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Supplemental Materials for "Distinct Ceramide Synthases Regulate Polarized Growth in the Filamentous Fungus *Aspergillus nidulans*^D"

Shaojie Li

University of Nebraska - Lincoln

Liangcheng Du

University of Nebraska - Lincoln, ldu3@unl.edu

Gary Yuen

University of Nebraska - Lincoln, gyuen1@unl.edu

Steven D. Harris

University of Nebraska - Lincoln, sharris2@unl.edu

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Distinct Ceramide Synthases Regulate Polarized Growth in the Filamentous Fungus *Aspergillus nidulans*

Mol. Biol. Cell Li et al. 17: 1218

Supplemental Material

The following supporting material pertains to the above article:

Supplemental Figure 1

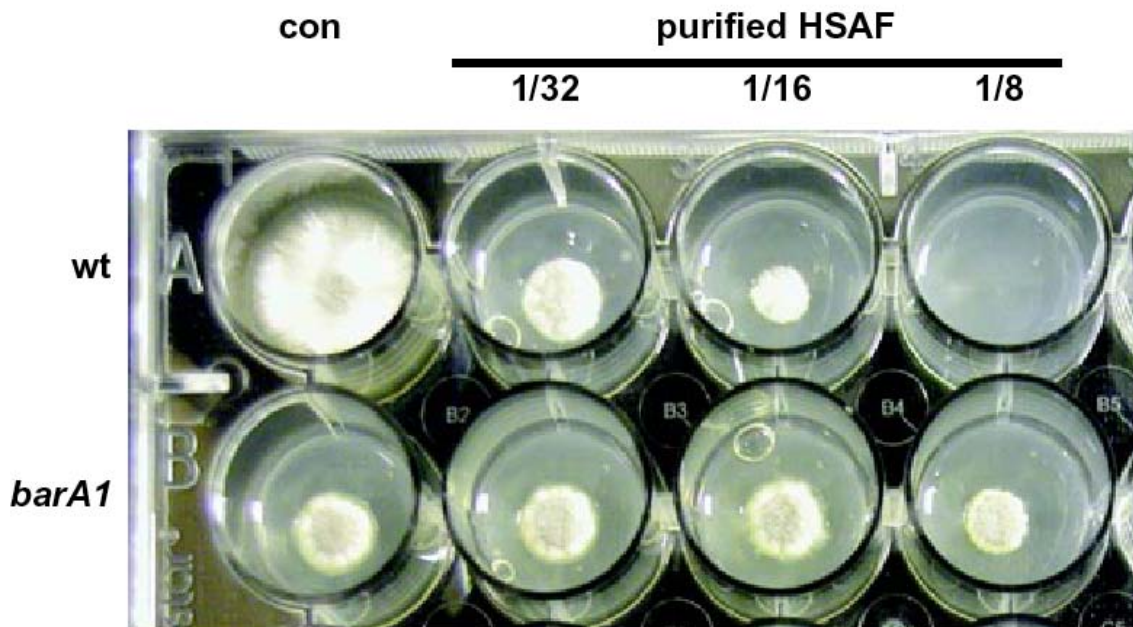


Figure 1 - *barA* mutants are resistant to the effects of HPLC-purified HSAF. Top row; purified HSAF inhibits the growth of wildtype hyphae in a dose-dependent manner. Bottom row, *barA1* mutants display equal growth at all tested doses of purified HSAF. Indicated doses represent dilutions of HPLC-purified HSAF.

Figure 2 (next page) - Alignment of Lag1 homologues. A multiple alignment was generated using ClustalW (MacVector v7.0). Identities are outlined in dark shaded boxes and similarities in light shaded boxes. Protein sequences used for the alignment include: An BarA (*A. nidulans* BarA, accession number: EAA60493); An LagA (*A. nidulans* Lag1 homologue, accession number: EAA64170); Sc Lag1 (*S. cerevisiae* Lag1p, accession number: UO8133); Sc Lac1 (*S. cerevisiae* Lac1p, accession number: NP_012917); and Hs Lag1 (*Homo sapiens* Lag1p homologue, accession number: BC032565). * indicates locations of nonsense mutations in *barA* mutants. The circled * indicates the *barA1* mutation.

