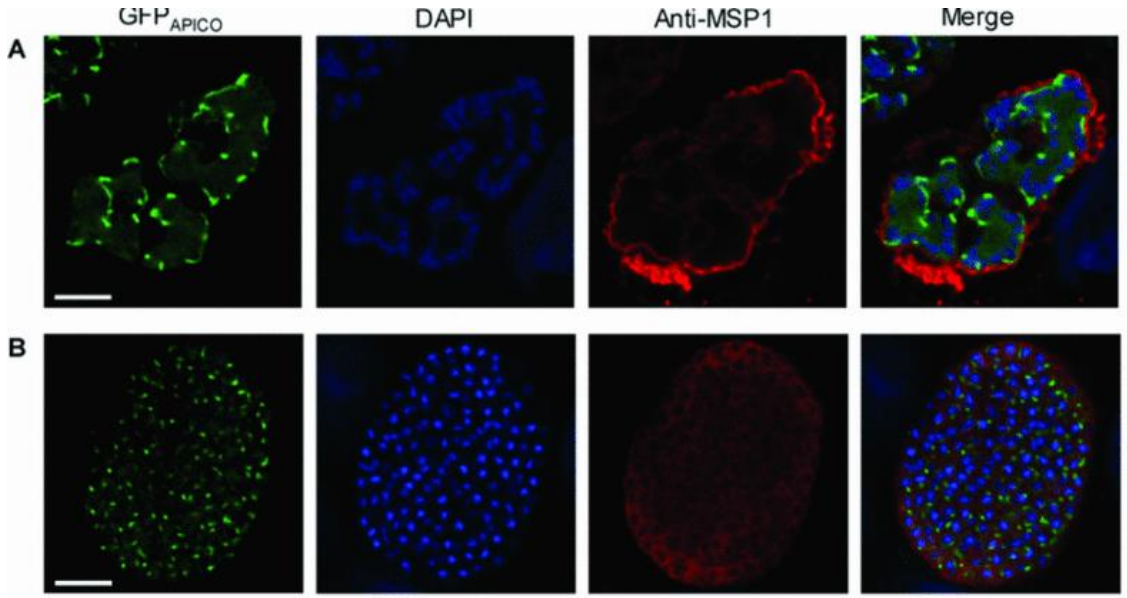
P. falciparum blood stages



van Dooren etal, Mol Microbiol, 2005



P. berghei liver stages



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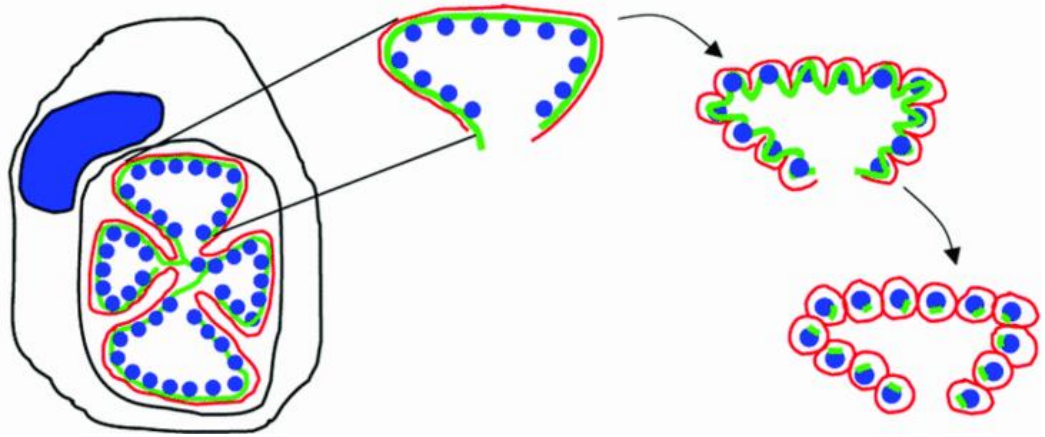
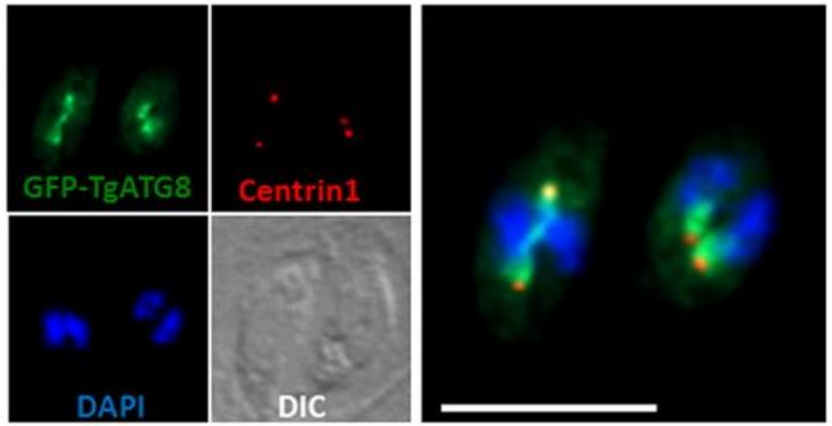


Stanway et al Biol Cell, 2012

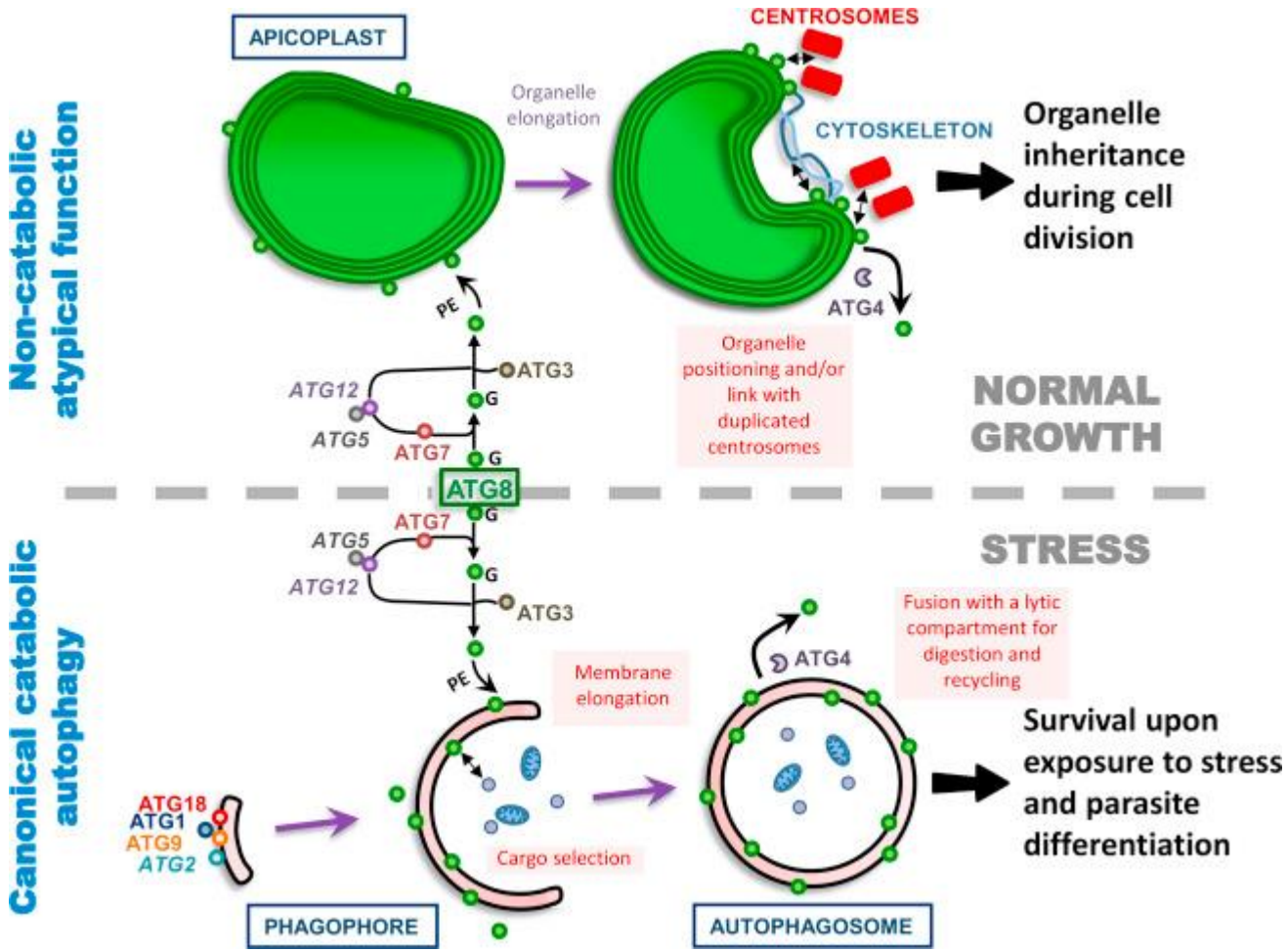
The unusual involvement of the autophagy machinery in apicoplast inheritance

Autophagy-related protein ATG8 and its associated membrane-conjugating machinery are **essential for apicoplast homeostasis** in *T. gondii* and *P. falciparum*

ATG8 is recruited to the end of elongating apicoplasts and might coordinate the organelle's **association with centrosomes**



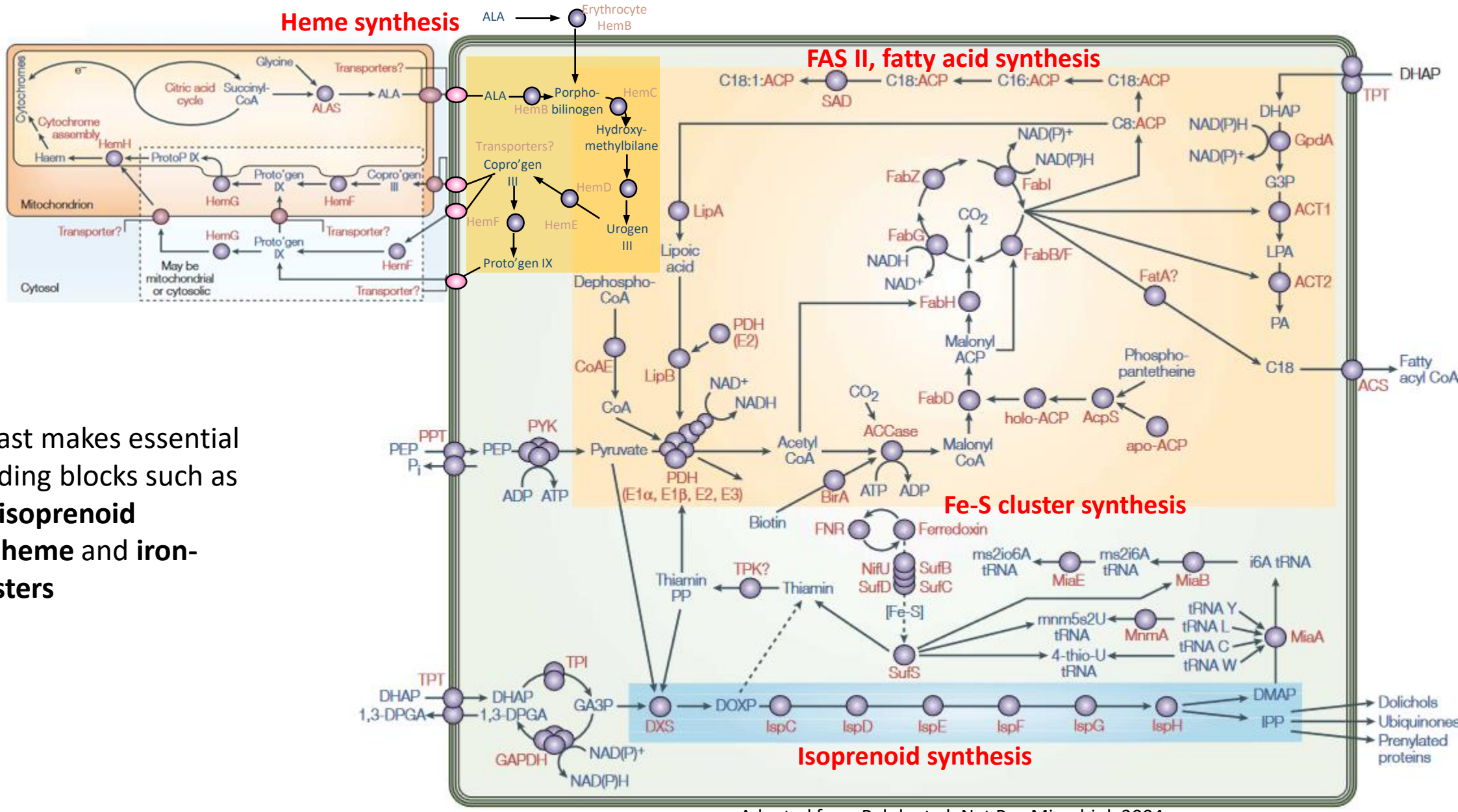
Lévêque et al, mBio, 2015



Besteiro S., Curr Opin Microbiol, 2017

The apicoplast is an important metabolic hub

The apicoplast makes essential cellular building blocks such as **fatty acids**, **isoprenoid precursors**, **heme** and **iron-sulphur clusters**

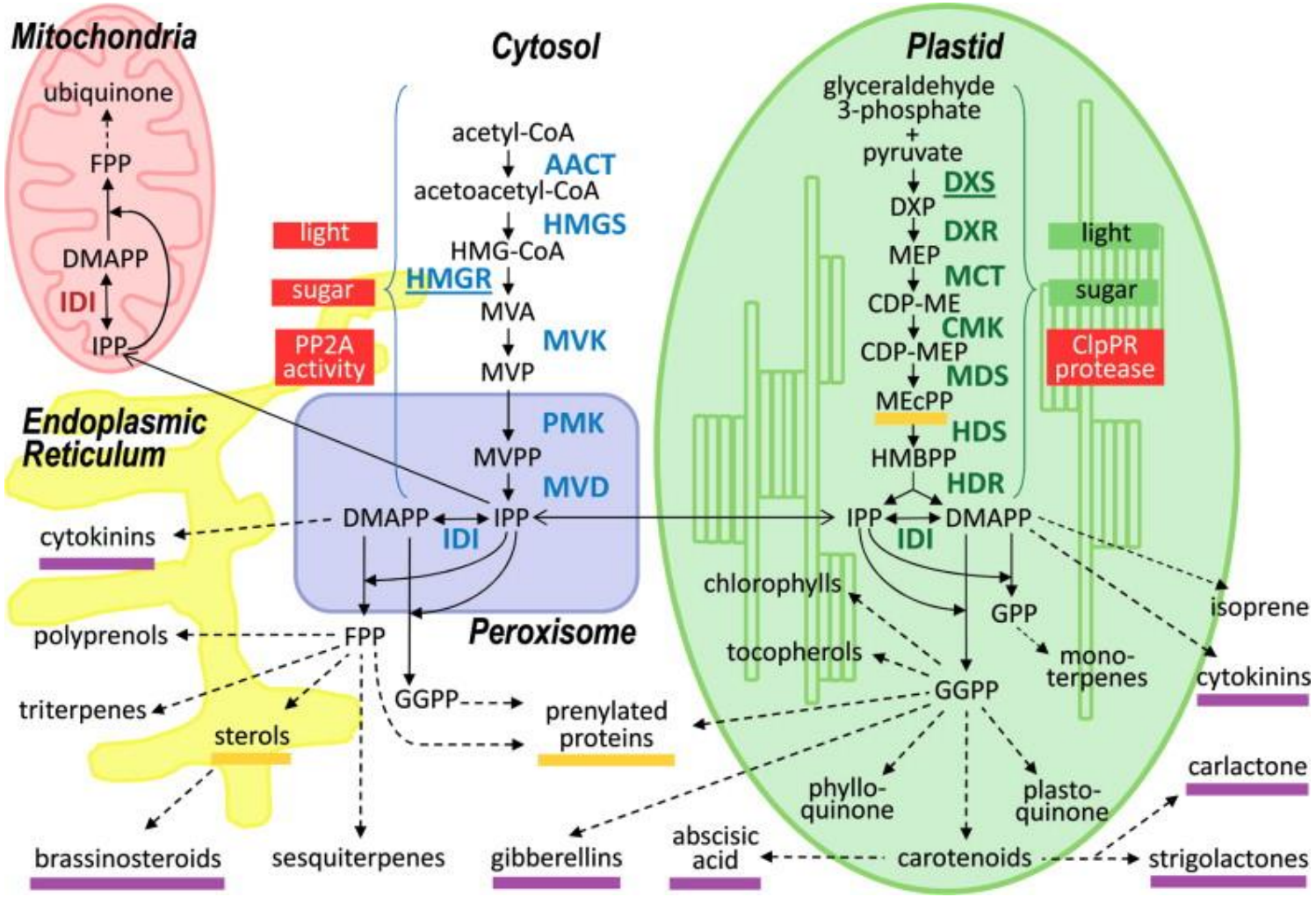


Adapted from Ralph et al, Nat Rev Microbiol, 2004

Essentiality of apicoplast pathways – isoprenoid synthesis

The pathway is required for the production of isopentenyl pyrophosphate (**IPP**) and dimethylallyl pyrophosphate (**DMAP**) that plays a role in the biosynthesis of molecules used in protein prenylation, ubiquinone, cell membrane maintenance, protein anchoring and *N*-glycosylation

