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Aberrant Synthesis of Indole-3-Acetic Acid in *Saccharomyces cerevisiae* Triggers Morphogenic Transition, a Virulence Trait of Pathogenic Fungi

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ABSTRACT

Many plant-associated microbes synthesize the auxin indole-3-acetic acid (IAA), and several IAA biosynthetic pathways have been identified in microbes and plants. *Saccharomyces cerevisiae* has previously been shown to respond to IAA by inducing pseudohyphal growth. We observed that IAA also induced hyphal growth in the human pathogen *Candida albicans* and thus may function as a secondary metabolite signal that regulates virulence traits such as hyphal transition in pathogenic fungi. Aldehyde dehydrogenase (Ald) is required for IAA synthesis from a tryptophan (Trp) precursor in *Ustilago maydis*. Mutant *S. cerevisiae* with deletions in two *ALD* genes are unable to convert radiolabeled Trp to IAA, yet produce IAA in the absence of exogenous Trp and at levels higher than wild type. These data suggest that yeast may have multiple pathways for IAA synthesis, one of which is not dependent on Trp.

THE auxin indole-3-acetic acid (IAA) is best known for its role in plant cell elongation, division, and differentiation (Halliday *et al.* 2009; Moller and Weijers 2009; Sundberg and Ostergaard 2009; Zazimalova *et al.* 2009; Abel and Athanosios 2010; McSteen 2010; Scarpella *et al.* 2010); however, IAA has been identified in numerous plant-associated bacteria (reviewed in Glick *et al.* 1999a,b) and several fungi, including *Rhizopus suinous* (Thimann 1935), Rhizoctonia (Furukawa *et al.* 1996), Colletotrichum (Robinson *et al.* 1998), and yeast (Nielsen 1931; Gruen 1959). Microbial IAA plays a significant role in plant–microbe interactions (Glick *et al.* 1999a), both pathogenic and symbiotic (Hirsch *et al.* 1989; Reineke *et al.* 2008). Plants infected with pathogenic microbes manifest phenotypes consistent with elevated levels of IAA, such as gall formation (a tumor resulting from cellular proliferation) and lengthening of the stem (Viglierchio 1971; Barash and Manulis-Sasson 2009; Stewart and Nemhauser 2009). The interplay between microbial-derived IAA and plant-derived IAA in plant disease is just beginning to be defined.

Exogenous IAA regulates filamentation in *Saccharomyces cerevisiae*, a fungus that is primarily associated with plants, by inducing expression of genes that mediate its morphological transition from a vegetative form to a pseudohyphal or filamentous form (Prusty *et al.* 2004). The fungal transcription factor, Yap1, regulates IAA homeostasis in *S. cerevisiae* (Prusty *et al.* 2004) by downregulating auxin permeases (Avt proteins) that import IAA in *S. cerevisiae* (Prusty *et al.* 2004). We show here that IAA stimulates filamentation in the human pathogen *Candida albicans* and that ***C.*** *albicans* **Yap1** (Cap1) also mediates IAA phenotypes. Filamentation often underlies the development of virulence of *C. albicans*. For example, the *C. albicans* double mutant *cph1*Δ/Δ *efg1*Δ/Δ is defective in the MAP kinase pathway through Cph1, as well as in the PKA pathway via Efg1. This mutant fails to switch from vegetative to filamentous form (Lo *et al.* 1997; Brown *et al.* 1999; Riggle *et al.* 1999; Liu 2001; Sohn *et al.* 2003) and is also avirulent (Dieterich *et al.* 2002). These studies suggest that the secondary metabolite IAA is a chemical signal that regulates fungal pathogenesis.

Plants have multiple pathways to synthesize, inactivate, and catabolize IAA (Delker *et al.* 2008; Lau *et al.* 2008; Normanly 2009). Molecular genetic studies in model systems such as *Arabidopsis thaliana* (reviewed in Normanly 2009), coupled with precise analytical methods (Barkawi *et al.* 2008), have helped expose some redundancy within this network. In fungi, IAA has been generally proposed as a metabolite of tryptophan (Trp) (Hazelwood *et al.* 2008) but this has been conclusively demonstrated only in *Ustilago maydis* (Reneke *et al.* 1988) and *S. uvarum* (Shin *et al.* 1991). Early studies used activity assays or qualitative colorimetric techniques to indicate the presence of IAA. Thin layer

