Cyst building mechanism in cyst/oocyst forming parasites

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What is encystation?

It is the usual response of many **protists** to environmental **stress,** where differentiation of actively motile feeding **trophozoites** into dormant walled **cysts** is occur**.**
The function of cysts not only the **survival**, but also the **dispersal** of the organism to new feeding grounds to complete its life cycle and its important for pathogenesis.

Cyst vs oocyst?

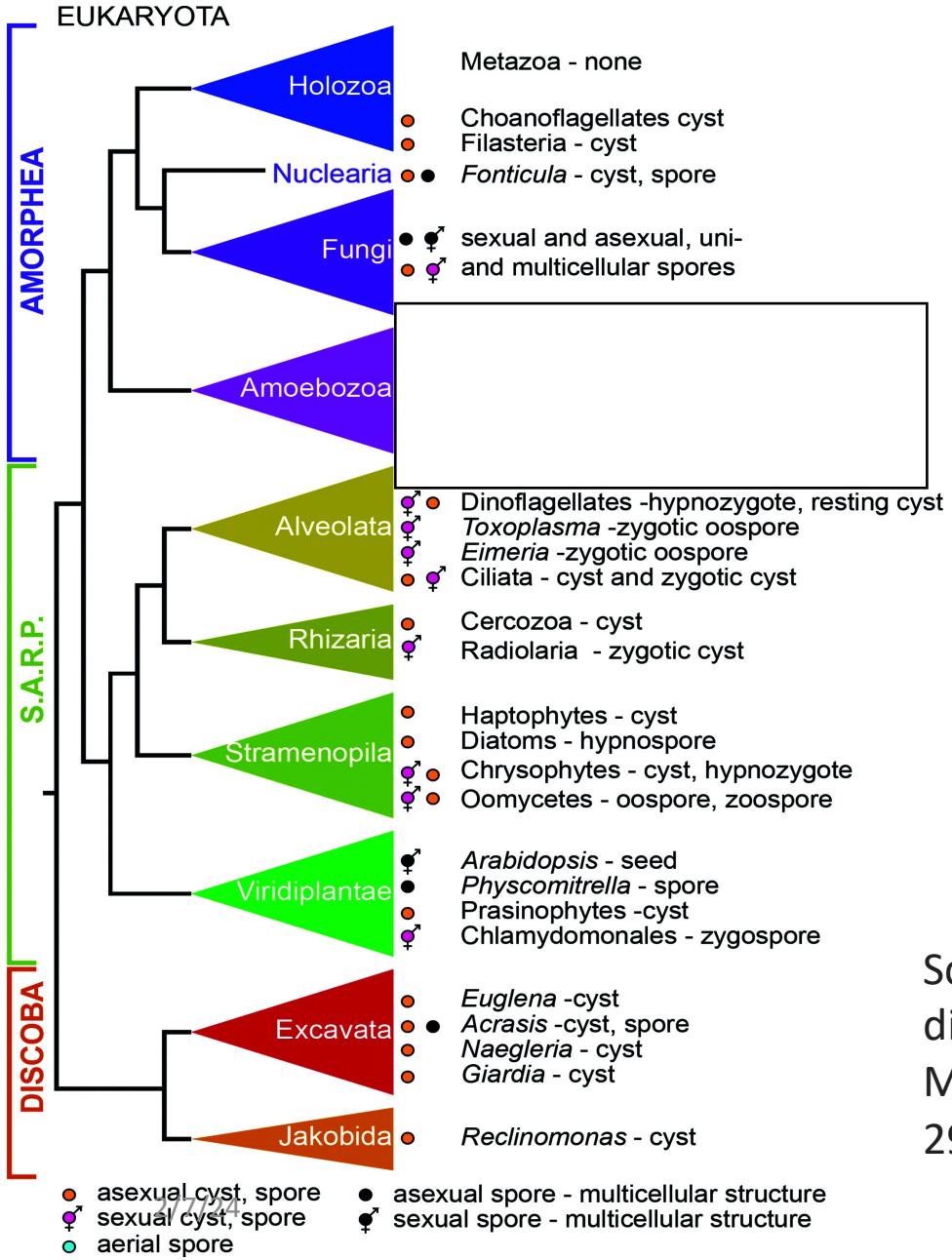
Cysts is asexual, resulting from the encapsulation of a single cell.