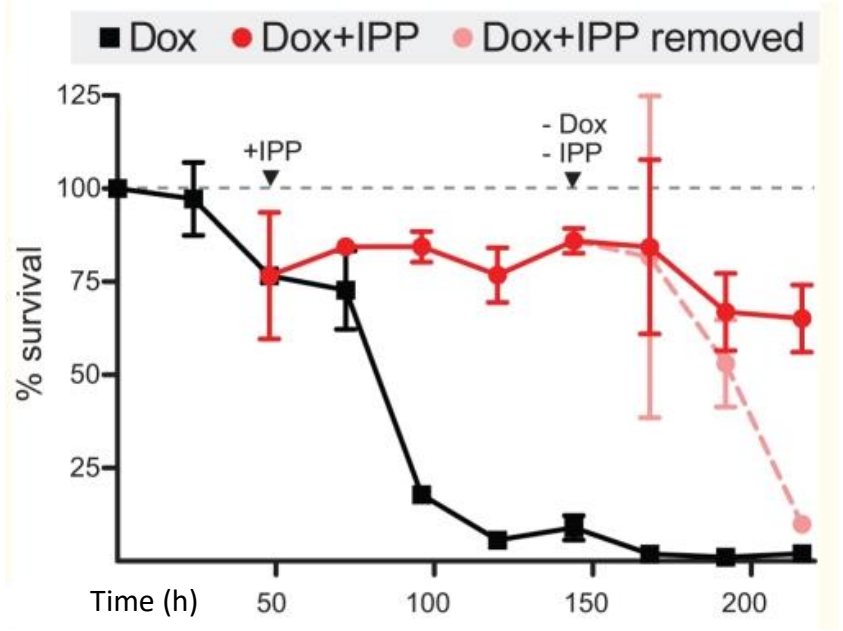
Apicomplexa, like plants, produce isoprenoids through a **pathway different from mammals** (non-mevalonate pathway)

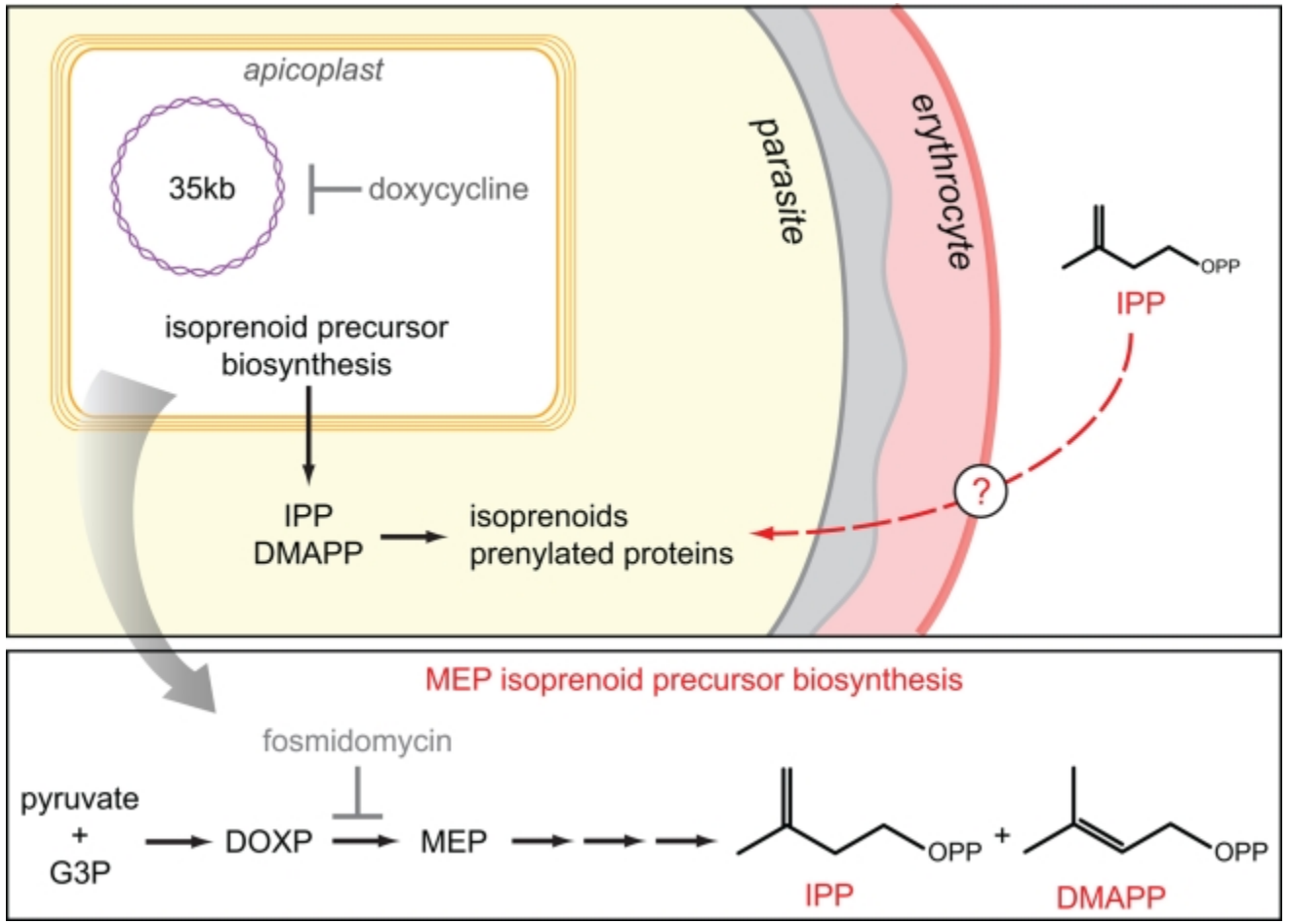


Pulido et al, Molecular Plant, 2012

Essentiality of apicoplast pathways – isoprenoid synthesis

While disrupting general apicoplast function with drugs like doxycycline is generally **lethal**, ***Plasmodium* blood stages** can be maintained in culture in spite of a complete loss of the apicoplast as long as **IPP is supplemented in the culture medium**





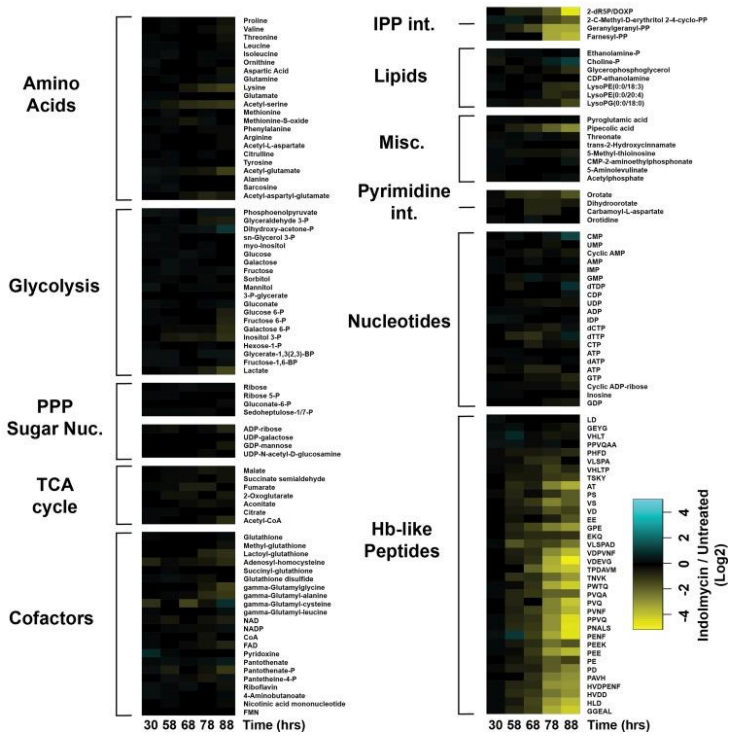
Yeh & DeRisi, PLoS Biol., 2011

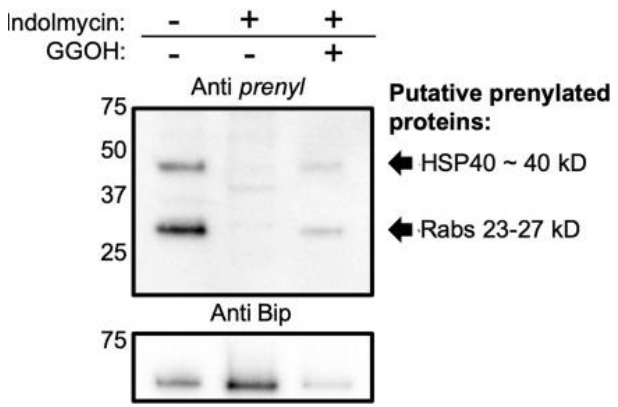
Isoprenoid precursor biosynthesis is the only essential metabolic role of the apicoplast for *P. falciparum* blood stages (and for gametogenesis)

Essentiality of apicoplast pathways – isoprenoid synthesis

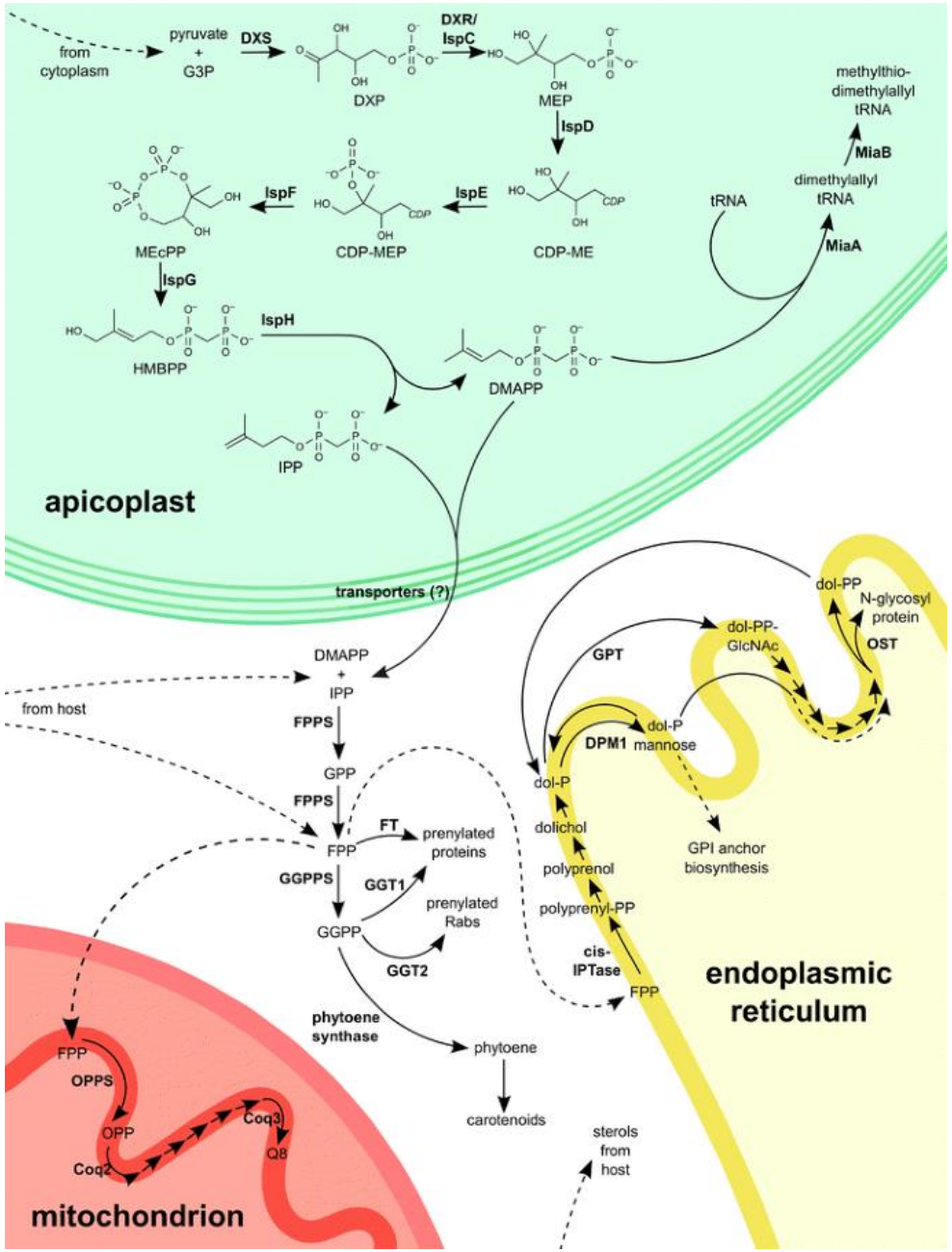
Disrupting general apicoplast function leads to an **accumulation of isoprenoids intermediates and hemoglobin-derived peptides**

Prenylated **Rab GTPases** are strongly impacted





Kennedy et al, PLoS Biology, 2019



Imlay & Odom, Curr Clin Microbiol Rep, 2014

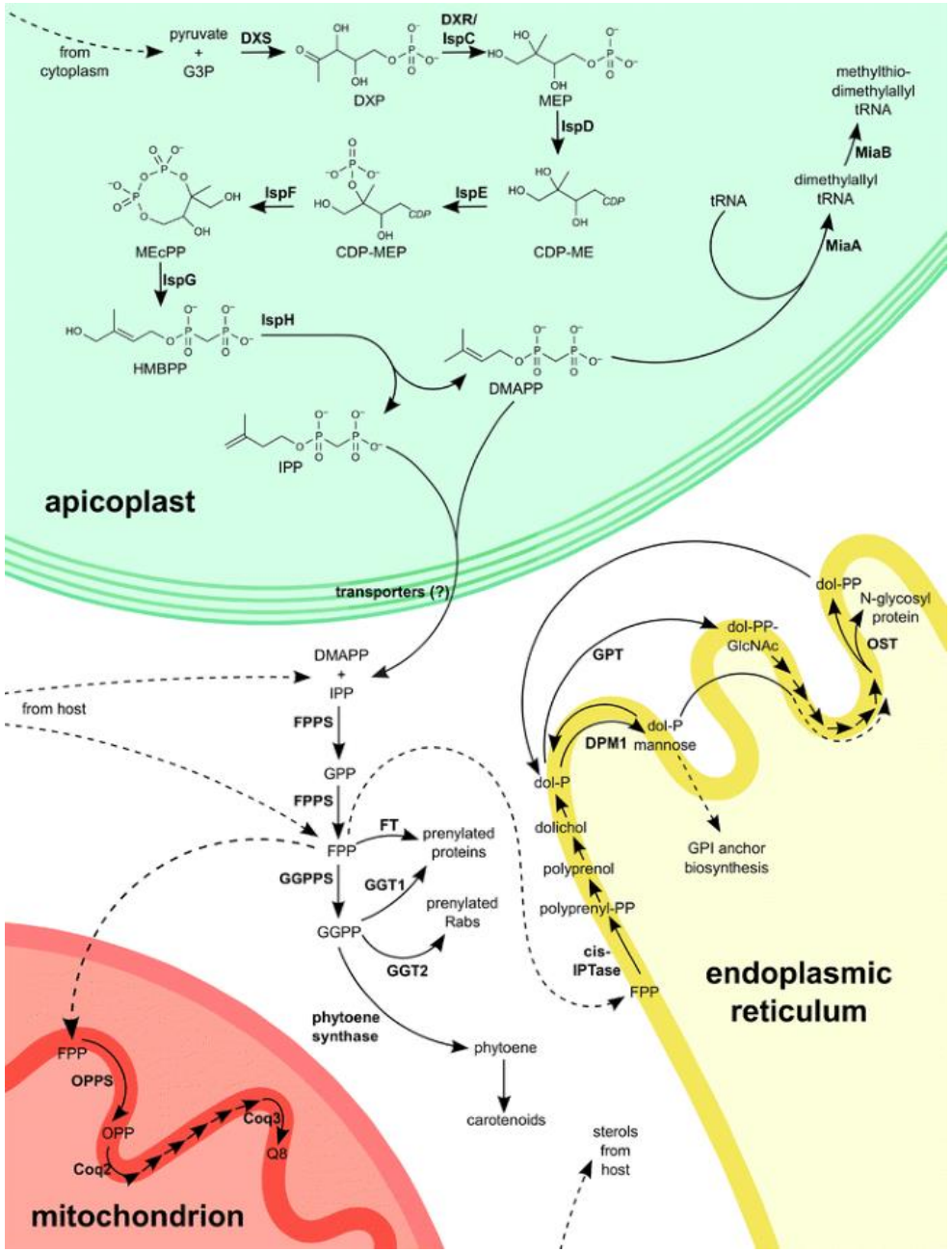
Essentiality of apicoplast pathways – isoprenoid synthesis

