Genetic Connection between Fatty Acid Metabolism and Sporulation in Aspergillus nidulans*

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In the Ascomycete fungus Aspergillus nidulans, the ratio of conidia (asexual spores) to ascospores (sexual spores) is affected by linoleic acid moieties including endogenous sporogenic factors called psi factors. Deletion of odeA (ΔodeA), encoding a Δ-12 desaturase that converts oleic acid to linoleic acid, resulted in a strain depleted of polyunsaturated fatty acids (18:2 and 18:3) but increased in oleic acid (18:1) and total percent fatty acid content. Linoleic acid-derived psi factors were absent in this strain but oleic acid-derived psi factors were increased relative to wild type. The ΔodeA strain was reduced in conidial production and mycelial growth; these effects were most noticeable when cultures were grown at 26 °C in the dark. Under these environmental conditions, the ΔodeA strain was delayed in ascospore production but produced more ascospores than wild type over time. This suggests a role for oleic acid-derived psi factors in affecting the asexual to sexual spore ratio in A. nidulans. Fatty acid composition and spore development were also affected by veA, a gene previously shown to control light driven conidial and ascospore development. Taken together our results indicate an interaction between veA and odeA alleles for fatty acid metabolism and sporulation in A. nidulans.

Several studies of filamentous fungi have suggested a role for 18:2 polyunsaturated fatty acids (i.e. linoleic acid) in fungal development, especially with regard to spore formation (1–4). In the filamentous fungus Aspergillus nidulans, linoleic acid-derived signal molecules called psi factors govern the development of cleistothecia (sexual bodies containing the sexual spores called ascospores) and conidiophores (asexual bodies producing the asexual spores called conidia) (5–8). Psi factor is a mixture of three hydroxylated linoleic molecules (PsiA1α, PsiB1α, and PsiC1α) and it has been reported that the proportion of these three compounds controls the ratio of asexual to sexual spore development in this fungus (5–8). Specifically, PsiB1α and PsiC1α are reported to stimulate sexual spore development whereas PsiA1α inhibits sexual spore development (6). Hydroxylated derivatives of oleic acid (PsiA1β, PsiB1β, and PsiC1β) have also been isolated from A. nidulans (7, 8) but their role in sporulation has not been characterized.

In addition to the effects of psi factor on Aspergillus development, recent studies have also shown that purified linoleic acid and hydroperoxy linoleic acids derived from seed also exhibit sporogenic activities toward several Aspergillus spp. including A. nidulans and the seed infecting fungi Aspergillus flavus and Aspergillus parasiticus (9). In all of these species, the primary effect of linoleic acid and hydroperoxy linoleic acids was to induce precocious and increased conidial development. Lower concentrations of linoleic acid and (9S)-hydroperoxy linoleic acid stimulated sexual spore development rather than conidial development in A. nidulans (9). These data suggest a relationship between linoleic acid and/or its derivatives and Aspergillus developmental processes.

The sporogenic effects of linoleic acid, hydroperoxy linoleic acids, and psi factor were demonstrated on A. nidulans strains with an intact velvet (veA) locus (5, 9). In A. nidulans veA strains, light delays and reduces sexual development and induces conidial production, while in the absence of light conidial production is repressed and the fungus develops cleistothecia (10). Strains with mutations in veA (veA1) exhibit light-independent development of conidia and ascospores (10). Furthermore, veA1 mutants do not develop spores in response to psi factor, linoleic acid, and other linoleic acid derivatives (5, 9). veA locus was originally identified by Käfer in 1965 (11), who also isolated the veA1 mutation. veA has been sequenced (accession number U95045).1 Currently the cellular function of VeA is not known.

As a first step in understanding the molecular genetics of psi factor formation and psi factor effects on Aspergillus development, we deleted the odeA gene encoding an oleate Δ-12 desaturase, which catalyzes the conversion of oleic acid into linoleic acid. The effects of the odeA deletion were examined in both veA and veA1 genetic backgrounds. The absence of odeA changed the fatty acid profile, including the composition of psi factor, and raised total percent fatty acids/fungal tissue. Interestingly, veA also affected fatty acid composition. Detailed examination of psi factor levels in the veA strains at 26 °C indicated that, in comparison to the wild type, the ΔodeA strain was delayed in psi factor biosynthesis; however, higher amounts of psi factor were found over time. This was paralleled by delayed but increased ascospore production in this strain compared with wild type. The ΔodeA strain also displayed delayed and decreased conidial production compared with wild type in both veA and veA1 backgrounds. Interactions between odeA and veA affected spor development, fatty acid composition, and psi factor composition.

