Role of the *Alternaria alternata* Blue-Light Receptor LreA (White-Collar 1) in Spore Formation and Secondary Metabolism

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*Alternaria alternata* is a filamentous fungus that causes considerable loss of crops of economically important feed and food worldwide. It produces more than 60 different secondary metabolites, among which alternariol (AOH) and altertoxin (ATX) are the most important mycotoxins. We found that mycotoxin production and spore formation are regulated by light in opposite ways. Whereas spore formation was largely decreased under light conditions, the production of AOH was stimulated 2- to 3-fold.

ATX production was even strictly dependent on light. All light effects observed could be triggered by blue light, whereas red light had only a minor effect. Inhibition of spore formation by light was reversible after 1 day of incubation in the dark. We identified orthologues of genes encoding the *Neurospora crassa* blue-light-perceiving white-collar proteins, a cryptochrome, a phytochrome, and an opsin-related protein in the genome of *A. alternata*. Deletion of the white-collar 1 (WC-1) gene (*lreA*) resulted in derepression of spore formation in dark and in light. ATX formation was strongly induced in the dark in the *lreA* mutant, suggesting a repressing function of LreA, which appears to be released in the wild type after blue-light exposure. In addition, light induction of AOH formation was partially dependent on LreA, suggesting also an activating function. *A. alternata ΔlreA* was still able to partially respond to blue light, indicating the action of another blue-light receptor system.

*A. alternata* is a filamentous ascomycetes containing many species of economic importance, including saprophytes, phytopathogens, and zoopathogens. Many *Alternaria* species are potent producers of mycotoxins and allergens, properties which may directly affect human life. Every year, large amounts of food and feed are contaminated and destroyed because of *Alternaria* growth and toxin production. Worldwide epidemiological studies indicate that *Alternaria* sensitivity is closely linked with the development of asthma (1). Despite the great importance of *Alternaria* species, little is known about the molecular biology underlying *Alternaria* biology.

*A. alternata* is a widespread saprophytic species which produces a wide variety of different secondary metabolites, among which are the mutagenic mycotoxins alternariol (AOH) and altertoxin (ATX) (2–4). For AOH and its monomethylether (AME), it has been shown recently that they have potential to cause DNA double-strand breaks in mammalian cells *in vitro* (5). The altertoxins ATX-I, -II, and -III are mutagenic in the Ames test and are more potent and acutely toxic to mice than AOH and AME (6).

Surprisingly, it is not clear yet how AOH or ATX is produced or how their production is regulated. Alternariol biosynthesis requires the activity of a polyketide synthase, 10 of which were identified recently in the genome. One of them, PksJ, was identified to catalyze the initial steps of alternariol biosynthesis (7). It was already reported 30 years ago that blue light reduced alternariol production in *A. alternata* (8). Blue light has also been reported to inhibit sporulation in *A. tomato* and *A. cichorii*. The effect was, in addition, temperature dependent and occurred only in mature conidiophores (9). These observations suggest that *Alternaria* species are able to sense and respond to light. It has been well established, meanwhile, that many fungi are able to respond to light and employ one or several photoreceptors (10–12). The existence of light receptors in filamentous fungi has been shown for *Neurospora crassa*, *Aspergillus nidulans*, *Coprinus cinereus*, *Cryptococcus neoformans*, *Physomyces blakesleeanus*, and *Physarum polycephalum* and for many others. Some of them possess receptors for blue, red, and green light and are therefore able to sense light over a broad spectral range. The perception of light in these fungi has been shown to influence asexual conidiation, sexual development, pigmentation, the circadian clock, and secondary metabolism (10, 12). The blue-light response often seems to be the most important one; e.g., all light-dependent processes in *N. crassa* are regulated by UV or blue light (13, 14). In contrast, *A. nidulans* responds well to red light in addition to blue light (15, 16). It was discovered recently that the blue- and red-light-sensing chromoproteins along with some additional proteins form a light-regulator complex in this fungus (17).

Here we studied the effect of light on the regulation of spore formation and mycotoxin production in *A. alternata* and assigned a function to LreA, the white-collar 1 (WC-1) orthologue, as the main photoreceptor in this fungus.

**MATERIALS AND METHODS**

**Culture conditions and harvesting of spores.** *Alternaria alternata* DSM 12633 cultures were grown on modified Czapek Dox broth (mCDB) agar if not stated differently and incubated 1 to 7 days at 28°C. For white-light experiments, a 10-W energy-saving lamp (Flair energy) was used; for red-

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and blue-light conditions, light-proof, ventilated boxes with wavelength (450- and 680-nm)-specific LEDs were used. The intensities of the blue- and red-light-emitting diodes (LEDs) were measured at 0.72 W/m² and 1.45 W/m², respectively.