The prenylome of *P. falciparum* identifies **important prenylated proteins potentially involved in trafficking**

High-confidence prenylated proteins identified by proteomics[a]

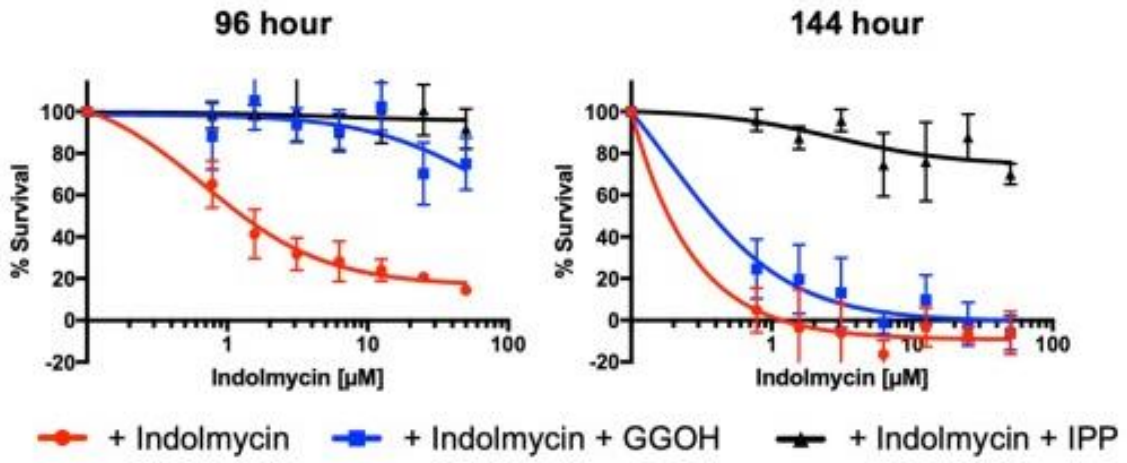
Gene ID	Annotation	Motif	PrePS prediction	PRENbase cluster
CaaX box proteins				
PF3D7_0322000	peptidyl-prolyl cis-trans isomerase (CYP19A)	CGEL	FT– GGT1–	Predicted conserved
PF3D7_0910600	SNARE protein, putative (YKT6.1)	CCSIM	FT+++ GGT1+++	Known conserved
PF3D7_1113100	protein tyrosine phosphatase (PRL)	CHFM	FT– GGT1–	Known conserved
PF3D7_1319100	Conserved DUF544 protein, unknown function	CTIM	FT+++ GGT1+++	Predicted conserved
PF3D7_1324700	SNARE protein, putative (YKT6.2)	CCSLY	FT+ GGT1–	Known conserved
PF3D7_1428700	Conserved protein, unknown function	CNFM	FT– GGT1–	Unknown Not conserved
PF3D7_1437900	HSP40, subfamily A, putative (ERdj3)	CAQQ	FT++ GGT1–	Known conserved
PF3D7_1460100	FYVE and coiled-coil domain-containing protein (FCP)	CNIM	FT+ GGT1++	Unknown Not conserved
Rab GTPases				
PF3D7_0106800	Ras related protein, Rab5c	KKCC	RabGGT+++	Known conserved
PF3D7_0211200	Ras related protein, Rab5a	KGCC	RabGGT+++	
PF3D7_0512600	Ras related protein, Rab1b	KKCC	RabGGT+++	
PF3D7_0807300	Ras related protein, Rab18	NCAC	RabGGT+++	
PF3D7_0903200	Ras related protein, Rab7	SRCC	RabGGT+++	
PF3D7_1144900	Ras related protein, Rab6	KCLC	RabGGT+++	
PF3D7_1231100	Ras related protein, Rab2	FSCC	RabGGT+++	
PF3D7_0513800	Ras related protein, Rab1a	FCSC	RabGGT+++	
PF3D7_1320600	Ras related protein, Rab11a	NKCC	RabGGT+++	
PF3D7_1340700	Ras related protein, Rab11b	VKCC	RabGGT++	
PF3D7_1310600	Ras related protein, Rab5b	None[b]	RabGGT –	

Gisselberg et al. Mol & Cell Proteomics, 2017



Imlay & Odom, Curr Clin Microbiol Rep, 2014

Essentiality of apicoplast pathways – isoprenoid synthesis

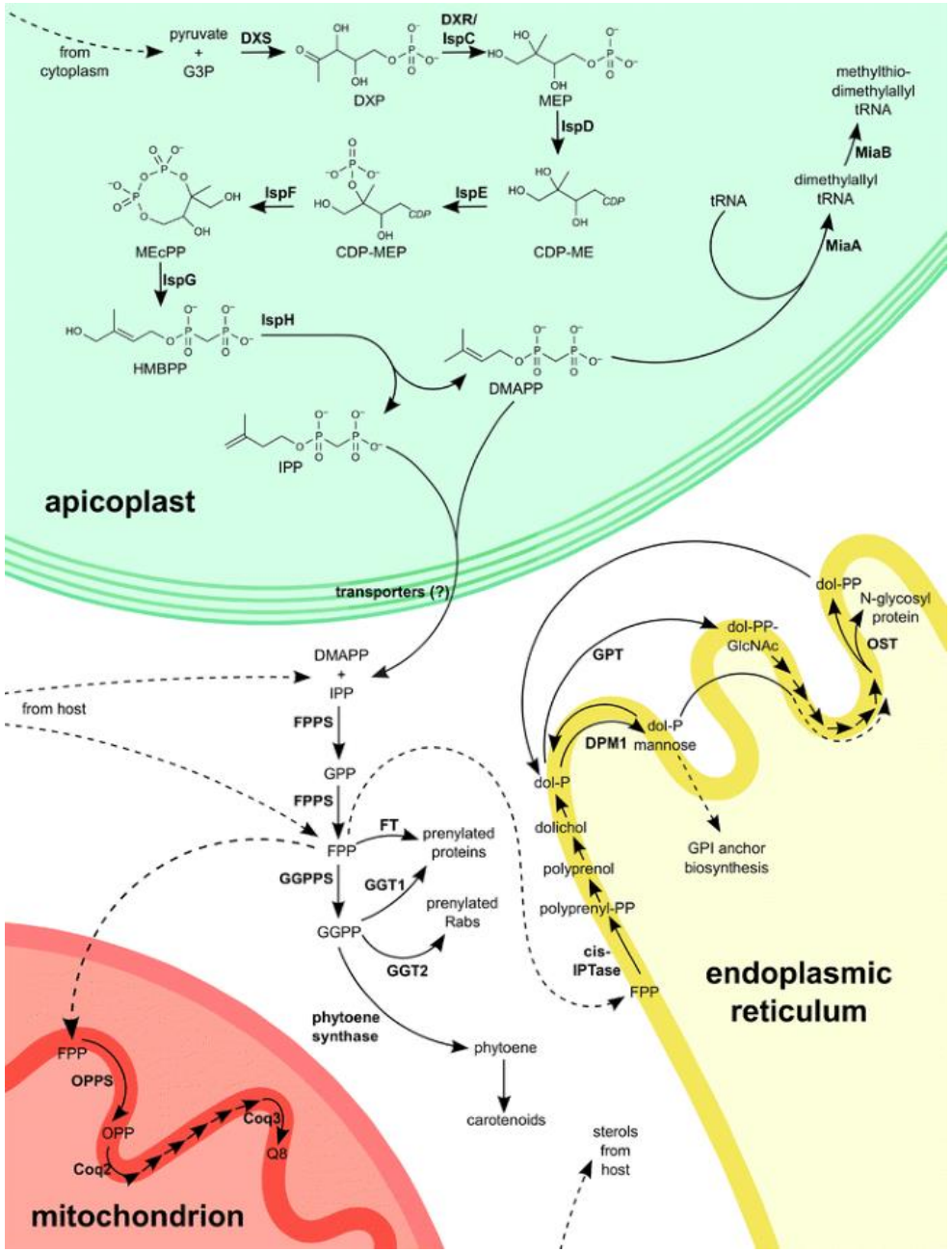


Kennedy et al, PLoS Biology, 2019

Provision of the polyprenol precursor GGOH **rescues parasites in the short term**, but the rescued parasites eventually succumb to the lethal effect of the drug

Dolichol-mediated GPI synthesis and/or ubiquinone electron transport, which are not chemically rescued by GGOH, are thus also likely essential

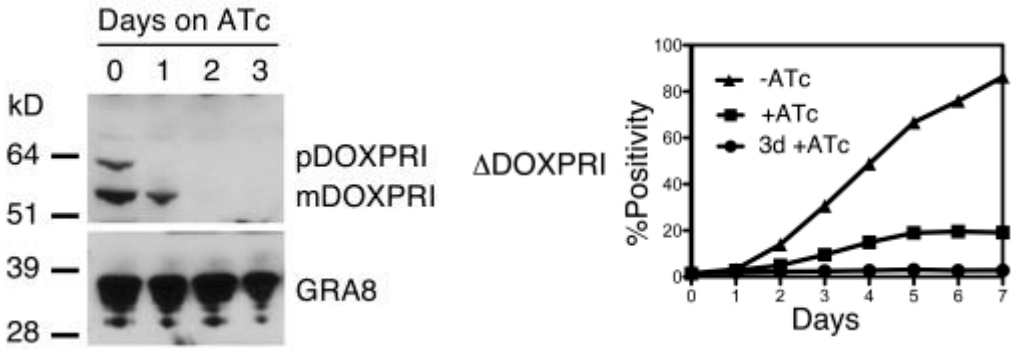
Intracellular trafficking pathways regulated by prenylated proteins are likely important apicoplast-dependent processes in blood stages



Imlay & Odom, Curr Clin Microbiol Rep, 2014

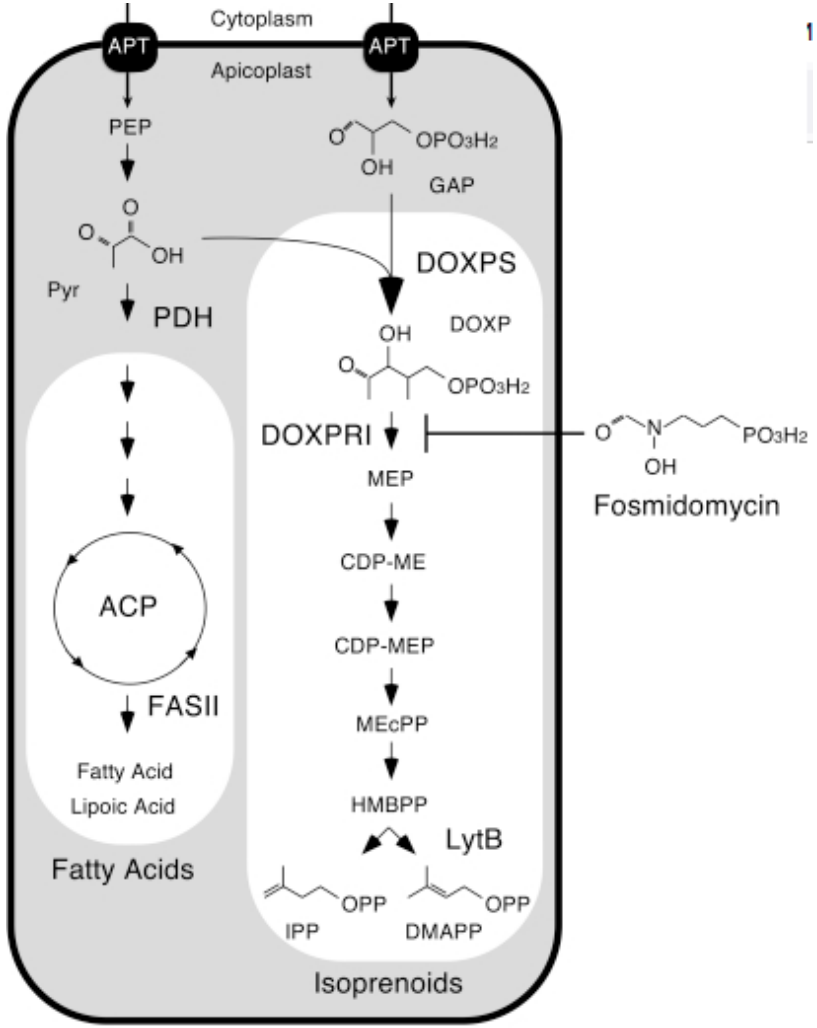
Essentiality of apicoplast pathways – isoprenoid synthesis

The DOXP pathway is also essential in ***T. gondii*** tachyzoites



Yet the *T. gondii* pathway **is not sensitive to fosmidomycin** due to differences with *Plasmodium* in membrane permeability

IPP rescue of apicoplast loss is not experimentally possible in *T. gondii*. This mays be due to differential membrane permeability to the precursor, or could be hinting **other apicoplast-hosted pathways are essential for tachyzoites**

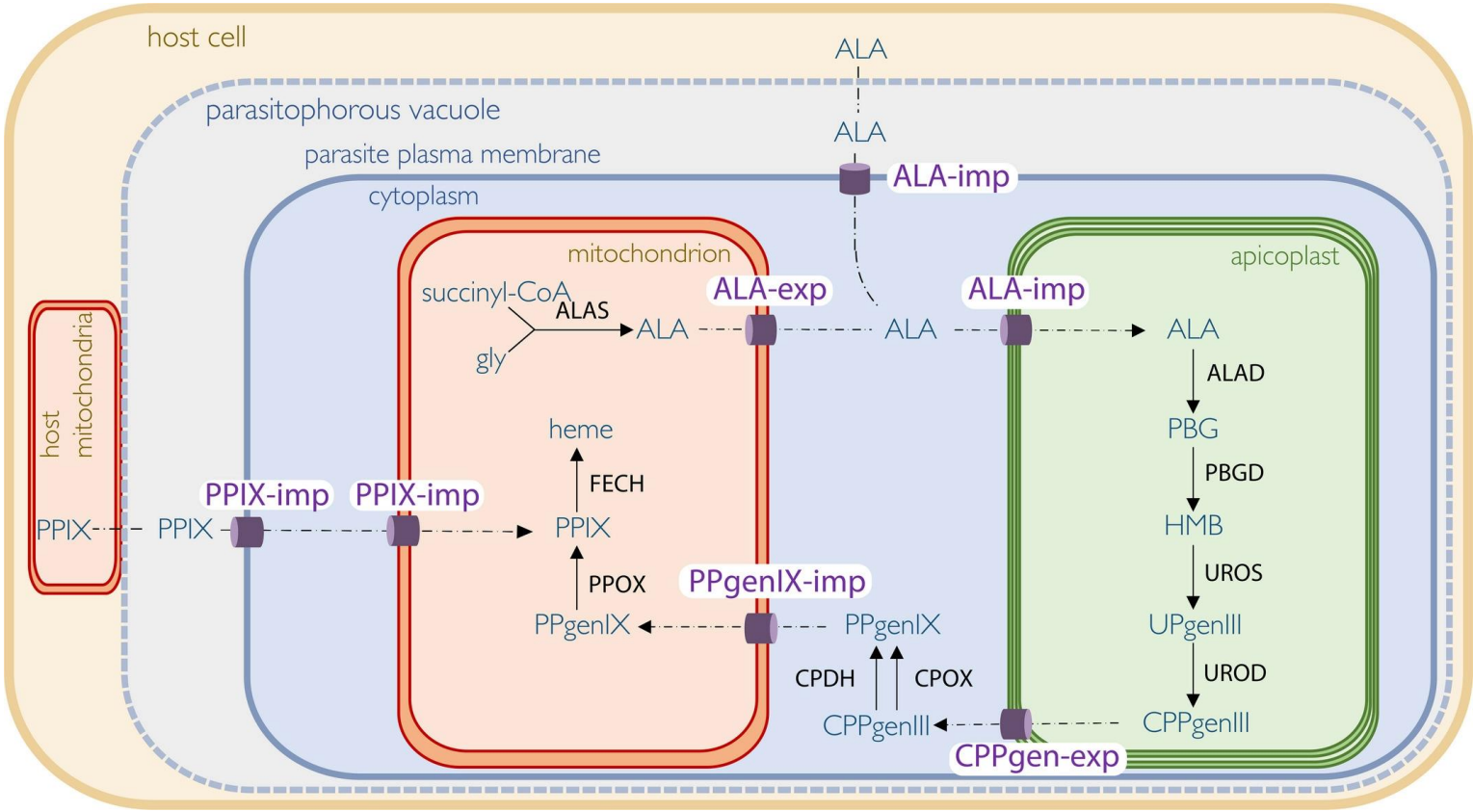


Nair et al. J Exp Med. , 2012

Essentiality of apicoplast pathways – heme synthesis

Heme is an important co-factor. It is the functional group in the antioxidant function of peroxidases and catalases, as well as the electron transfer reactions of cytochromes in the mitochondrial respiratory chain