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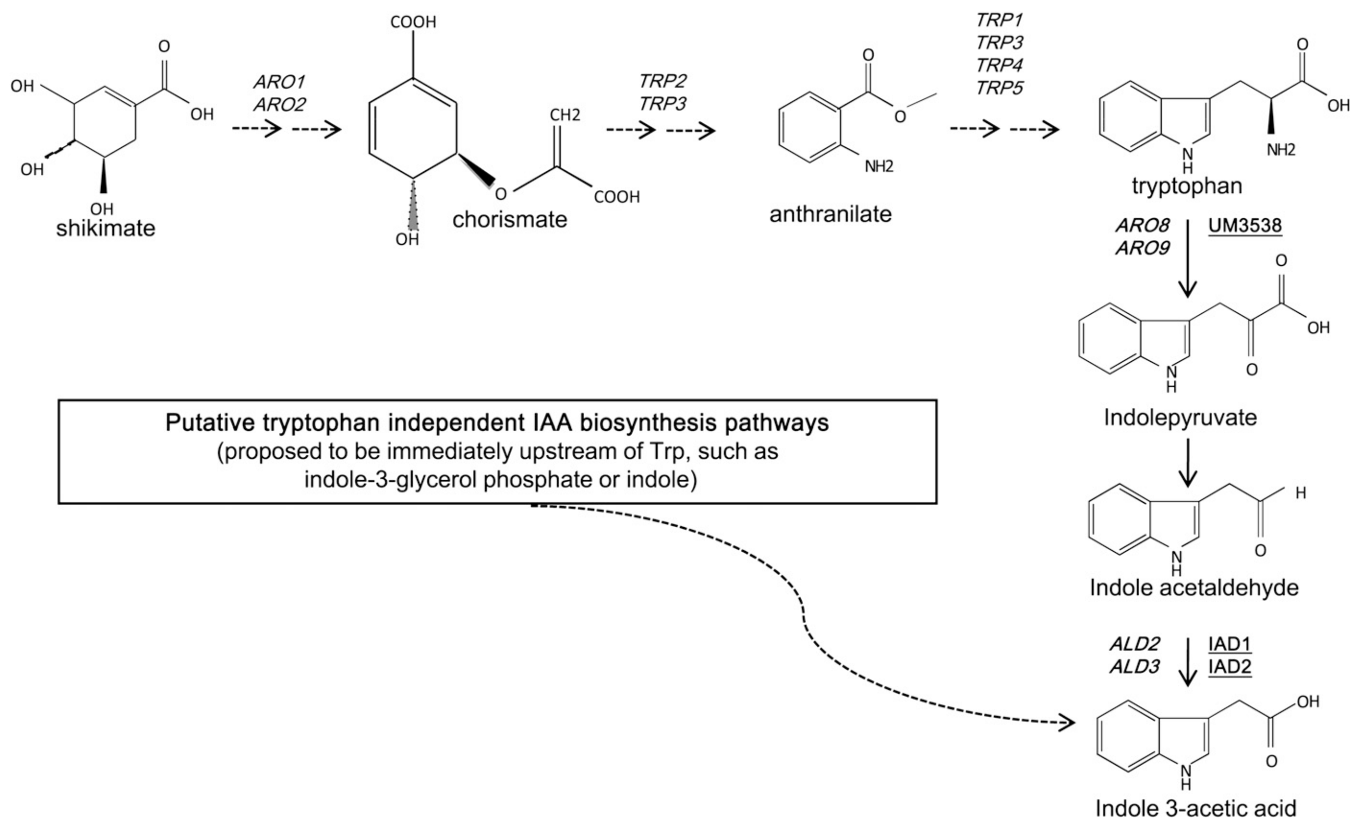

Figure 1.—The IAA biosynthetic pathway identified in this study (in boldface type) and the analogous pathway identified in *U. maydis* (right, underlined) where the homologs of Ald2 and Ald3 have been shown to catalyze the conversion of indole-3-acetaldehyde to indole-3-acetic acid.

chromatography (TLC) and high performance liquid chromatography (HPLC) were subsequently employed for the detection of IAA, where the bioactive compound was shown to chromatograph with authentic IAA. Definitive isotope dilution quantification of IAA was first carried out with [^{14}C]IAA and extracts from *U. zeae* tumors (Turian and Hamilton 1960).

Here, we used gas-chromatography mass spectrometry (GC-MS) coupled with stable isotope dilution to demonstrate that *S. cerevisiae* synthesizes IAA. We identified genes homologous to the aldehyde dehydrogenase that functions in a Trp-dependent IAA biosynthetic pathway in *U. maydis* (Figure 1) (Basse *et al.* 1996; Reineke *et al.* 2008). Our results are consistent with the presence of a Trp-independent IAA biosynthetic pathway in yeast as well.

MATERIALS AND METHODS

Strains, media, and growth conditions: Table 1 lists the strains used in this study. Deletion strains were derived from the yeast-deletion set (Winzeler *et al.* 1999) and subsequently reconstructed by replacement of the relevant ORF with a dominant drug resistance marker (Wach *et al.* 1994). Analytical and phenotypic studies were performed in cognate deletion mutants, made in the Σ1278b background. A [^{14}C]Trp incorporation assay was performed to verify that phenotype observed in the library strain could be recapitulated in the newly constructed Σ1278b strain. Typically three independent transformants were isolated, confirmed by PCR, and used for further studies. Standard culture conditions were used (Sherman *et al.* 1986) and analysis of IAA-associated phenotypes was performed as described earlier (Prusty *et al.* 2004).

[^{14}C]Trp incorporation assay: Yeast strains were grown in 5-ml overnight cultures with aeration at 30° in synthetic complete medium (Sigma, St. Louis) (Guthrie and Fink 1991). To estimate cell density, the absorbance at 600 nm was measured and the culture was adjusted to an OD_{600} of 1 ($\sim 2 \times 10^7$ cfu/ml). Cells (1 ml) were harvested by centrifugation at 3000 rpm for 5 min on an Eppendorf table-top microfuge at room temperature. Cells were washed twice by resuspending pellets in water and then harvested by centrifugation. Cell pellets were resuspended in 200 μl of SD medium supplemented with auxotrophic amino acids (Guthrie and Fink 1991). Samples were incubated with rocking (Thermolyne, speci mix) at 30° for ~18 hr in media containing 400 μm Trp and 0.5 μCi of [^{14}C]Trp (Trp L-[side chain-3-^{14}C], specific activity 50 mCi/mmol; American Radiochemicals). Cells were removed by centrifugation (3000 rpm in an Eppendorf table-top microfuge) at room temperature and the conditioned medium (CM) was transferred to new tubes for TLC. Control samples were prepared identically but without the addition of cells to the SD medium. Ten microliters of the CM was spotted on TLC plates. The [^{14}C]Trp metabolites in the CM were resolved on a silica gel 60 F_{254} (20 × 20 cm, 250 μm thick, precoated) TLC plate (EMD Chemicals). A mixture of 85% chloroform, 14% methanol, and 1% water was used as the eluting solvent. IAA that had incorporated label from [^{14}C]Trp was visualized by autoradiography. Commercially available [^{14}C]IAA (American Radiochemicals) was used as a standard.