All plates were inoculated with 5 × 10⁴ spores. For quantification, the spores were harvested in sterile H₂O, filtered for separation from the mycelium, and centrifuged. The pellet was resuspended in 5 ml H₂O, and the optical density at 600 nm (OD₆₀₀) was measured with a spectrophotometer (Pharmacia Ultraspec III). The relation of the OD₆₀₀ to the amount of spores was calculated before via counting spores in a Helber chamber. All experiments were done with three technical replicates.

Analysis of mycotoxins using TLC and LC/MS. For the extraction of mycotoxins, three disks from each plate were excised with the back of a blue pipette tip and extracted by shaking with 1 ml ethyl acetate for 1 h. The solvent was vaporized in a SpeedVac and the pellet resolved in 60 μl ethyl acetate. A 20-μl volume was used for thin-layer chromatography (TLC) with a mobile phase composed of toluene, ethyl acetate, and formic acid (5:4:1) on silica plates (Merck TLC silica gel 60) and visualized under UV light at 365 nm. As a standard, TLC-prepared AOH was used. For liquid chromatography-mass spectrometry (LC/MS) analysis, the same extracts were used but the ethyl acetate was evaporated and the pellet resolved in 100 μl methanol.

LC-DAD-MS analysis. A LXX Linear Ion Trap multiple-stage MS (MSn) system (Thermo Fisher Scientific, Waltham, MA) together with a Finnigan Surveyor high-performance LC (HPLC) system equipped with a binary pump, autosampler, diode array detector (DAD), and Xcalibur (MSn) system (Thermo Fisher Scientific, Waltham, MA) together with a capillary voltage (ESI) mode. Nitrogen was used as the sheath gas, auxiliary gas, and sweep gas, with flow rates of 40.0, 10.0, and 0.02 liters/min, respectively. Spray voltage was 5.5 kV, spray current 0.05 μA, capillary voltage −1.0 V, capillary temperature 300°C, and tube lens voltage −69.89 V. For MSn analysis, the collision-induced dissociation (CID) voltage was set to 1.75 V.

Generation of an IreA deletion strain by fusion PCR. For the fusion of IreA upstream and downstream regions to a selection marker cassette, the protocol of Szewczyk and coworkers was essentially followed (18). One-kilobase upstream and downstream regions of the IreA open reading frame (ORF) were amplified with primers wc1_LB_fq_P1, wc1_LB_rv_P3, wc1_RB_fq_P4, and wc1_RB_rv_P6. P3 and P4 contain overhangs complementary to the ends of the hygromycin B cassette used as the selection marker and amplified with primers hph_sfa_fq_neu2 and hph_sib_rv_neu2 from the pPK2 vector (kindly provided by N. Requena, Karlsruhe, Germany). The sequences of all primers are listed in Table 1. The cassette consists of the gpdA promoter from A. nidulans, the hygromycin phosphotransferase gene from Escherichia coli, and the trpC terminator from A. nidulans. The borders and primers were amplified via fusion PCR in one step with nested primers wc1_P2 and wc1_P5. As the polymerase, PrimeStar polymerase (TaKaRa Bio Inc., Japan) was used. PCR conditions were as follows: an initial denaturing step at 98°C for 2 min, followed by 25 cycles of 10 s of denaturation at 98°C, 7 s of annealing at 60°C, 55 s of elongation at 72°C with an increase of 10 s per cycle, and a final elongation at 72°C for 7 min. The purified product was directly used for transformation.

For control PCRs, Tag polymerase was used with primers wc1_up_LB_fq_P6 and Hyg_end_fq for the left border and Hyg_end_fq and wc1_p6 for the right border. Southern blot analyses were performed following standard procedures (19).

### Table 1 Primers used in this study

<table>
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<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>wc1_LB_fq_P1</td>
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<td>wc1_P2</td>
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<td>wc1_LB_rv_P3</td>
<td>GTATTTCTACGTTGCAAAATCTGTGAAG</td>
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<tr>
<td>wc1_RB_fq_P4</td>
<td>GCACTTCCACCATCCTGATTTTCC</td>
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<td>wc1_P5</td>
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<td>GGGCCACCTAGGCCGCGCGACTGAGGAGA</td>
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<td>hph_sfa_fq_neu2</td>
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<td>hph_sib_rv_neu2</td>
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Orientation, 5′→3′. Overhangs and restriction sites are indicated in italics.