Forward Alignment

10 20 30 40 50 60

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

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M I S A T D K S I D R L V W M A X I R R R R M S S V G X I D L G D T V F G F A A M P E S A A S X M E A X X R M X A L I G D S K X
M S S I X P S P S M M L X V R S R P R R X - S S I G X I D L G D T V F S L G T M P E T X E S X T A A X R R M Q R L S E A T K M
M I S L V A R I R R M S S G . . G D T S . P . . M E A S X A . . S .
  
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70 80 90 100 110 120 130

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

```

D T I P R R E W V V S M Q I G I S L I T I L S M L L A V H H L Y - P S L S P Y I T P E P Q L S Y - - - - Y Q P S Q
R S - - - - F L R R F K D I C L K H I T W V L P L L I L I V L L A G Y A V M - P T P S M P L H Y A I P L S Y P E P P K T P G G P
D G - - - - Y G Y P R G R H I L S V P F L A A G I P F V R L L P E - R P I A X P C A L C I G I E D - - - - S G P Y
D S D L L W K V W P S Y R E M Y R H S W L I T P P F I L V C V Y S A Y P L S G M R T E S M P L H M P V A I S Y - - - - Q V D G T
D S D L V X K I W P S P R E I S Y R H A W I A P L M I L I A V Y S A Y P I S G M T T X T M V L H R P V A V S Y - - - - Q I G D T
D S D L X W P P R E . R H W . P L I L . . . . Y . G M T S M P L H P . . S Y P E P P K G
  
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140 150 160 170 180 190

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

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G V Y I Q G W D D I Y F V I S S V F A F T A I R A I A - - M E W V F W P L A R W S G L X R K - - - - A S I R L A E Q G W M W L
V M Y G X G P K D I A F V S F Y M V V L S P I R E P L - - M Q R M I R P F A V Y C G I R G K G - - - - K T A R P M E Q V Y T A I
Q A Q P M A I L E K V F I S I T X Y P D K X R L E G L S X Q L D W M V R X I Q C W F R H R M Q D K P P T I L I X P C E S M W R F T
D S Y A X G I X D L S F V F F Y M I P F I P L R E P L - - M D V V I R P P I V Y L M V I S E H - - - - R Q X R M L E Q M Y A I P
M A Y G X G I M D L C F V F F Y M I P F I P L R E P L - - M D V V I R P P A I R L H V I S K H - - - - R I X R I M E Q M Y A I P
. Y . X G I D . F V . Y M . F I P . R E P L S X Q M D V I R P P A . . . . X D X P P . R . E Q M Y .
  
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200 210 220 * 230 240 250 260

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

```

Y Y A V F W I V G M Y I W S Q S D Y W M - D F K A I W A H W P A R G V S G L M K W Y L L A Q L A F W V Q Q - - I P V I M I E E R R
Y P A I P G P Y G L Y W S R I M I W Y E M I I A M P E G F H R E H E G L F K A Y Y L L Q A S Y W A Q Q A I V L L L Q L E K P R
F Y L C I F C Y G I R F L W S S - P W F W D I R Q C W H V Y P F Q P L S S G L Y H Y Y I M E L A F Y W S L - - M F S Q P I D I X R
Y C G V S G P F G L Y I M Y H S D L W L F X I X P M Y R I Y P V I T M P F L P X I P Y L G Q A A F W A Q Q A C V L V L Q L E X P R
Y T G V S G P F G I Y C M Y H S D L W P P M I X A M Y R I Y P D P T M P F L P X V F Y L G Q A A F W A Q Q A C I L V L Q L E X P R
Y . . V G P . G . Y . M . . S D . W . F I X A M . . Y P . . L P X . Y Y L . Q A A F W A Q Q A C . L V L Q L E X P R
  