In Apicomplexa, heme is synthesized through a **collaboration between the mitochondrion and the apicoplast**

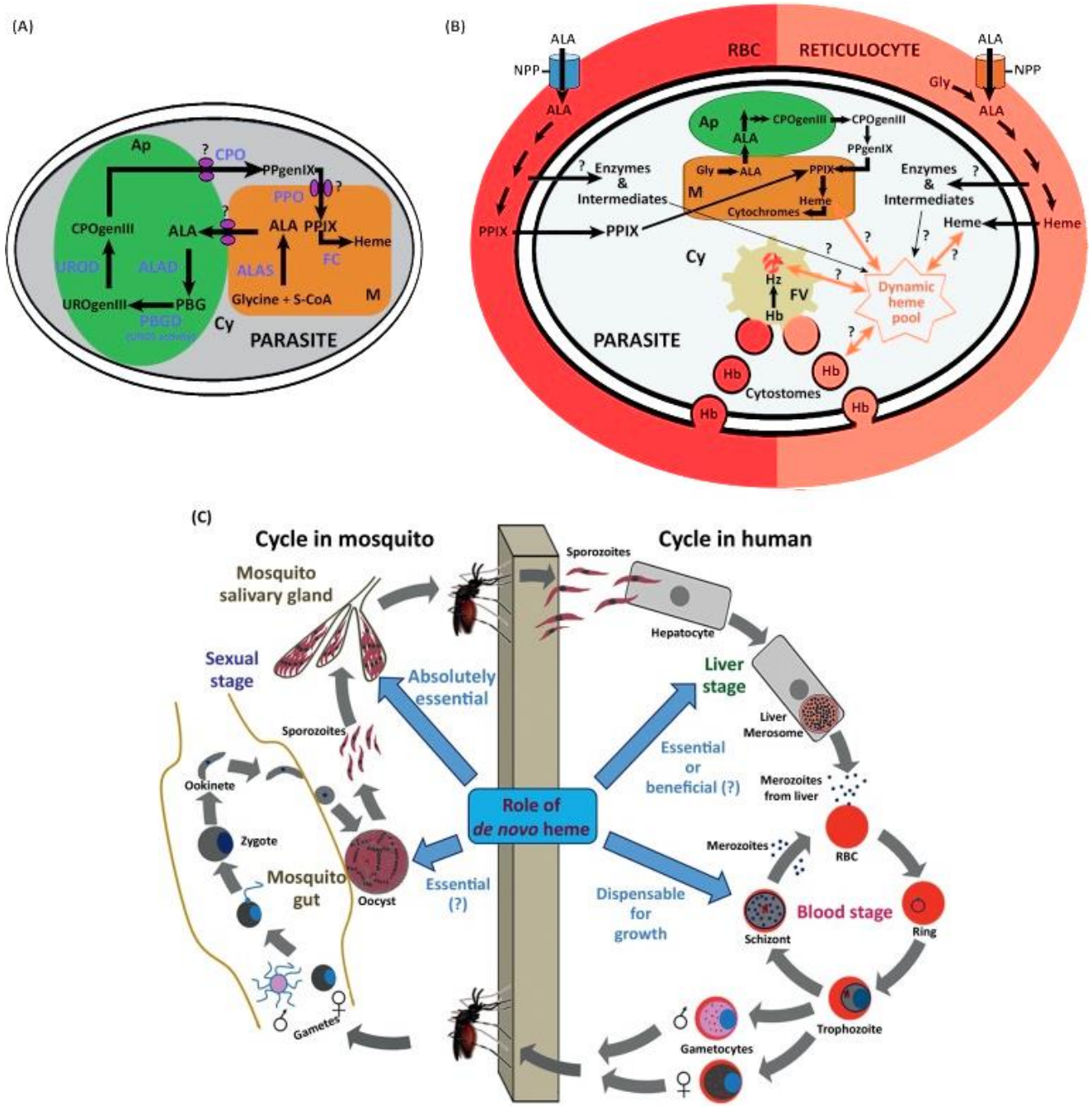


Kloehn et al, FEBS J., 2020

Essentiality of apicoplast pathways – heme synthesis

Plasmodium **acquires host hemoglobin in the blood stages** and digests it in the food vacuole to generate amino acids. Since accumulation of free heme is toxic, it stores the excess heme derived from hemoglobin as hemozoin pigment, a biocrystallized form of heme-aggregates

The parasite heme pathway is dispensable for blood stages, since the parasite has back-up mechanisms to satisfy heme requirements, but **it is absolutely essential for the development of sporozoites in the mosquito**



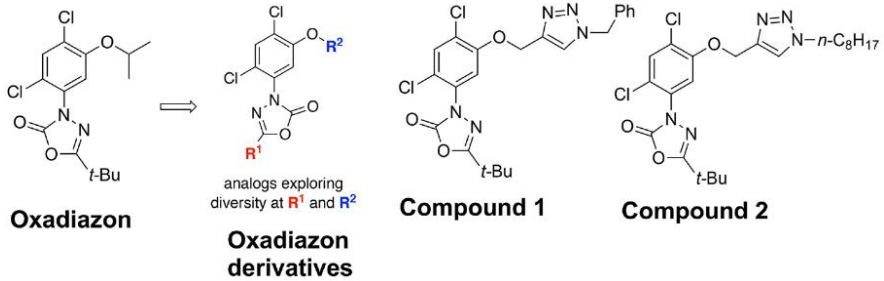
Nagaraj & Padmanaban, Trends Parasitol, 2017

Essentiality of apicoplast pathways – heme synthesis

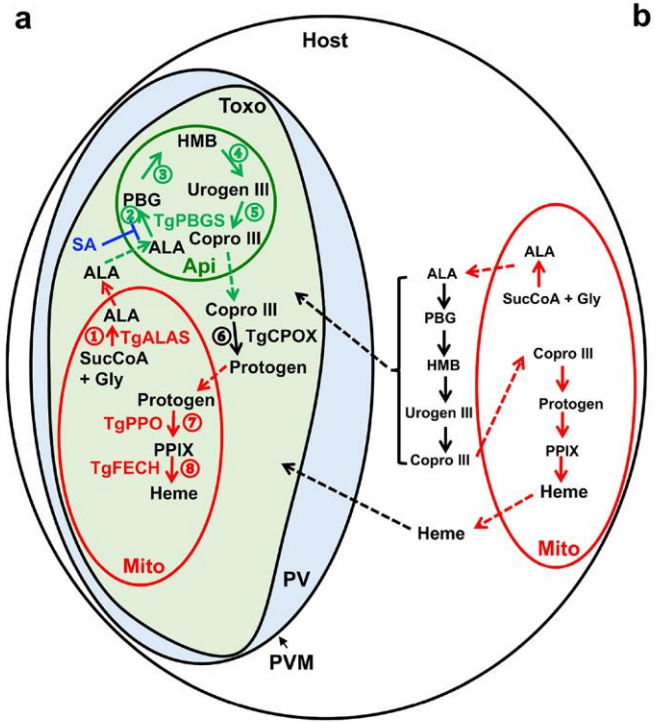
De novo heme production **is essential for *T. gondii*** intracellular growth and pathogenesis

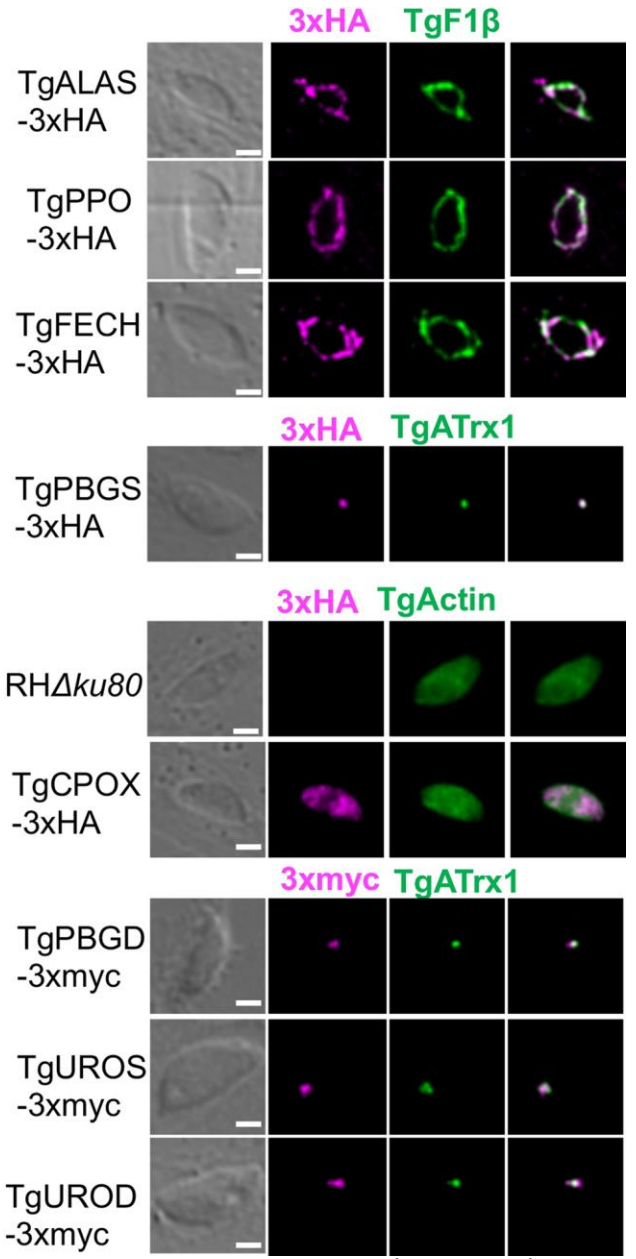
The herbicide oxadiazon significantly impaired *T. gondii* growth, consistent with phylogenetic analyses that show parasite protoporphyrinogen oxidase is more closely related to plants than mammals

Targeting *T. gondii* heme synthesis shows some potential for a future therapeutic intervention



Strains	Oxadiazon (μM)	Compound 1 (μM)	Compound 2 (μM)	Pyrimethamine (μM)
WT::*NLuc*	131.4 ± 3.9	5.0 ± 1.5	8.3 ± 1.3	0.75 ± 0.02
Δ*ppo* ::*NLuc*	874.8 ± 102.2	232.5 ± 46.0	360.8 ± 66.2	0.72 ± 0.16
Δ*ppoPPO* ::*NLuc*	109.9 ± 5.9	5.6 ± 1.3	8.6 ± 3.0	8.72 ± 1.93
WT::*NLuc -pTub-TgPPO*	203.0 ± 17.6	13.9 ± 1.1	26.6 ± 2.8	Not determined



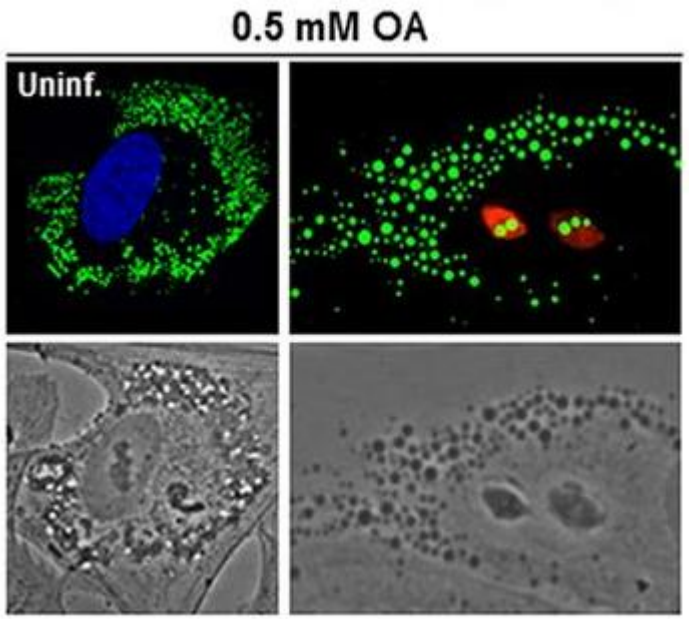


Bergmann et al, PLoS Path, 2020

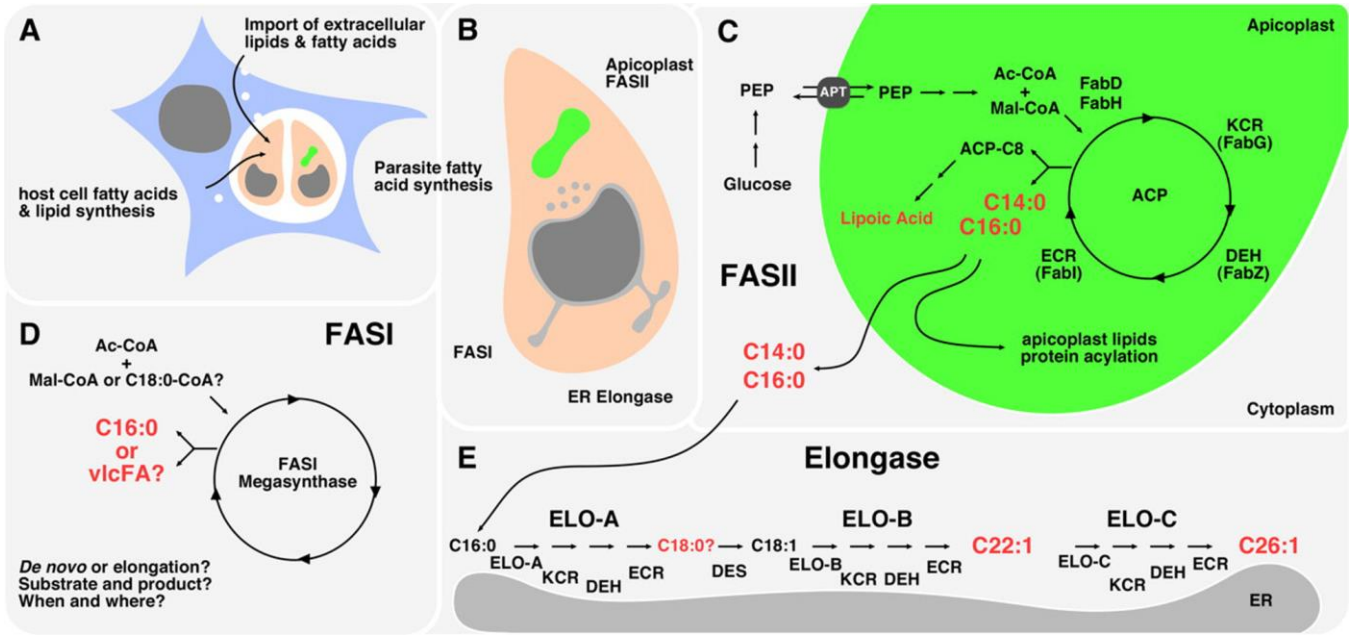
Essentiality of apicoplast pathways – fatty acid synthesis

Apicomplexa parasites can **synthesize fatty acids *de novo* in a cooperation between the apicoplast and the ER**: short chain FAs are made in the apicoplast and elongated in the ER

