

Dtr1p, a Multidrug Resistance Transporter of the Major Facilitator Superfamily, Plays an Essential Role in Spore Wall Maturation in *Saccharomyces cerevisiae*

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Presented by Amanda Howard
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(SEM Yeast Tetrad)

Spore wall structure & formation

- Diploid a/a yeast cells can form spores that are protective against harsh environmental conditions
- Authors' GOAL:** clarify mechanism of spore wall formation
- Spore wall synthesis:
 - prospore membrane (vesicle-derived) starts to form @ spindle pole bodies during the second meiotic division
 - extends along outer surface of the nuclear envelope
 - each n nucleus covered by prospore membrane by end meiosis II
 - spore wall material deposited in luminal space of prospore membrane
- Mature spore wall:** 4 layers with outermost consisting of dityrosine macromolecule

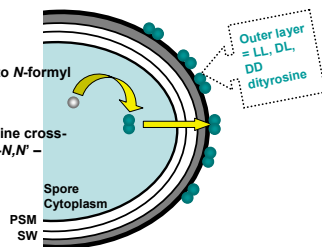
Mature spore wall surface composed of dityrosine macromolecule

1st Step:

free L-tyrosine modified into N-formyl tyrosine (Dit1p)

2nd Step:

2 molecules N-formyl tyrosine cross-linked by Dtr2p forming LL-N,N'-bisformyl dityrosine

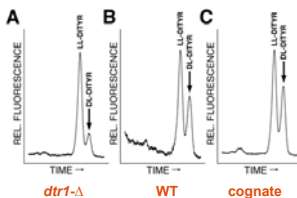


Elucidating the mechanism of spore wall formation: library screen & dityrosine analysis

- HCL liberates LL- & DL- dityrosine (+ precursors) from sporulated yeast**
 - Can be separated by Reverse Phase (RP)-HPLC (dityrosine fluorescent) & determine ratio by peak integration
 - WT strains have fixed ratio of LL- & DL-dityrosine (3:2)
 - Dityrosine epimerized (DL-) in spore wall & soluble precursors are LL-**
 - Thus, deviations in ratio serve as markers for perturbations in spore wall formation
- Yeast deletion library screen**
 - Assayed for change in LL:DL
 - 191 with altered ratio; 1 with strong phenotype = **YBR180w ORF deletion in several strains (DL/LL ratio 0.2 vs. 0.6 in WT)**
 - YBR180w encodes predicted 63.4kDa protein w. 12 TM spans & is homologous to family of drug:H⁺ antiporters (DHA12)**

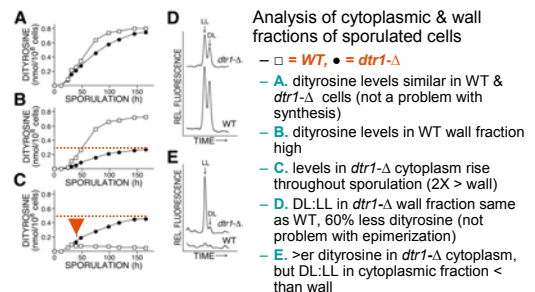
Disruption of *YBR180w* (*DTR1*) leads to decrease in DL-dityrosine levels in asci

- DTR1* disruption causes decrease of DL- in sporulated total cell hydrolysates
- Chromatograms show DL/LL ratios
 - A. *dtr1-Δ* DL/LL ratio 0.2
 - B. WT 0.6
 - C. complementation with *DTR1* restores WT ratio

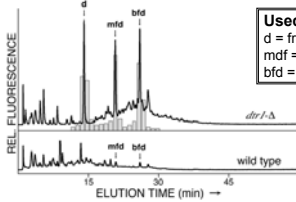


Defect in epimerization or accumulation unincorporated LL-dityrosine in cytoplasm?

dtr1-Δ spores accumulate soluble, unincorporated bisformyl dityrosine



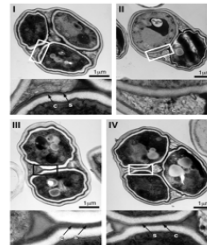
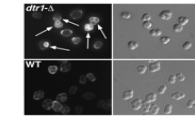
Accumulation of dityrosine-containing compounds in the cytoplasm of *dtr1*-Δ spores



Used gradient RP-HPLC:
d = free dityrosine
mdf = *N*-monoformyl dityrosine
bfd = *N,N*-bisformyl dityrosine

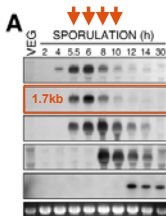
Hypothesis: *Dtr1p* is a dityrosine transporter

dtr1-Δ spores have an aberrant spore surface



- A. Fluorescence microscopy:** fluorescent dye (Calcofluor white) binds 2nd layer (chitosan) = disrupted outer spore layer
 - WT = no fluorescence
 - dtr1*-Δ = mix of WT & aberrant spore surfaces (arrow)
- B. EM (OsO₄ stain):** dityrosine layer (s) electron-dense & chitosan diffuse
 - (I) 25% asci with WT-like spore wall
 - (II) ascus with 1-WT & 1-lacking outer layer (15% all asci had 1 or more)
 - (III & IV) 60% had less electron-dense surface layer
- Confirmed with biochemical tests
- Dityrosine outer layer altered by deletion of *DTR1*

DTR1 expression sporulation-specific

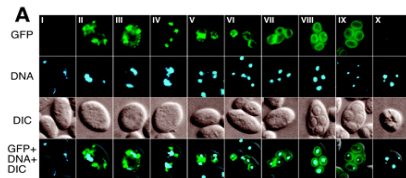


Sporulation-specific genes expressed step-wise through sporulation...