**EXPERIMENTAL PROCEDURES**

_Fungal Strains and Growth Conditions—_A. nidulans strains used in this study are listed in Table I. Cultures were grown on A. nidulans

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1 L. Yager, personal communication.
Aspergillus nidulans Δ-12 Desaturase Gene

Table I

| Strain        | Genotype                        | Source         |
|---------------|---------------------------------|----------------|
| FGC536        | (biA1; veA1)                    | FGC536         |
| FGC539        | (biA1; argB2; veA1)             | FGC539         |
| TAMC31.65     | (biA1; veA1; ΔodeA)             | This study     |
| FGC533        | (pyrA4; veA1)                   | This study     |
| RAMC25        | (pyrA4; veA1; ΔodeA; odeA; pyrA) | This study     |
| TAMC34        | (pyrA4; veA1; ΔodeA; pyrA)      | This study     |
| WIM126        | (pabaA1, yA2; veA)              | L. Yager       |
| RAMC22.1      | (biA1; veA)                     | This study     |
| RAMC29.24     | (biA1; veA, ΔodeA1)             | This study     |

* Fungal Genetics Stock Center (Kansas City, KS). veA, velvet gene; veA1, mutation in veA; odeA, delta-12 desaturase gene; ΔodeA, deletion of odeA; biA1, mutation in biA causing biotin-deficient auxotrophy; argB2, mutation in argB causing arginine-deficient auxotrophy; pyrA4, mutation in pyrA causing pyrimidine-deficient auxotrophy; pabaA1, mutation in pabaA causing 4-amino benzoic acid-deficient auxotrophy.

glucose minimal medium (GMM) unless otherwise indicated. GMM consists of 10 g of glucose, 6 g of NaNO3, 0.52 g of KCl, 0.52 g of MgSO4·7H2O, 1.52 g of KH2PO4, 1 ml of trace elements (2.2 g of ZnSO4·7H2O, 1.1 g of H3BO3, 0.5 g of MnCl2·4H2O, 0.5 g of FeSO4·7H2O, 0.162 g of CaCl2·H2O, 0.16 g of CuSO4·5H2O, 0.19 g of NH4MoO4·4H2O, 5 g of Na3EDTA, and 2 ml of distilled H2O). O2 level was adjusted to 6.5 with a 10% NaOH solution. Appropriate supplements corresponding to the auxotrophic markers were added to the medium (12). Agar (15 g/liter) was added to obtain solid medium. Temperature of incubation was 37 °C, unless indicated otherwise. Cultures were grown in continuous white light or in the dark. Cultures requiring white light in an incubator equipped with General Electric 15-W broad-spectrum fluorescent light bulbs (F15T12CW) positioned at a distance of 20 cm from the agar surface, with a light intensity of 66 mE/m²/s.

Identification and Cloning of the A. nidulans Δ-12 Desaturase Gene—An A. nidulans cosmid library (pWE15, Fungal Genetics Stock Center, Kansas City, KS) and A. parasiticus cosmid library (pA1, provided by J. Bernheimer and P. MaGe) were screened using the A. parasiticus cosmids library yielded a cosmid, pMAC8, containing the putative Δ-12 desaturase gene. A 1.2-kb BamHI fragment from pMAC8 containing the carboxyl-terminal coding region of the A. parasiticus Δ-12 desaturase gene was used as a probe to screen the A. nidulans pWE15 genomic cosmid library, and a single cosmid, pWEO2H5, was obtained. Fragments from these cosmids were subcloned to facilitate sequencing and construction of transformation vectors.

Sequence Analysis—Fragments from the cosmid pWEO2H5 were subcloned into the plasmid pK19 (14), obtaining pMAC15 (4.6-kb HindIII insert), pMAC16 (2.4-kb KpnI insert), pMAC17 (6-kb SalI insert), pMAC18 (1.8-kb BamHI insert), and pMAC19 (1.3-kb BamHI/HindIII insert). DNA sequencing of both strands was performed using synthetic primers and ABI PRISM DNA Sequencing kit (PerkinElmer Life Science). Sequences were assembled with the Sequencher 3.1 program. Nucleotide sequence was translated in all six reading frames using FLAST (20) and compared with the sequences in GenBankTM (15). The GenBank32° sequence accession number of A. nidulans odeA is AF262955.