TABLE 1

Strains used in this study

Strain	Description	Source
BY4741	*MAT***a**; *his3*Δ1; *leu2*Δ0; *met15*Δ0; *ura3*Δ0	YDL[a]
*ald2*Δ	*ald2*Δ in BY4741	YDL
*ald3*Δ	*ald3*Δ in BY4741	YDL
*ald4*Δ	*ald4*Δ in BY4741	YDL
*ald5*Δ	*ald5*Δ in BY4741	YDL
*ald6*Δ	*ald6*Δ in BY4741	YDL
*ald2*Δ *ald3*Δ	*ald2*Δ *ald3*Δ in BY4741	This study
Σ1278b	*MAT***a**; *ura3*Δ0	J. Heitman, Duke University
*ald2*Δ/Δ *ald3*Δ/Δ	*ald2*Δ *ald3*Δ in Σ1278b, *MAT***a**/α	This study
Caf2-1	*Candida albicans* wild type	G. Fink, MIT
*cph1*Δ/Δ	Homozygous *cph1*Δ in Caf2-1	G. Fink, MIT
*efg1*Δ/Δ	Homozygous *efg1*Δ in Caf2-1	G. Fink, MIT
*cph1*Δ/Δ *efg1*Δ/Δ	Homozygous *cph1*Δ *efg1*Δ in Caf2-1	G. Fink, MIT
*cap1*Δ/+	Heterozygous *cap1*Δ in Caf2-1	M. Raymond, University of Montreal
*cap1*Δ/Δ	Homozygous *cap1*Δ in Caf2-1	M. Raymond, University of Montreal

[a] Yeast Deletion Library.

To screen the yeast deletion set, this assay was adapted for use in 96-well microtiter dishes by scaling down the reaction volume to 50 μl containing 0.1 μCi [^{14}C]Trp.

Quantification of IAA from yeast: To confirm that IAA was present in the CM, 5-ml cultures were harvested and stored at −80°. The supernatants were thawed on ice and 38.4 ng of [$^{13}C_6$]IAA (99 atom%, Cambridge Isotope Laboratories) in 10 μl of 2-propanol was added as an internal standard. Additionally, 500 μl of 0.2 m imidazole (pH 7.0) was added. The sample was mixed and left to equilibrate on ice for 1 hr. The sample was loaded onto a 200-mg NH_2 solid phase extraction (SPE) column (aka amino columns, Alltech) that was preconditioned with sequential applications of 2 ml each hexane, acetonitrile, and water and 0.2 m imidazole (pH 7.0) followed by 6 ml of water on a vacuum manifold (Fisher Scientific, Pittsburgh, PA). After loading the sample, the column was allowed to aspirate under vacuum for an additional 30 sec at 3–5 psi. Next, the column was washed with sequential additions of 1 ml each of hexane, ethyl acetate, acetonitrile, and methanol. IAA was eluted in ∼6 ml of methanol that was 5% acetic acid. Dried samples were resuspended in 1.3 ml of a mixture (∼6:1 to reach a pH between 3 and 3.5) of 0.25% phosphoric acid and 0.1 m succinic acid, pH 6.0. The sample was placed in a 2-ml capacity 96-well plate and subjected to an additional SPE step with polymethymethacrylate epoxide resin, using a Gilson 215 SPE automated liquid handler (ALH) as described in Barkawi *et al.* (2008). The epoxide SPE column eluate was transferred to 2-ml amber vials, and ∼1 ml of ethereal diazomethane (prepared as described in Cohen 1984) was added. After 5 min incubation at room temperature, the sample was dried to a residue under a stream of N_2 gas in a 45° sand bath. The methylated IAA was resuspended in 45 μl of ethyl acetate and subjected to GC-MS analysis as described in Barkawi *et al.* (2008), except that a full scan spectrum was obtained. For mutant analysis this protocol was scaled down to 1-ml cultures containing the same amount of [$^{13}C_6$]IAA internal standard but only 0.2 ml of 0.2 m imidazole, pH 7.0.

RESULTS

S. cerevisiae **secretes IAA:** To confirm that *S. cerevisiae* synthesizes IAA, we analyzed CM from yeast cultures. Thin layer chromatography of CM from *S. cerevisiae* grown in the presence of [^{14}C]Trp revealed a radiolabeled product that comigrated with commercially available [^{14}C]IAA (Figure 2A). UV shadow of the fluor-impregnated TLC plate showed a UV absorbing compound with the same retention profile as the pure unlabeled IAA that was used as a standard (data not shown but position marked with asterisk in Figure 2A). GC-MS analysis of IAA that was extracted from the CM along with [$^{13}C_6$]IAA internal standard and methylated for GC analysis (Figure 2, B–D) confirmed the presence of IAA in the CM. Figure 2B (left) shows the total ion chromatogram (TIC) of pure methyl (Me)-IAA, which shows a GC retention time for authentic Me-IAA to be between 7.322 and 7.380 min. Figure 2B (right) shows the full scan spectrum corresponding to this retention time. The predominant ions for pure Me-IAA (also shown in Figure 2C, top) are m/z 189 (intact Me-IAA, aka molecular ion) and mass to charge ration (m/z) 130 (fragment ion). The observed fragment ions of m/z 103 and 77 were consistent with pure Me-IAA as well, but were lower in abundance and not typically used for quantification. Figure 2C (bottom) shows the molecular and fragment ions for Me-[$^{13}C_6$]IAA. Figure 2D (left) shows the TIC of IAA that was extracted from 5 ml of CM (to which [$^{13}C_6$]IAA had been added), methylated, and run on GC-MS. The four predominant ions for Me-IAA and Me-[$^{13}C_6$]IAA are shown. Figure 2D (bottom) shows the full scan spectrum for the retention time that corresponds to authentic Me-IAA, demonstrating that yeast secretes IAA.