They can also **scavenge FAs from the host cell**



Nolan et al. Antimicrob Agents & Chemother, 2018



Ramakrishnan et al J. Biol. Chem, 2012

FASII is absent from the mammalian host and thus offers a potentially new opportunity for drug design

Essentiality of apicoplast pathways – fatty acid synthesis

Fatty acids are required for membrane lipid synthesis and other essential cellular processes, and their production represents a central aspect of parasite lipid metabolism

Existing **compounds already established as FASII inhibitors** in other organisms showed promising results on ***Plasmodium* blood stages** but turned out to be **unspecific**

Core components of pathway were then targeted for **deletion** in *Plasmodium* blood stages and found to be **dispensable**

Subsequent studies, however, showed that **FASII is required for *Plasmodium* liver stage development**

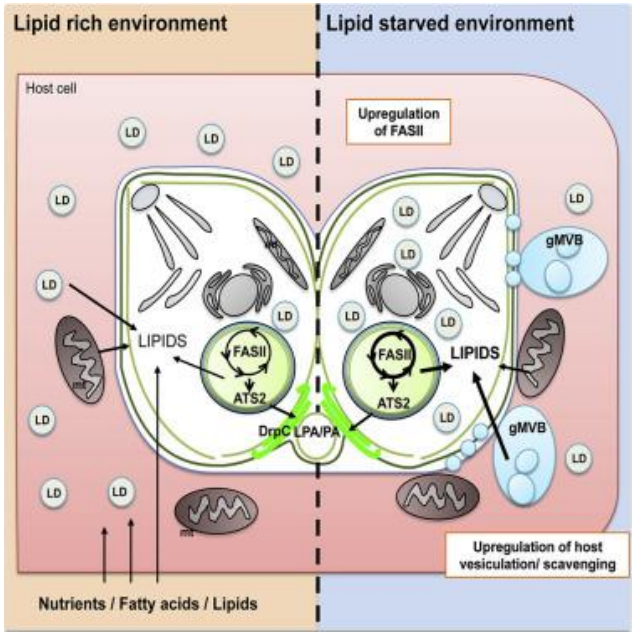
		Localization				Activity				Knockout			
	Enzyme	*Pf*	*Pb*	*Py*	*Tg*	*Pf*	*Pb*	*Py*	*Tg*	*Pf*	*Pb*	*Py*	*Tg*
Fatty acid synthesis (FASII)	Phosphate transporter (pPT) of outer membrane[#] PF3D7_0508300[^]	✓			✓	✓			✓		✓ *ML*		✓
	Phosphate transporter (pPT) of inner membrane PF3D7_0530200	✓	✓			✓					✓ *B*		
	Pyruvate kinase (PKII) PF3D7_1037100	✓			✓				✓				
	Pyruvate dehydrogenase E1α subunit (PDH E1α) PF3D7_1124500	✓		✓	✓					✓ *M*	✓ *L*	✓ *L*	
	Pyruvate dehydrogenase E1β subunit (PDH E1β) PF3D7_1446400				✓								
	Pyruvate dehydrogenase E2 subunit (PDH E2) PF3D7_1020800	✓			✓	✓							
	Pyruvate dehydrogenase E3 subunit (PDH E3) PF3D7_0815900	✓		✓	✓							✓ *L*	
	Acetyl-CoA carboxylase (ACC) PF3D7_1469600	✓			✓				✓	✓			
	Malonyl-CoA:ACP transacylase (FabD) PF3D7_1312000					✓							
	Acyl carrier protein (ACP) PF3D7_0208500	✓	✓	✓	✓	✓							✓
	Acyl carrier protein synthase (ACPS) PF3D7_0420200												
	β-ketoacyl-ACP synthase III (FabH) PF3D7_0211400	✓				✓							
	β-ketoacyl-ACP synthase I/II (FabB/F) PF3D7_0626300					✓				✓ *M*	✓ *L*	✓ *L*	
	β-ketoacyl-ACP reductase (FabG) PF3D7_0922900			✓		✓							
	β-hydroxyacyl-ACP dehydratase (FabZ) PF3D7_1323000			✓	✓	✓						✓ *L*	
	Enoyl-ACP reductase (FabI) PF3D7_0615100	✓	✓	✓		✓			✓	✓ *M*	✓ *L*		
Utilization and export	Octanoyl-ACP:protein transferase (LipB) PF3D7_0823600	✓				✓				✓	✓ *L*		
	Lipoic acid synthase (LipA) PF3D7_1344600	✓			✓	✓			✓	✓ *			
	Lipoate protein ligase (LplA2) PF3D7_0923600	✓				✓					✓ *M*		
	Glycerol-3-phosphate dehydrogenase (G3PDH) PF3D7_1114800			✓								✓ *L*	
	Glycerol-3-phosphate acyltransferase (G3PAT) PF3D7_1318200			✓				✓ *L*				✓ *L*	
	Acetyl-CoA synthase (ACS) PF3D7_0215000, PF3D7_0215300												

Shears et al Mol & Biochem Parasitol, 2015

Essentiality of apicoplast pathways – fatty acid synthesis

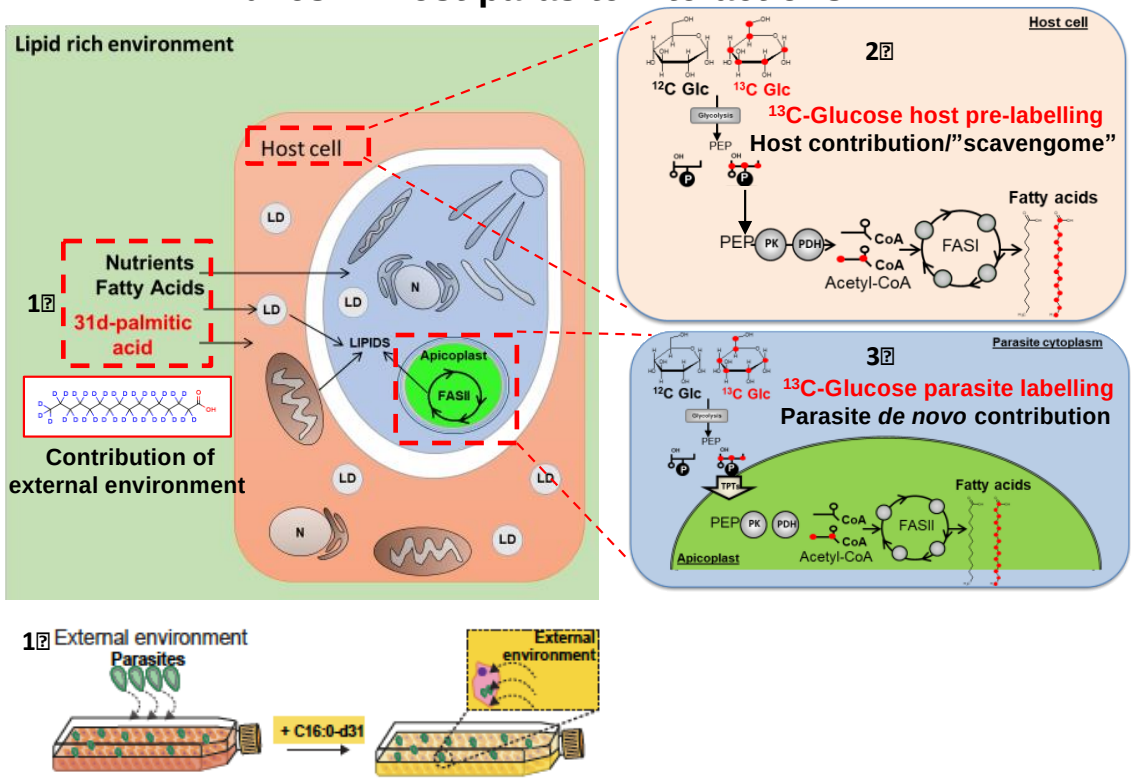
In ***T. gondii*** **tachyzoites**, there are **conflicting results in the literature** as to whether or not the apicoplast-hosted FASII system is absolutely essential for parasite viability, but it seems at least **important for parasite fitness**

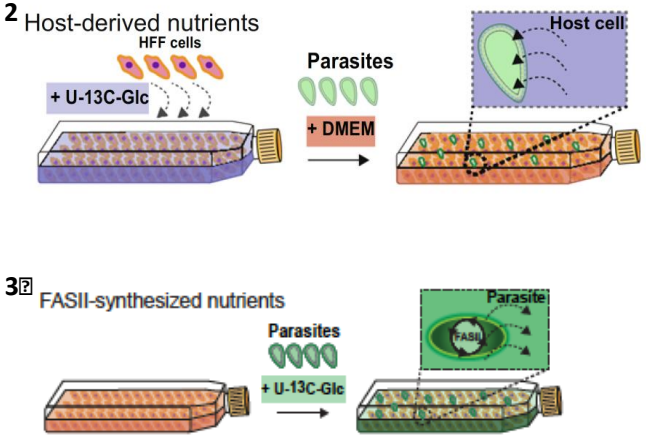
Parasites have a remarkable **capacity to compensate for defects in fatty acid synthesis through increased salvage from the host**



Amiar et al, Cell Rep, 2020

Metabolomics/fluxomics approaches to analyse metabolic fluxes in Host-parasite interactions





Phenotype severity likely depends on the **parasite environment and lipid availability**

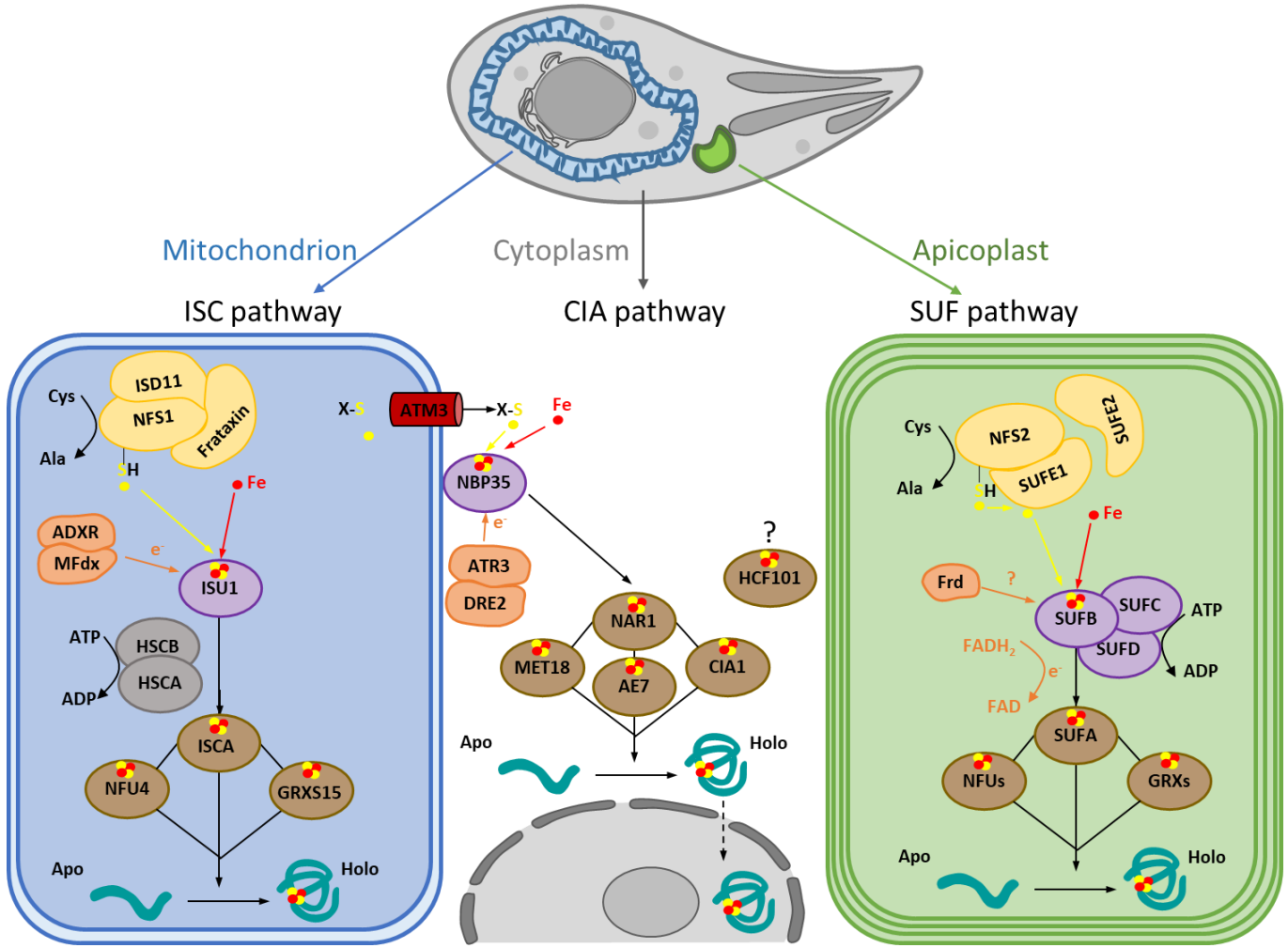
Metabolic labelling and lipidomics can help understand different contributions of de novo synthesized vs scavenged lipids

Essentiality of apicoplast pathways – Fe-S cluster synthesis

Iron–sulfur clusters are ancient and essential cofactors required for performing important functions including nitrogen fixation, ribosome assembly, DNA repair, mitochondrial respiration, and metabolite catabolism

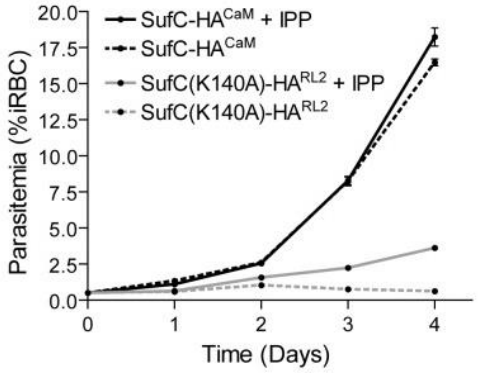
Apicomplexa, like plants, possess three pathways located in the mitochondrion (ISC), the cytoplasm (CIA), and the plastid (SUF)

Fe-S cluster-containing proteins of the plastid include **tRNA modification enzyme MiaB**, as well as **isoprenoid synthesizing enzymes IspG and IspH** and **lipoate synthase LipA**

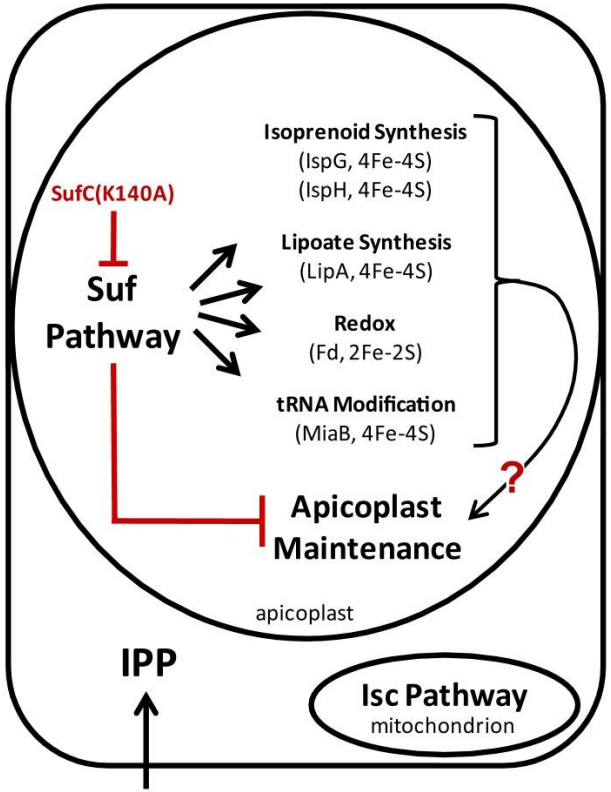


Essentiality of apicoplast pathways – Fe-S cluster synthesis

In ***Plasmodium*** **blood stages**, dominant negative mutant parasites of SufC have **dysfunctional apicoplasts and are not viable**