Deletion of A. nidulans odeA—The transformation vector utilized to delete odeA was called pMAC31.3. This plasmid included the argB marker gene and odeA flanking sequences without the odeA encoding region. pMAC31.3 was constructed as follows: first, the plasmid pMAC26.6 was obtained by insertion of a 7.8-kb SphI-SalI fragment from pWEO2H5 into the SphI-SalI sites in pK19 (14). Another plasmid, pMAC30, was generated by digestion of pBlueScript SK2 (Stratagene) with EcoRI and XhoI, followed by a blunt-end reaction and religation of the plasmid. A 4.3-kb Smal fragment, containing the 1.3-kb odeA encoding region, was released from pMAC26.6 and ligated into the SmaI site in pMAC30. The entire odeA encoding region plus 389 base pairs downstream from the putative stop codon was then removed from pMAC30.4 by a SalI double digest. This left two 1.3-kb genomic DNA fragments that were on either side of the excised odeA gene. The remaining linear vector was blunt-ended and ligated to a blunt-ended 1.8-kb fragment containing the A. nidulans argB gene to obtain the final transformation vector pMAC31.3. Using standard procedures (16), A. nidulans FM8 (biA1; argB2) (Table I) was transformed with pMAC31.3 to create TAMC31.65 (biA1; veA1; ΔodeA). Replacement of odeA by argB is denoted by the symbol ΔodeA.

Complementation of odeA Deletion Strain—The transformation vector pMAC33.3 was used to complement the odeA deletion strain RAMC25 (pyrA4; veA1; ΔodeA). pMAC33.3 was constructed by inserting

2°The abbreviations used are: GMM, glucose minimal medium; kb, kilobase(s); FAME, fatty acid methyl esters; HFAME, hydroxy fatty acid methyl esters; FID-GC, flame ionization detection-gas chromatography; OTMIS, trimethylsilylox; PUFAs, polyunsaturated fatty acids; HPLC, high performance liquid chromatography; GC-MS, gas chromatography-mass spectrometry; H NMR, proton nuclear resonance; 8-HOE, 8-hydroxy-9(Z)-octadecenoic acid; 5,8-diHODE, 5,8-dihydroxy-9(Z)-octadecadienoic acid; 8-HODE, 8-hydroxy-9(Z,12(Z)-octadecadienoic acid.

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Aspergillus nidulans Δ-12 Desaturase Gene

Results

Sequence Analysis of odeA and Composition of the ΔodeA Deletion with a Wild Type odeA Gene

Sequence analysis of A. nidulans odeA at both the nucleotide and amino acid level revealed high similarity with other Δ-12 desaturases from plants and fungi. OdeA contained the conserved His-rich regions found in other Δ-12 desaturases (data not shown) (20–22). Deletion of odeA resulted in loss of polyunsaturated fatty acid biosynthesis and alterations in spore production (described below). Transformation of the ΔodeA strain with the odeA gene recovered the wild type phenotype as revealed by fatty acid analysis and physiological studies (data not shown). This supports the conclusion that the defects in the ΔodeA phenotype are solely due to loss of the odeA gene.

Effect of odeA, veA, and Environment on A. nidulans Fatty Acid Composition

Due to the extensive use of veA1 strains as research models throughout the international research community, the odeA deletion was placed in both a veA and veA1 genetic backgrounds, although our main interest was examining sporulation and psi factor composition in the veA strains. Also, because fatty acid composition has been shown to change with temperature (23, 24), fatty acid composition was compared in the veA; veA1; veA, ΔodeA; and veA1, ΔodeA strains at both 26 and 37 °C. Fatty acids were also examined under light and dark regimes at 37 °C due to the importance of light in conidial versus ascospor development in veA strains (10).

Fatty acid composition was first examined under culture conditions known to promote asexual spore development (72 h growth and GMM medium). Table II shows the fatty acid composition of veA; veA1; veA, ΔodeA; and veA1, ΔodeA under the environmental conditions (treatments) tested. OdeA allele, veA allele, and treatment had a significant effect on fatty acid composition (p < 0.01). There was a near loss of polyunsaturated fatty acids (PUFA, 18:2 and 18:3) in ΔodeA strains (Table II). Their presence was, however, confirmed independently by silver-ion HPLC followed by GC-MS of the HPLC-purified FAME (not shown). To determine whether these low levels of PUFA were of fungal or exogenous origin in the ΔodeA strains, GMM medium was examined for the presence of linoleic acid. The medium contained trace amounts of linoleic acid (0.8 μg), but this amount was negligible in comparison to the total amount found in ΔodeA mycelia (average of 23 μg/culture). Levels of other fatty acids also changed in ΔodeA mycelia (Table II). For example, the percentage of saturated fatty acids, palmitic acid (16:0), and stearic acid (18:0) was reduced. In addition, the total percentage of FAME per g of mycelium was approximately 2–3-fold higher in the ΔodeA strains. These effects were conserved independently of veA or veA1 alleles, illumination regimen, and temperature (Table II).