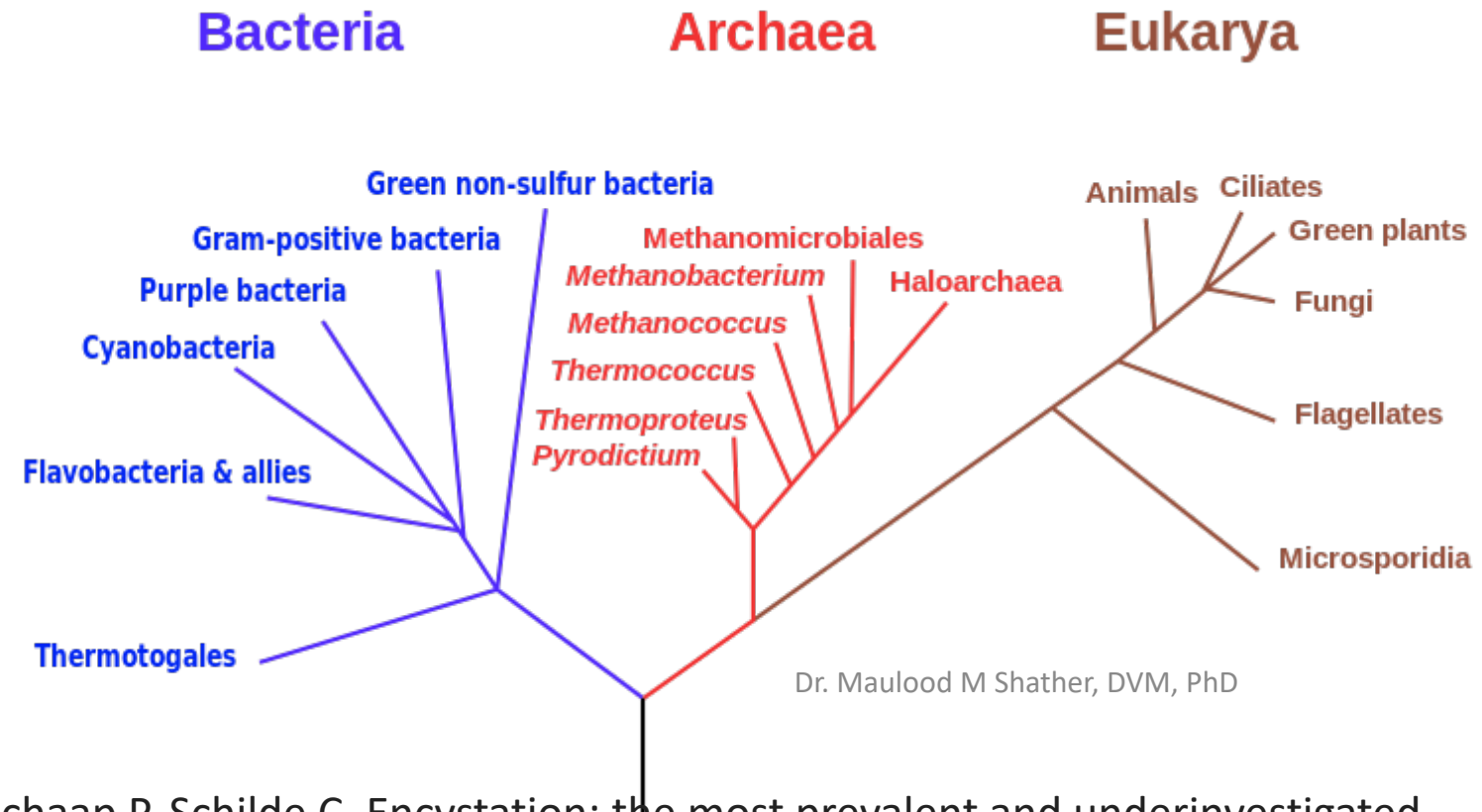
Oocyst is sexual, resulting from the encapsulation of a zygote, formed by fusion of two cells of opposite mating types

Spors??

A sexual encystment of some **protists**, allowing organisms to survive **long winter darkness** and all **protists** at high **latitude** and **altitude** the **freezing** of their habitats, even for **thousands of years.**

Encystation occurs in all eukaryote domains



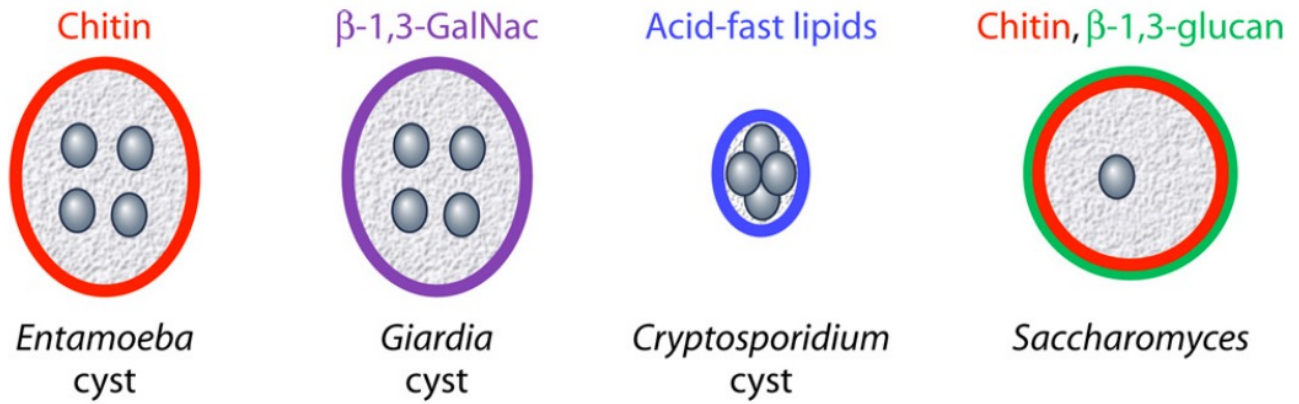


Schaap P, Schilde C. Encystation: the most prevalent and underinvestigated differentiation pathway of eukaryotes. Microbiology (Reading). 2018 May;164(5):727-739. doi: 10.1099/mic.0.000653. Epub 2018 Apr 5. PMID: 29620506.

Cyst/Oocyst component of major Protists

Entamoeba and ***Giardia***: Proteins and sugars are the major components of cyst wall.

Cryptosporidium and ***Toxoplsma***: Proteins, carbohydrates, and *lipids* are important structural components of Oocyst wall.



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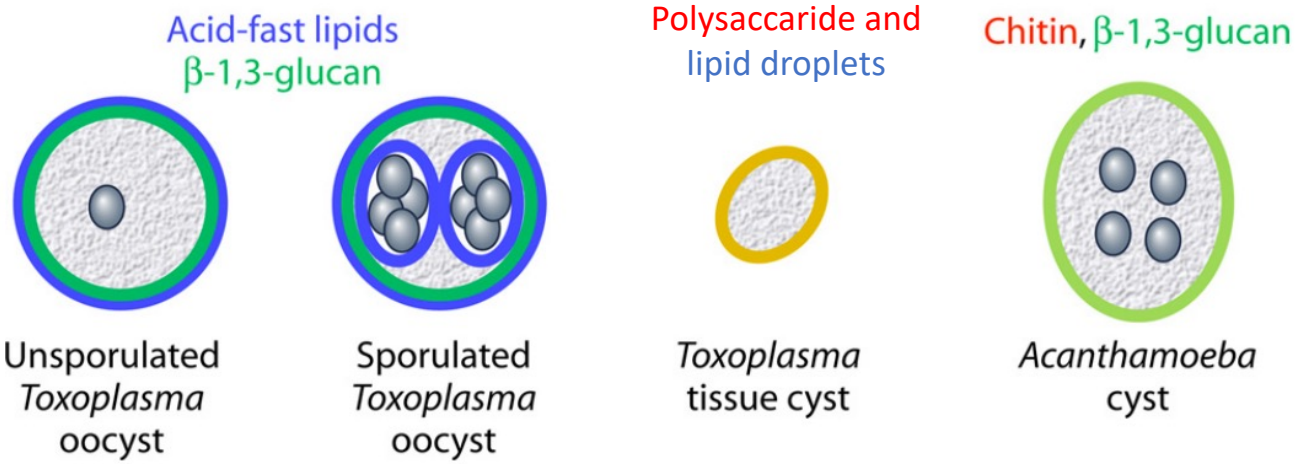