The accumulation of IAA in the CM reached its highest level after cultures entered stationary phase. To correlate the production of IAA with cell density, cells from a high-density culture (10^8 cells/ml) were diluted to either low (5×10^5 cells/ml) or high density (5×10^7 cells/ml) in fresh medium. IAA secreted into

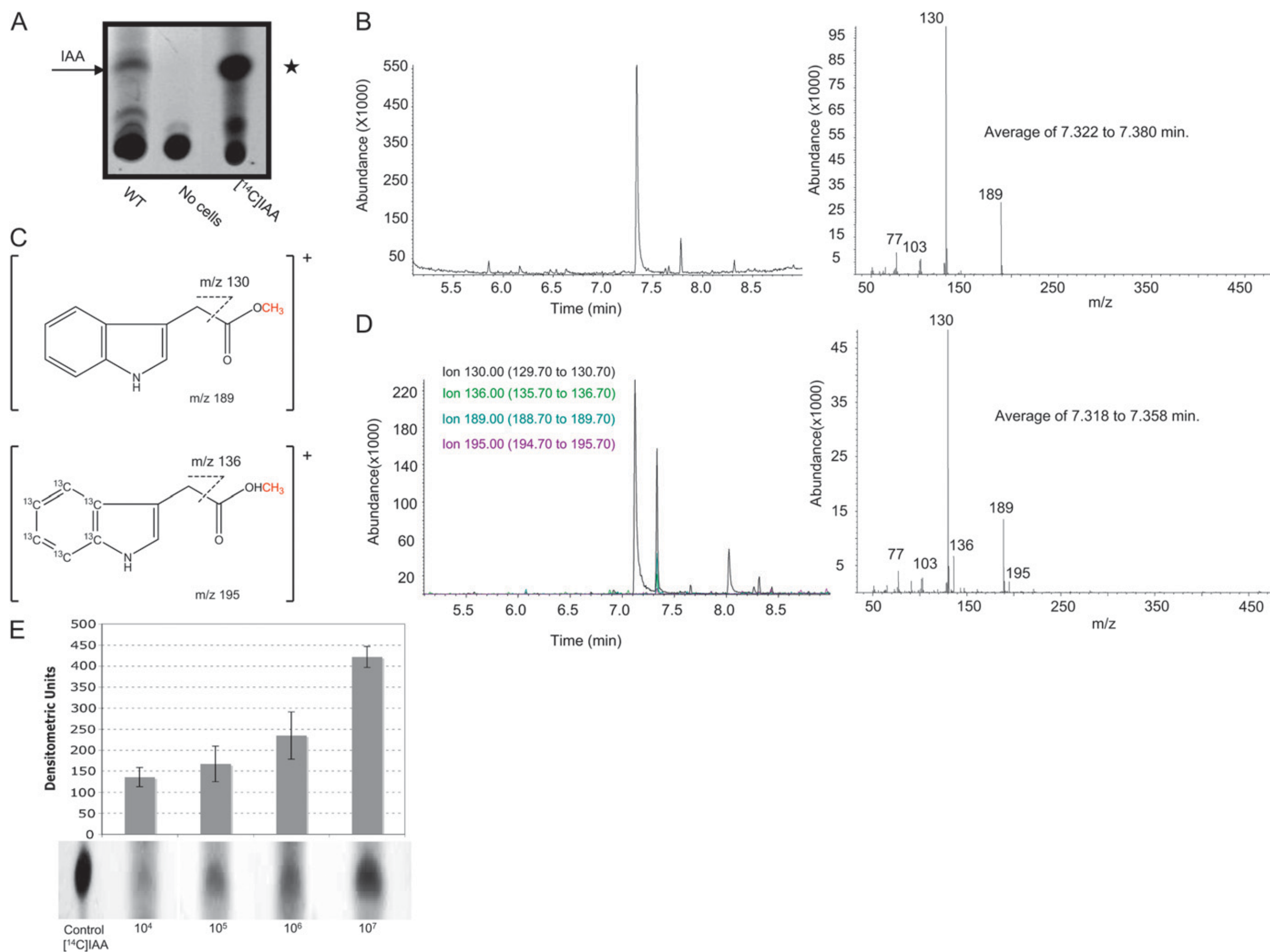

FIGURE 2.—(A) *S. cerevisiae* produces a molecule that comigrates with commercially available IAA. Wild-type yeast cells were incubated with [^{14}C]Trp, and products of the conditioned media were resolved by thin layer chromatography (TLC). Commercially available IAA and [^{14}C]IAA were used as controls. The position (marked with the asterisk) of the nonradiolabeled IAA control was determined by UV shadowing. (B) Total ion chromatogram (TIC, left) and full scan spectrum (right) of authentic methyl-IAA. (C) Top, methyl-IAA molecular ion m/z 189 and fragment ion m/z 130 (the site of fragmentation to form the fragment ion is indicated by dashed lines). Bottom, methyl-[$^{13}C_6$]IAA molecular ion m/z 195 and fragment ion m/z 136. For each compound, the derivatization moiety (the methyl group) is shown in red. (D) TIC (left) and corresponding full-scan spectrum (right) of IAA (methylated prior to GC-MS analysis) that was purified from the culture medium of wild-type yeast that had been grown in the presence of Trp. The TIC shows four selected ions; m/z 130 and m/z 189 are the fragment ion and the molecular ion, respectively, of endogenous IAA (methylated prior to GC-MS analysis). Ions with m/z 136 and 195 are the fragment ion and the molecular ion, respectively, of [$^{13}C_6$]IAA (methylated prior to GC-MS analysis) that was added to the yeast culture medium supernatant prior to extraction of IAA. The large peak in the TIC (left) with a retention time of ~7.15 min was determined to be tryptophol by full-scan spectra analysis (not shown). (E) The CM taken from a high-density culture contained a much greater concentration of IAA than CM from a low-density culture as determined by TLC (bottom) and densitometry of the autoradiograph (top).

the medium was assessed by TLC (Figure 2E). After normalizing for the difference in cell number, we found that CM taken from a high-density culture contained more IAA than CM from a low-density culture (Figure 2E), indicating that IAA accumulation is directly proportional to cell density. In *S. cerevisiae*, IAA is perceived, is imported, and stimulates diploid pseudohyphal growth and haploid invasive growth by regulating the cell surface glycoprotein Flo11. Together these studies suggest that IAA accumulates in the growth environment of yeast where it may act as a chemical signal that regulates virulence traits.