The veA allele also had a significant effect on the fatty acid profile (p < 0.01). The veA strain contained less linoleic acid and linolenic acid but more oleic and stearic acid than veA1 (Table II). An interaction between veA and odeA alleles on the relative percent of each fatty acid except stearic acid was illustrated by the fact that variations in these fatty acids between veA and veA1 strains were not maintained in the ΔodeA back-
The analysis was carried out on 72-h old mycelia grown in liquid glucose minimum medium under stationary conditions.

| Sample       | % FAME | Wt % of individual FAME |
|--------------|--------|-------------------------|
|              | 16:0   | 18:0                    | 18:1 | 18:2 | 18:3 | Others |
| veA 26D      | 5.88 ± 0.05 | 12.1 ± 0.2          | 13.6 ± 0.1 | 14.5 ± 0.3 | 48.4 ± 0.5 | 8.02 ± 0.36 | 2.40 ± 0.08 |
| veA, odeA 26D| 2.71 ± 0.17 | 12.7 ± 0.1          | 15.2 ± 0.6 | 22.0 ± 0.7 | 43.1 ± 0.6 | 3.32 ± 0.24 | 3.59 ± 1.07 |
| veA, DodeA 26D| 6.40 ± 0.35 | 6.02 ± 0.19        | 10.7 ± 0.6 | 79.1 ± 0.4 | 62.0 ± 0.8 | TR           | 3.63 ± 0.43 |
| veA 37D      | 1.81 ± 0.16 | 15.4 ± 0.06         | 8.90 ± 0.19 | 15.0 ± 0.3 | 55.7 ± 0.2 | 1.77 ± 0.13 | 3.18 ± 0.44 |
| veA, DodeA 37D| 5.68 ± 0.06 | 8.77 ± 0.20         | 6.46 ± 0.46 | 81.4 ± 0.4 | 47.4 ± 0.5 | ND           | 2.89 ± 0.04 |
| veA, DodeA 37D| 2.38 ± 0.09 | 16.0 ± 0.3          | 9.42 ± 0.46 | 23.7 ± 0.8 | 46.5 ± 1.0 | 0.46 ± 0.12 | 3.88 ± 0.12 |
| veA, 37L     | 5.32 ± 0.37 | 9.25 ± 0.05         | 7.24 ± 0.17 | 79.7 ± 0.5 | 39.0 ± 0.8 | ND           | 3.43 ± 0.28 |
| veA, DodeA 37L| 2.06 ± 0.20 | 16.7 ± 0.1          | 7.34 ± 0.79 | 16.1 ± 0.1 | 55.0 ± 1.3 | 0.60 ± 0.04 | 4.02 ± 0.19 |
| veA, DodeA 37L| 4.84 ± 0.21 | 8.62 ± 0.48         | 4.99 ± 0.62 | 83.0 ± 1.3 | 30.3 ± 0.6 | ND           | 3.04 ± 0.16 |
| veA 37L      | 2.07 ± 0.22 | 15.6 ± 0.3          | 8.46 ± 0.89 | 22.8 ± 1.0 | 49.4 ± 1.3 | 0.28 ± 0.07 | 3.35 ± 0.30 |

* The abbreviations used in this table are: FAME, fatty acid methyl esters (*) weight percent FAME based on lyophilized weight of mycelia; Wt., weight; 16:0, palmitic acid; 18:1, oleic acid; 18:2, linoleic acid; 26D, 26 °C; 37D, 37 °C in the dark; 37L, 37 °C in the light. Values are means of three replicates. Standard deviation is shown.

** ND, not detected.

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Other observations included detection of 10-hydroxy-8,12-octadecadienoic acid in veA strains and 10-hydroxy-8,12-octadecadienoic acid (16:0) decreased, regardless of veA or odeA alleles. This compound has not been previously identified in A. nidulans, and we give it the term PsiB1. No PsiB1 was detected in any samples. This was expected, as PsiA1 and veA were also apparent by the high amount of psiClp found only in the veA, DodeA strain at 26 °C. An interaction between psiA and veA was also apparent by the high amount of psiClp found only in the veA, DodeA strain (Table III).