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270 280 * 290 300 310 320

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

```

K D H Y Q M L I T H H P I T S C L L I S A X V Y G P Y W V S M V V L M M D I V D L L L P T A X I L X Y L X P E M S C M I A P G V P
K D P R E L V G H H I I T L A L I A L S Y R F H P T Y L G L A V Y I I H D V S D P F L A T S K I L M Y L D A Y I I A - P Y P G V P
K D P L I M F V H H L V T I G L I S F S Y I M M M V R V G T L I M C L H D V S D P L L E A A X L A M Y A X Y Q R L C D I L P V I P
K D Y X E L V F H H I V I L L L I W S S Y V H P T X M G L A I Y I T M D V S D P F L S L S K I L M Y L M S V P I P - P V P G L P
K D H M E L T F H H I V I L L L I W S S Y V H P T X M G L P I Y I T M D V S D P L L S P S K I L M Y L D S G L A P - P S A I P
K D . E L . H H I V I L . L I S S Y V H P T . . G L . I Y I T M D V S D P L L S K I L M Y L . . . . P G . P
  
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330 340 350 360 370 380 390

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

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M V V W A I S R H I M Y P L L C W S I F K D V P A V M P W G C Y S G A T G E L I S I M G Y P D R V M H L P S P F L M I D G P I C M
V C V W I Y L R H P L M L K P F L W A V L I E F R I V G P F - E L M W E I Q Q X K - - - - - - - - - - - - - - - - C W
S A V F M V I R L G I Y P - - P W I L M T I L P S W E I - - - - - I G P Y A - - - - - - - - - - - - - - - - C W
V P P W I Y L R H V V M I R I L W S V L I E F R H E G M Y - V L M F A T I Q Q Y X - - - - - - - - - - - - - - - - C W
W V A W I Y L R H Y I M L K I L W S V L I Q P R I E G M Y - V L M F A T I Q Q Y X - - - - - - - - - - - - - - - - C W
V . V W I Y L R H . . M . . . L W S V L I . P R . G . G L M . A I T Q Q Y X I M G Y P D R V M H L P S P F L M I D G P I C W
  
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400 410 420 * 430 440 450

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

```

M R I I X W I F L S S L L A L Q V L S I I W F S M V I R V A I G V L R I G M A - E D I R S D S E E X D E E E V D P X S - - A A M G
I S - - Q Y I I E A L L A S L Q A V M A F W L P L I L R I L K M Y L F S M I X - K D E R S D E E D E E E E E I E Q S I M S T A A L
S W - - W L L M G L L L I L Q L L H V I W S Y L I A R I A L X A L I R G X V S K D R S D V E S S E E E E D - - - - - - - - - -
I S - - L P I V F V L I A A L Q L V M L Y W L F L I R I L Y R I L V G I Q - K D E R S D S D S E S A E M - - - - - - - - - -
I S - - L P I V F V L I G A L Q L V M L Y W L F L I P R V L Y R I L W R G I L - K D D R S D S S D E E S D E S S T I - - - - -
I S T I . I . P . L L . A I Q L V M . . W L P L I . R I L . . L . G I . S K D . R S D S E S . E E E E . I M S A
  
```

460 470 480 490 500 510 520

An BarA
 An LagA
 Hs LagI
 Sc LagI
 Sc Lact

