Rhomboids and proteolysis in the Dicty mitochondrion

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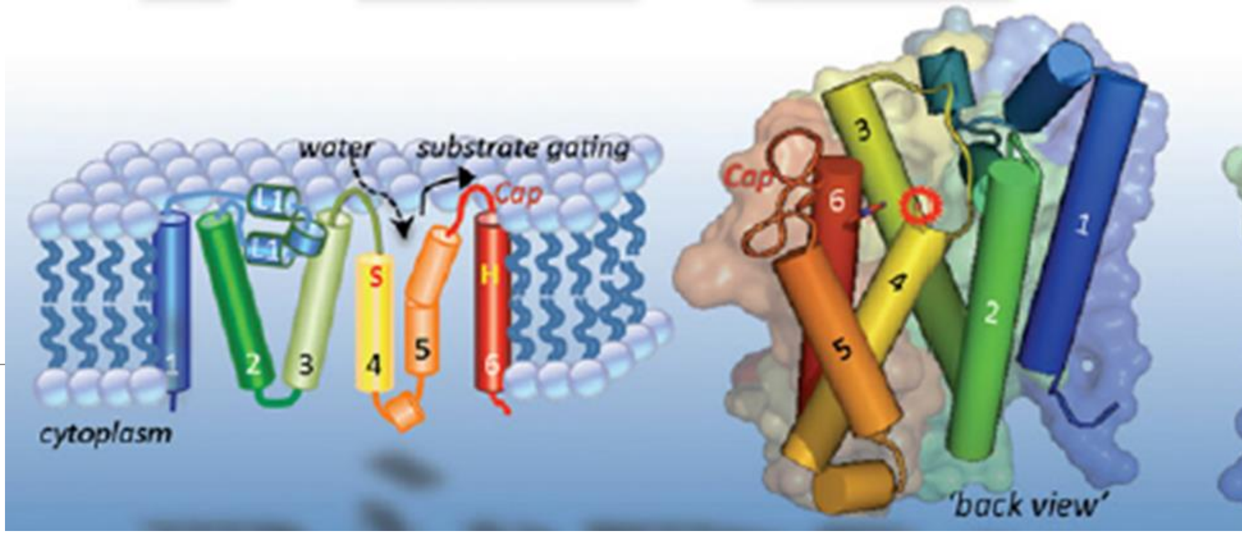
Rhomboids



Membrane located proteases

- 'Unusual'

Ubiquitous across evolution

- 'One' in prokaryotes: multigene families in eukaryotes

Well-conserved structure

- 6 or 7 t/m; catalytic dyad …but poor sequence conservation
    - Necessity of conserved motifs not yet fully understood but sequence and structure predicts mitochondrial location of a subfamily
    - And functional vs non-enzymatically active subtypes

Influence development, signalling and infection in a range of eukaryotes and prokaryotes

- e.g., necessary for *Plasmodium* and *Toxoplasma* infection, EGF signalling in *Drosophila*

Substrate prediction is hard

Rhomboid philosophy – why are they interesting?

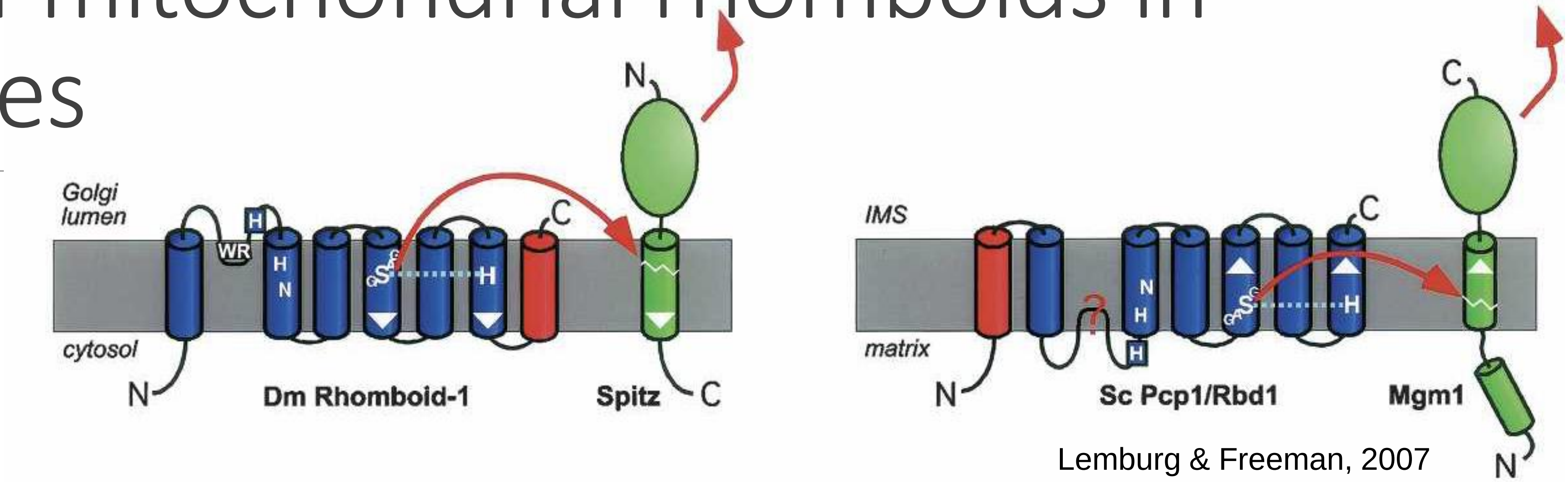
Proteolysis is not just housekeeping: a key regulatory mechanism in cell biology

- Proteases comprise 2-5% of organism genomes across evolution
- Protein activation, localisation, exposure of cryptic binding sites and release of neoproteins…
- Pathogenesis of disease: altered protease expression and substrate-proteolysis, e.g., in Parkinson’s and other neurodegenerative diseases

Proteolysis **in cell membranes** occurs via several families of ‘new’ intermembrane proteases:

- <u>R</u>egulated <u>I</u>ntramembrane <u>P</u>roteolysis
- Includes the rhomboid family

Subset of mitochondrial rhomboids in eukaryotes



Lemburg & Freeman, 2007

In Drosophila EGFR ligand (e.g., Spitz) cleaved and to release product outside cell for signalling

Orientation reversed in mitochondrial rhomboids

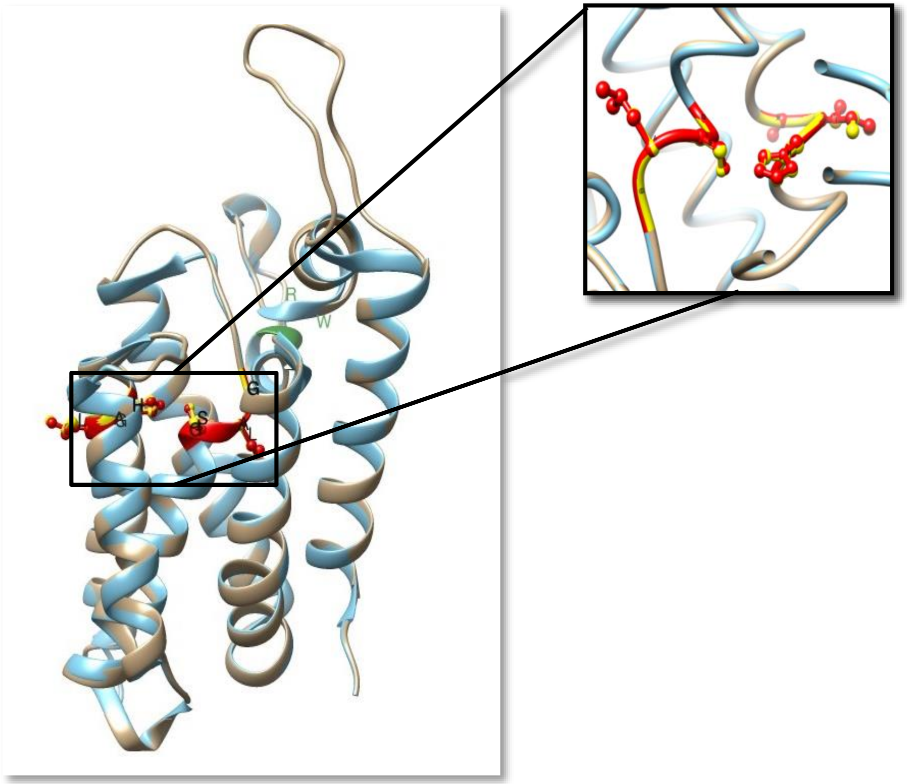
- Rhomboid cleavage is on opposite side of membrane
- Prokaryotes?

Rhomboid subsets in *Dictyostelium*

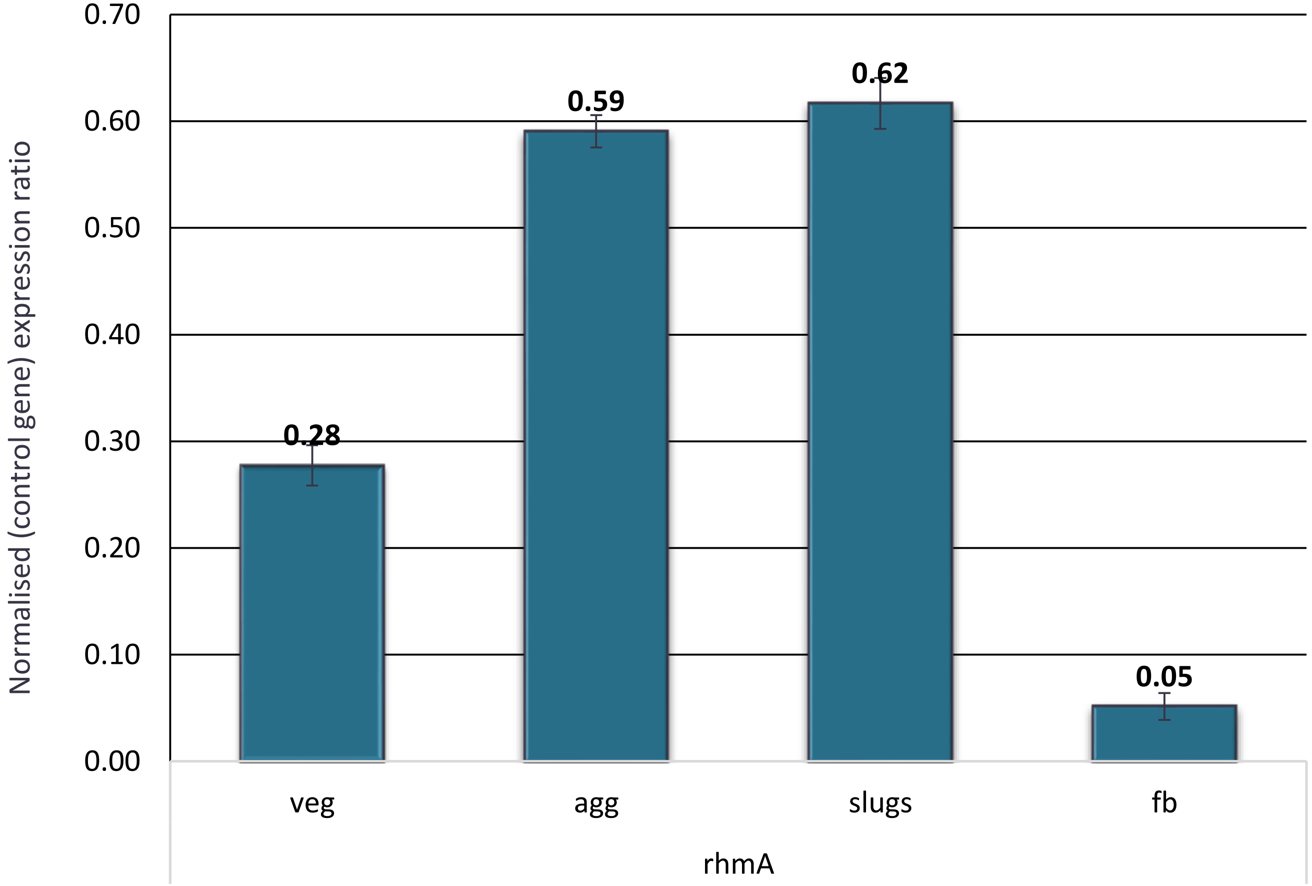
Predicted *Dictyostelium* active rhomboids