Other observations included detection of 10-hydroxy-8,12-octadecadienoic acid in veA and veA strains and 10-hydroxy-8-octadecenoic acid in all the strains (data not shown). This is the first report of their presence in A. nidulans although these PUFAs have been detected in other fungi (25–27). Currently, it is not known if they also act as sporogenic elements.

** Fatty Acid and Psi Factor Composition during Sexual Stage Development Is Affected by odeA

Because sexual development has typically been assessed by growth of veA strains on YGT medium and we are attempting to examine a possible role of psi factor composition in sexual development, we looked at psi factor composition in veA strains (wild type odeA) and wild type in veA1 and veA genetic backgrounds) grown on this medium. Both psi factor analysis and microscopic observations were performed at the same time points for these strains.

Fig. 1 shows the changes in relative percent of FAME composition over time in veA (Fig. 1A) and the veA, DodeA strains (Fig. 1B). In both strains, the most unsaturated fatty acid (linoleic in veA and oleic in veA, DodeA) showed a similar trend of decreasing in percentage composition at 66 and 114 h but increasing in percentage at 162 and 240 h. The percent composition of the other detectable fatty acids showed an opposite pattern in both strains. The FAME weight/mycelium weight peaked at 66 h in both

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The FAME weight/mycelium weight peaked at 66 h in both
the veA strain and the veA, ΔodeA strain. As major components of the total FAME, both linoleic acid and oleic acid reflected the trend of the total FAME weight/mycelium weight (Fig. 1C). On a weight basis/g of mycelium, there was a correlation in linoleic acid versus PsiB1β and PsiC1α, as well as a correlation in oleic acid versus PsiB1β plus PsiC1β. It was also noteworthy that compared with linoleic acid, oleic acid appeared to yield a much greater quantity of psi factors. This greater accumulation of oleic acid-derived psi factor proved to be valid when all values obtained in this study were examined. Linear plots of oleic acid versus PsiB1β plus PsiC1β (r = 0.873) and linoleic acid versus PsiB1α plus PsiC1α (r = 0.715) showed that oleic acid-derived psi factor accumulated 2.5-fold greater per quantity of oleic acid compared with linoleic acid-derived psi factor from linoleic acid. In general, about 5–5.8 μg of PsiB1β plus PsiC1β were found per mg of oleic acid, and about 2.2 μg of PsiB1α plus PsiC1α accumulated per mg of linoleic acid. Our examination also showed that although psi factor accumulation was delayed in the veA, ΔodeA strain, more psi factor was found in this strain at time points later than 42 h (Fig. 1D).

Effect of odeA, veA, and Environment on A. nidulans Developmental Processes

Vegetative growth and asexual spore production were assessed in both veA and veAI backgrounds. However, sexual development was assessed solely in veA strains because the veA allele is required for response to psi factor, and because sexual development has been better characterized in veA strains (5–10).

Vegetative Growth—Fig. 2A shows that environmental treatment, veA allele and odeA allele had a significant effect (p < 0.01) on A. nidulans colony diameter. In wild type odeA backgrounds, the veA strain had a larger colony diameter than the veAI strain. This was not true in ΔodeA backgrounds, demonstrating an interaction between veA and odeA alleles (p < 0.01).
odeA and sdeA Are Temperature and Light Regulated

Positive regulation of desaturase genes by low temperatures and light has been reported in other organisms (24, 28–32). We studied the effect of temperature and light on odeA and sdeA expression in A. nidulans. As previously mentioned, sdeA encodes a putative Δ-9 desaturase in A. nidulans, that is responsible for the conversion of stearic acid into oleic acid. Fig. 4 shows that both odeA and sdeA transcript accumulation was induced by low temperatures (26 and 20 °C) in both veA and veA1 strains and by light (only examined at 37 °C) in veA1 strains. As expected, no odeA transcripts were observed in the

The lack of the odeA allele led to significant reduction in colony diameter. These effects were maintained over 5 days of incubation (data not shown).

