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:

- Regulated Intramembrane Proteolysis
- Includes the rhomboid family
Subset of mitochondrial rhomboids in eukaryotes

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?

Lemburg & Freeman, 2007
Rhomboid subsets in *Dictyostelium*

Predicted *Dictyostelium* active rhomboids

<table>
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<tr>
<td><strong>Rho like</strong></td>
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<td><em>rhmA</em></td>
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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

Oxygen Consumption

- AX2
- rhm A⁻
ATP luciferase assay

![Graph showing ATP measurement in a bioluminescence assay](image-url)
Where is RhmA? GFP-RhmA in contractile vacuole/ cytoplasmic spots

No co-localisation with Mitotracker red

Reduced Mitotracker uptake in rhmA- (MMP?)
rhmA- mitochondrial morphology defect

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-C CELLS RELEASE CYTC AND HAVE MORE OPEN CRISTAE

REGULATING MITOCHONDRIAL FUSION DYNAMICS IN YEAST
Veg cell RTPCR (no diff in agg cells) - dynamin-like proteins.

Meanwhile...

Pulldown in RhmA-GFP yields band

Some ideas...

Activity assay ongoing in Prague

Transcription levels of dynamin related genes in vegetative cells.
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.
Oxygen Consumption

Time (Min)

O$_2$ (nmol/ml)

AX2
rh_mB$^-$

Amount of ATP measured in bioluminescence assay

mol ATP x 10$^{-8}$

AX2
rh_mB$^-$

ns
Substrate fishing...

RTPCR with dynamin-related proteins
Substrate and activity assays ongoing

RhmA/B double mutant
No growth on bacterial lawns

RhmD essential
RhmcC 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.
A tendency for specialisation?

~15 Arabidopsis rhomboids
G, H mutants
Arabidopsis organellar rhomboid

RBL10-GFP chloroplast rhomboid $\rightarrow$ organelle outer membrane

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

*Thompson et al. 2012*
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

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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