		Sequence ID
Rho like	**rhmA**	DDB_G0295849
Mito like	**rhmB**	DDB_G0284937
Rho like	**rhmC**	DDB_G0281359
Mito like	**rhmD**	DDB_G0292430

rhmA – ‘active’, transcription peaks from unicellular-slug stages

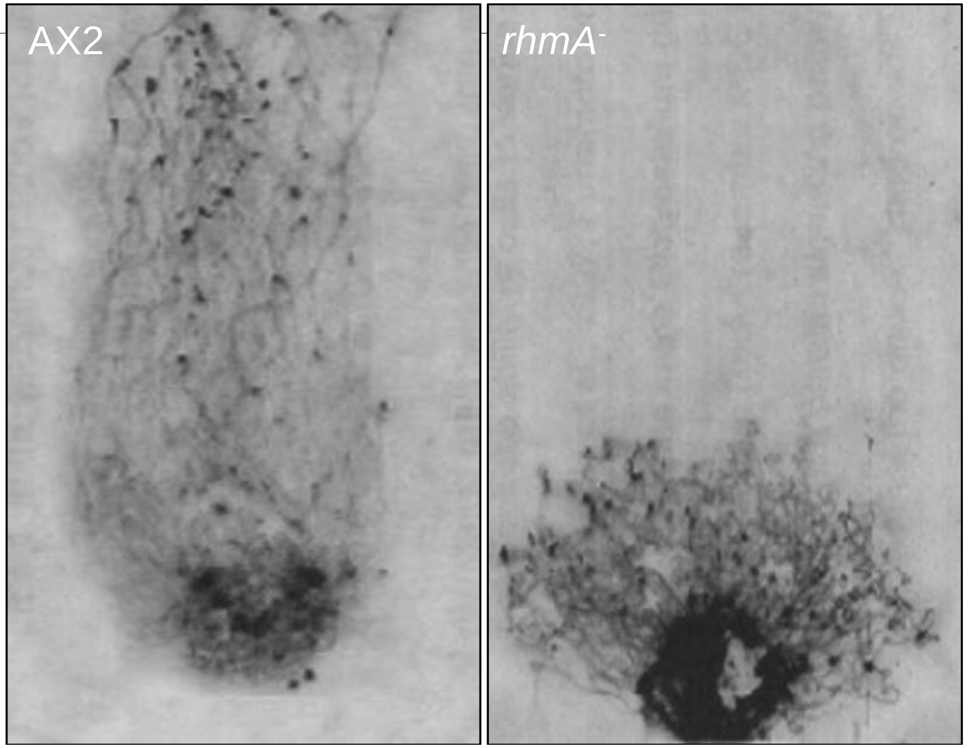


RhmA (brown) vs. *E. coli* GlpG (2IC8, blue). WR (green); G*SG (red)

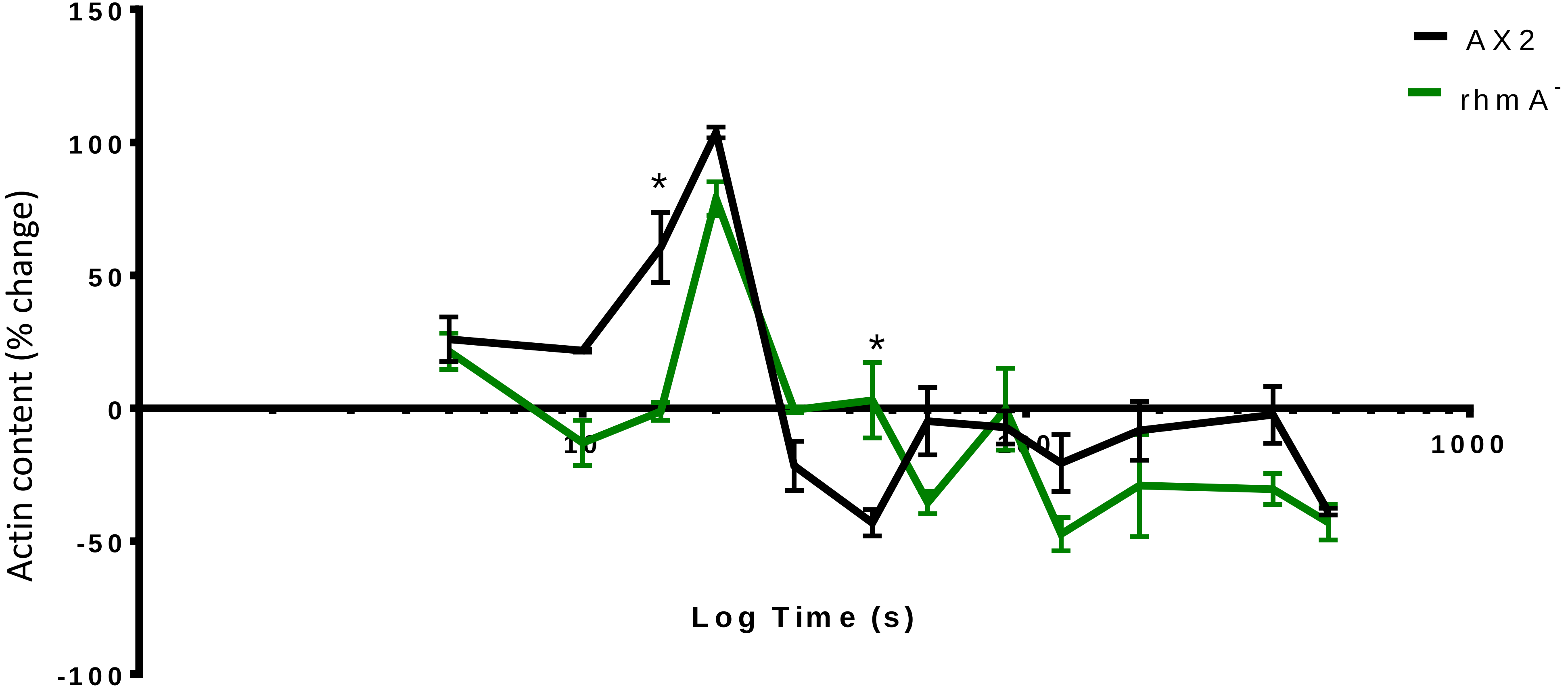


RhmA- phenotype

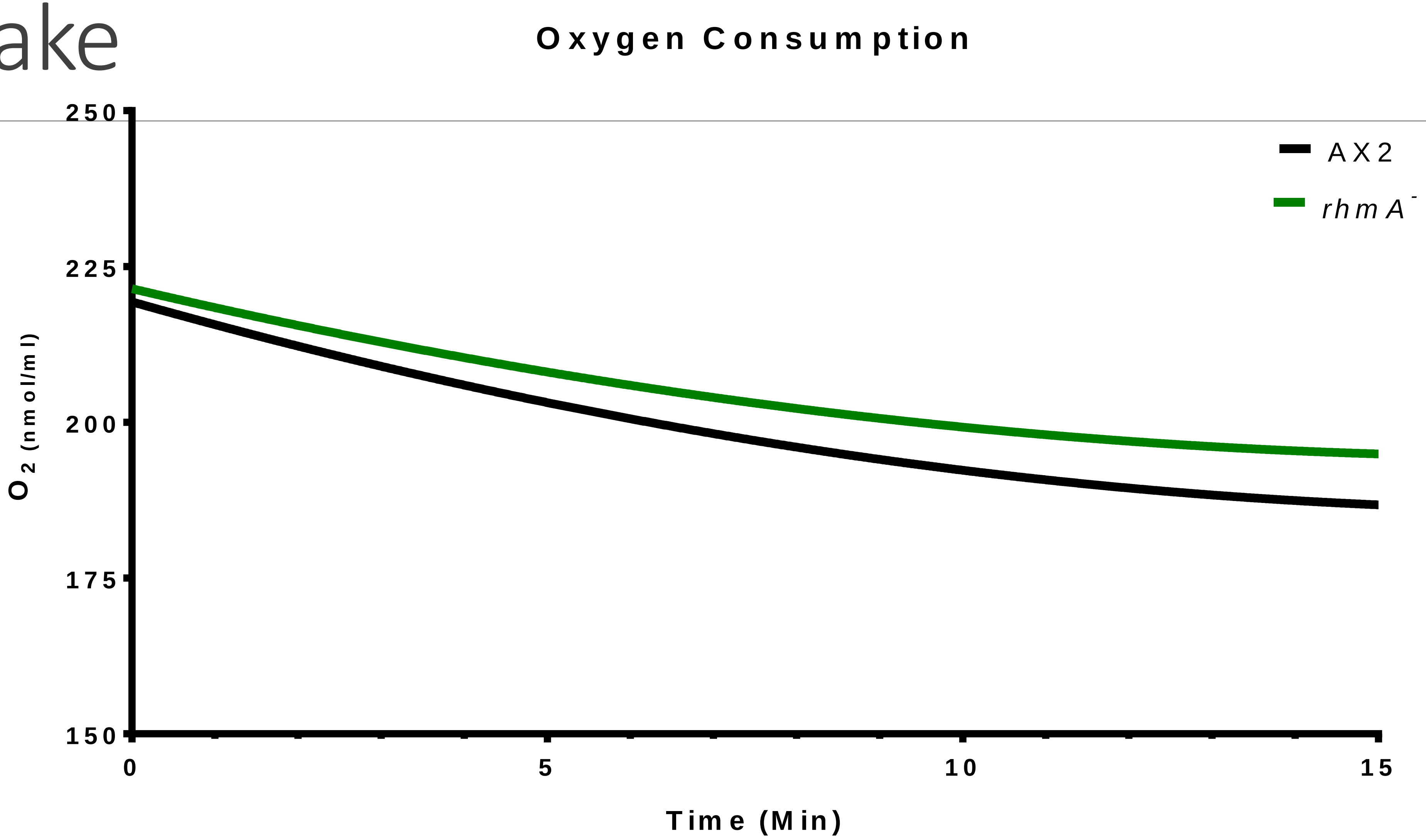
- aberrant phototaxis,
- slower chemotaxis to both cAMP and folate,
- significant although small reduction in directed movement (random cell movement the same)