Cyst/Oocyst component of mjor protists

TABLE 1 Structural components of fungal, cyst, and oocyst walls

	Structural component(s)				
Molecule(s)	*Saccharomyces*	*Entamoeba*	*Giardia*	*Toxoplasma* (oocyst)	*Cryptosporidium*
Sugar polymer(s)	Chitin, β-1,3-glucan	Chitin	β-1,3-GalNAc	β-1,3-Glucan	None
Lipids	None	None	None	Acid-fast lipids	Acid-fast lipids
Proteins	Glucanase, chitinase, dityrosines (spores), ~100 proteins with modified GPI anchors	Chitinase, Jacob lectin, Jessie lectin	CWP1 to CWP3 (GalNAc-binding lectins)	Glucanase, Tyr-rich proteins, Cys- and His-rich OWPs, Cys-rich repeat protein	Cys- and His-rich OWPs, POWPs, Ser- and Thr-rich tethers
Abundant glycans	Mannans (high-mannose N-glycans), Man-rich O-glycans	Dextran-like O-P-glycans, N-glycans with galactose	Very short N-glycans	GalNAc- and fucose-rich O-glycans	GalNAc- and fucose-rich O-glycans

Samuelson J, Bushkin GG, Chatterjee A, Robbins PW. Strategies to discover the structural components of cyst and oocyst walls. Eukaryot Cell. 2013 Dec;12(12):1578-87. doi: 10.1128/EC.00213-13. Epub 2013 Oct 4. PMID: 24096907; PMCID: PMC3889564.

Stimuli triggering encystation

Entamoeba	Glucose depletion, hypo-osmolarity, cholesteryl sulfate and catecholamines such as adrenaline and noradrenaline.
Giardia	pH elevation and lipid depletion or by increasing bile and lactic acid
Cryptosporidium	Elevated temperature and low pH.
Toxoplasma	Bile salts, nutrient depletionand catecholamines such as adrenaline and noradrenaline.

Entamoeba encystation

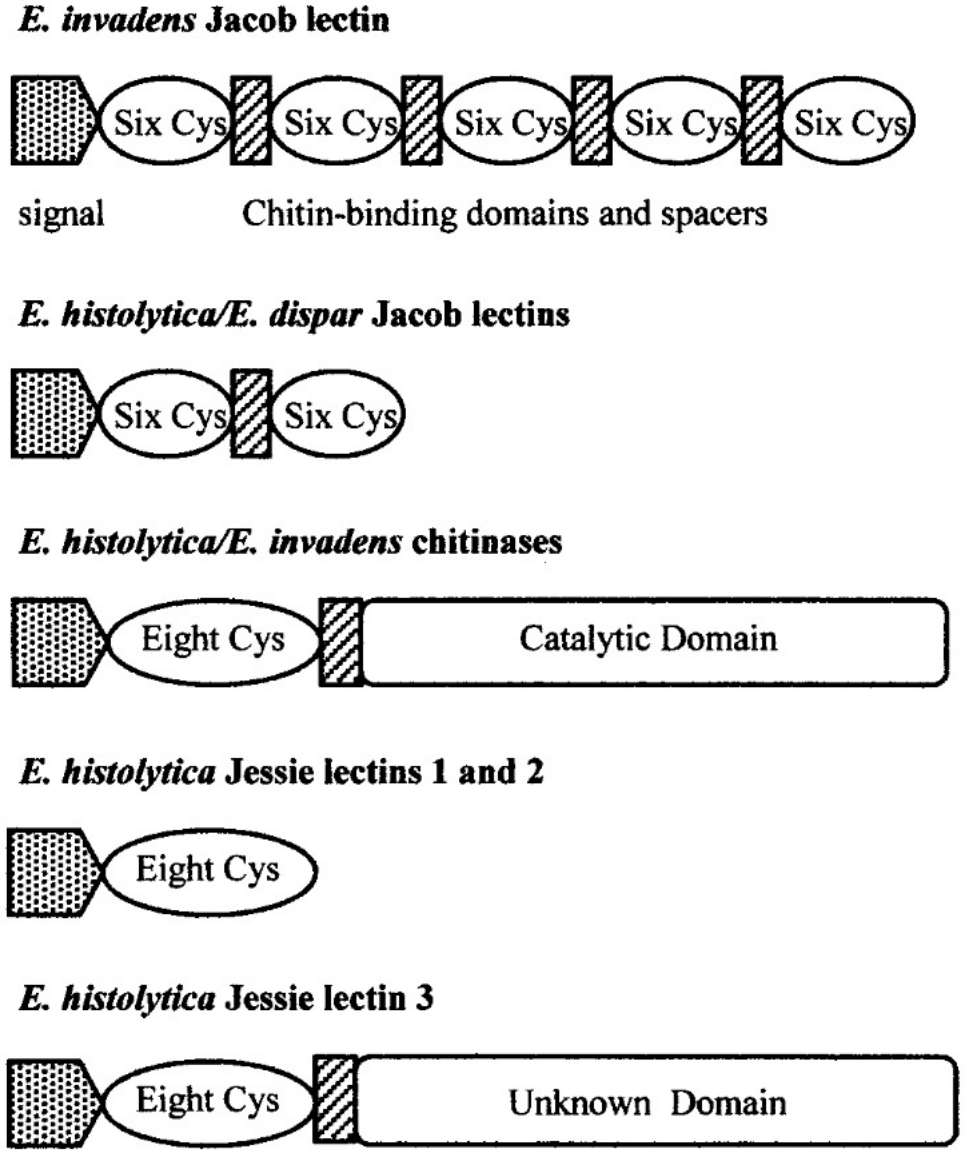
- Encystation involves significant **changes** in **morphology**, **metabolism**, **transcription** and **DNA** content.
- Before the initiation of encystation, the trophozoites **cease feeding**, become **rounded** following one **cycle of nuclear division** resulting in **four-nuclei**.
- Non-motile **trophozoites** enclosed in a **rigid wall** that gives resistance against harsh environmental conditions.
- The intermediate forms of the **cyst** (**pre-cyst**) is observable at the **early stages** of encystation, following the rounding up of the trophozoites and the disposal of ingested particles.
- In **trophozoites**, **D- fructose-6P** is the key metabolite in **glycolysis**, converting **glucose** to **ethanol**, the fuel required for **trophozoite motility.**
- However, **glycolysis interruption** occurs during encystation in which **glucose** is redirected toward **chitin synthesis**, where *Entamoeba* transforms **D-fructose-6-phosphate** into **chitin biosynthesis.**
- On the other hand, amino acids **aspartate** and **asparagine** are used as a **substitute source of energy**.