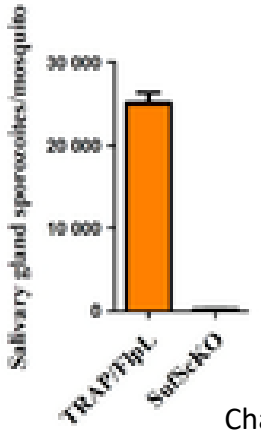


Gisselberg et al. PLoS Pathog, 2013



Gisselberg et al. PLoS Pathog, 2013

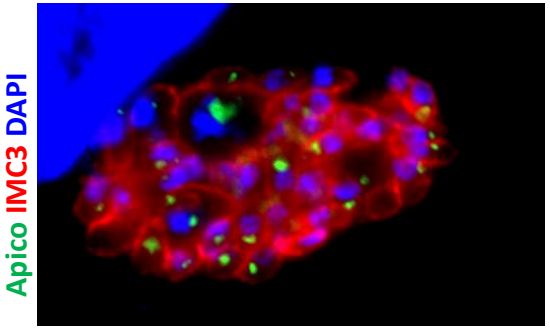
Mutating SufS also leads to a **severely impaired development of sporozoites in oocysts**, establishing essentiality of the SUF machinery in the vector



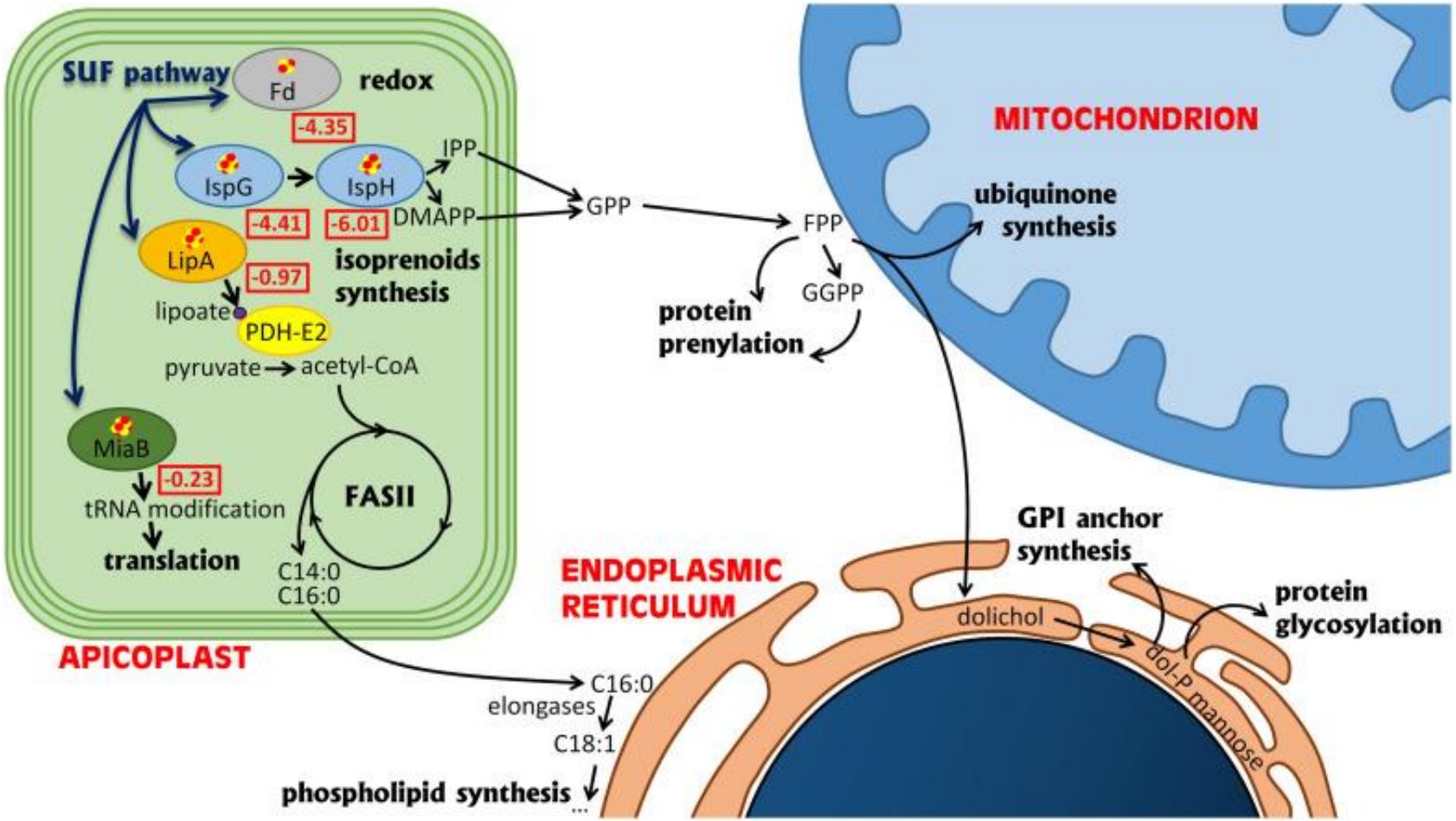
Charan et al, FEBS J, 2017

Essentiality of apicoplast pathways – Fe-S cluster synthesis

In ***T. gondii*** **tachyzoites**, disrupting the SUF pathway leads to a **loss of parasite viability**, a late loss of the apicoplast and perturbation in membrane homeostasis



Pamukcu et al. PLoS Pathog, 2021



Renaud et al. et al. J. Biol. Chem, 2022

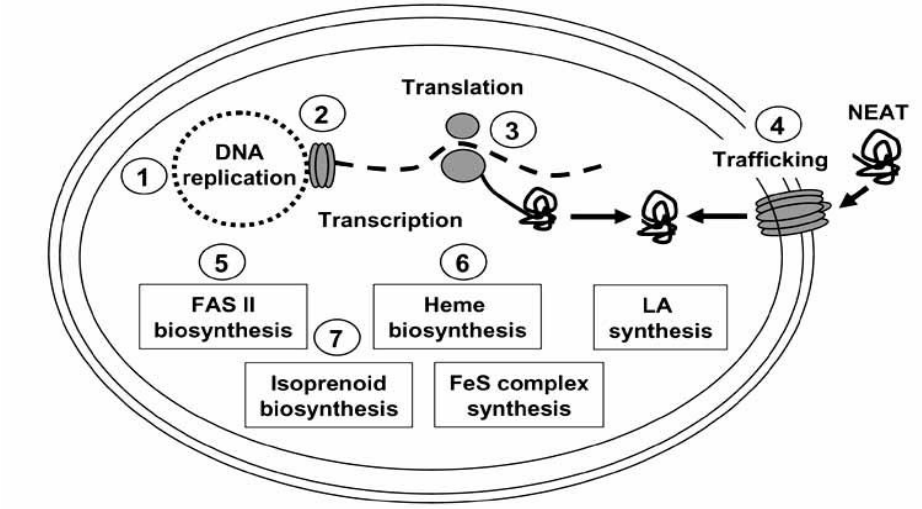
As **two important apicoplast pathways** (FASII and isoprenoid synthesis) are dependent on Fe-S clusters and the SUF pathway is of **bacterial origin**, it constitutes a **good potential target for drug design**

The apicoplast as a drug target

Several drugs targeting the apicoplast are already in clinical use for both malaria and toxoplasmosis, like clindamycin, azithromycin and spiramycin, **which inhibit protein translation in the organelle**

High concentrations of these types of compounds can also harm mitochondrial translation in the host. Hence, **drugs targeting other essential pathways of the apicoplast** are also being considered

Especially for **malaria**, apicoplast-targeting drugs are rather used together with other drugs as a **combination therapy**. If used alone, they **do not lead to the immediate death** and clearance of the parasite



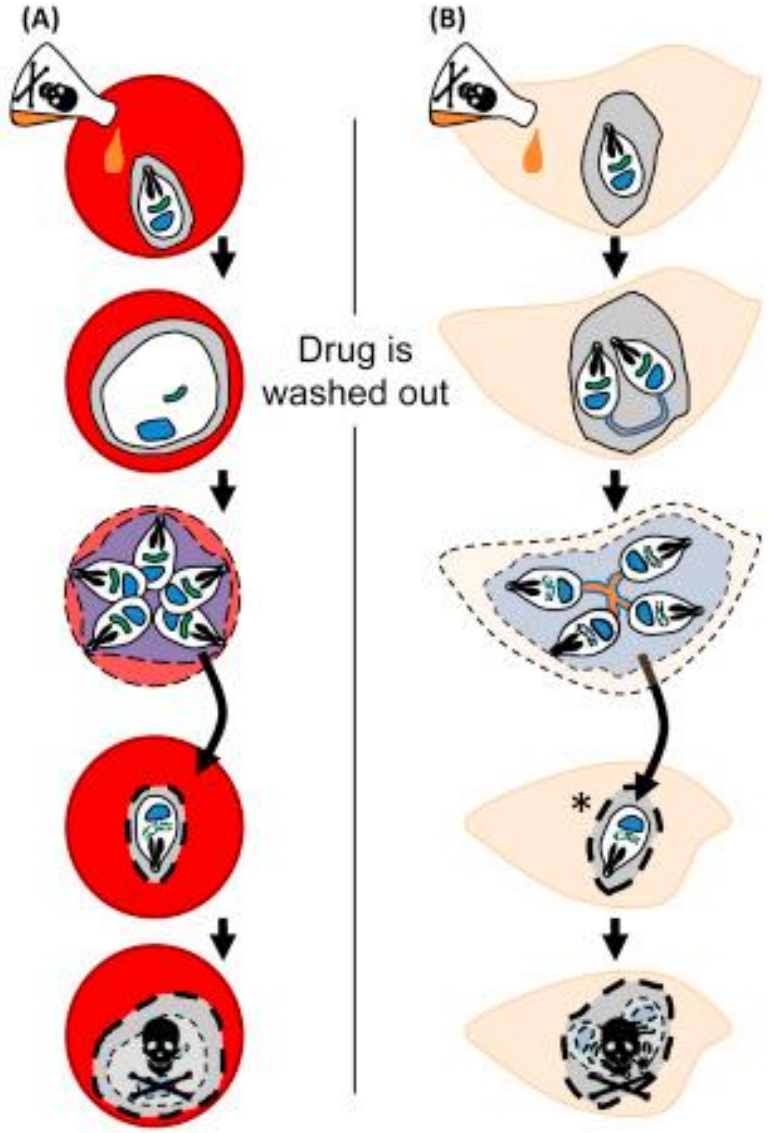
Drug	Site of action	pathway	effect	
ciprofloxacin	DNA gyrase (**1**)	replication	immediate killing (?)	[10]
rifampicin	RNA polymerase (**2**)	transcription	delayed death (?)	[9, 10, 79]
thiostrepton	LSU rRNA (**3**)	translation	immediate killing	[10, 11]
clindamycin	LSU rRNA (**3**)	translation	delayed death	[10, 79]
telithromycin	LSU rRNA (**3**)	translation	delayed death	[12]
azithromycin	LSU rRNA (**3**)	translation	delayed death	[11, 12]
chloramphenicol	LSU rRNA (**3**)	translation	delayed death	[79]
tetracycline	SSU rRNA (**3**)	translation	delayed death	[9, 10, 79]
doxycycline	SSU rRNA (**3**)	translation	delayed death	[9, 12]
quinupr.-dalfopristin	SSU rRNA (**3**)	translation	delayed death	[12]
15-DSG	HSP70 (?) (**4**)	trafficking	delayed death	[70]
thiolactomycin	Fab B/F (**5**)	FAS II	immediate killing	[82]
cerulenin	Fab B/F (**5**)	FAS II	immediate killing	[79]
triclosan	Fab I (**5**)	FAS II	immediate killing	[11, 79]
syccinyl acetone	ALAD (**6**)	heme S	immediate killing	[79]
fosmidomycin	DOXP RI (**7**)	IPS	immediate killing	[84]

Pradel & Schlitzer, Curr Mol Med 2010

Delayed death in apicoplast mutants

Inhibition of apicoplast **housekeeping** leads to **delayed death**, while inhibition of apicoplast **anabolic pathways** leads to a **more rapid death**

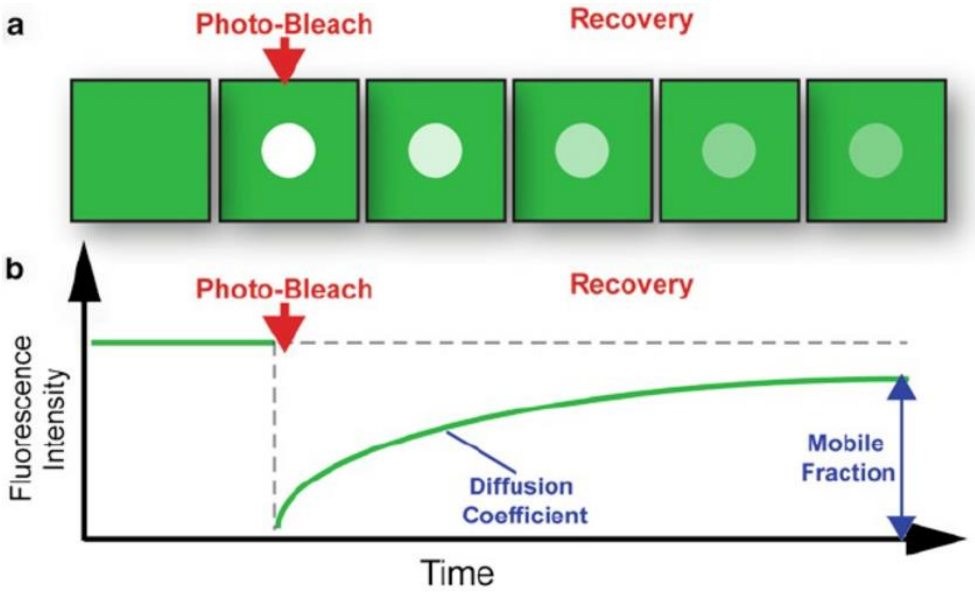
It is believed interfering with apicoplast inheritance initially only leads to a **partial loss of the organelle**. Interconnection of dividing parasites appears to facilitate **sharing of apicoplast-derived metabolites**, and apicoplast-deficient parasites are only blocked for replication **after a subsequent round of invasion**

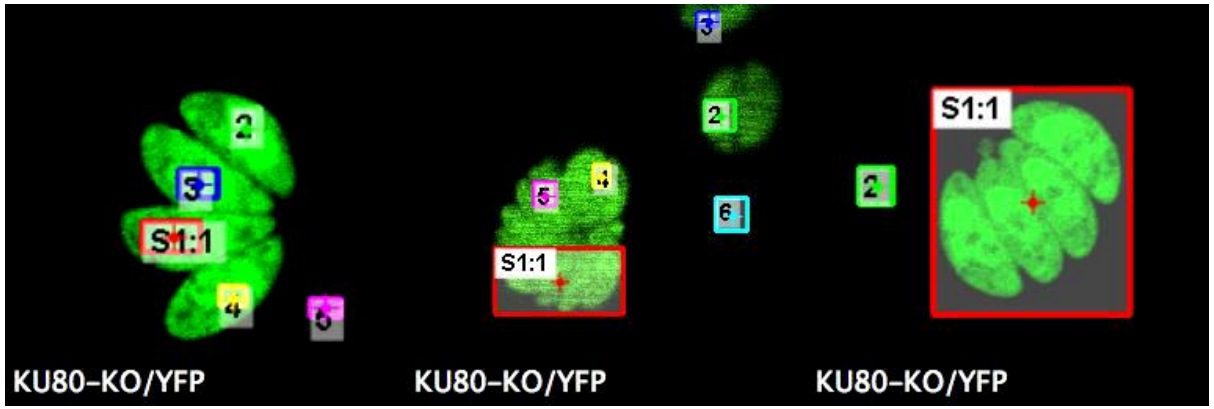


Kennedy et al, Trends Parasitol, 2019

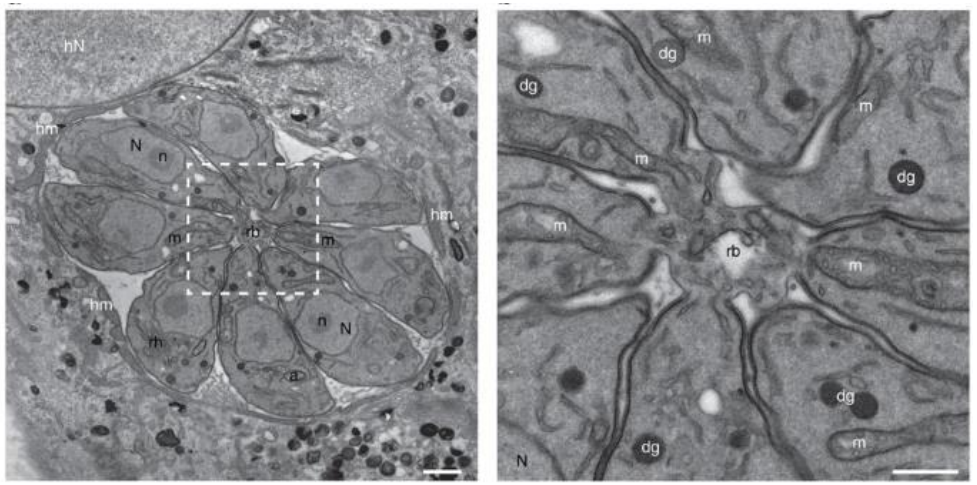
Delayed death in apicoplast mutants

Fluorescence Recovery After Photobleaching (**FRAP**) experiments have shown that parasites within the same vacuole **can share molecules**