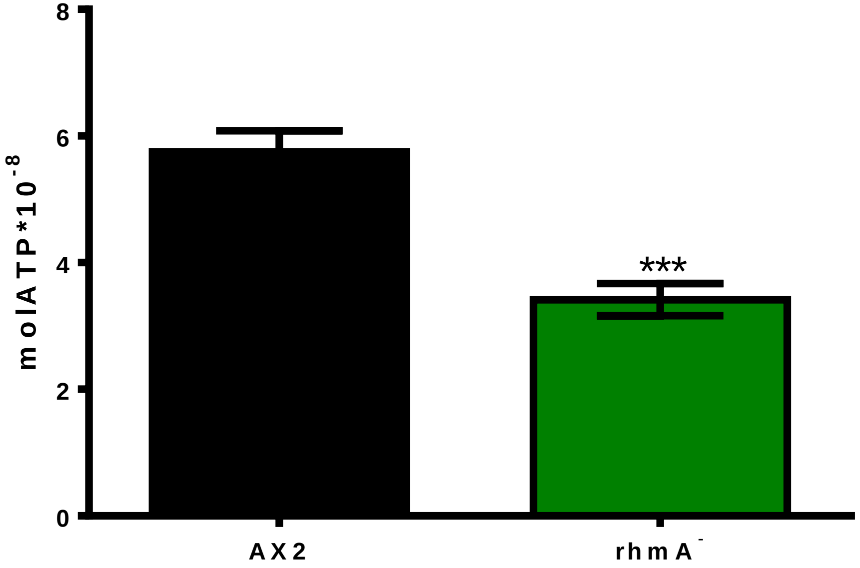
Why defective motility – no actin defect



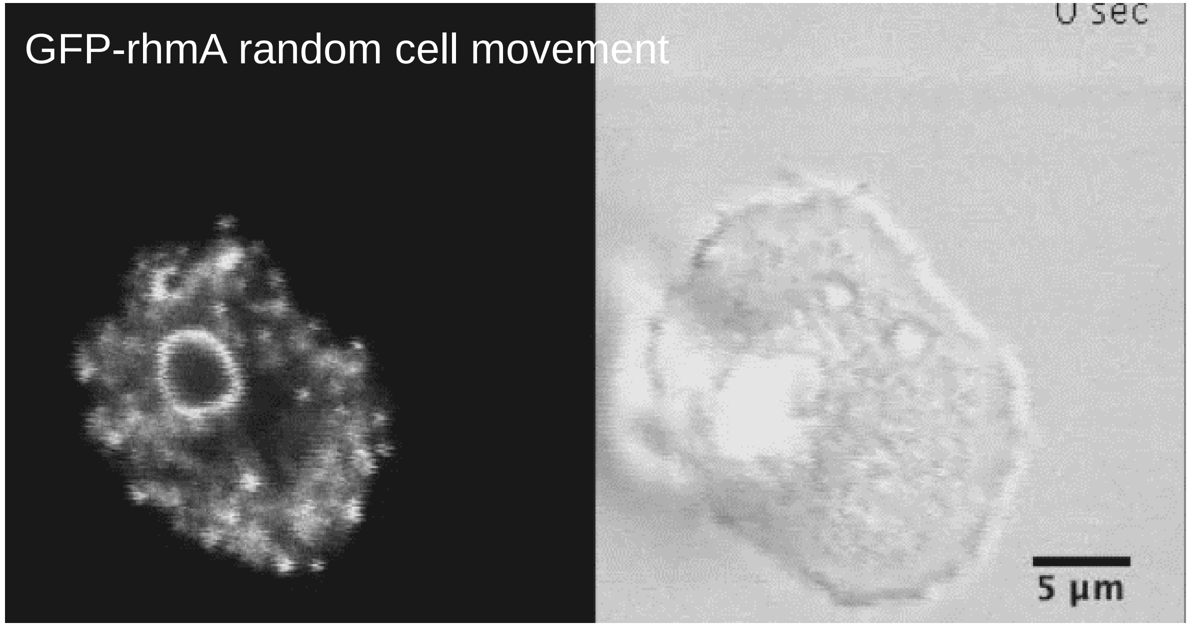
Respiratory defect? Not apparent via O2 uptake



ATP luciferase assay

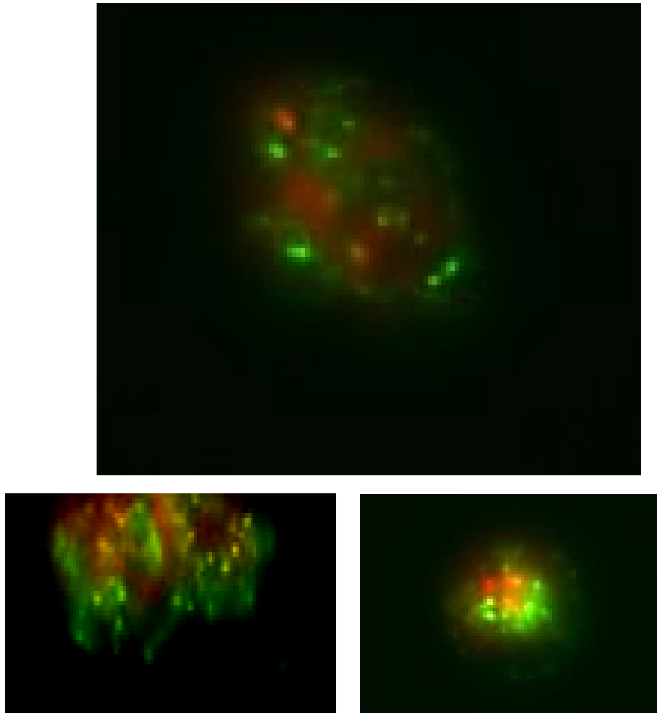


Where is RhmA? GFP-RhmA in contractile vacuole/ cytoplasmic spots


GFP-rhmA random cell movement

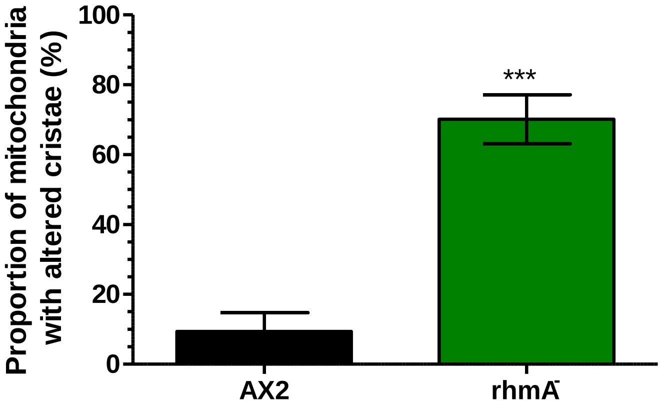
No co-localisation with Mitotracker red

Reduced Mitotracker uptake in rhmA- (MMP?)

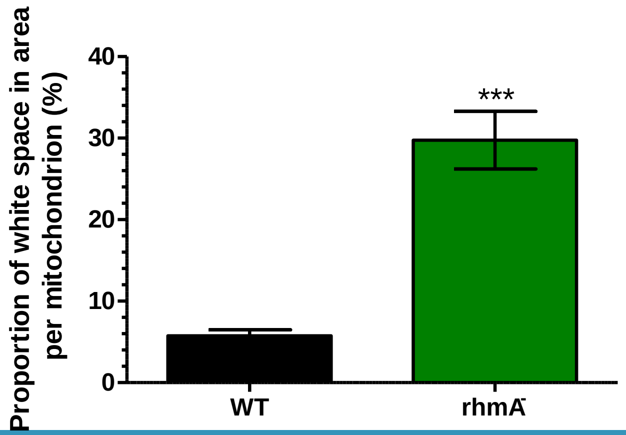

GFP-rhmA (green) co visualised with Mitotracker red

rhmA- mitochondrial morphology defect

A

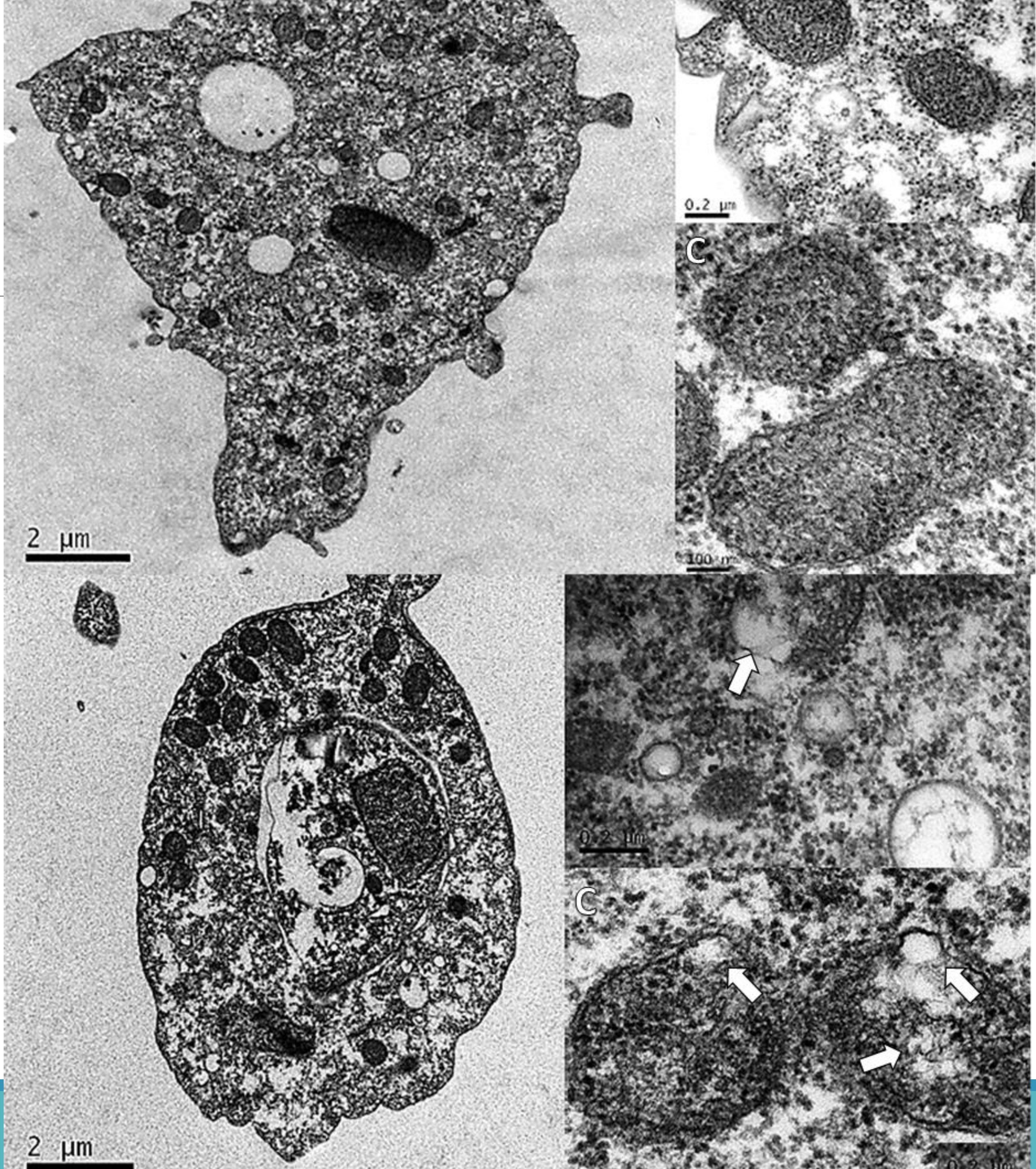


B



Most rhmA- mito show same ultrastructural abnormality

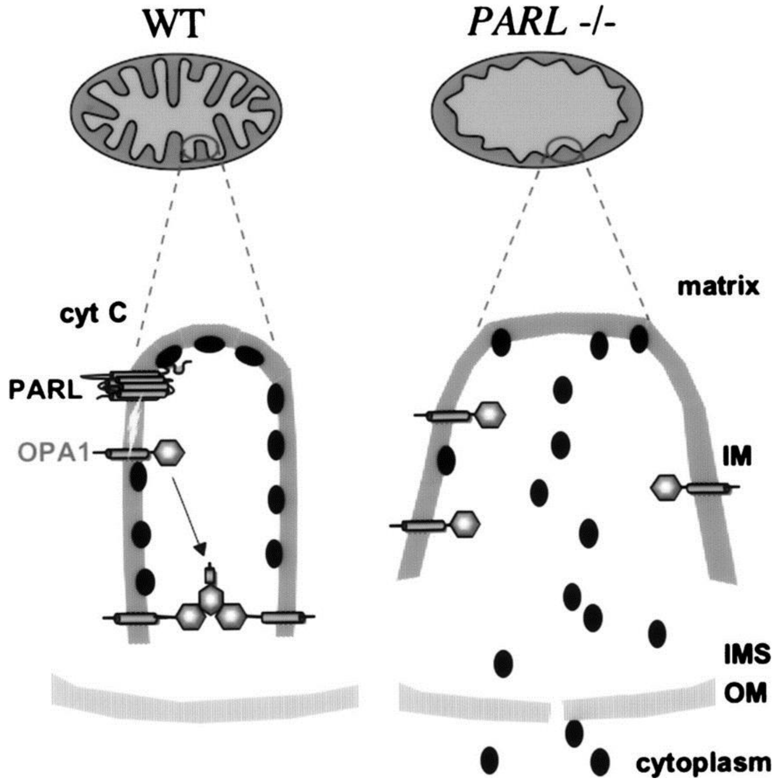
RhmA not predicted/located in the mitochondrion