Transcriptional regulation and signal transduction in encystation

- The transmembrane protein **Gal/GalNAc lectin** has been proposed as a trigger for the signal transduction leading to encystation.
- **Gal/GalNAc lectin** is vital in the **cytolysis**, **invasion** and **resistance** to the innate host immunity including the complement system.
- Singh *et al.*, (2015), demonstrated the negative role of **heat shock protein 90 (Hsp90)** on *Entamoeba* encystation and **inhibition of Hsp90 stimulates encystation** in both *Entamoeba* (Singh *et al*., 2015) and *G. intestinalis.*
- One of the possible **promising drug targets** is **chitin**, which is a significant **cyst wall component**. As aforementioned, during encystation the **glucose** is diverted into **chitin** production and chitin is synthesised during the early phase of stage transition.
- **Dynamins** are large GTPases and function in **membrane remodelling**, essential for **encystation**, and **organelles fission**, involved in membrane dynamics. *E. histolytica* has four dynamins called dynamin related proteins (Drps).

Cyst wall compoents in *Entamoeba*

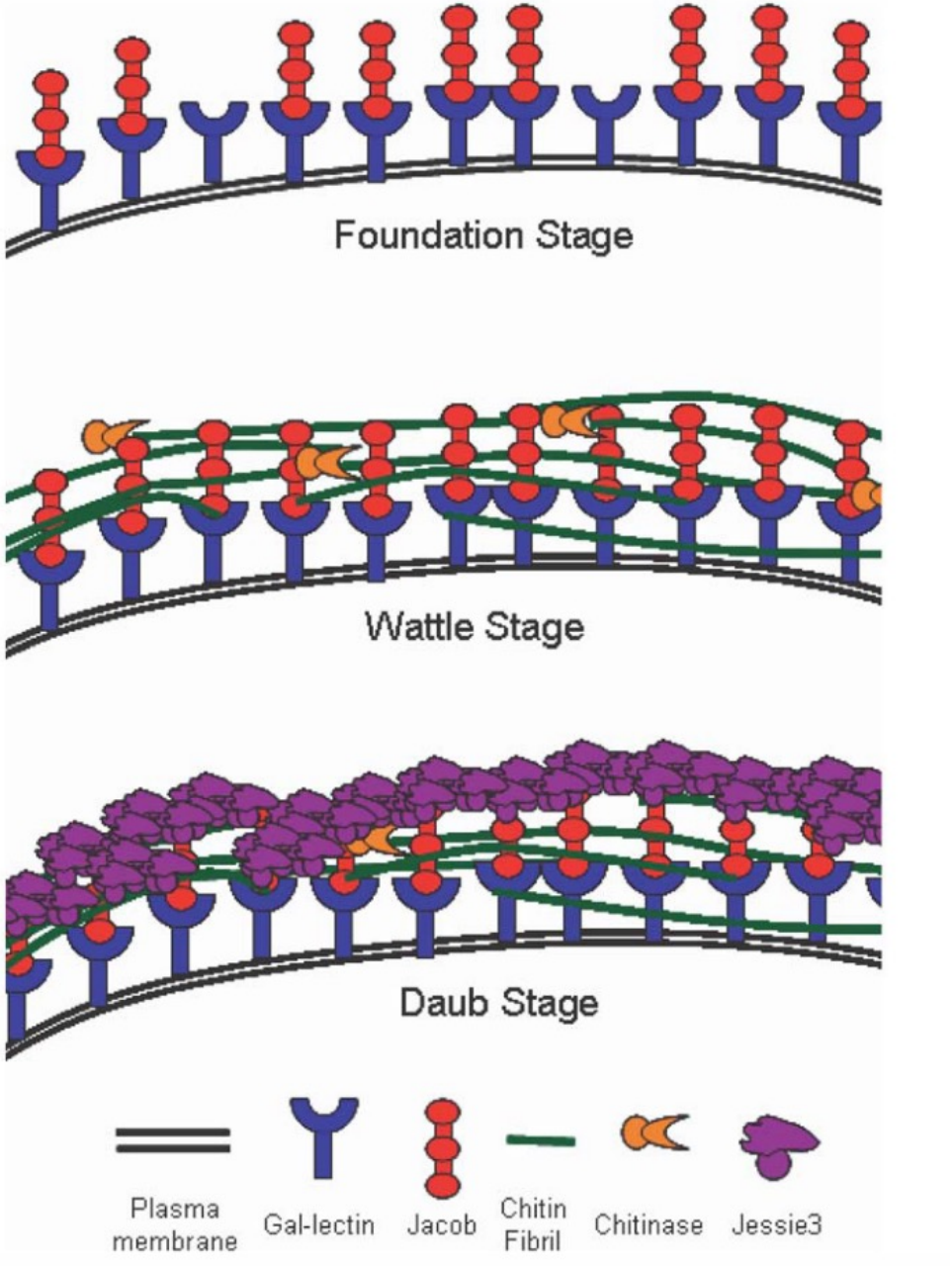
- The cyst wall is mainly made of **chitin** and its deacetylated form **chitosan**, with **three proteins** containing **chitin-binding domains** (CBDs).
- **Chitin** is a polymer of N-acetylglucosamine, GlcNAC.
- The **three proteins** are: **Jacob** with **6-Cys** CBDs, **Jessie** and **chitinase** with a single **8-Cys CBD.**