- A. Northern analysis of total RNA (SK1) from different stages of sporulation**
 - compared with marker genes: *NDT80* (delayed early); *SPS1* (middle); *DIT1* (mid-late); *SPS100* (late)
 - DTR1* (early-middle); peak @ 6h
- B. Onset of dityrosine synthesis correlates with max accumulation *DTR1***
 - dityrosine synthesis
 - densitometry *DTR1* expression
- C. *DTR1* expression among *MATa/a*, *a/a*, & *a/a* (northern blot)**
 - NOT expressed in asporogenous cells

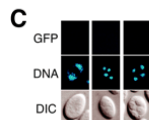
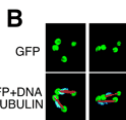
Dtr1p localizes to prospore membrane

Fluorescence microscopy of *DTR1*-GFP, DAPI; DIC; & merge in dityrosine (-) SK1 derivative



- I. vegetative cell
- II. - III. end Meiosis I fluorescent dots are PSM precursors
- IV. 4 foci @ end microtubule* bundles where precursors form PSM
- V. - VIII. *Dtr1p*-GFP outlines immature spores, evenly distributed in PSM throughout sporulation (IX., X)

Dtr1p localizes to prospore membrane



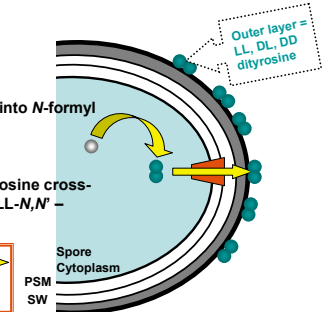
- B. Confirmation that *Dtr1p* localizes to prospore membrane, which form at ends of microtubule bundles (@ meiosis II)**
 - Dtr1p*-GFP co-localizes with microtubules (anti-tubulin = red)
- C. Untagged *DTR1*-transformed cells @ different stages of meiosis (control)**

Hypothesis: *Dtr1p* is a dityrosine transporter

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Dtr1p transports dityrosine to the outer layer of the spore wall



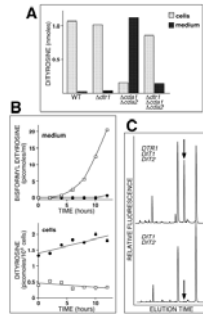
Dtr1p is a dityrosine transporter

A. Dityrosine transport in sporulating cells (cells vs. media)

- *cda1, cda2*-Δ strain (lacks outer 2 layers) = media has 85% dityrosine
- WT, *dtr1*-Δ = trace amounts in media
- *cda1, cda2, dtr1*-Δ = 85% in cells

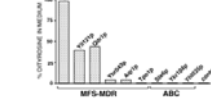
B/C. Dityrosine transport in vegetative cells, which normally lack dityrosine (*DIT1/2* not expressed) (switched promoter to express *DIT1/2* and/or ectopically expressed *DTR1*)

- □ = *DIT1/2* & *DTR1*, ● = *DIT1/2*
- RP-HPLC: bisformyl dityrosine peaks



Substrate specificity of Dtr1p & other MDRPs

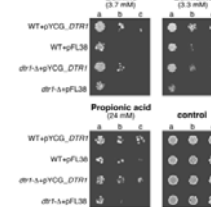
A



A. Is dityrosine transport restricted to *Dtrp*?

- Dtr1p & MDR proteins from MFS & ABC families expressed with *DIT1/2* in vegetative cells
- Measured dityrosine in media by RP-HPLC
- 2 other MFS-MDR (same cluster) proteins transported w. 50% efficiency

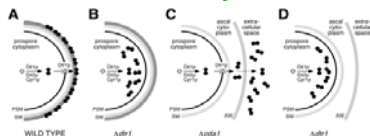
B



B. Is *Dtr1p* a drug R determinant?

- b & c dilutions of cell suspension a
- measured susceptibility of WT & isogenic *dtr1*-Δ strains transformed with *DTR1*- or empty-vectors
- Dtr1p confers R to propionic, benzoic, & butyric acids (preservatives) & quinine & quinidine

Summary



- Spore wall deficient *cda1/2*-Δ strain (dityrosine not epimerized into surface layer): Dtr1p pumps dityrosine into media (C)
- Dityrosine in cells in *cda1/2, dtr1*-Δ strains: Dtr1p absent thus not pumped into media (D)
- *DTR1* mRNA sporulation-specific & max accumulation coincides with prospore membrane formation prior to dityrosine synthesis
- Dtr1p localizes to prospore membrane through spore wall formation
- Dtr1p confers resistance to some toxic compounds

DTR1 1st MFS-MDR protein w/ described normal physiological role