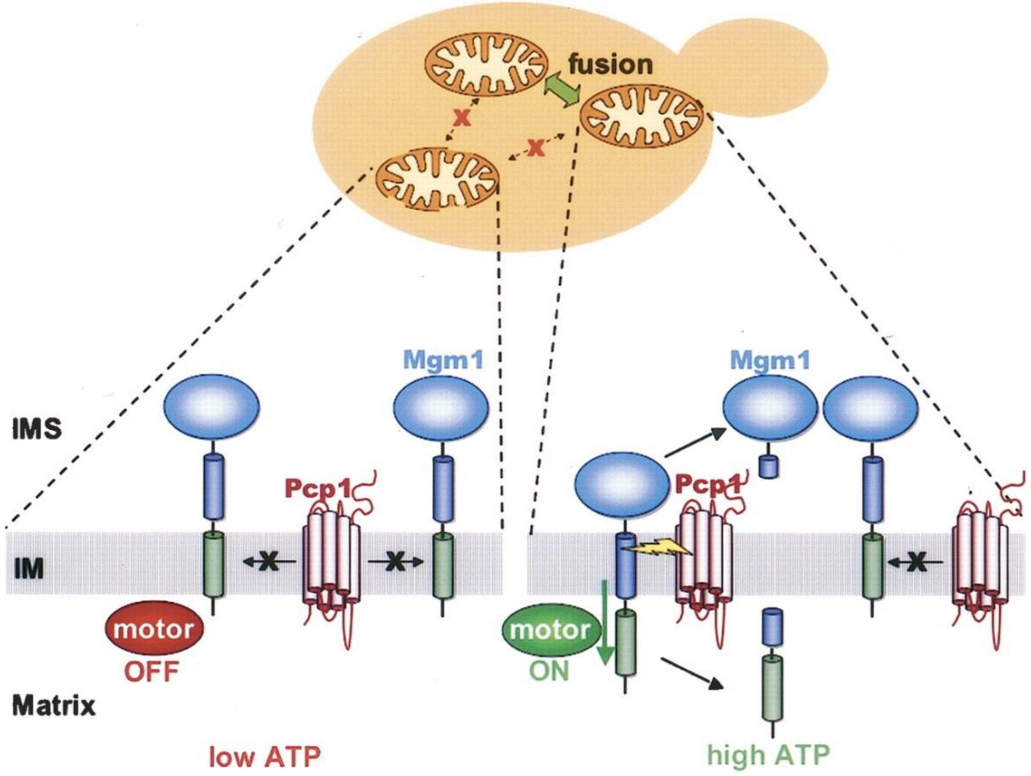
Rhomboid function in mitochondria – dynamin family GTPase substrates

MAINTAINING TIGHT MITOCHONDRIAL CRISTAE IN VERTEBRATES

PARL RBD- CELLS RELEASE CYTC AND HAVE MORE OPEN CRISTAE

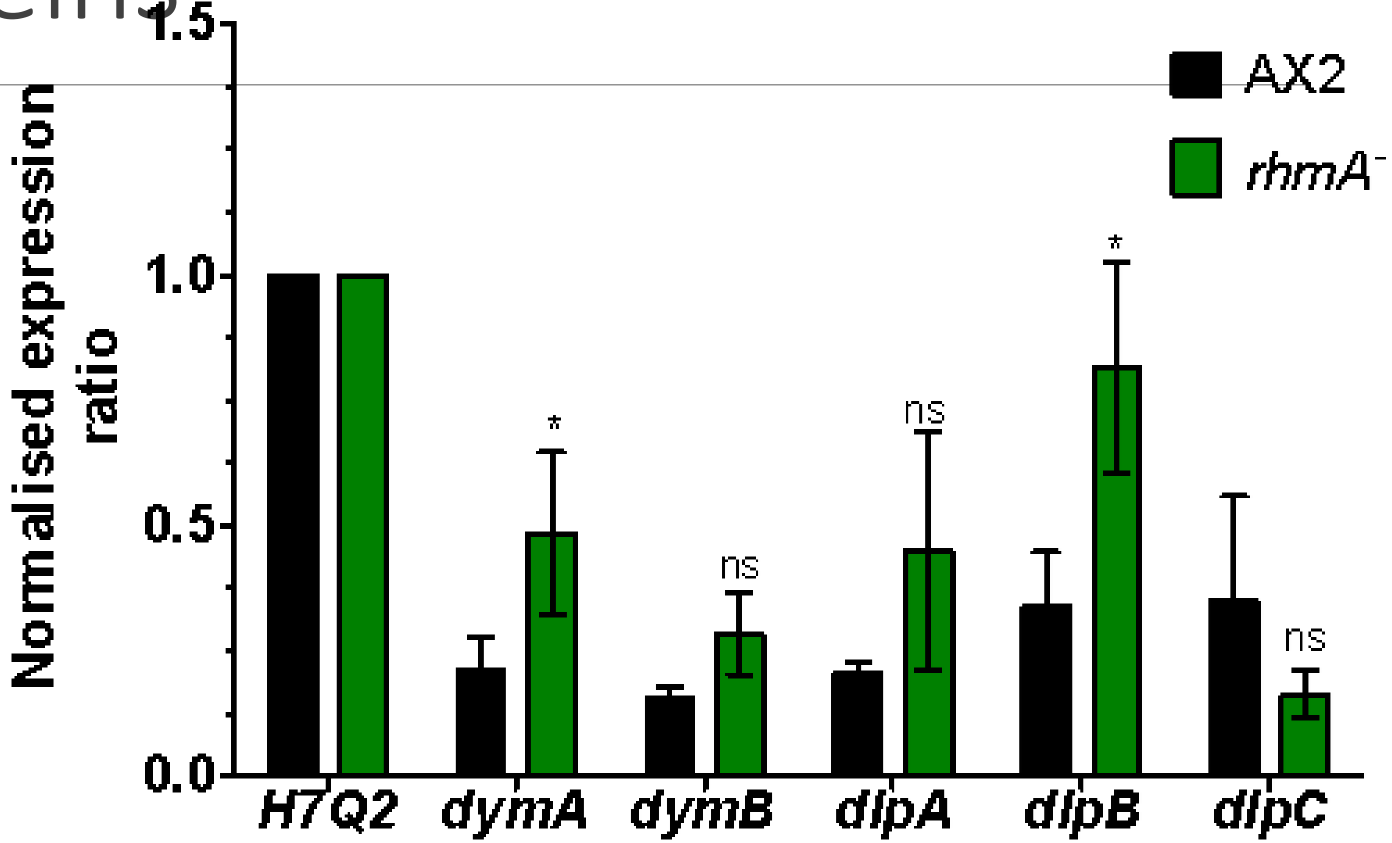


REGULATING MITOCHONDRIAL FUSION DYNAMICS IN YEAST



Sinisa Urban Genes Dev. 2006;20:3054-3068

Veg cell RTPCR (no diff in agg cells) -dynamin-like proteins



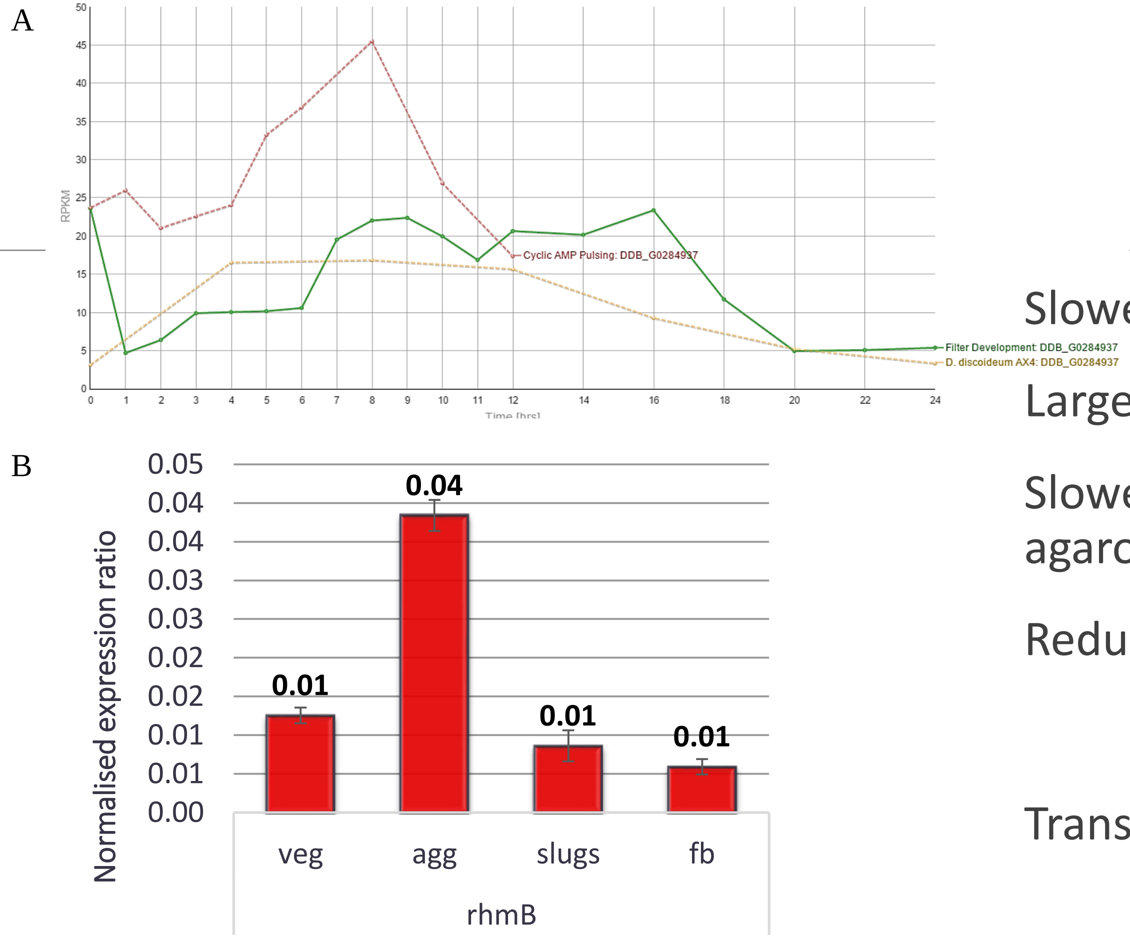
Transcription levels of dynamin related genes in vegetative cells.