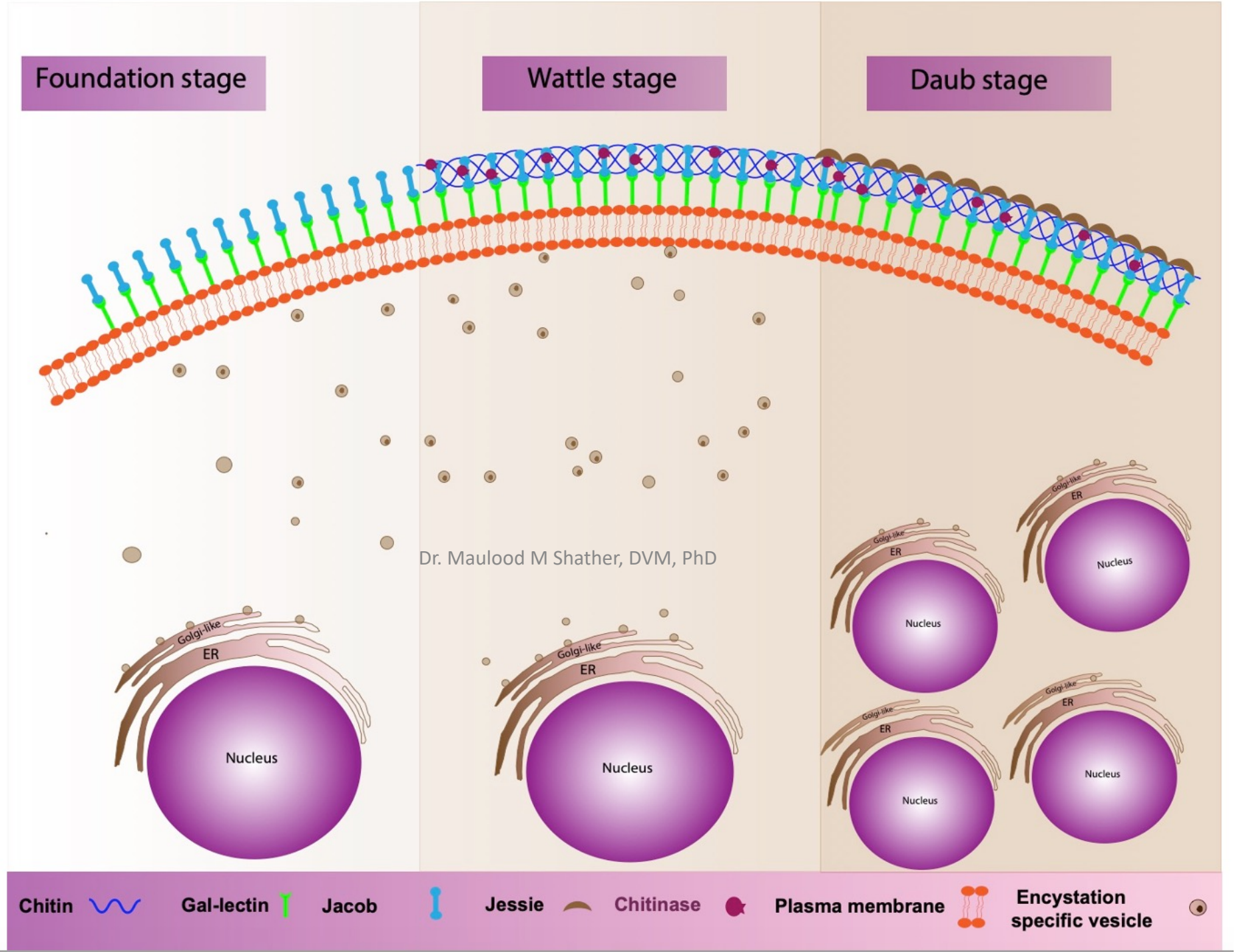


"Wattle and Daub hypothesis"

The formation of the cyst is carried out under three phases referred to as the "wattle-and-daub" model and is dependent on the specific, ordered secretion of cyst wall proteins.

- **The foundation phase** (also referred to as the initial encystation phase) involves the binding of the **glycoprotein containing** Gal (the Jacob lectins) to the **surface** of the encysting amebae following the constitutive expression of the plasma membrane **Gal/GalNAc lectins**.
- **The Wattle phase**, the **Jacob** lectins cross-link with **chitin fibrils** deposited on the surface of encysting amebae.
- **In the Daub phase**, the cyst wall becomes solidified as a result of self-aggregation caused by the addition of the **Jessie3** lectin, that binds the **chitin** at the C-terminal domain, making them impenetrable by minute molecules.