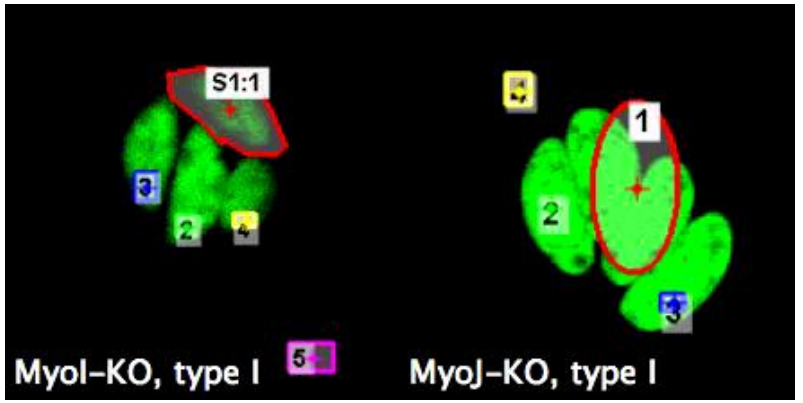




Frénal et al, Nature Comm, 2017

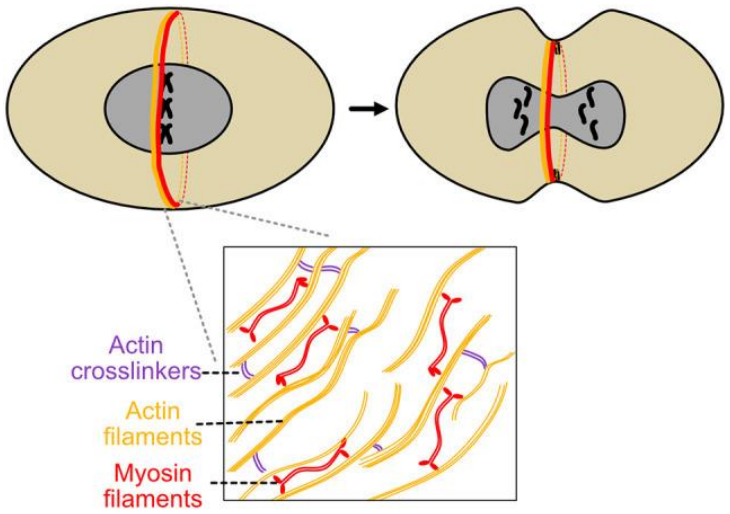


Parasites remain connected during division



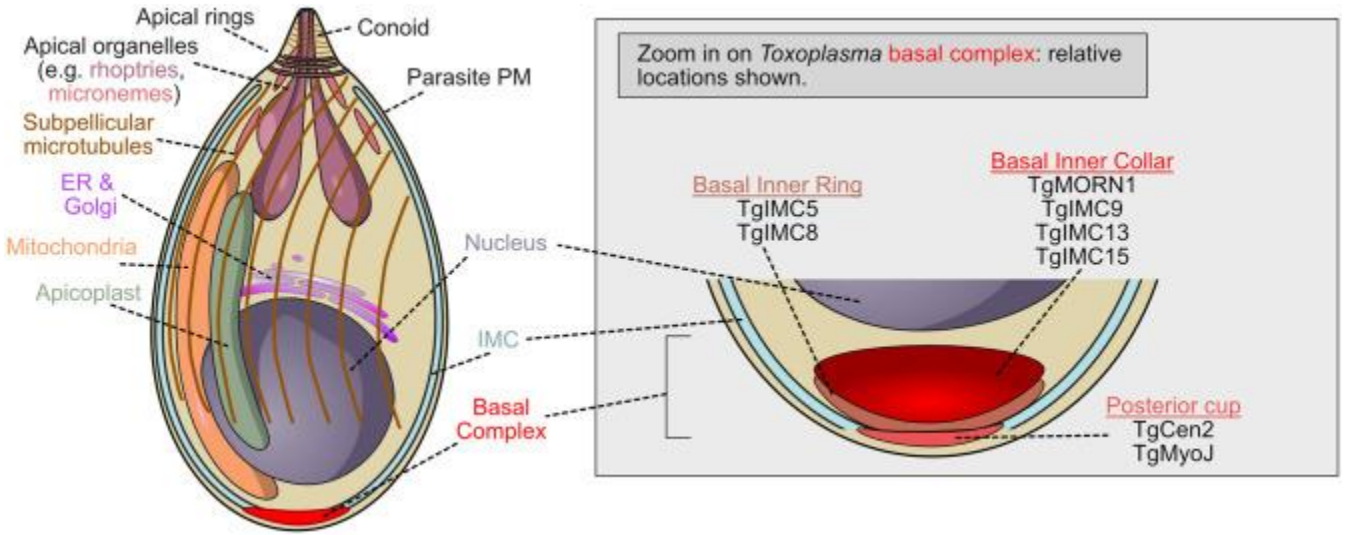
Some myosin mutants lose that connection

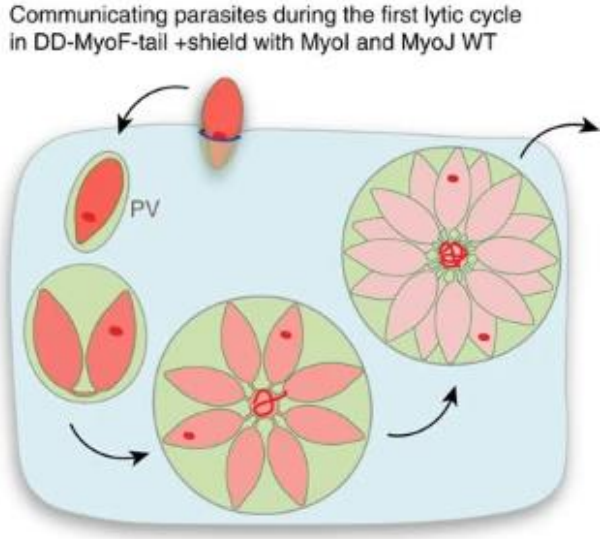
Delayed death in apicoplast mutants



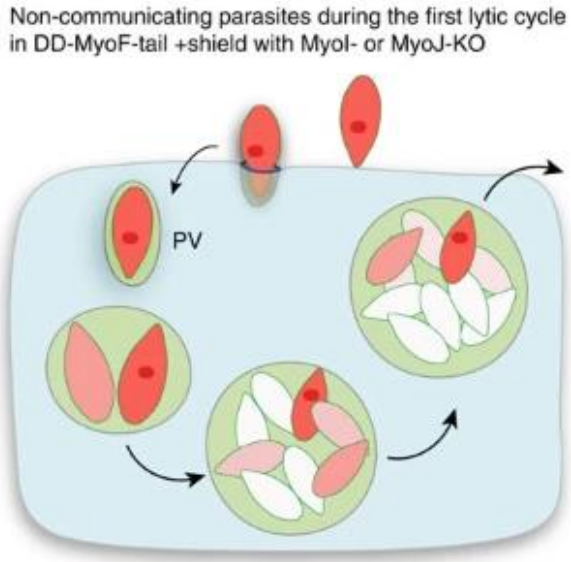
Actomyosin ring constriction during *S. pombe* cytokinesis

Morano & Dvorin, Front Cell Infect Microbiol. 2021





Frénal et al, Nature Comm, 2017



Delayed death in apicoplast mutants

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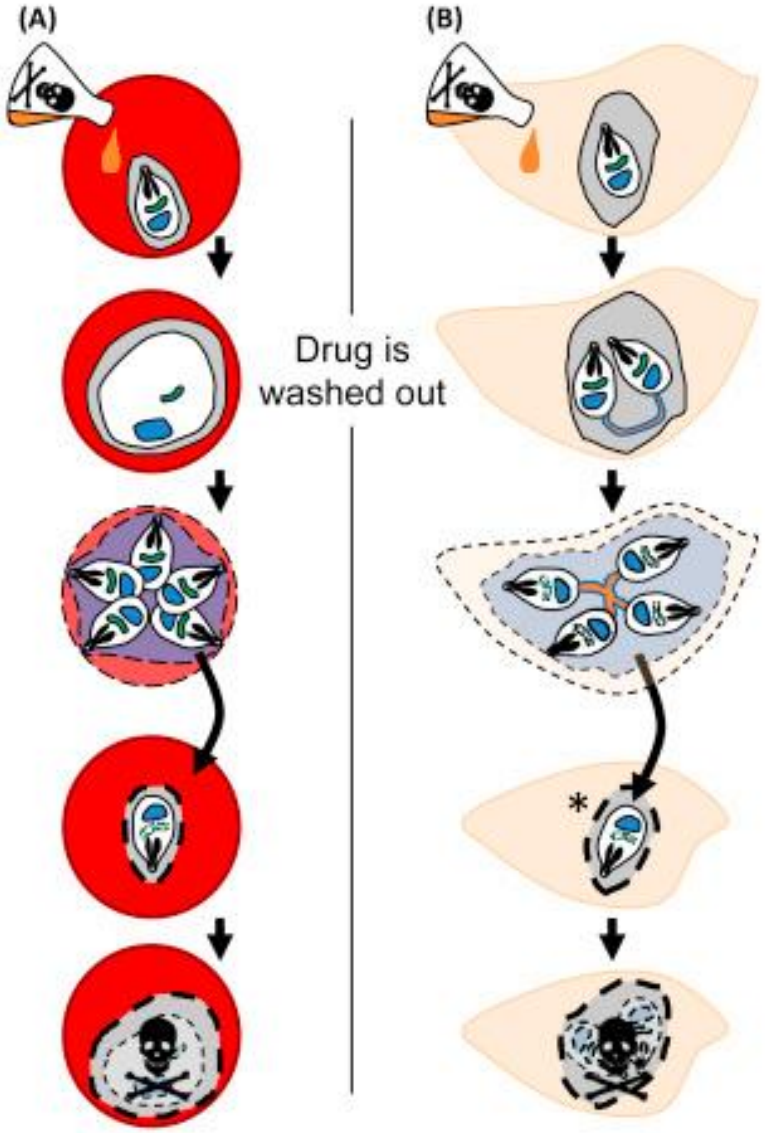
It is believed interfering with apicoplast inheritance initially only leads to a **partial loss of the organelle**. Interconnection of dividing parasites appears to facilitate **sharing of apicoplast-derived metabolites**, and apicoplast-deficient parasites are only blocked for replication **after a subsequent round of invasion**

Delayed death is also likely modulated by the ability of the parasites to **acquire metabolites from the host**



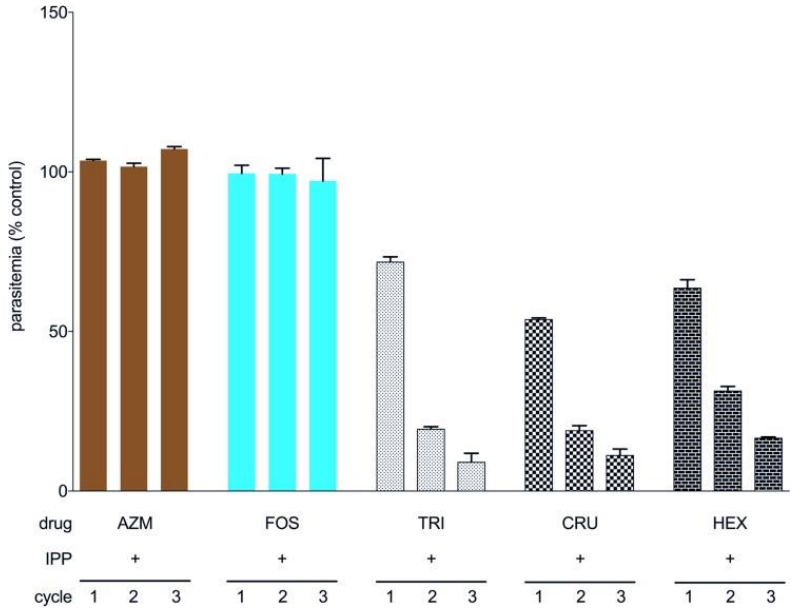
Kennedy et al, Trends Parasitol, 2019

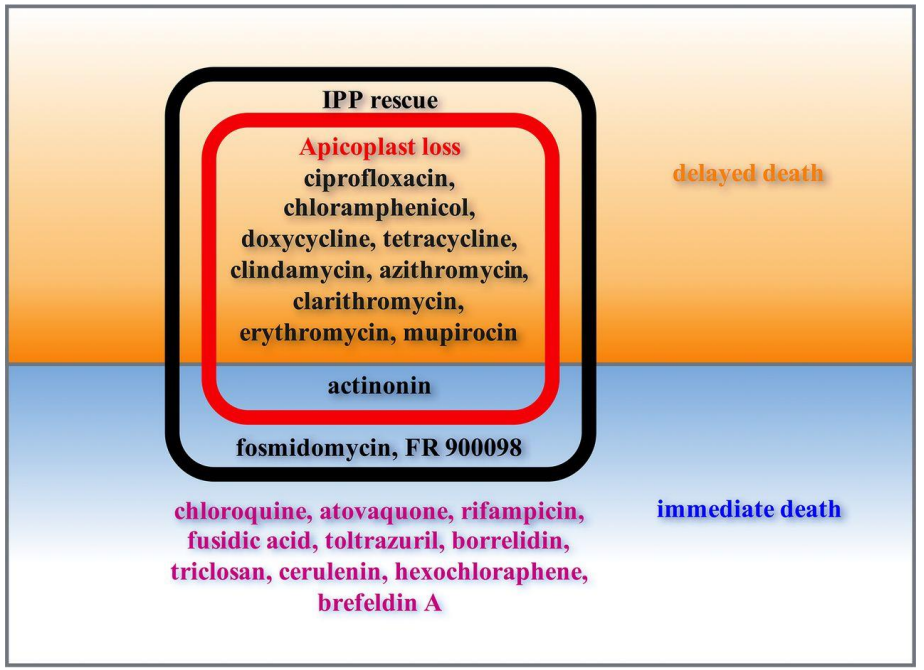
IPP helps resolving the specificity of “apicoplast-targeting” drugs

A number of supposedly apicoplast-targeting drugs potentially also have other **secondary targets** in the parasites

The fact that **IPP completely rescues the lethal effect induced by apicoplast loss** in malaria blood stages offers the opportunity to investigate the **specificity** of these drugs

FASII inhibitors triclosan, cerulenin, and hexachlorophene likely target other pathways. This is confirmed by the lack of essentiality of FASII in blood stage malaria parasites as demonstrated by genetic approaches





Uddin et al Antimicrob Agents Chemother 2018

Differential essentiality for different parasites

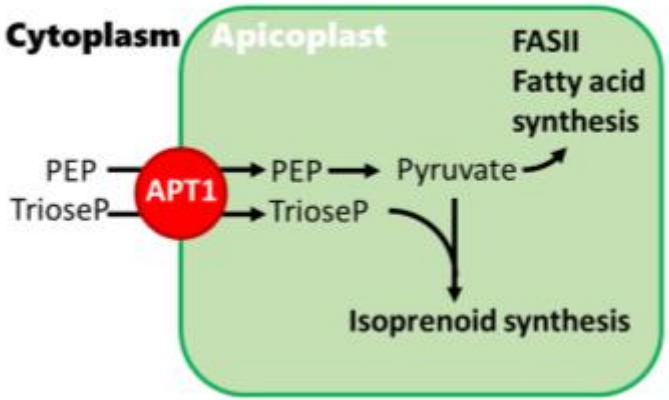
Parasite species and specific developmental stages **do not rely to the same extent on apicoplast-hosted pathways**