Meanwhile…

Pulldown in RhmA-GFP yields band

Some ideas…

Activity assay ongoing in Prague



RhmB… succinctly

Slower growth axenic and reduced phagocytosis

Larger cells

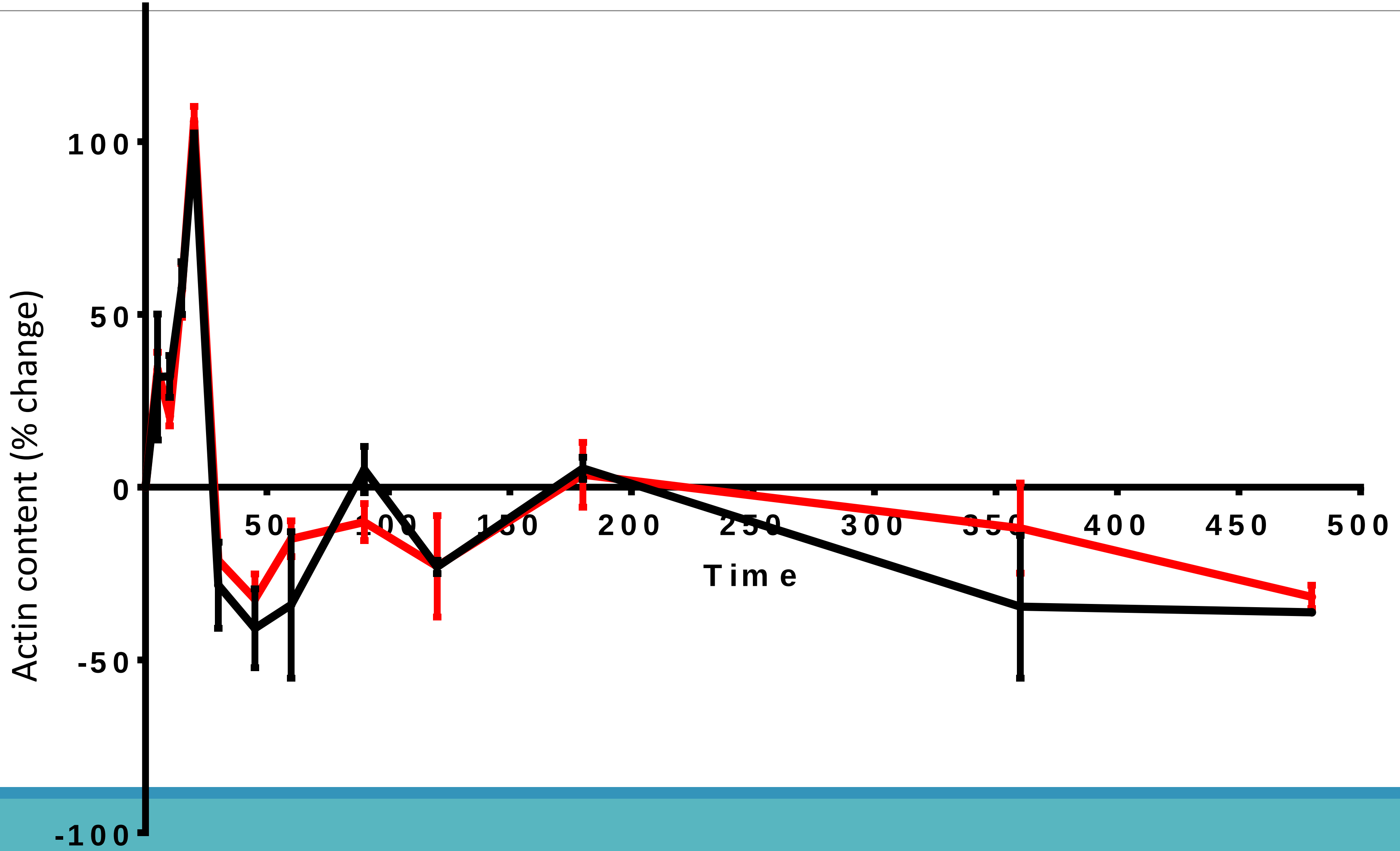
Slower response to folate in one-drop and under-agarose assays

Reduced adhesion – unicellular stage

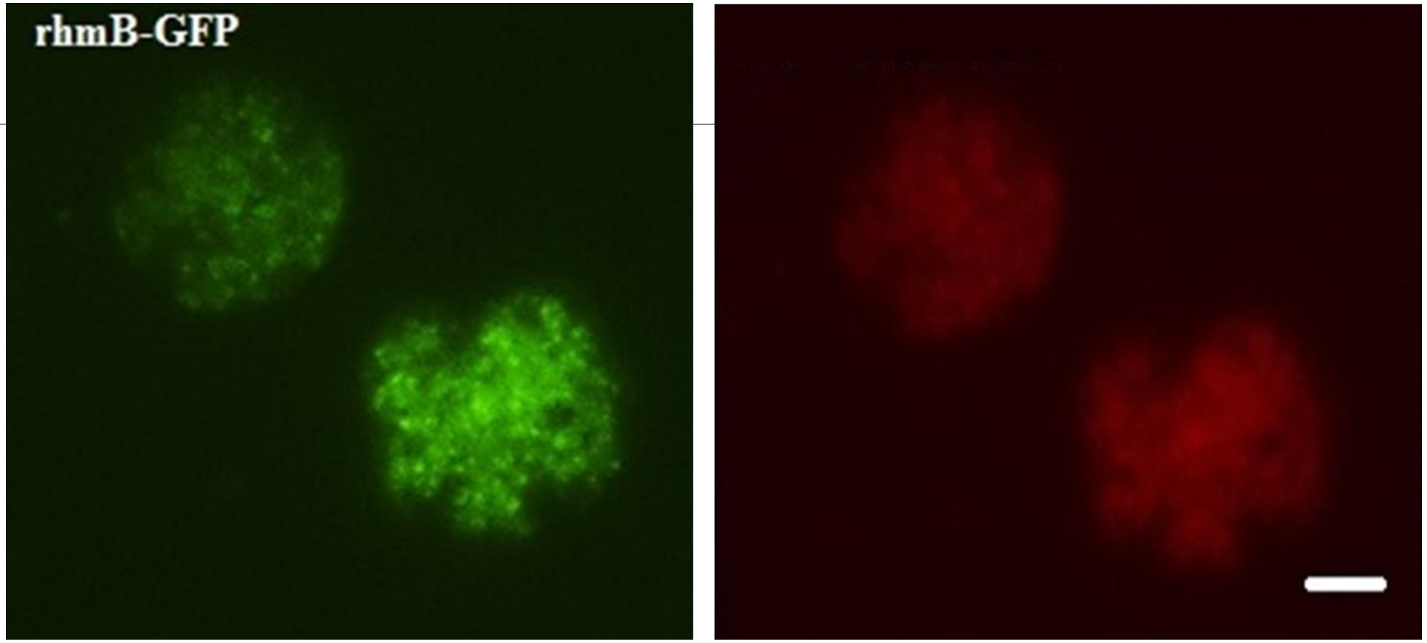
Transcription peak at aggregation (as Dictybase)