Foundation stage
Wattle stage
Daub stage
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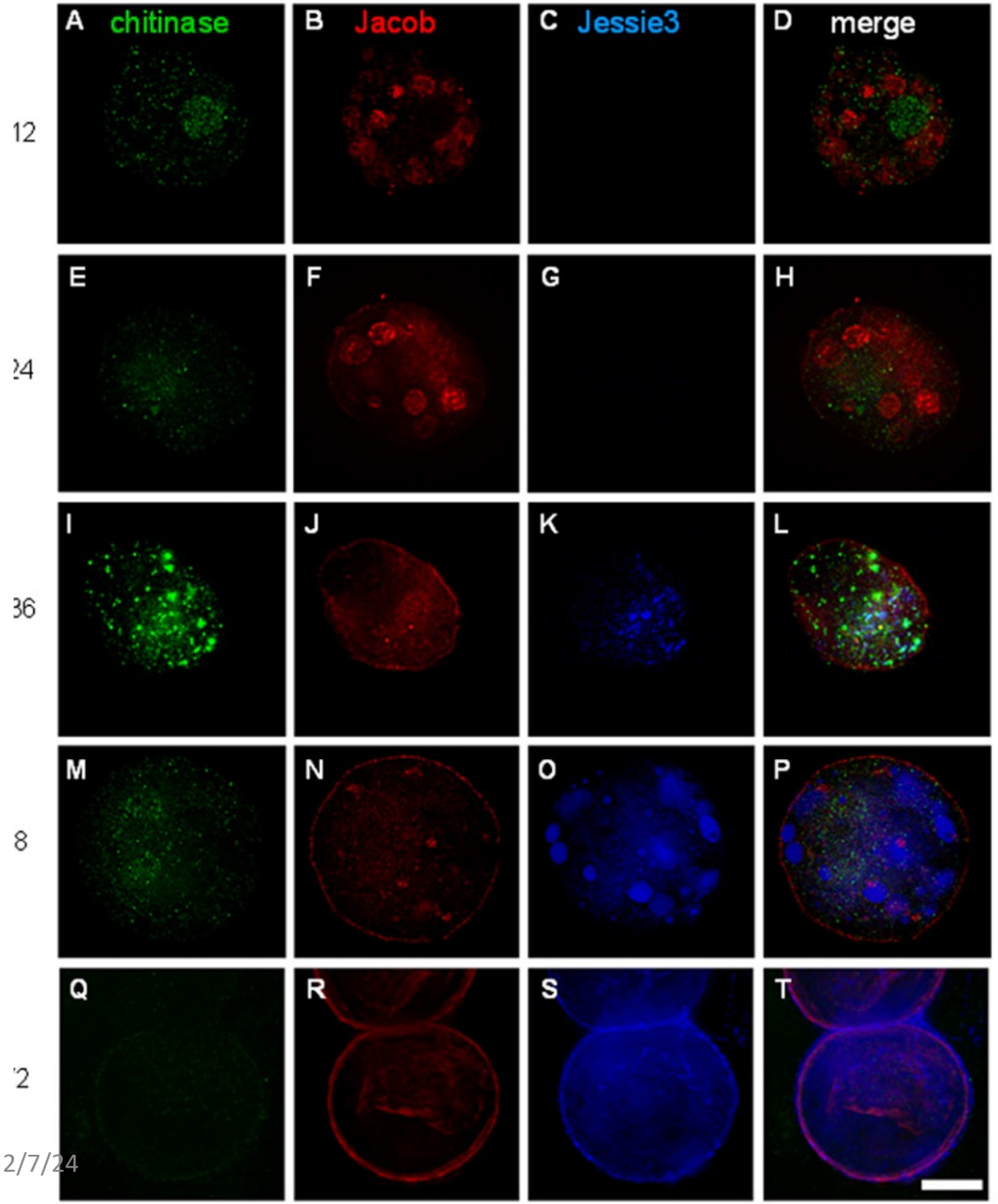
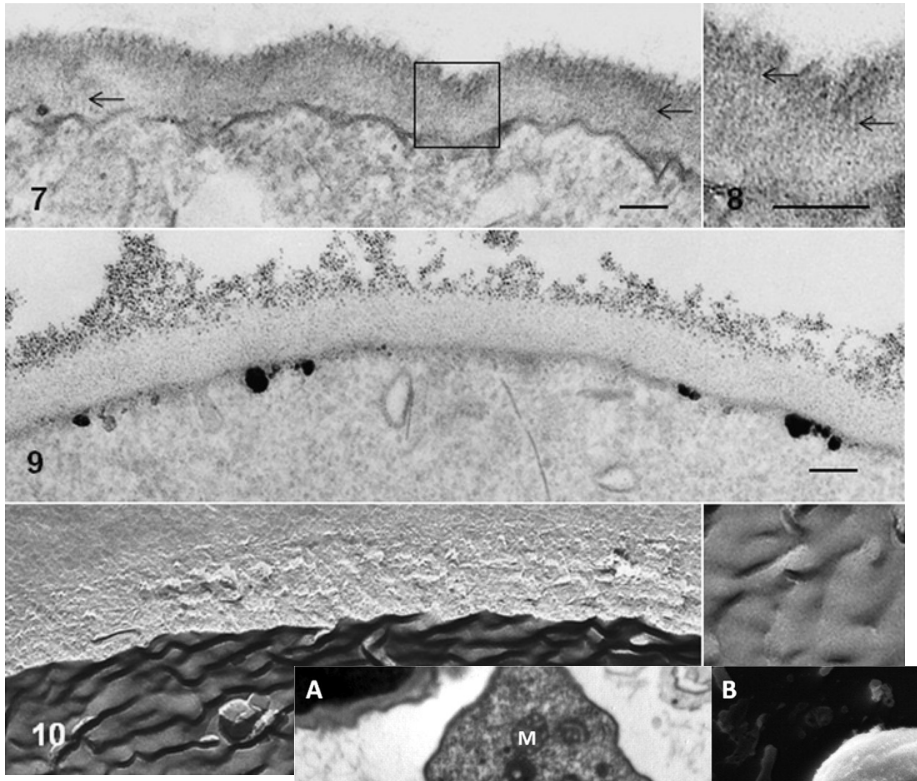
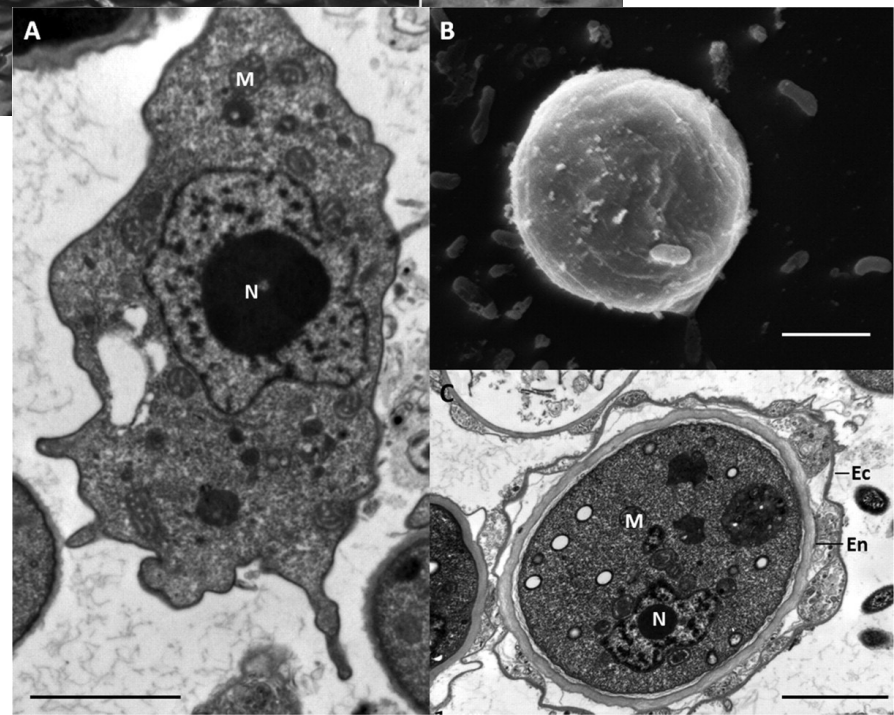
Golgi-like
ER
Nucleus
Chitin
Gal-lectin
Jacob
Jessie
Chitinase
Plasma membrane
Encystation specific vesicle