```

M A I A A E V S S A E W R R M G P S S I R S R R S D R X A L L G R I G C D I R I V
A I G I E P A S L I A R S V M V E X - - - - - R I P H V L V W G Q P V X R
- - - - - V I T C I X S P C D S S S M - - - - - G A N R V M G H M G S Y W A E E
- - - - - E E S X E X C E
- - - - - P I D S I P I X X D I
. I . . . S S R S R R S R . G G . . . E I R
  
```

Formatted alignment

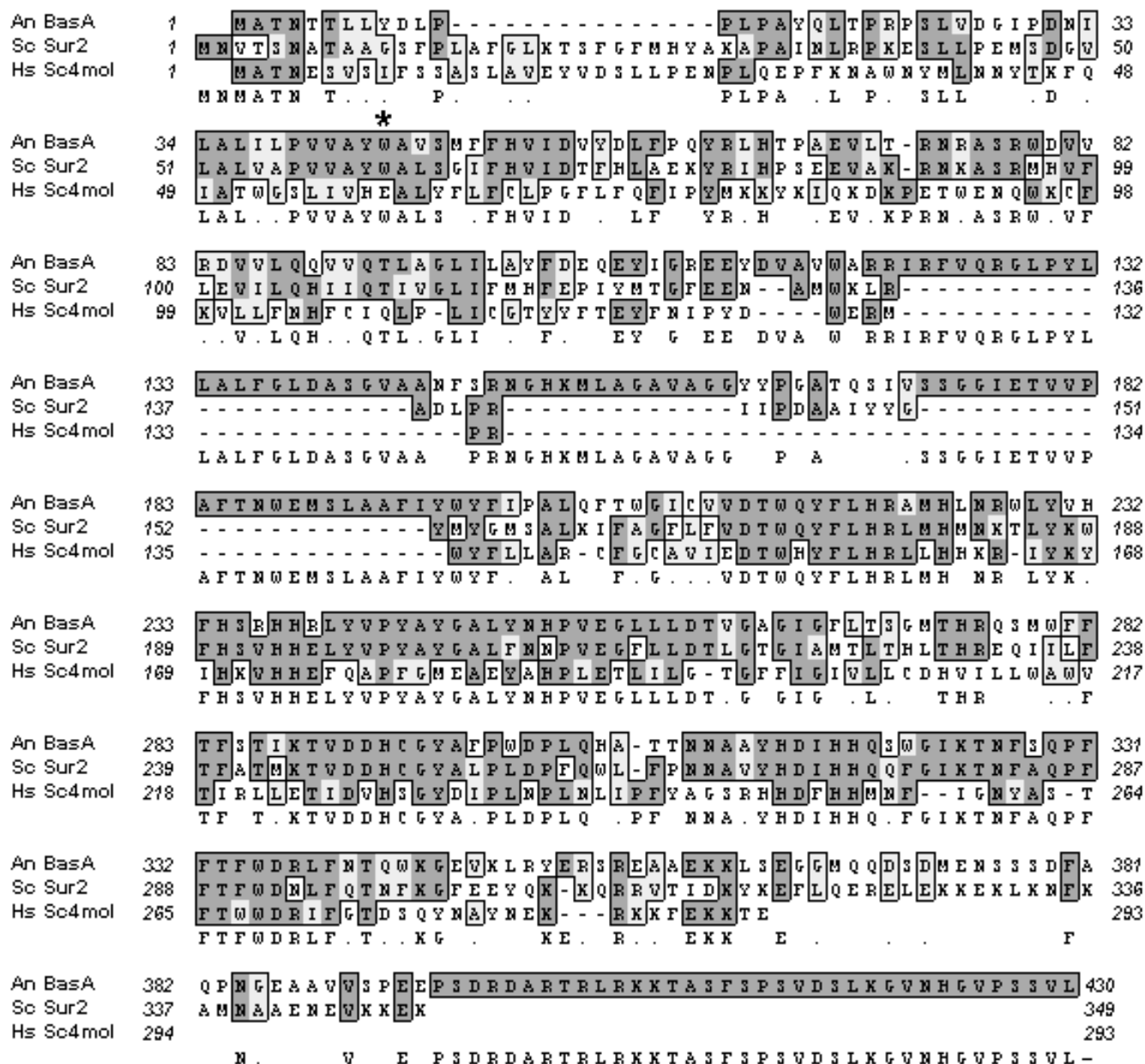


Figure 3 - Alignments of BasA homologues. A multiple alignment was generated using ClustalW (MacVector v7.0). Identities are outlined in dark shaded boxes and similarities in light shaded boxes. Protein sequences used for the