Phototaxis as WT

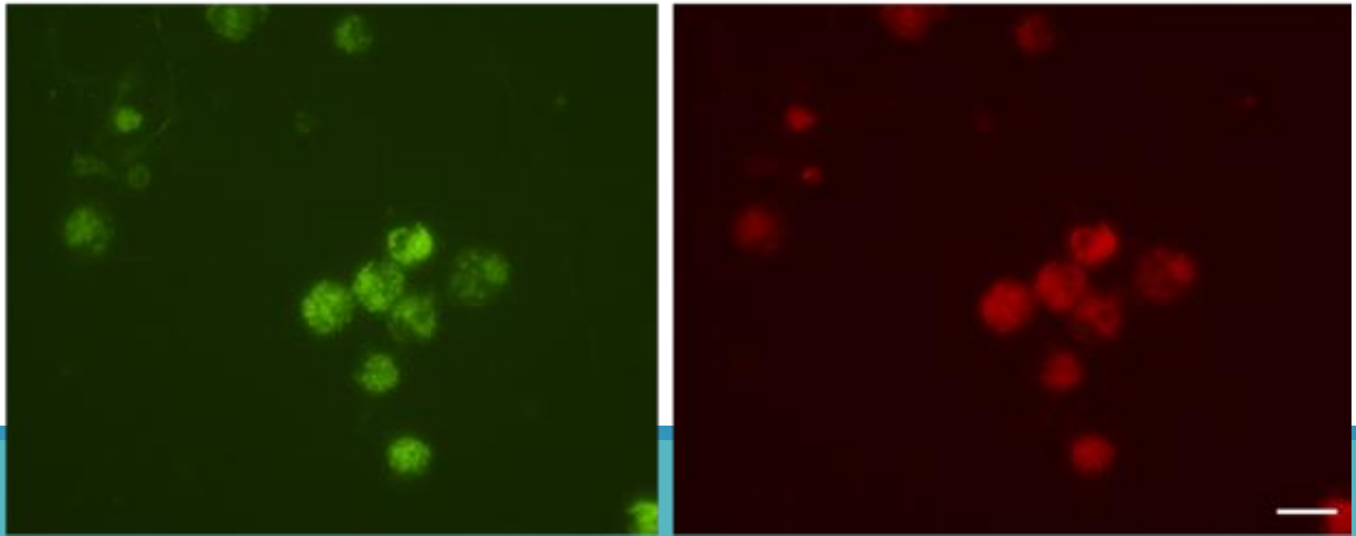
Motility via actin assay

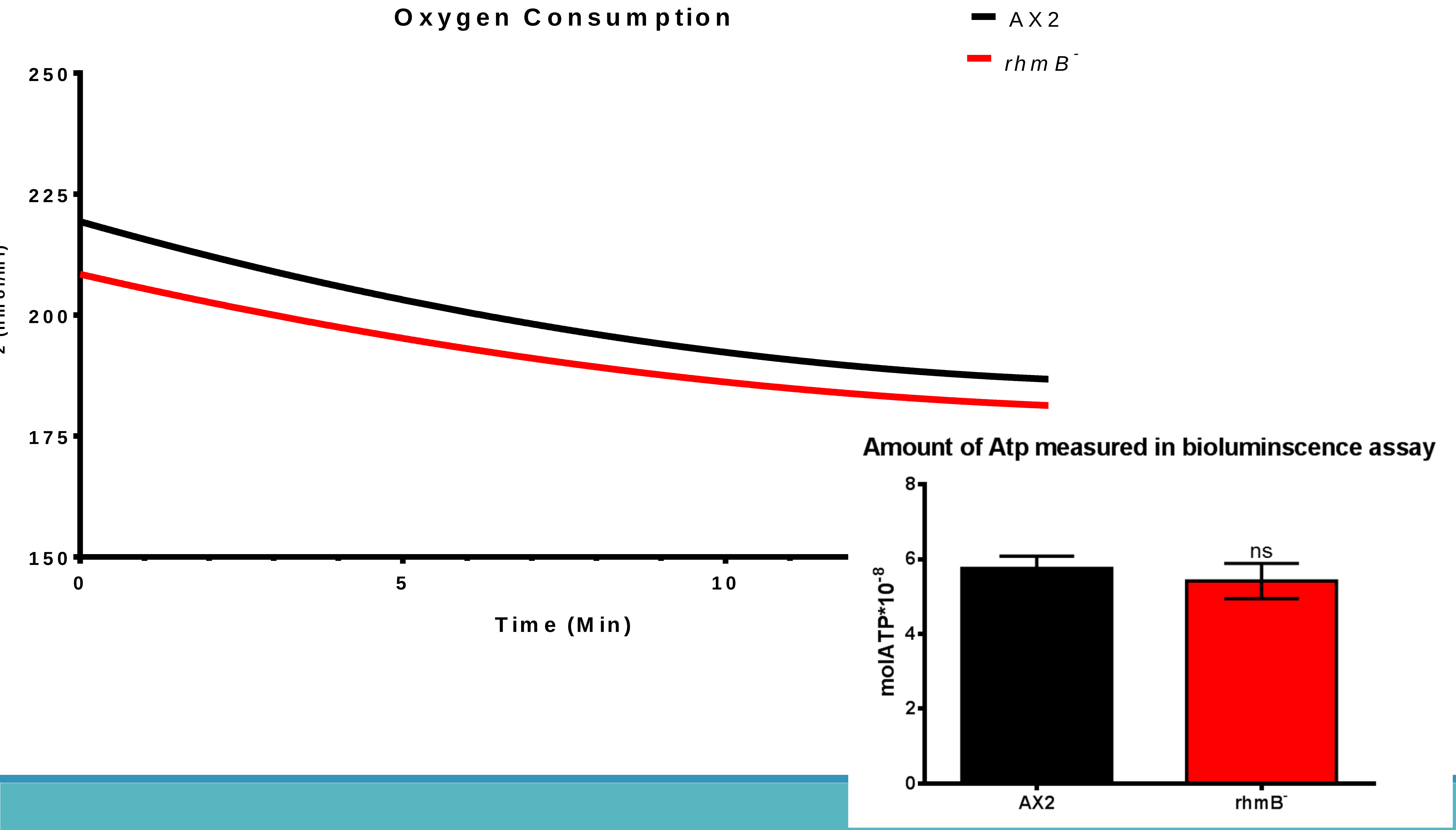


RhmB-GFP fusion protein located in mitochondria ?OM



pJSK543 RFP-GemA mito outer membrane marker




Oxygen Consumption
AX2
rhmB⁻
250
225
200
175
150
0
5
10
Time (Min)
Amount of Atp measured in bioluminscence assay
molATP*10⁻⁸
8
6
4
2
0
ns
AX2
rhmB⁻

Substrate fishing…

RTPCR with dynamin-related proteins

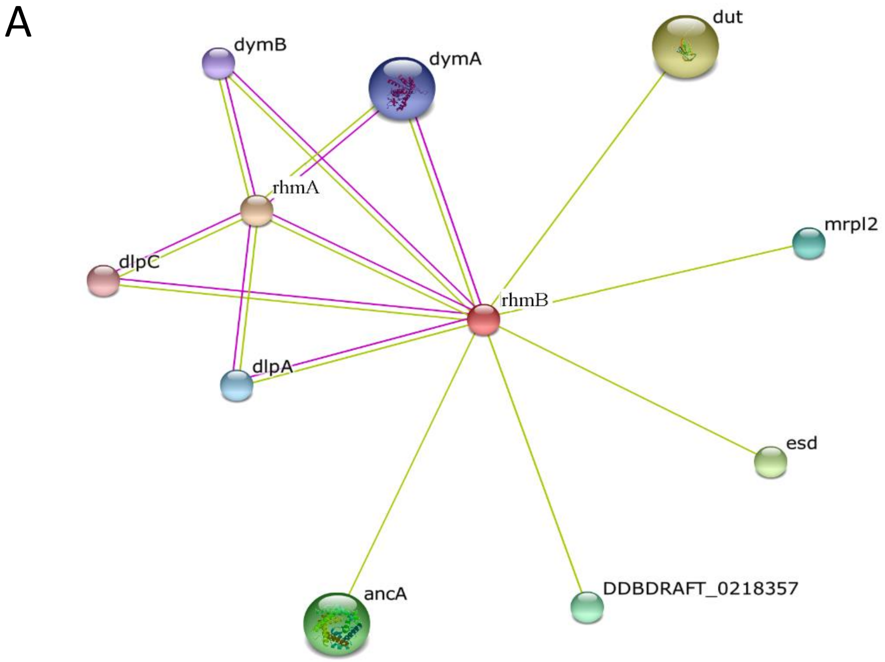
Substrate and activity assays ongoing

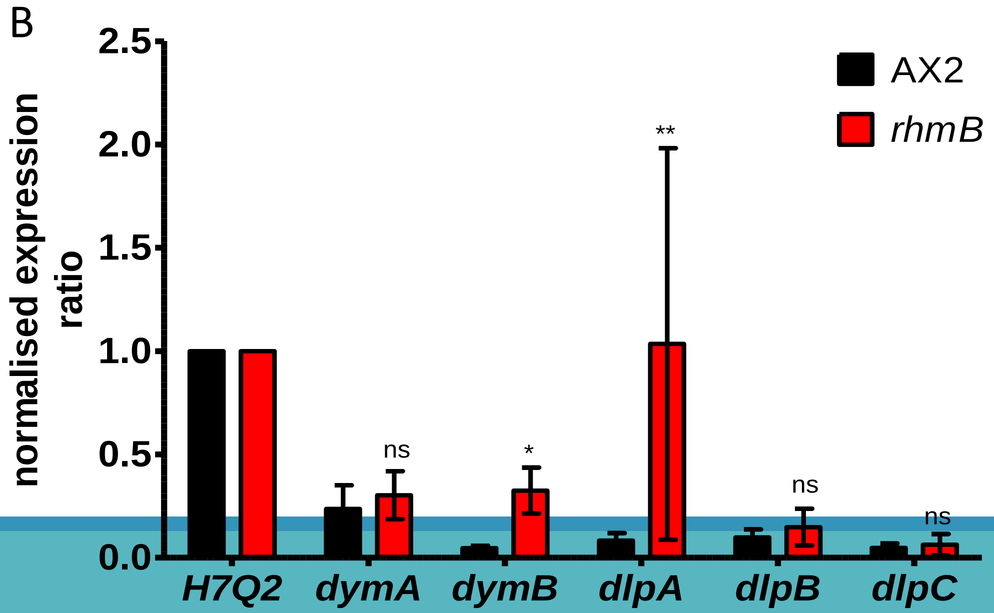
RhmA/B double mutant

No growth on bacterial lawns

RhmD essential

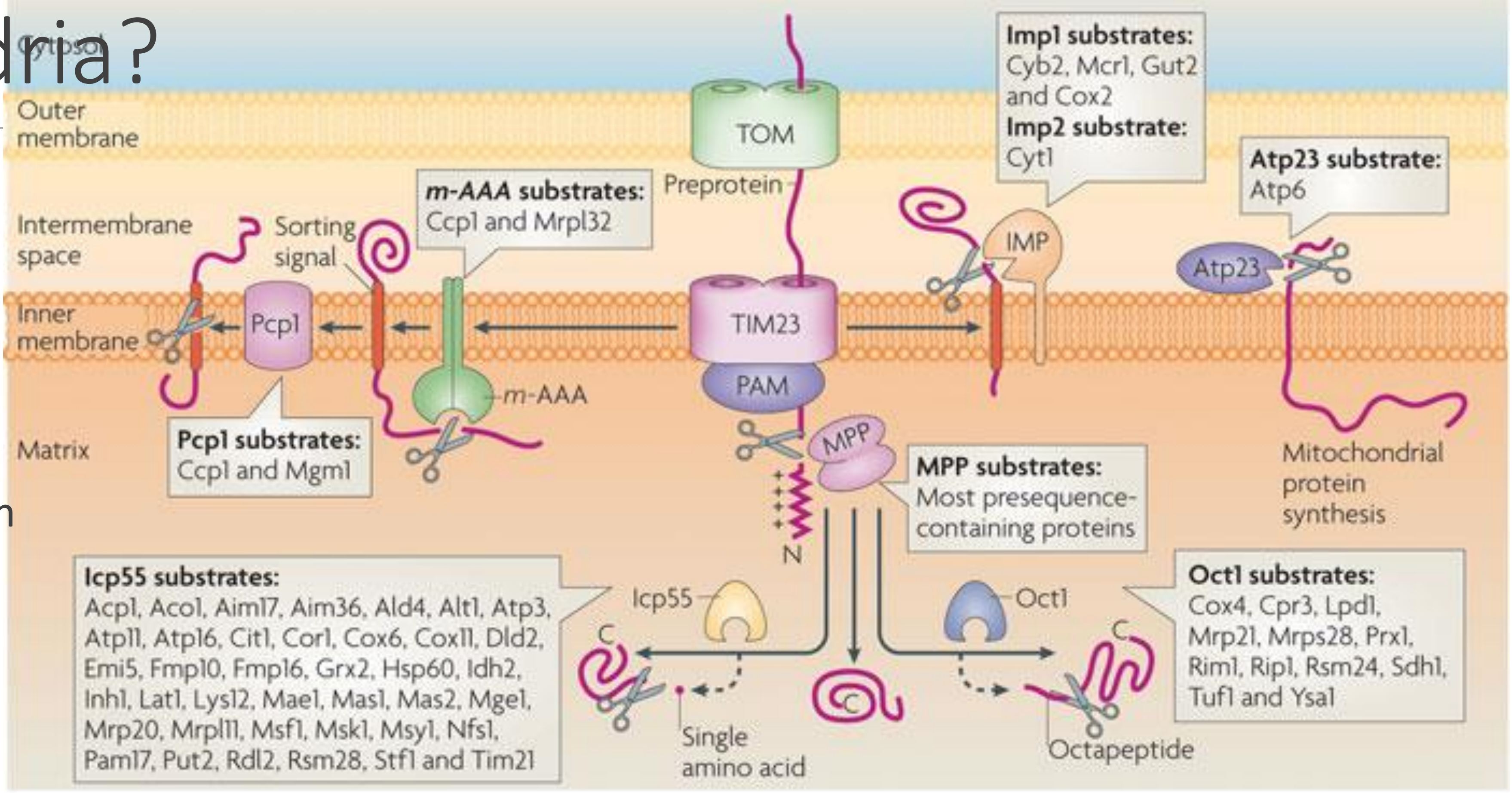
RhmC no pheno?





Add rhomboids to the proteolytic proteome of Dicty mitochondria?