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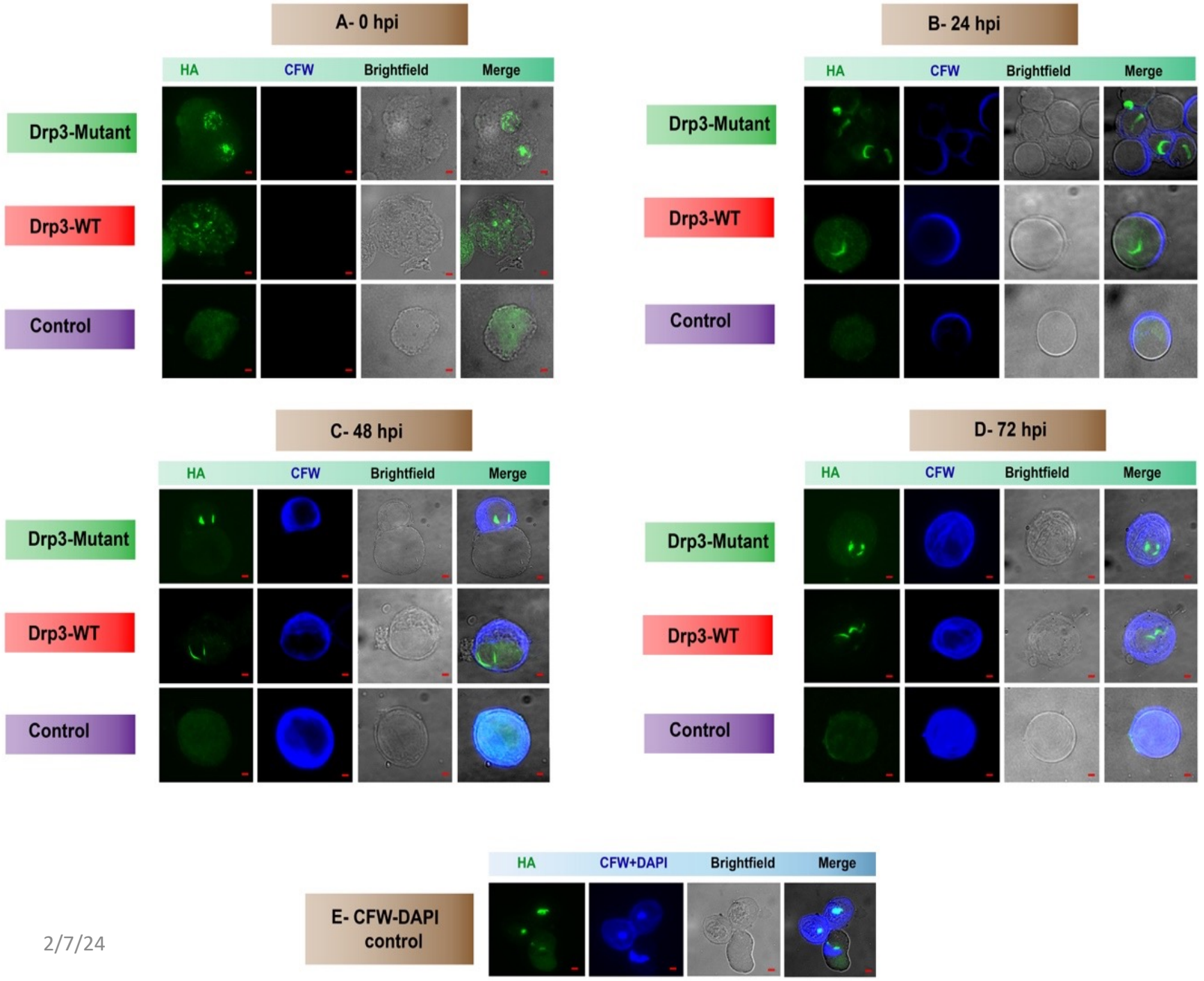

A- 0 hpi
HA
CFW
Brightfield
Merge
Drp3-Mutant
Drp3-WT
Control
B- 24 hpi
HA
CFW
Brightfield
Merge
Drp3-Mutant
Drp3-WT
Control
C- 48 hpi
HA
CFW
Brightfield
Merge
Drp3-Mutant
Drp3-WT
Control
D- 72 hpi
HA
CFW
Brightfield
Merge
Drp3-Mutant
Drp3-WT
Control
E- CFW-DAPI control
HA
CFW+DAPI
Brightfield
Merge