Protoplast transformation of A. alternata. The transformation procedures based on the protocol of A. brassicicola (20) were used, with modifications. Fungal spores were harvested from a mCD culture plate, filtered, and inoculated into 100 ml Richard’s liquid medium (sucrose at 20 g/liter, KO₃ at 10 g/liter, KH₂PO₄ at 5 g/liter, MgSO₄·6H₂O at 2.5 g/liter, yeast extract at 1 g/liter) for 19 to 24 h at 30°C and 150 rpm. The mycelium was harvested by filtering, washed with 0.7 M NaCl, and digested in a Katalase (Wako Chemicals) suspension (60 mg in 6 ml 0.7 M NaCl) for 1 h with soft shaking at 80 rpm and 30°C. Protoplast quality and quantity were checked via microscopy. Protoplasts were separated by filtering through miracloth and glass wool and washed with 0.7 M NaCl by centrifugation at 7,000 rpm and 4°C for 10 min, followed by a second washing step with STC (1 M sorbitol, 50 mM CaCl₂, 50 mM Tris-Cl, pH 8.0). The pellet was resuspended in 200 to 500 μl STC, and protoplasts were counted in a Helber chamber. Protoplasts (4 × 10⁴) were prepared for DNA absorption by 1 min of incubation at 37°C. A 4-μg volume of DNA was added, and the mixture was incubated on ice for 30 min. Cells were subjected to heat shock for 2 min at 42°C and, after addition of 2 ml PEG (40% polyethylene glycol [PEG] 4000, 50 ml Tris-Cl [pH 8.0], 50 mM CaCl₂), incubated for 20 min at room temperature. The suspension was spread on regeneration medium containing hygromycin B (HygB) (1 M sucrose, 0.5% caseic acids, 0.5% yeast extract, 80 μg/ml HygB) and incubated for 3 days at 28°C.

Purification of transformants. Transformation results were confirmed via PCR and Southern blot analysis with probes against hygromycin phosphotransferase and IreA. Because only heterokaryotic strains were obtained, strains were purified via 3 rounds of single-spore isolation and 1 round of protoplast isolation.
Expression analysis. Cultures were grown for 2 days in the dark in static liquid cultures at 28°C and subsequently for 1 h in blue light (450-nm wavelength). The mycelium was dried, frozen in liquid nitrogen, and crushed. RNA was then extracted with a Qiagen RNeasy Plant minikit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Gene expression in the wild-type strain and in the transformants was analyzed by quantitative real-time PCR in an iCycler iQ detection system from Bio-Rad. For expression analysis of the polyketide synthases, cultures of the wild-type and trans- 1ated protein. This composition of photoreceptors resembles that 1ated protein. This composition of photoreceptors resembles that 1ated protein. This composition of photoreceptors resembles that
bring it into relation to the putative photoreceptors in *A. alternata*, we studied the effect of light on sporulation. We inoculated agar plates with a defined number of conidiospores and incubated the plates for 1 to 7 days in constant white light as well as constant dark and, later, additionally in blue (450 nm) and red light (680 nm). The cultures showed different colors of the mycelia, and a view under the light microscope revealed a drastic decrease of spore numbers on the plates incubated in constant blue light in relation to dark (Fig. 2). The experiment was done with two different media, mCDB and rice flour medium. The effect was the same on both, but because the fungus grows faster on rice flour medium, it produced more spores in the same time period. The larger number of spores in the dark-grown cultures was already visible by the naked eye, because the spores are black pigmented and thus the agar plates appear dark. To quantify this inhibitory effect, we harvested the spores and determined their numbers via optical density measurements (Fig. 3A). Blue light had nearly the same effect as white light, whereas the effect of red light was similar to that of dark conditions, with the exception of day 7 (Fig. 3B).

Further on, we were interested in the time of blue-light exposure that is necessary for the inhibition of sporulation and tested different exposure times from 1 min to 24 h. Before the cultures were exposed to blue light, they were grown for 2 days in the dark, and after irradiation, the plates were put back in the dark for 18 h. A significant inhibition occurred after 2 or more h of blue-light irradiation (data not shown).

To address the issue of whether the inhibition effect persists or is reversible in the dark, we compared cultures that were incubated for 4 days in constant blue light or for 4 days in constant darkness to cultures that were grown for the first 3 days in blue light and afterward for 1 day in the dark. Whereas the blue-light cultures showed the expected sporulation reduction of about 50%, the sporulation in the dark-after-blue-light culture was nearly fully restored (92%; data not shown). This reversibility is not surprising if it is assumed that the phenotypic effects are caused by a blue-light regulator acting in the way WC-1 does.