- Rhomboids having regulatory roles in *Dictyostelium* mitochondria fits in with our increasing appreciation of the importance of proteolysis in signalling and development – not just house keeping function

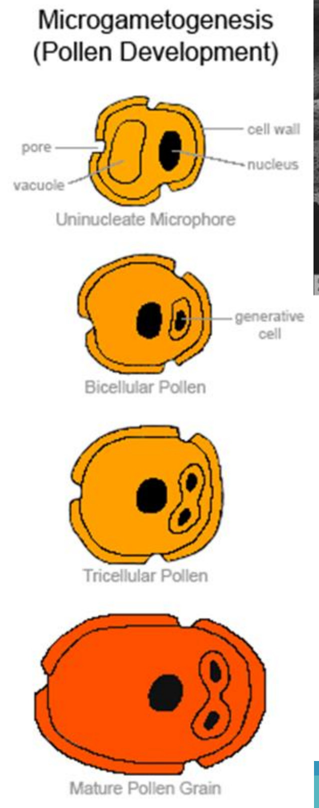


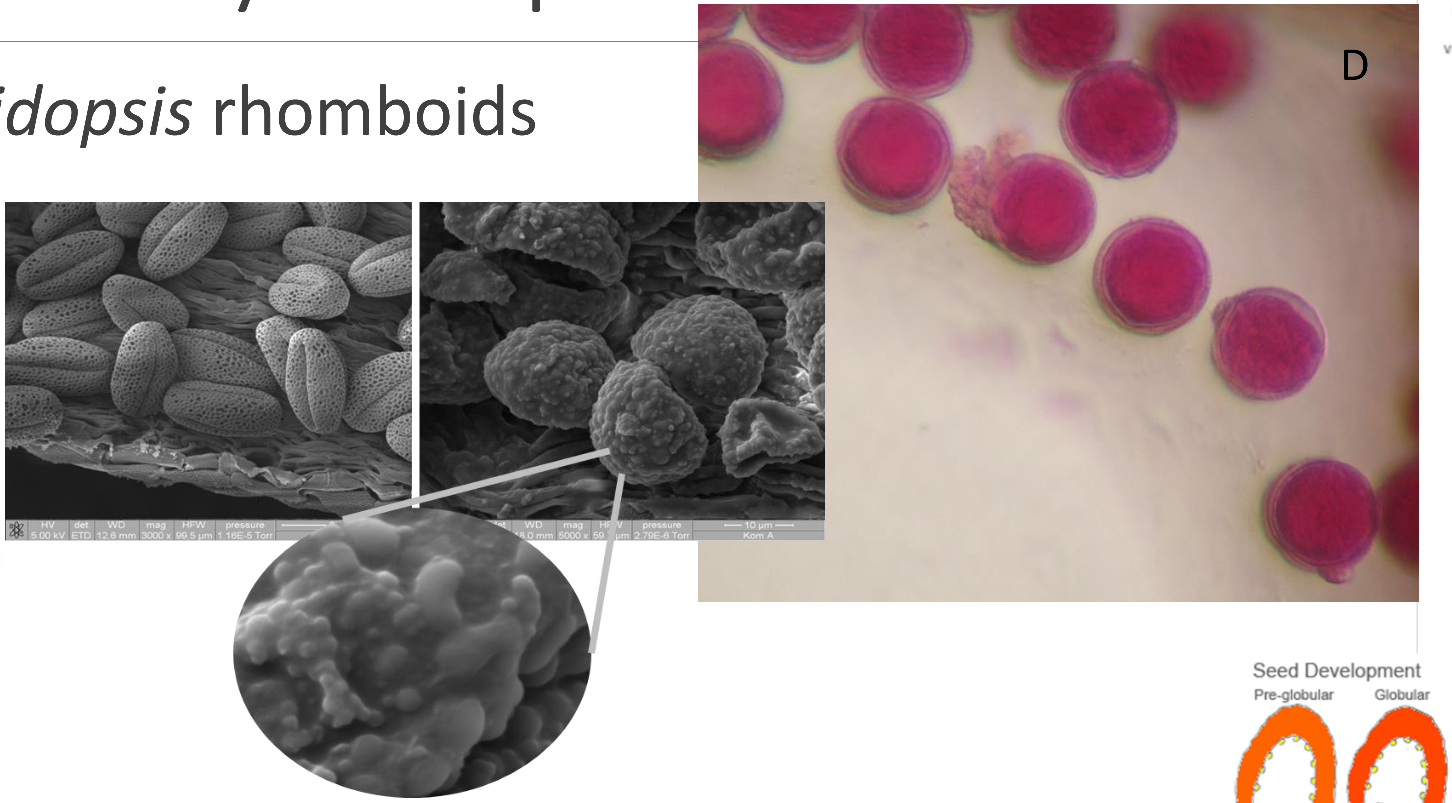
Nature Reviews | Molecular Cell Biology

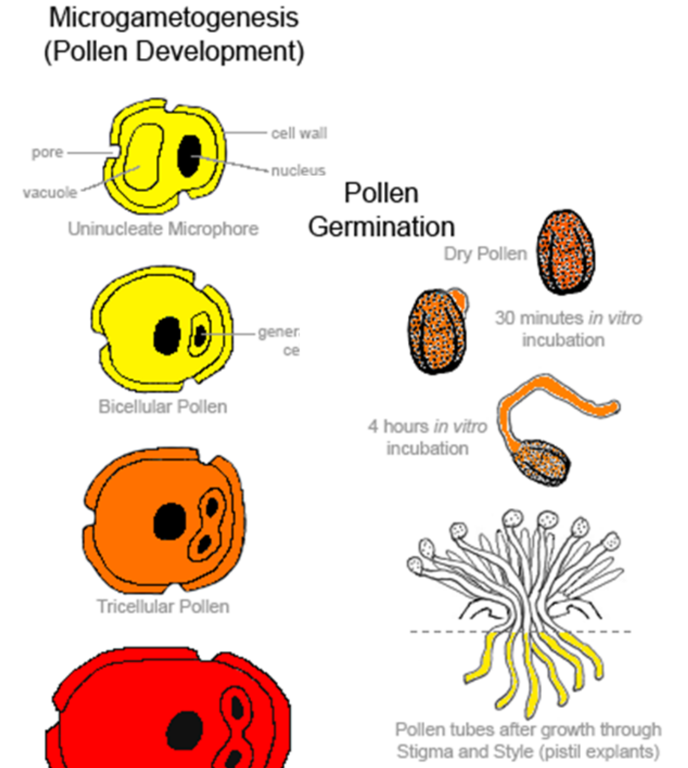
A tendency for specialisation?

~15 *Arabidopsis* rhomboids

KOM

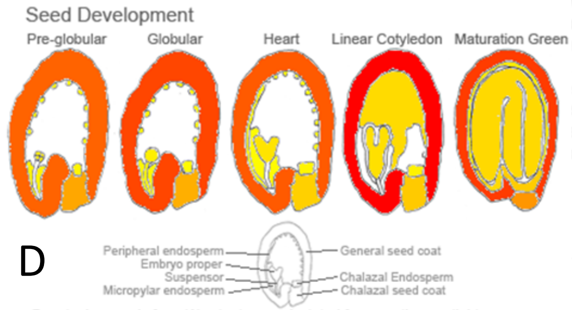






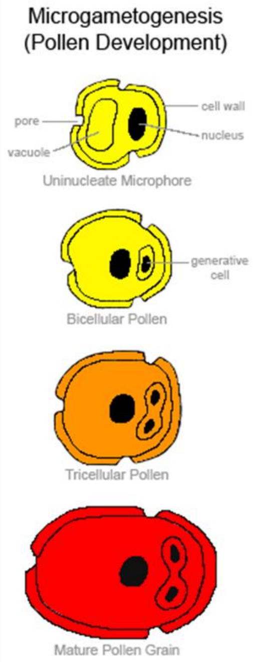
- Dry pollen grains were harvested by vacuum
was germinated using an *in vitro* system,
nples were taken at 30 minutes or 4 hours
ubes were also harvested after germination
semi-*in vivo* pistil explant system
s were taken in quadruplicate or triplicate,
rage signal is shown
as isolated and hybridized to the ATH1 chip
rmalized by GCOS/MAS5, TGT 100

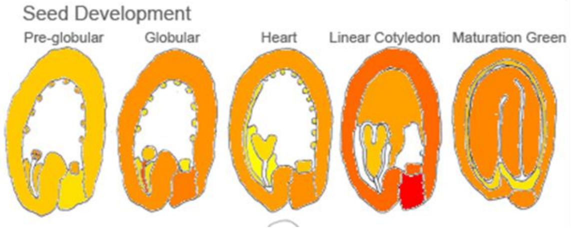
(2009) PLoS Genetics 5: e1000621.



- Developing seeds from Ws plants were isolated from continuous light-grown plants and the indicated parts were excised by laser-capture microdissection.
- RNA was extracted and amplified, before hybridization to the ATH1 GeneChip.

G, H mutants


Microgametogenesis (Pollen Development)