A genomic scale screen for IAA homeostasis mutants: To identify genes involved in IAA synthesis, specifically the conversion of Trp to IAA, we initiated an unbiased, systematic genomic screen of the yeast deletion library (Brachmann *et al.* 1998; Winzeler *et al.* 1999). The haploid deletion library in *S. cerevisiae* consists of ~4940 clones representing every single viable gene disruption. A [^{14}C]Trp incorporation assay was

developed and optimized to facilitate a large-scale screen using microtiter plates. An aliquot of the CM from each reaction was loaded onto a TLC plate and components of the CM were resolved and compared with a ^{14}C-IAA standard. A total of 1425 deletion strains (29% of the library) have been screened to date. A secondary screen was performed in triplicate on putative mutants and related gene families, using the [^{14}C]Trp incorporation assay.

This screen identified three genes, *ALD2*, *ARO9*, and *ADH2*, representing families of particular interest with respect to IAA biosynthesis in yeast: the **ALD**ehyde dehydrogenases, the **ARO**matic transaminases, and the **A**lcohol **D**e**H**ydrogenases (Figure 1). In *S. cerevisiae*, the aromatic transaminases Aro8 and Aro9 have been implicated in the conversion of Trp to indole pyruvate (IPA) (Chen and Fink 2006). As expected, *aro8*Δ and *aro9*Δ mutants show decreased conversion of labeled Trp to labeled IAA compared to the cognate wild type but are not the focus of this study (data not shown). Alcohol dehydrogenases are proposed to convert indole acetaldehyde (IAAld) to indole-3-ethanol (aka tryptophol) (Chen and Fink 2006). Interestingly, *adh2*Δ, identified in the screen, was the only member of the *ADH* family to show decreased [^{14}C]IAA accumulation (data not shown). One explanation for this result is that Adh2 preferentially catalyzes the conversion of ethanol to acetylaldehyde. Therefore *adh2*Δ mutants are unable to convert indole-3-ethanol to IAAld, ultimately leading to decreased IAA accumulation. Deletion mutants of members of the *ALD* family accumulated lower levels of radioactive IAA from radioactive Trp than did wild type. We focused our study on the aldehyde dehydrogenase (*ALD*) genes hypothesized to catalyze the ultimate step in the production of IAA and set out to test whether altering IAA production affects filamentation. Multiple sequence alignment and phylogenetic analysis (data not shown) indicate that *S. cerevisiae* Ald2 and Ald3 share identity with *U. maydis* Iad1. Ald2 and Ald3 are nearly identical to each other and have 50% (Ald3) and 49% (Ald2) protein sequence identity with *U. maydis* Iad1, a NAD-dependent aldehyde dehydrogenase. Ald2 and Ald3 have less sequence identity with the NADH-dependent aldehyde dehydrogenases such as lez O (data not shown). Single and double deletions of the *ALD* genes showed decreased IAA production from [^{14}C]Trp when compared with wild-type cells on TLC (Figure 3). These results together with previous enzymatic studies in *U. maydis* (Reineke *et al.* 2008) suggest that these genes are involved in IAA synthesis. *ALD2* and *ALD3* are also required for synthesis of a nonproteinogenic amino acid, β-alanine in *S. cerevisiae* (White *et al.* 2003).

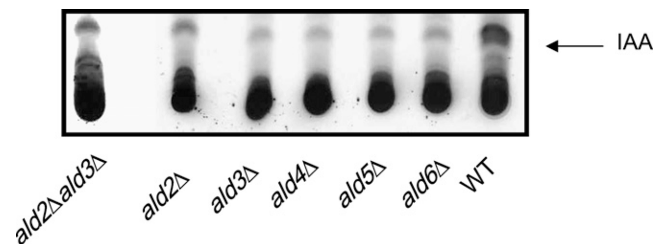

Figure 3.—Products of the CM of *ald* single deletion mutants and *ald2*Δ*ald3*Δ double deletion incubated with [^{14}C]Trp were resolved by TLC and compared to the isogenic wild-type strain. Each experiment was performed a minimum of three times. Three and two independent transformants were tested for the single and double mutants, respectively. One representative transformant for each mutant is shown.

The *ald2*Δ*ald3*Δ deletion mutant exhibits virulence traits: IAA regulates dimorphic transition in *S. cerevisiae* by inducing adhesion and filamentation (Prusty *et al.* 2004). The ability of a fungus to perceive a small molecule signal that causes it to differentiate into an invasive form has important implications for host–pathogen interactions. To test the hypothesis that mutants with aberrant IAA accumulation also affect dimorphism, we examined diploid filamentation and haploid invasive growth in all *ald* single mutants and selected combinations of double mutants. The *ald2*Δ*ald3*Δ double mutant demonstrated increased filamentation (Figure 4A) and invasive growth (Figure 4B) as compared wild type. We also tested a previously reported growth inhibition phenotype associated with exposure to IAA (Figure 4C). This IAA-associated growth inhibition phenotype exhibits a direct proportionality between IAA concentration and growth inhibition. Deletion of both *ald2* and *ald3* caused an increase in sensitivity to IAA (Figure 4C, right) whereas single deletion of an *ALD* gene did not affect IAA sensitivity when compared with wild-type cells (data not shown). Together, these data suggest that a perturbation in the IAA secretion profile alters substrate adhesion and filamentation of *S. cerevisiae*. However, these phenotypes are consistent with *ald2*Δ*ald3*Δ mutants producing more IAA than isogenic wild-type strains.