Asexual Spore Development—Environmental treatment, veA allele, and odeA allele had a significant effect on conidial production (p < 0.01; Fig. 2B). There were also significant interactions between veA and odeA, veA, and environmental treatment, and odeA and environmental treatment (p < 0.01). There was a significant decrease in conidial production in the veAI, ΔodeA and veA, ΔodeA strains at 26 °C (Fig. 2B). However, at 37 °C only the veAI, ΔodeA strain showed a reduction in conidial production in comparison to the wild type. Both veA and veA, ΔodeA strains exhibited the light/dark response characteristic of a veA wild type allele which was to produce more conidia in the light than in the dark. No significant differences in conidial production were observed in veAI strains, wild type odeA or ΔodeA, with respect to illumination conditions (Fig. 2B). Lower temperature decreased conidial production in all veAI strains, but only in the veA strain containing the ΔodeA allele.

Sexual Spore Development—Our studies indicated that light and temperature and the odeA interaction with light and temperature significantly affected ascospore development in A. nidulans (Fig. 3A and data not shown). As observed before (5, 9, 10), the wild type veA strain produced more ascospores in the dark than in the light at 37 °C (p < 0.01). This effect was also observed in the ΔodeA background (p < 0.05). The ΔodeA strain showed an increase in ascospore production relative to the wild type odeA strain in 10-day-old cultures grown at 26 °C (Fig. 3A, p < 0.01), but no significant differences between these strains were observed at 37 °C in light or dark grown cultures.

To further investigate the effect of the odeA deletion on sexual development at 26 °C, we made microscopic observations of sexual development from 42 to 162 h on the wild type and ΔodeA strain. At 42 h, only hyphal growth was present in both cultures. Hüille cells were observed at 66 and 90 h in both strains. At 114 and 138 h, the cleistothecial walls were forming. At 162 h ascis (at different stages of maturity) and free ascopores were present. Quantitative analysis showed that the numbers of mature ascis and free ascopores were higher in the wild type than in the ΔodeA strain at 162 h (Fig. 3B). This was in contrast to the ΔodeA 10-day-old culture, which showed an increase in ascospore production with respect to the wild type odeA strain (as mentioned above, Fig. 3A).

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FIG. 2. Deletion of odeA decreases colony growth (A) and conidial production (B). Cultures of veAI, veAI, ΔodeA, veA, and veA, ΔodeA strains were grown at 26 °C in the dark, 37 °C in the dark, and 37 °C in the light in glucose minimum medium. D, cultures grown in dark. L, cultures grown in light. Panel A, 5-day-old cultures. Panel B, 72-h-old cultures. Values are the means of three replicates.

FIG. 3. Ascospore production. Panel A, cultures of veA and veA, ΔodeA strains were grown at 26 °C in the dark, 37 °C in the dark and 37 °C in the light for 240 h in YGT medium. D, cultures grown in dark. L, cultures grown in light. Panel B, cultures of veA and veA, ΔodeA strains were grown at 26 °C in the dark for 162 h in YGT medium. Values are the means of four replicates.


**Aspergillus nidulans Δ-12 Desaturase Gene**

**Fig. 4. Temperature-dependent and light-dependent expression of Δ-12 desaturase (odeA) and the Δ-9 desaturase (sdeA) genes from A. nidulans.** Total RNA (20 μg) was isolated from mycelia after growing for 72 h on glucose minimum medium at different temperatures in a range from 40 to 20 °C. Light regulation was studied at 37 °C. Panel A, veA strain. Panel B, veA, ΔodeA strain. Panel C, veAI strain. Panel D, veAI, ΔodeA strain. D, cultures grown in light. An ethidium bromide stained picture of rRNA is shown to indicate RNA loading. Arrows indicate the accumulation of odcA and sdeA transcripts.

**Fig. 5. Unsaturated fatty acid-mediated regulation of Δ-12 desaturase (odeA) and Δ-9 desaturase (sdeA) gene expression in A. nidulans.** After growing the strains in GMM in liquid shaken cultures for 16 h, the mycelia were transferred to a different second medium: GMM, GMMOLA (GMM plus sodium linoleate in tergitol), GMMOA (GMM plus sodium oleate in tergitol), GMMLA (GMM plus sodium linoleate in tergitol), MMOA (MM plus sodium oleate in tergitol), and MMT (MM plus with tergitol control). Total RNA (20 μg) was isolated from mycelia 8 h after the shift. Panel A, veA strain. Panel B, veA, ΔodeA strain. Panel C, veAI strain. Panel D, veAI, ΔodeA strain. An ethidium bromide-stained picture of rRNA is shown to indicate RNA loading. The arrows indicate the accumulation of odcA and sdeA transcripts.

\[\Delta odeA\] strains (Fig. 4, B and D). sdeA transcripts were elevated in the \(\Delta odeA\) strains (Fig. 4).