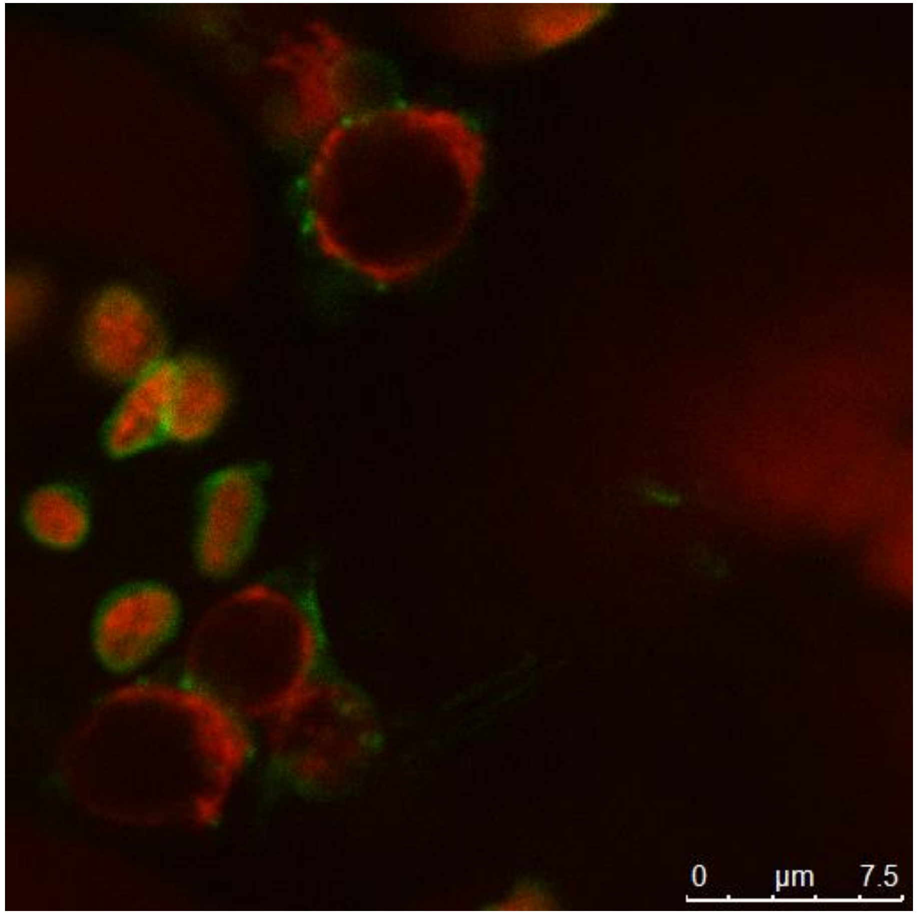

Seed Development

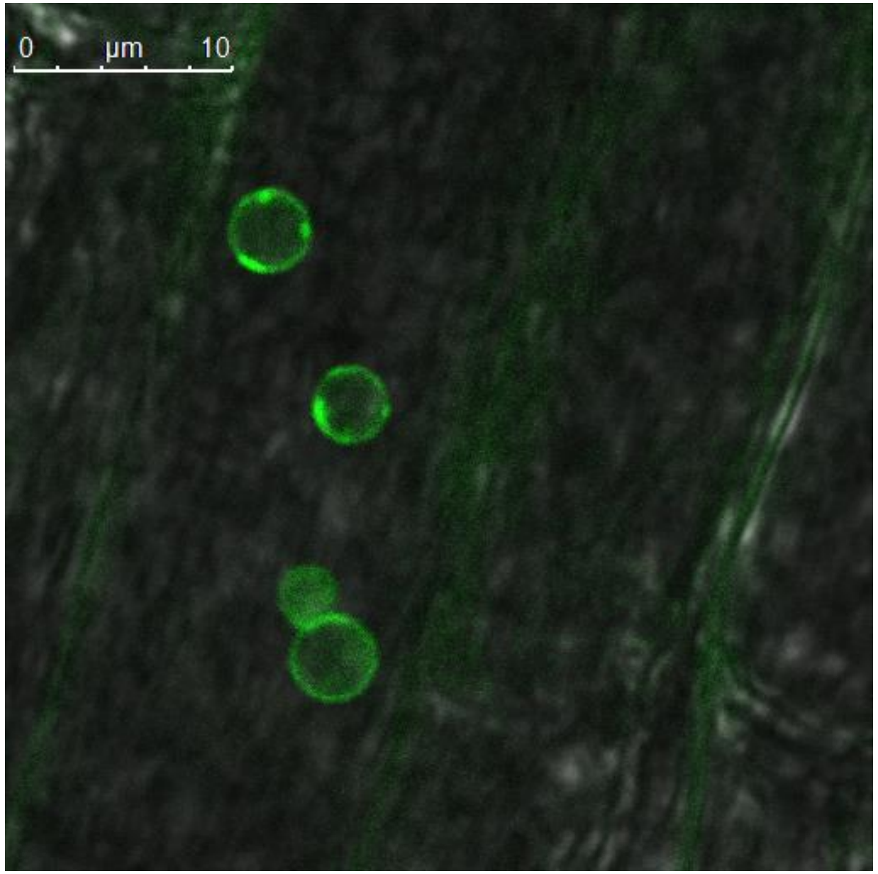
Arabidopsis organellar rhomboid

RBL10-GFP chloroplast rhomboid –> organelle outer membrane

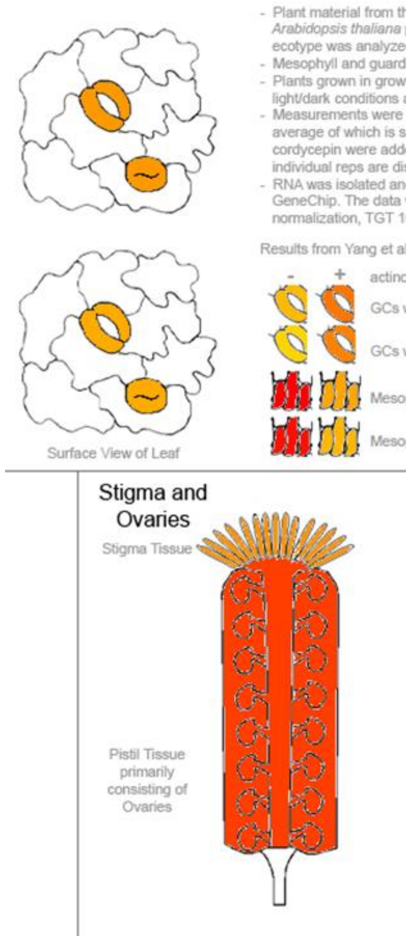
Like other rhomboids, positioned to activate a protein within signalling cascade?

Thompson et al. 2012

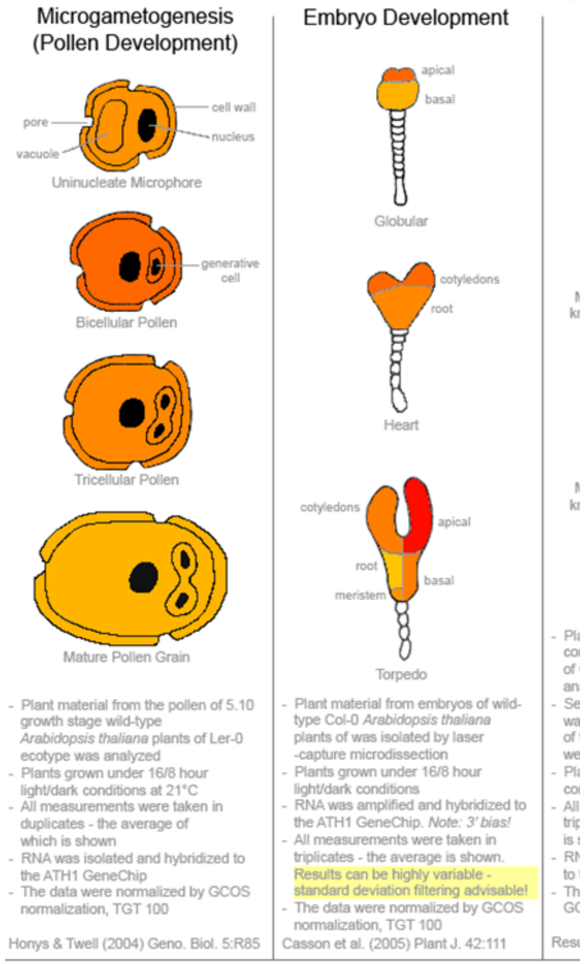




RBL10


- Plant material from th
Arabidopsis thaliana
ecotype was analyze
- Mesophyll and guard
- Plants grown in grow
light/dark conditions
- Measurements were
average of which is s
cordycepin were add
individual reps are di
- RNA was isolated an
GeneChip. The data
normalization, TGT 1

Results from Yang et al

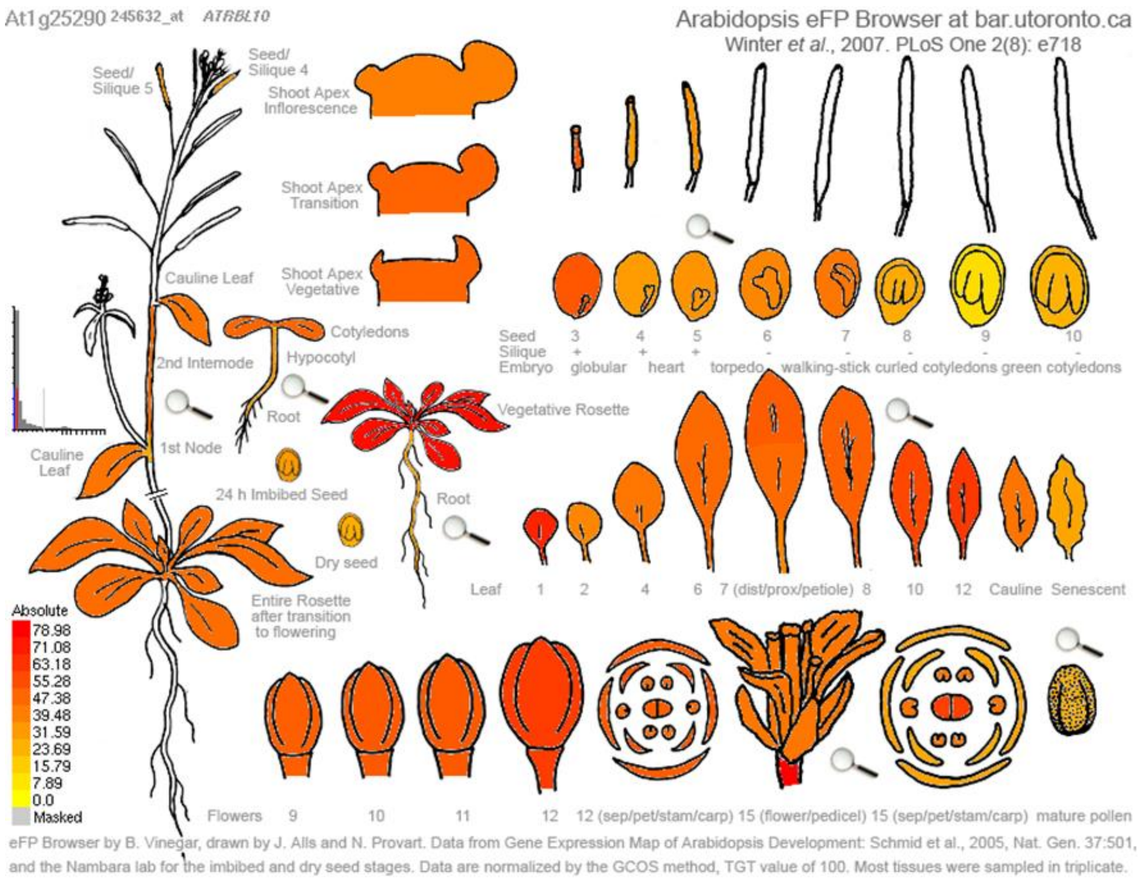


- Plant material from the pollen of 5.10 growth stage wild-type *Arabidopsis thaliana* plants of Ler-0 ecotype was analyzed
- Plants grown under 16/8 hour light/dark conditions at 21°C
- All measurements were taken in duplicates - the average of which is shown
- RNA was isolated and hybridized to the ATH1 GeneChip
- The data were normalized by GCOS normalization, TGT 100

Honys & Twell (2004) Geno. Biol. 5:R85

- Plant material from embryos of wild-type Col-0 *Arabidopsis thaliana* plants of was isolated by laser -capture microdissection
- Plants grown under 16/8 hour light/dark conditions
- RNA was amplified and hybridized to the ATH1 GeneChip. *Note: 3' bias!*
- All measurements were taken in triplicates - the average is shown. Results can be highly variable - standard deviation filtering advisable!
- The data were normalized by GCOS normalization, TGT 100

Casson et al. (2005) Plant J. 42:111



eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

Chloroplast RBL10 transcription in vegetative and floral tissues

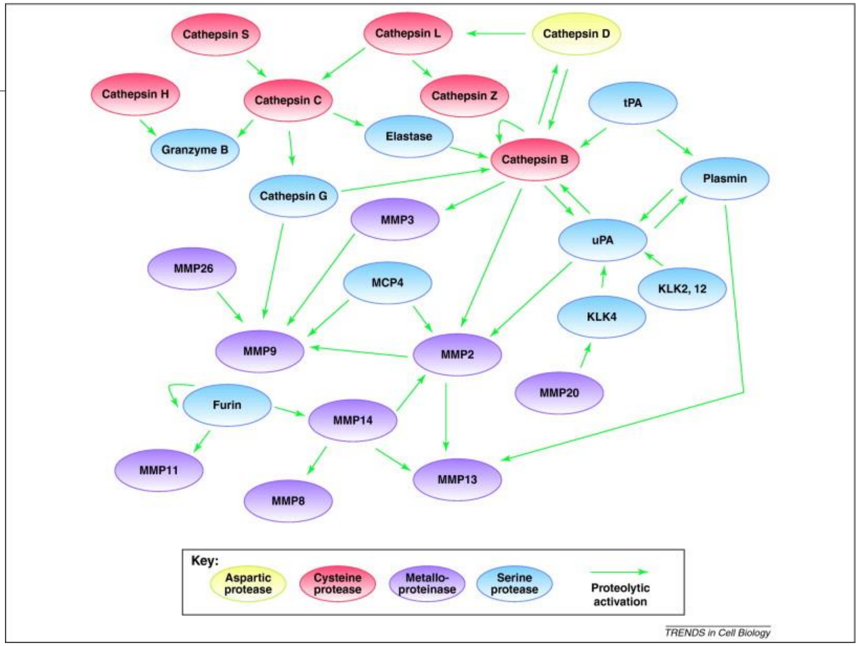
RBL10 floral phenotype

Do rhomboids often act in concert/related pathways?

RhmA, B and D (of four ‘active’ RBDs in Dicty) are in/affect mitochondria

RTPCR suggests overlap

Double A/B mutant is very sick



TRENDS in Cell Biology

Literature relates pathways utilising multiple proteolytic events

- Bacterial stress signalling --DegS and YaeL cleaving RseA
- Mitochondrial apoptotic pathways and stress /unfolded protein response
- Higher euk RBDL4 clipping in ER -> proteasome
- Photosystem II repair: DegP/FtsH sequential cleave events

- “members of the small subfamily of type II transmembrane serine proteases …of particular interest … compartmentalized expression patterns localizing activity to a limited number of cell types… demonstrated roles as direct contributors to cancer progression”
- “tumor-promoting proteases function as part of an extensive multidirectional network of proteolytic interactions”

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RHUL

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Greenwich

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Rebecca Vestal