The *ald2*Δ*ald3*Δ mutant uncovers an IAA biosynthetic pathway that is independent of exogenous Trp: The *ALD* genes were identified on the basis of a radiolabeled [^{14}C]Trp incorporation assay. IAA accumulation in the CM of the double mutant was quantified using GC-MS and [$^{13}C_6$]IAA as an internal standard. These measurements revealed that the CM of the *ald2*Δ*ald3*Δ deletion mutant contained fourfold more IAA (240.3 ng/ml ± 71.9 ng/ml) than the wild type (59.8 ng/ml ± 3.8 ng/ml). The amount of IAA present in the conditioned media is adequate to induce filamentation in an *in vitro* plate assay. Together these analytical data correlate well with the phenotypic data, suggesting that the *ald2*Δ*ald3*Δ double mutant makes more IAA and thus exhibits enhanced virulence traits as compared to its wild-type counterpart.

While the radiolabeled [^{14}C]Trp incorporation assay detects the pool of IAA synthesized from labeled Trp,

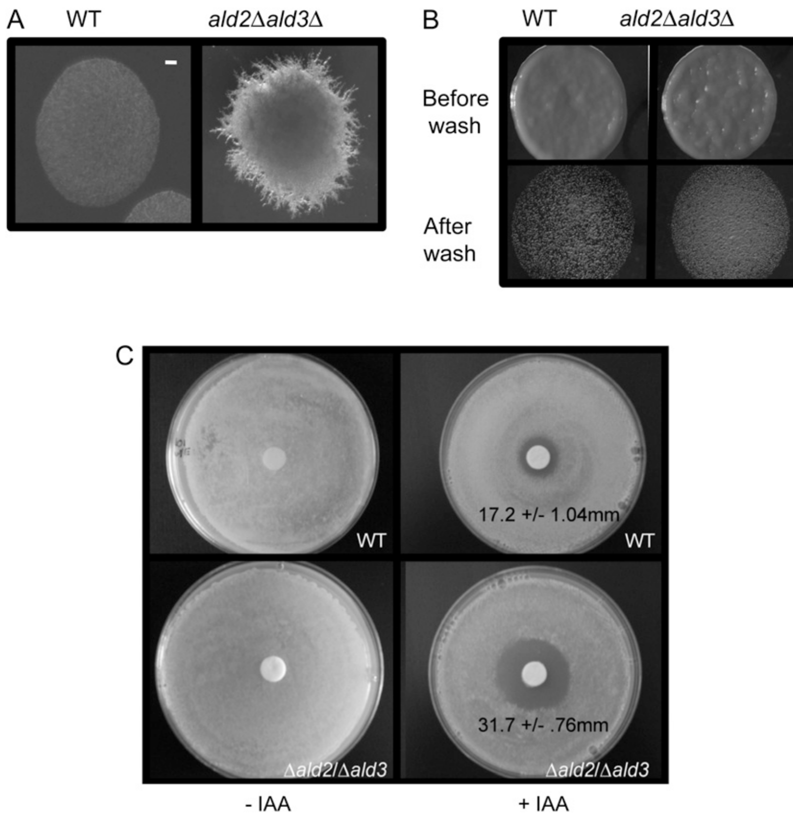

FIGURE 4.—(A) A representative diploid *ald2*Δ/Δ *ald3*Δ/Δ colony was grown on filamentation-inducing media and photographed after 3 days of growth (bar, 1 μm). (B) Haploid *ald2*Δ*ald3*Δ strains were spotted onto SC media and washed. Before wash, unwashed plates; after wash, the plates after washing. (C) A filter disk saturated with IAA (right) was placed on a lawn of *ald2*Δ*ald3*Δ mutant cells (bottom) and compared to the wild-type cells (top). Control disks (left) do not contain IAA. Plates were incubated for 3 days in the dark. The clear area around the IAA-containing filter disks indicates a zone of growth inhibition.

the GC-MS analysis allowed us to detect any unlabeled (endogenous) IAA that was present. We grew the *ald2*Δ*ald3*Δ double mutant in the absence of exogenous Trp and quantified IAA from the CM using GC-MS. These measurements revealed that the *ald2*Δ*ald3*Δ mutant was able to synthesize a modest amount of IAA (9.48 ng/ml ± 0.22 ng/ml) in the absence of exogenous Trp. Wild-type yeast also produced similar amount of IAA in the absence of Trp (9.81 ng/ml ± 0.77 ng/ml).

IAA induces filamentation in *C. albicans*: The effects of the secondary metabolites identified in fungi appear to be largely species specific (CHEN and FINK 2006). Previous work suggests that IAA induces invasive growth in *S. cerevisiae* (PRUSTY *et al.* 2004). To test whether the IAA effects could cross species barriers, we exposed wild-type *Candida albicans*, a human pathogen, as well as attenuated mutants in the mitogen-activated protein (MAP) kinase and the cAMP-dependent protein kinase pathways (Figure 5) to IAA. The *cph1*Δ/Δ *efg1*Δ/Δ double mutant, which fails to switch from the vegetative to the filamentous form, was filamentous in the presence of IAA (compare Figure 5A with 5E). The single mutants *efg1*Δ/Δ or *cph1*Δ/Δ that normally show reduced filamentation also showed a robust filamentation when exposed to IAA (compare Figure 5B with 5F and 5C with 5G). Wild-type strains also filamented more when treated with IAA as compared to untreated cells (compare Figure 5D and 5H). These results indicate that IAA enhances filamentation of the human pathogen *C. albicans*. Furthermore, the IAA-mediated filamentation signal does not require components of the MAPK or PKA pathways. The *cph1*Δ/Δ *efg1*Δ/Δ double mutant, which is nonfilamentous under standard laboratory conditions and avirulent in mice, filaments in the oral cavity of immunosuppressed piglets and when embedded in agar (RIGGLE *et al.* 1999). Together these results suggest that IAA-mediated filamentation in *C. albicans* occurs via an Efg1p- and Cph1p-independent mechanism and confirm prior findings of the existence of an alternate filamentation pathway in *C. albicans*.