alignment include: An BasA (*A. nidulans* BasA, accession number: XM_404777); Sc Sur2 (*S. cerevisiae* Sur2p, accession number: UO7171); Hs SC4MOL (*H. sapiens* methyl oxidase-like protein, accession number: NM_006745). * indicates location of the mutation in *basA1* mutant 8-145.

Supplemental Figure 4

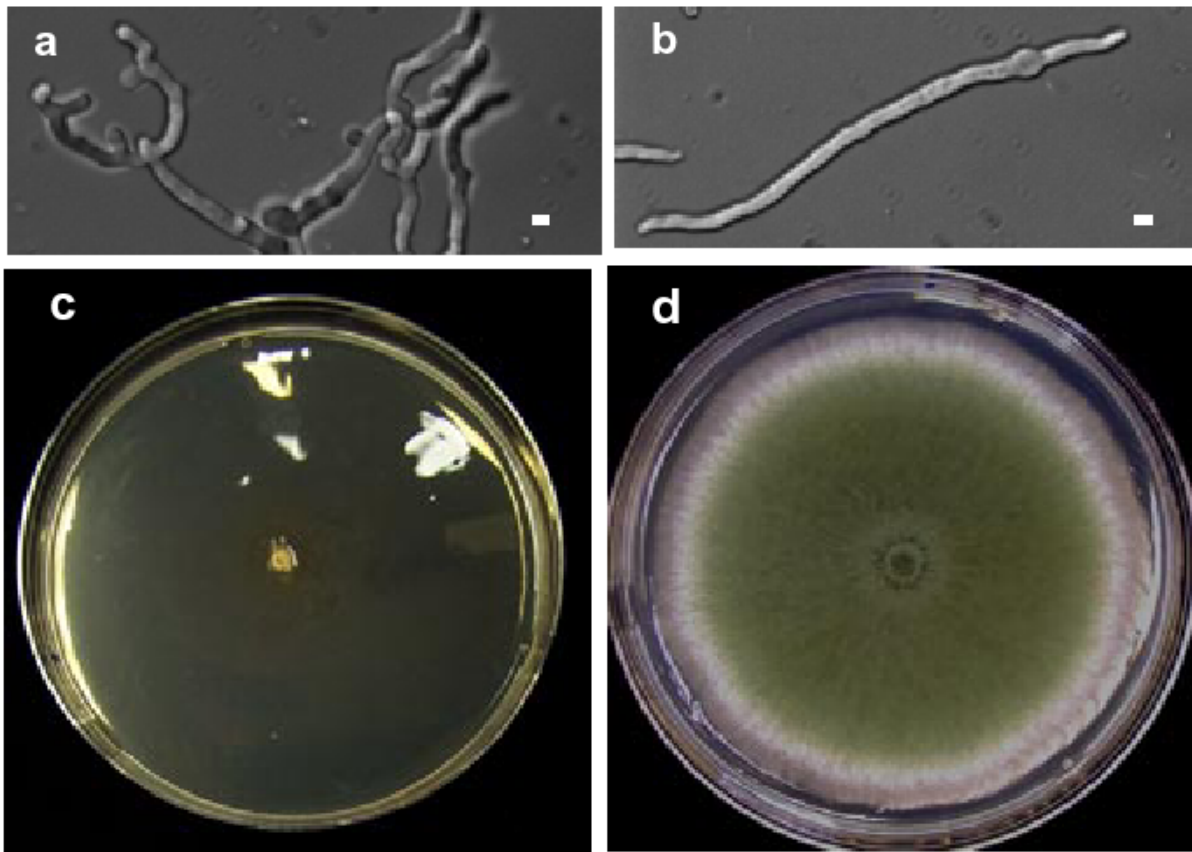


Figure 4 - Growth defects of *lagA* mutants. (A,B) Conidia from *lagA* deletion mutant ASL11 (A) and wildtype strain A28 (B) were germinated in YGV media for 12 h. (C,D) The *lagA* deletion mutant ASL11 (C) and wild type A28 (D) were incubated on MAG plates at 28°C for 10 days. Bar, 3 μ m.