Metabolic and signaling pathways regulating biosynthesis of CW components

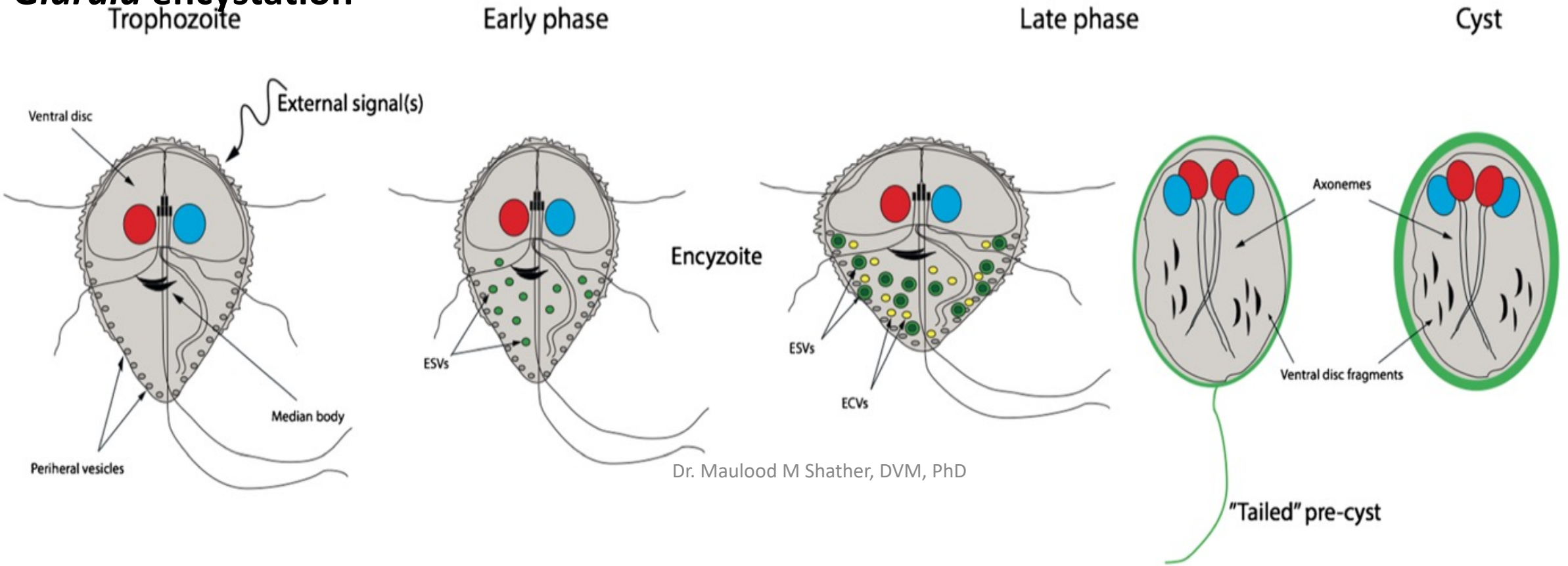
- **Genes** associated with the **membrane trafficking system** (**MTS**) were reported to be significantly **upregulated** during encystation.
- **MTS** involves the **secretory pathway** and **TGN-endosomes recycling**.
- The **MTS** is an organised **flow of cargo** such as **proteins** and other **macromolecules** in membrane-bound vesicles from **membranous organelles** to **various destinations** inside or outside the cell.
- It is essential for **cellular homeostasis** and **signal transduction**, and it can be described as **intracellular highways**.
- The **secretory pathway** is associated with the secretion of **cyst wall components** such as **Jacob**, **Jessie** and **chitinase**, and it is controlled by **small GTPases** such as **Arf** and **Rab proteins**.
- Genes coding for **dynamin-related proteins** (Drps), such as **Drp3** and **Drp4**, are **upregulated** during **encystation** process.
- **Dynamins** are **large GTPases** and function in **membrane remodelling** and **organelles fission.**

Giardia cyst induction

Encystation starts when the parasite **travels** further down in the **small intestine**. The change in the **surrounding environment** in the intestine, such as **high PH,** and **low lipid,** act as trigger signal for **encystation induction**. **Encystation process** can be divided into an **early** and **late phase**.

- The **early phase** involves the activation encystation-specific genes that activate the biogenesis of **encystation-specific vesicles (ESVs)** to carry **cyst wall proteins** (CWPs) to the **plasma membrane** for building of the cyst wall. The ESV containing cell (**encyzoite**) starts to **change shape**, and the **adhesive disc** together with **the flagella** is internalized. These **ESV** undergo **maturation** steps after leaving the endoplasmic reticulum ER (where synthesis of CWPs occur) toward the plasma membrane to deposit CWPs.
- In the **late phase** of **encystation**, the **nuclei divide** and undergo **a round of DNA replication**, giving the mature cyst **four tetraploid nuclei**. The **last step of encystation** is a **maturation step** where the **cyst wall filaments are cross-linked** to generate a compact, **mature cyst wall.**

Giardia encystation



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Drug targets approaches

- The **pharmaceutical industries** invest approximately **25 million dollars** per year developing new **anti-protozoan drugs** for use in the medical field.

- However, a **small number of drugs are commercialized** every decade, and few of these are designed specifically against **parasitic molecules**.

- Most new anti-parasitic drugs are focused on **interfering** with **parasite survival**; nevertheless, they must be **safe** for the host and **avoid cross-resistance** for other existent drugs as well.

- Regarding **cyst proteins**, some attempts have been made in experimental (murine) **giardiasis** using CWP2 as an **immunogen coupled to bacterial vectors**, reducing cyst shedding by up to 70% with these **transmission-blocking vaccines.**

- The fact that many of the protozoan **genomes** are currently being sequenced opens a new window in the knowledge of the **molecular mechanisms involved in the encystment process**, as well as in the design of **different transcriptome maps** which potentially explain the interac- tion and expression among genes related to each parasite stage and the inter-conversion process, **encystment** and **excystment**.

- In this context, it is important to **understand the biological role** of **signal transduction pathways** during each process, and the use of **potential inhibitors of these pathways** in trophozoites, cysts and during **encystment**, for optimal protection.

References

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Thank you