To test if other aspects of IAA regulation in *S. cerevisiae* were also conserved in *C. albicans*, we tested Cap1, the *C. albicans* homolog of Yap, for its sensitivity to IAA. The amino acid auxin permeases genes are upregulated in the *yap1* mutant, which is sensitive to growth on IAA because it retains more IAA (PRUSTY *et al.* 2004). Heterozygous and homozygous deletion mutants of *CAP1* (ALARCO and RAYMOND 1999) (obtained from M. Raymond, University of Montreal) to grew less well on media containing IAA as compared to the isogenic wild type (Figure 5I), suggesting that the *cap1*Δ/Δ mutant was more sensitive to IAA. The heterozygous mutant, *cap1*Δ/+ exhibited an intermediate sensitivity to IAA as compared to the wild-type *CAP1*+/+ strain or the homozygous *cap1*Δ/Δ deletion strain. These results suggest that *cap1* mutants are hypersensitive to IAA, further supporting our hypothesis that the molecular

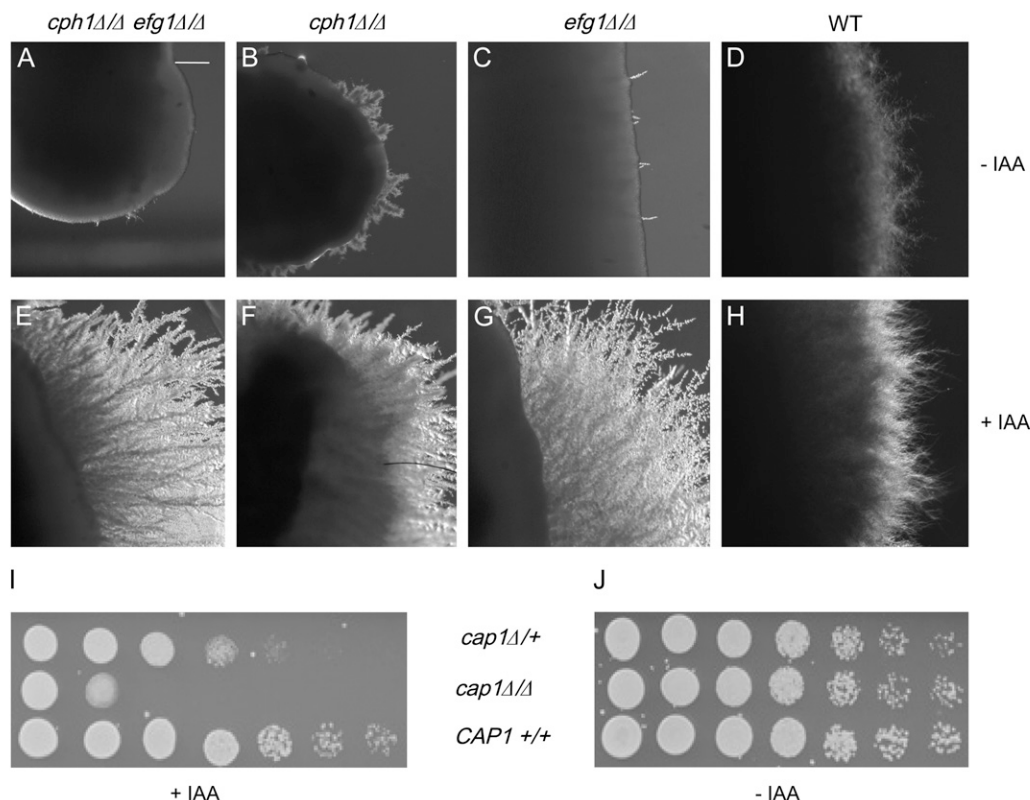

Figure 5.—The human pathogen *Candida albicans* was exposed to IAA [experimental plates (E–H) contain 50 μm IAA and control plates (A–D) contain no IAA; bar in A, 10 μm]. A–H show the edge of a patch of *C. albicans* (A and E, *cph1efg1*; B and F, *cph1*; C and G, *efg1*; and D and H, isogenic wild-type control strains) growing on synthetic low ammonium media with xylose as a carbon source. Plates were incubated in the dark to prevent photodegradation of IAA. I and J show the IAA sensitivity profile of a *cap1* homozygous deletion mutant as compared to an isogenic wild-type and a heterozygous mutant [experimental plates (I) contain 120 μm IAA, and control plates (J) contain no IAA].

mechanism of IAA response is likely to be conserved between *S. cerevisiae* and *C. albicans*.

DISCUSSION

The quantitative GC-MS analysis in this study confirmed that *S. cerevisiae* synthesizes and secretes IAA into the culture environment where it is available to function as a signal that regulates filamentation. Filamentation is a pathogenic trait because it contributes directly to virulence of pathogenic fungi like *C. albicans*. Pathogenic bacteria and fungi are known to produce IAA, but a direct link to pathogenicity has not been demonstrated in these pathogens.

IAA is a small molecule capable of stimulating the developmental transition from the vegetative yeast form to the filamentous form in *S. cerevisiae* (Prusty *et al.* 2004). The current study provides strong support for a connection between fungal dimorphism and IAA synthesis, because the *ald2*Δ*ald3*Δ strain that accumulates more IAA is also more filamentous. IAA was also able to stimulate dimorphic transition in the human pathogen *C. albicans*. Deletion of a key regulator of the IAA responses had the same effect in both organisms. Homologs of enzymes that transport and synthesize IAA in *S. cerevisiae* are present in *C. albicans*. We suggest that IAA is an important signal that triggers dimorphic transition—a virulence trait.