**Polyunsaturated Fatty Acid-regulated Expression of odcA and sdeA**

The increase in sdeA transcript in the \(\Delta odeA\) strains suggested a possible regulation of PUFA on sdeA expression. To further investigate this possibility, all four strains were grown in various carbon sources including unsaturated fatty acids. In the odcA wild type strains, odcA and sdeA expression was notably higher in the presence of glucose than in its absence, however, this was not observed in \(\Delta odeA\) strains (Fig. 5, B and D). When the \(\Delta odeA\) strains were grown in linoleic acid as a sole carbon source there was a noticeable decrease in sdeA transcript accumulation in both veA and veAI strains. This decrease was attenuated by the addition of glucose in the medium (Fig. 5, B and D). miRNA analysis also showed that the accumulation of odcA transcripts was higher in the veA strain than in veAI strain (Fig. 5, A and C). In the veA strain, a slight reduction in odcA and sdeA transcript accumulation was also observed when exogenous linoleic acid was added (Fig. 5A).

**DISCUSSION**

The genus *Aspergillus* contains many industrially, medically, and agriculturally important species whose mode of reproduction depends primarily on the production of asexual spores called conidia and, for some species, sexual spores called ascospores. Factors contributing to spore development of this genus include linoleic acid (9) and various oxidized derivatives of linoleic acid. These include endogenous *A. nidulans* sporogenic molecules called psi factor (5–8) and plant defense metabolites, 9S- and 13S- hydroperoxylinoleic acid (9). This latter point is of significance as many *Aspergillus* spp. are seed infesting fungi that elicit hydroperoxylinoleic acid production in higher plants (33). The sporogenic response to linoleic acid moieties requires the presence of an intact veA gene. However, as veAI mutant strains have been historically used by the research community due to their convenient trait of developing asexually in the dark, we have investigated the role of linoleic acid and psi factor on fungal development through character-

ization of both *A. nidulans* veA and veAI strains deficient in linoleic acid biosynthesis.

As expected, chemical analysis of \(\Delta odeA\) strains demonstrated the absolute requirement of OdeA for normal fatty acid metabolism in *A. nidulans* (Table II and Fig. 1, A and B). In contrast to odcA strains, where linoleic acid content was ~50% of FAME, the \(\Delta odeA\) strains presented only trace amounts of linoleic acid. The odcA deletion also resulted in a 2–3-fold increase in total fatty acids/weight of fungal biomass. Moreover, the chemical makeup of the fatty acid profile was altered in these strains: palmitic acid content was decreased and both stearic and oleic acid content increased compared with wild type strains. The extraordinarily high amount of oleic acid was likely due not only to the block in the fatty acid pathway, but also to the increase in sdeA transcript accumulation in the \(\Delta odeA\) strains (Figs. 4 and 5).

We also found that fatty acid composition was affected by veA. The differences observed between veA and veAI strains with respect to the fatty acid profile were medium-dependent. Lower amounts of PUFA and higher amounts of monounsaturated fatty acids were found in veA strains compared with those found in veAI strains in glucose minimum medium, a medium which promotes asexual spore development (Table II). The inverse was observed when the fungal strains were grown in YGT (data not shown), the medium used for promoting the sexual stage in *A. nidulans* (5–8). Furthermore, there were significant interactions between veA and odcA alleles on fatty acid metabolism as detailed under “Results” (Tables II and III; Figs. 4 and 5). This suggests a complex regulation of fatty acid metabolism involving veA and fatty acid biosynthetic genes.

Elimination of odcA also led to changes in both % psi factor/weight of fungal biomass and psi factor composition. Both PsiB1α and PsiB1β were found in the wild type strain, but the \(\Delta odeA\) strain was crippled in its ability to synthesize PsiB1α (Table III). Instead, high levels of PsiB1β and PsiC1β were found in the mutant strain. Furthermore, an interaction between veA and odcA alleles was demonstrated by the fact that the PsiB1β and PsiC1β levels were statistically greater in the veAI, \(\Delta odeA\) strain versus the veA, \(\Delta odeA\) strain (Table III).
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Also, when grown at 26 °C in YGT medium, psi factor was not detected in the ΔodeA strain until 66 h, at which time the level of psi factor was 5-fold above that of wild type (Fig. 1D). Although there were differences in the amount of psi factor found dependent on experiment (Fig. 1D, Table III), in general the total amount of psi factor detected in ΔodeA strains was several fold greater than that of wild type odeA strains.