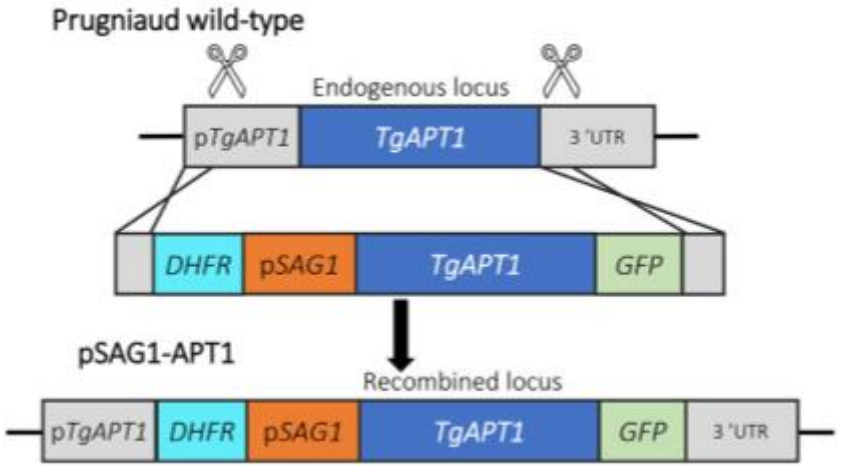
There are differences in their ability to **salvage metabolites from the host**, or in their **membrane permeability properties** that render them differently sensitive to specific apicoplast-targeting drugs

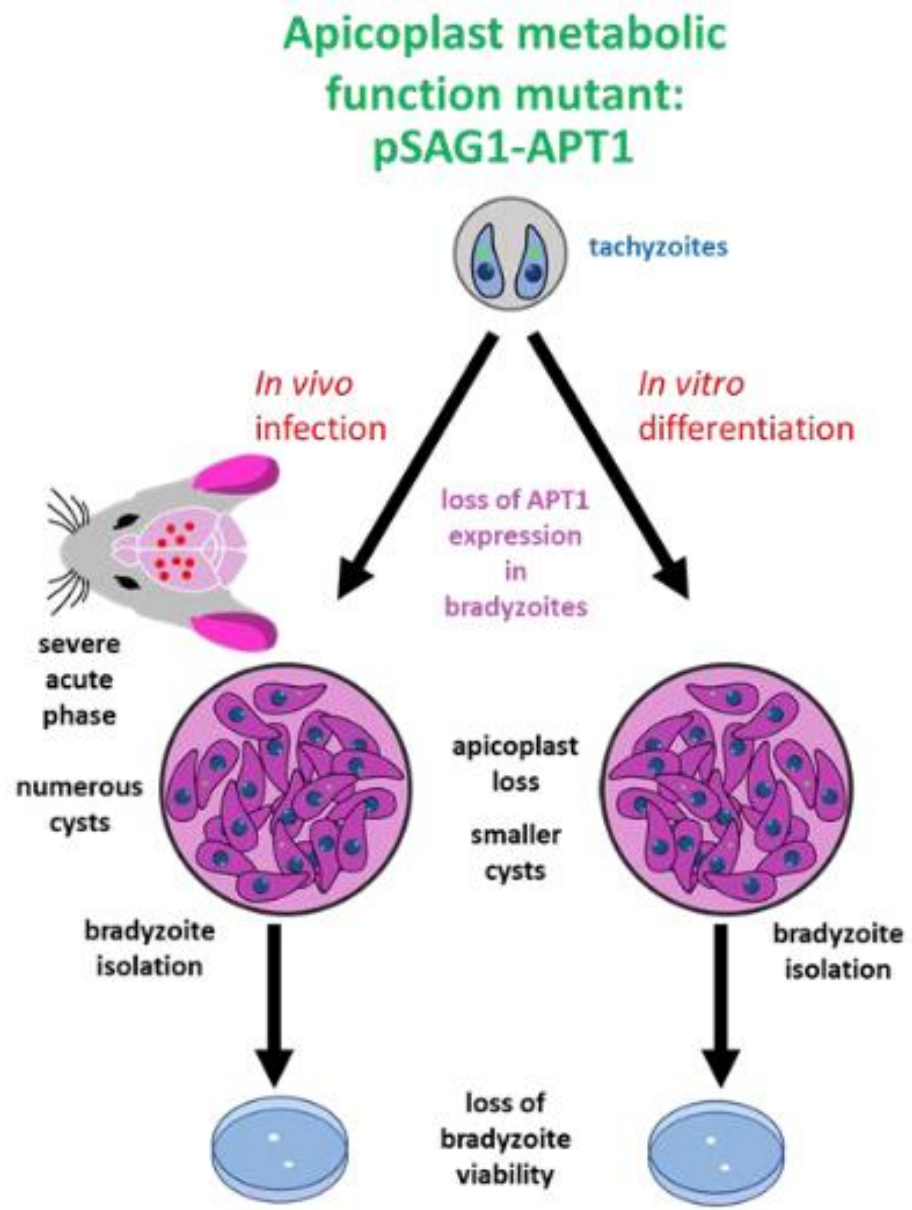
	Plasmodium			***Toxoplasma***	
Pathway	blood stages	sporozoites	liver stages	tachyzoites	bradyzoites
Isoprenoid	yes	?	?	yes	?
Heme	no	yes	yes	yes	?
Fe-S	yes	yes	?	yes	?
FASII	no	yes	yes	yes	?

The essentiality of the organelle remains **completely unexplored in some developmental stages** (ie *T. gondii* bradyzoites) and may reveal avenues for developing novel therapeutic approaches

The apicoplast is also essential for *T. gondii* bradyzoites

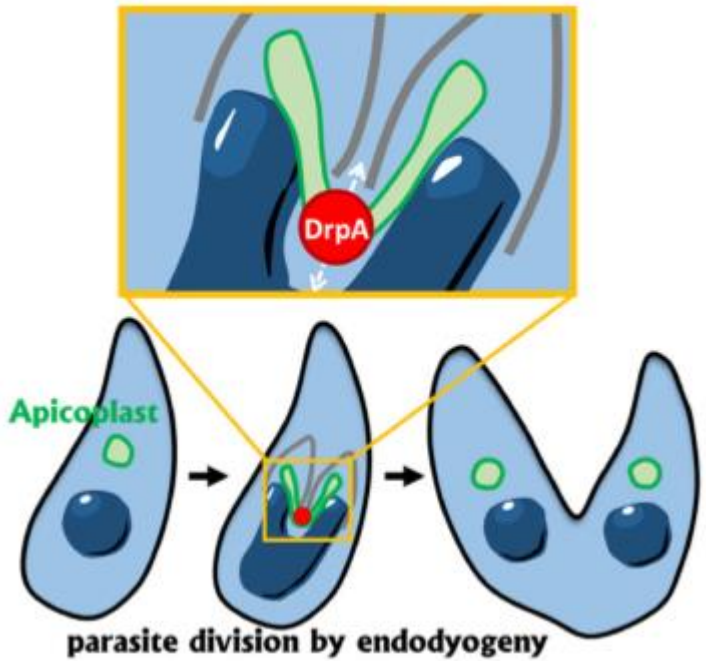


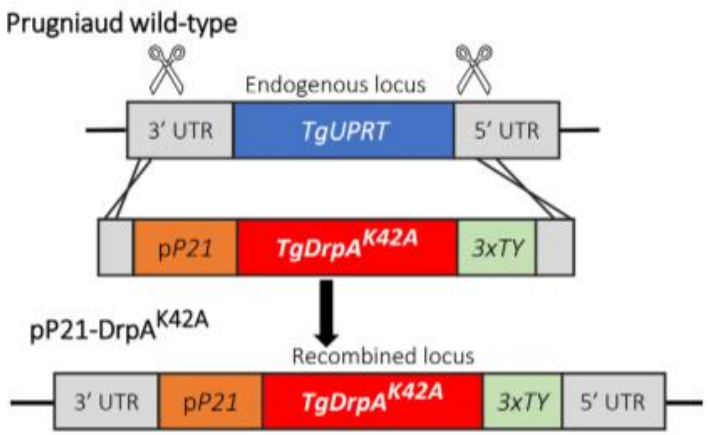


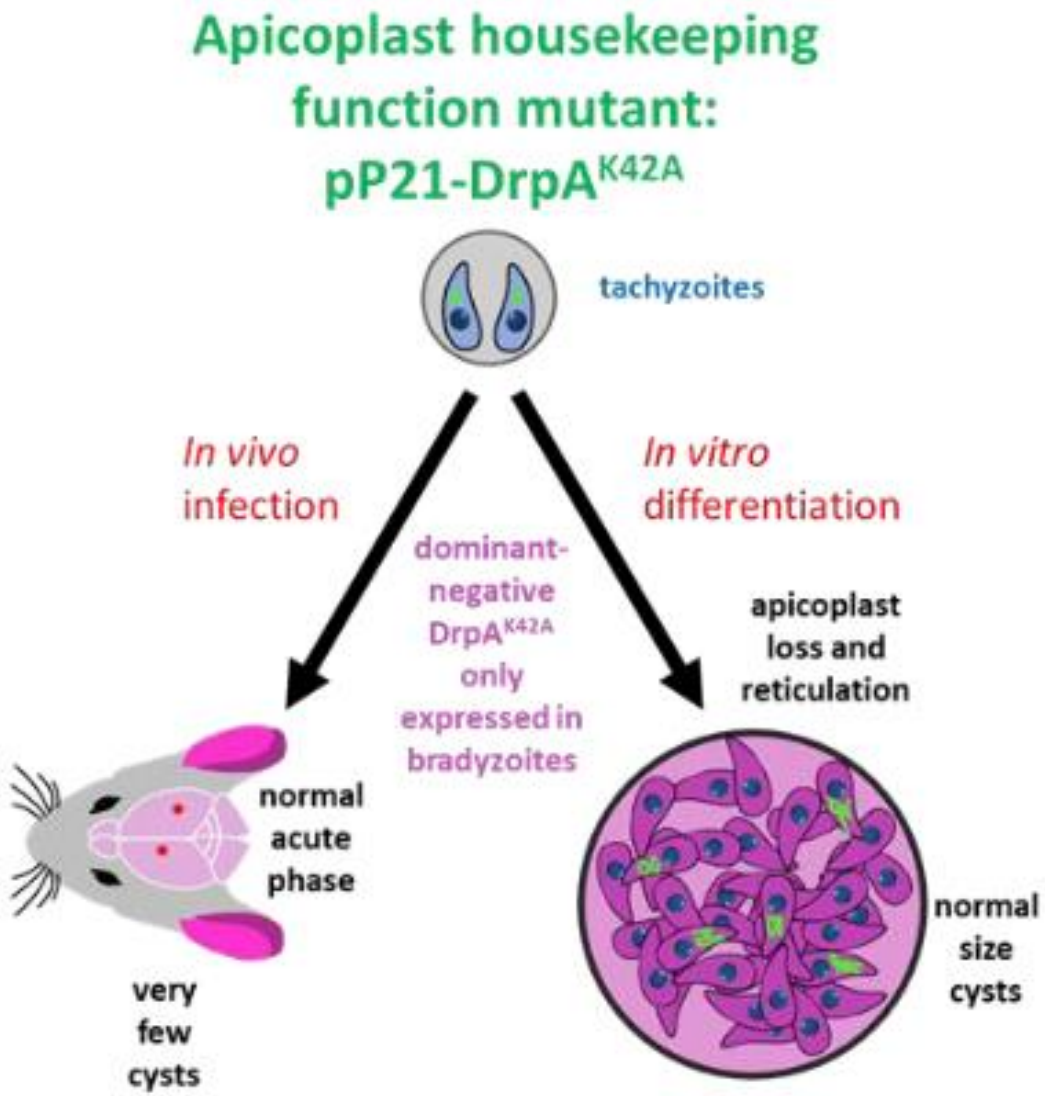


Sanchez et al PNAS 2023

The apicoplast is also essential for *T. gondii* bradyzoites







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Conclusion

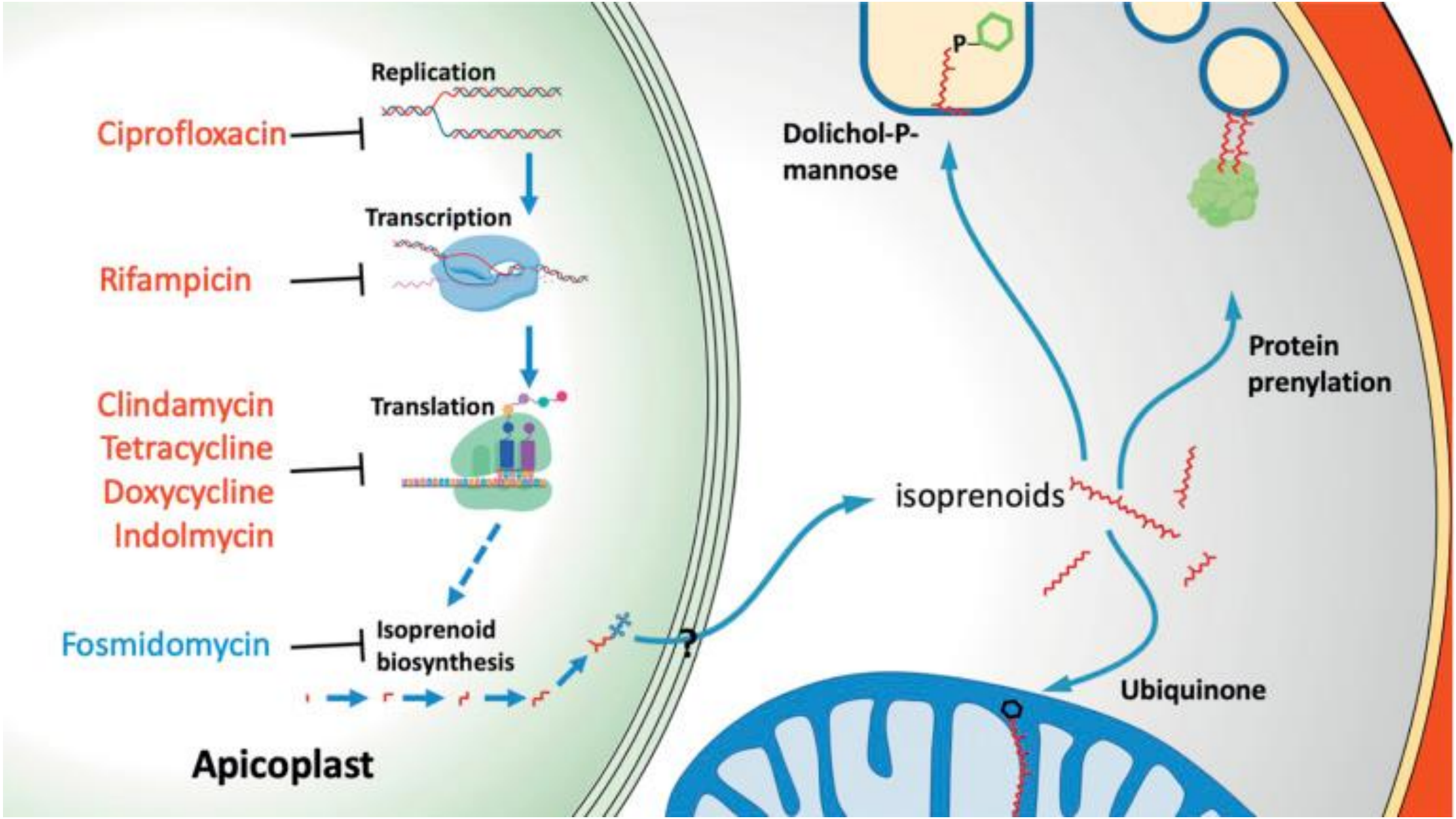
- many Apicomplexa contain a non-photosynthetic plastid named **the apicoplast**

- this plastid is **not photosynthetic** but contains metabolic **pathways which are crucial to the fitness** of important apicomplexan pathogens such as *Plasmodium* or *Toxoplasma*

- the four main metabolic pathways hosted by the apicoplast are for the synthesis of **heme, isoprenoid precursors, iron-sulfur clusters and fatty acids** (FASII)

- because of their **metabolic importance** and their **divergent evolutionary origin**, these pathways may be **exploited for designing drugs** specifically targeting the parasites

- **not all of these pathways have the same importance in all Apicomplexa or developmental stages**, and care should then be taken to study them and assess their functional importance individually



Kennedy et al. Trends Parasitol, 2019