A genomic scale screen for IAA homeostasis mutants implicated the aldehyde dehydrogenases, Ald2 and Ald3 in the final step of IAA synthesis from Trp. Ald2 and Ald3 share significant sequence similarity with Iad1, the *U. maydis* aldehyde dehydrogenase that has been shown to catalyze the conversion of IAAld to IAA (Basse *et al.* 1996; Akamatsu *et al.* 2000; Mizuno *et al.* 2006; Pigeau and Inglis 2007; Reineke *et al.* 2008). The *ALD* genes are responsible for acetate formation during anaerobic fermentation (Saint-Prix *et al.* 2004; Pigeau and Inglis 2007) and are hence of interest to the brewing industry. They have previously been implicated in mediating a variety of stress responses and are regulated by general-stress transcription factors Msn2 and -4 (Miralles and Serrano 1995; Navarro-Avino *et al.* 1999; Aranda and del Olmo 2003). Ald activity is required in the synthesis of two amino acid derivatives, IAA and β-alanine in *U. maydis* and *S. cerevisiae*, respectively (White *et al.* 2003; Reineke *et al.* 2008). This screen identified other members of the pathway (Aro9), which has previously been implicated in the first step of IAA synthesis (Chen and Fink 2006). In the process of characterizing mutants in a Trp-dependent IAA synthesis pathway, we uncovered another pathway that did not rely on exogenous Trp for IAA biosynthesis. Trp-independent synthesis of IAA has been demonstrated in several plant species, but the intermediates, intermediate steps, and genes involved in this pathway remain undefined (Woodward and Bartel 2005; Normanly 2009). The observation that *S. cerevisiae* has an analogous pathway provides a much simpler system to employ in the characterization of Trp-independent IAA synthesis.

There is precedence for multiple IAA biosynthetic pathways in microbes, particularly plant-associated bacteria (Clark *et al.* 1993; Costacurta and Vanderleyden 1995; Glick *et al.* 1999b; Lambrecht *et al.* 2000). An interesting example of differential utilization of multiple IAA biosynthetic pathways in microbes is found in *Erwinia herbicola*, which requires a functional indole acetamide (IAM) pathway (Trp is converted to IAM and then to IAA) to be pathogenic to plants and requires a functional IPA pathway (Figure 1) to exist as a plant epiphyte (Manulis *et al.* 1998). We note that while aldehyde dehydrogenase has been implicated in IAA synthesis in *U. maydis*, this pathway is not involved in tumorigenesis (Reineke *et al.* 2008). This result is consistent with our observation that *ALD2* and *ALD3* are not necessary for IAA-induced filamentation and that an alternate IAA synthesis pathway likely exists in yeast.

The coexistence of both Trp-dependent and Trp-independent IAA-biosynthetic pathways has been documented in plants (Normanly *et al.* 2004; Woodward and Bartel 2005) and microbes (Prinsen *et al.* 1993). In plants, Trp-independent IAA synthesis is proposed to branch from either indole or indole glycerol phosphate, both precursors of Trp (Normanly *et al.* 2004). One of the proposed Trp-dependent IAA biosynthetic pathways for plants converts Trp to IPA (reviewed in Woodward and Bartel 2005 and Normanly 2009). The Arabidopsis TAA-1 protein can convert Trp to IPA *in vitro*, and mutations in the TAA-1 gene produce less IAA when the plant is subjected to simulated shade (Tao *et al.* 2008), high temperature (Yamada *et al.* 2009), or ethylene (Stepanova *et al.* 2008). IAAld has been proposed as an intermediate of the Trp-dependent IAA synthetic pathway in plants, but this has yet to be confirmed, and plant orthologs of *ALD* genes have not been identified. One putative aldehyde oxidase from Arabidopsis shows a substrate preference for IAAld *in vitro*, but the relevance of this gene to IAA biosynthesis *in vivo* has yet to be confirmed (Seo *et al.* 1998). Future studies will involve using differential stable isotope labeling coupled with genetic mutants to identify components of alternate IAA biosynthetic pathways in *S. cerevisiae*.

Secondary metabolites are recognized as important signals. *Aspergillus fumigatus* hyphae release a small molecule, gliotoxin, which can exacerbate the pathogenesis of invasive aspergillosis (Sutton *et al.* 1996). *Pseudomonas aeruginosa* produces a signaling molecule, homoserine lactone, which inhibits *C. albicans* filamentation (Hogan *et al.* 2004). Two predominant types of small molecules, acyl homoserine lactones (AHLs) (Fuqua *et al.* 2001; Danhorn *et al.* 2004; Akimkina *et al.* 2006) and modified oligopeptides (Kleerebezem *et al.* 1997), are used by gram-negative and gram-positive bacteria, respectively, to regulate phenotypes that lead to virulence such as antibiotic production and biofilm formation. *C. albicans* has been shown to produce secondary metabolites such as tyrosol and farnesol that regulate dimorphic transition (Shchepin *et al.* 2003; Chen *et al.* 2004). Aromatic alcohols such as tryptophol and phenylalanol, a catabolic product of Phe, are produced by both *S. cerevisiae* and *C. albicans* but exert different effects on their morphogenesis, suggesting that they have distinct species-specific effects. IAA differs from these previously described signaling molecules because its effects appear to cross species barriers. Diverse fungal species respond to IAA; therefore, defining the pathways by which IAA regulates filamentation in *C. albicans* will yield a better understanding of its pathogenesis and potentially the development of broad-spectrum antifungal therapies. Furthermore, auxin permeases that import IAA in *S. cerevisiae* are homologous to the Arabidopsis IAA importer, Aux1 (Prusty *et al.* 2004). Therefore, defining IAA synthesis and regulation in yeast, a simple eukaryote, will yield a better understanding of IAA regulation in plants.

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