Experiments by Champe et al. (5, 6) led to the hypothesis that PsiB1α and PsiC1α play a prominent role in increasing the sexual to asexual spore ratio in A. nidulans but no experiments were conducted with PsiB1β and PsiC1β to determine if they also had a role in spore development. Although we did not directly assess the effect of PsiB1β or PsiC1β on Aspergillus development, our results suggest that these derivatives may also act as sexual sporogenic factors as the increase in ascospore numbers in the ΔodeA, veA strain at 240 h and 26 °C (Figs. 1D and 3A) was accompanied by an increase in Psiβ level. Our data also suggested that the oleic acid:linoleic acid ratio may be playing a role in the relative development of conidia and ascospores. We note that in Neurospora crassa oleic acid is the predominant fatty acid found in developing asc and mature ascospores, whereas linoleic acid is the predominant fatty acid in asexual tissue in this fungus (34).

The ΔodeA strains produced less conidia than the odeA wild type strains, especially at low temperatures (Fig. 2B). Aside from some possible role of the oleic acid:linoleic acid ratio on directing asexual to sexual spore development, this decrease could also be explained as a need for high PUFA content for conidial formation in cold environments. Temperature had a decided effect on odeA and sdeA transcript accumulation; both were more abundant when the fungus was grown at lower temperatures (Fig. 4). The adaptation of cells to maintain the membrane fluidity in response to a downward shift in temperature by desaturating fatty acids has been studied in higher plants (35–37), animals (38), and in cyanobacteria (39–41). Low-temperature induction of desaturase genes has been reported in cyanobacterium species (24, 30, 31). Positive regulation of a fungal Δ-9 desaturase gene by low temperature has been described previously in fungi of Mucor rouxii (32). Considering the increase in linoleic acid (18:3) in cultures grown at 26 °C (Table II), it is also likely that A. nidulans contains an ω-3 desaturase positively regulated by low temperature in a similar manner as described in cyanobacteria (24, 30, 31).

A most interesting observation in this study was the response of the odeA and sdeA alleles to light. There was light induction of odeA and sdeA transcription but only in the strains containing the veA1 allele. These results also indicate another possible genetic link between veA and odeA. Perhaps this response is part of the reason that there are differences seen in the fatty acid profile between veA and veA1 strains. Light-induced transcription of green algae and plant desaturases have been recorded (28, 29) but this is the first report of a fungal desaturase that responds to light.

The increased expression of the sdeA gene in the ΔodeA strain (Figs. 4 and 5) and the high levels of oleic acid in the ΔodeA strain suggest a role of OdeA and/or linoleic acid in regulating fatty acid desaturation in A. nidulans. Additionally, we found that exogenous linoleic acid partially repressed sdeA expression (Fig. 5, A, B, and D). Feedback regulation of Δ-9 desaturase activity has also been noted in mammal (42–45) and yeast (19). The attenuation of the negative regulation of sdeA transcript in ΔodeA strains when grown in medium containing glucose indicates interactions between carbon metabolism and PUFA metabolism. PUFA have also been shown to negatively regulate fatty acid synthase, the first committed step in fatty acid metabolism, in mammals (46). We suggest that depletion of PUFA in the ΔodeA strain derepresses PUFA regulation of fatty acid metabolic genes leading to the observed 3-fold increase in total fatty acid content of this strain.

In conclusion, we have shown that fatty acid composition in A. nidulans varies during spore development and is influenced by odeA, veA, temperature, and light. Both odeA and veA alleles are required for normal asexual and sexual spore development. Although fatty acid and psi factor composition alters with mutations in both of these alleles, it is not yet possible to attribute asexual or sexual spore production to the presence of specific fatty acids as other aspects of fungal physiology also changed. We hope by characterizing sdeA mutants and genes involved in psi factor formation to further elucidate the role of oleic acid or linoleic acid psi factors in spore development. We also found that odeA and sdeA expression, like that of other desaturase genes, is responsive to environmental factors including temperature and light and that OdeA and/or linoleic acid play a role in regulating fatty acid desaturation. In addition, it is important to note that even though most of the Aspergillus research community investigates on veA1 strains, the veA1 mutation leads to major changes in sporulation, fatty acid and psi factor profiles. These results open the possibility that previous findings in veA1 might not always apply in veA strains.

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