**Blue light stimulates mycotoxin production.** Because *A. alternata* is known as producer of a large spectrum of secondary metabolites, we analyzed the effect of light on the amount of secondary metabolites by thin-layer chromatography (TLC). Häggblom and Unestam described an inhibitory effect of blue light on the production of alternariol (AOH) and alternariol monomethyl ether (AME) in 1979 (8). We inoculated mCDB agar plates in the way described above and incubated them for 7 days. Both mycelium and agar medium were extracted with ethyl acetate and an-
AME production was completely and AOH production nearly production when the strain was incubated at 22, 25, or 28°C. At 30°C, temperatures and found no difference in the AOH or AME production. We tested four incubation cause the parameters for mycotoxin production in fungi may be dependent on light and temperature. (A) Extracts from cultures grown for 7 days on mCDB agar at 28°C under conditions of constant darkness and of white, blue, and red light, respectively. AOH is marked by an arrow, the circle marks AME, and the asterisk marks ATX-I. The latter two are visible only under white- and blue-light conditions. For AOH, the amount is increased under these conditions. (A) Cultures grown 7 days on mCDB agar in darkness at different temperatures. AOH and AME are produced in the same amounts at 22, 25, and 28°C, but the production of both was strongly reduced at 30°C. Growth was not reduced at 30°C.

Deletion of the ileA white-collar homologue. To start the molecular analysis of light regulation in A. alternata, the role of ileA, the white-collar 1 orthologue, has been studied by gene deletion. One-kilobase ileA upstream (left) and downstream (right border) regions were PCR amplified and fused to the hygromycin B (hph) resistance cassette by fusion PCR (18). The 5.9-kb-long PCR product (4 µg) was directly used for protoplast transformation and homologous replacement of the ileA open reading frame (Fig. 5A to C). Transformants were analyzed by PCR and Southern blotting. The integration of the construct into the genome was confirmed by PCR with primers for the 1-kb hph gene (data not shown). Homologous integration of the construct was demonstrated by PCR using primers derived from the hygromycin B cassette and primers outside the left or the right border sequences (Fig. 5D). For two of the three transformants, the PCR results showed the expected bands for both sides. Southern blot analysis with a probe for hygromycin B confirmed the results (data not shown). However, a wild-type copy of ileA was still detectable by PCR and Southern blotting in all 3 transformants (data not shown). We anticipated that the transformants still harbored non-transformed wild-type nuclei as a heterokaryon. The other possibility of multiple copies in the genome was excluded because there was only one band detected in the Southern blot. To purify the mutants, 3 rounds of single-spore isolation were performed with no effect concerning the existence of the wild-type band. Therefore, protoplasts were generated and regrown to single colonies, 18 of which were further analyzed. In two of them, the wild-type band in the PCR with ileA primers was lost and the mycelium of these two strains appeared much darker on agar plates than that of the wild type (Fig. 5B, no. 4 and 5). The other 16 analyzed strains still showed the wild-type signal in the PCR and essentially the same phenotype as the wild type, as shown for no. 2 and 6 in Fig. 5B. For further studies, strain no. 4 was chosen. In Southern blot analyses with the ileA probe, the wild-type band was no longer visible in this strain and the hph probe still confirmed the homologous integration (Fig. 5E). This strain was recomplemented as described in Materials and Methods (Fig. 5C). As a final proof for the deletion of the ileA gene, real-time reverse transcription-PCR (RT-PCR) was performed also to identify small amounts of wild-type mRNA in the deletion strain. In comparison to wild-type results, ileA mRNA could no longer be detected. In contrast, the nonpurified heterokaryotic transformant showed a reduction in the expression level. Expression analysis of the recomplemented strain showed ileA expression like that of the wild type (Fig. 6F).

Spore formation in blue light is restored in the ileA mutant and increased in the dark. With confirmed deletion mutant no. 4 and the recomplemented strain, we analyzed the spore numbers in darkness and blue light in comparison to wild-type numbers. Given that spore formation is inhibited by blue light and that LreA is likely to be involved in blue-light signaling, we expected a release of the repression in the ileA mutant. Interestingly, after 7 days of culturing, the amount of spores in the ileA mutant strain grown under blue light was slightly higher than in the cultures kept in the dark. Surprisingly, the number of spores obtained in the dark in the ileA mutant was 1.61 higher than the number seen with the wild type. The recomplemented strain behaved like the wild type (Fig. 6). These results suggest that LreA acts as a repressor independently of light conditions. Evidence for LreA as a light sensor and activator came from the study of AOH formation.

Blue-light induction of AOH depends partially on LreA. The qualitative detection of the mycotoxins in the TLC assay already

FIG 4 Thin-layer chromatography analysis of secondary metabolites in dependence of light and temperature. (A) Extracts from cultures grown for 7 days on mCDB agar at 28°C under conditions of constant darkness and of white, blue, and red light, respectively. AOH is marked by an arrow, the circle marks AME, and the asterisk marks ATX-I. The latter two are visible only under white- and blue-light conditions. For AOH, the amount is increased under these conditions. (B) Cultures grown 7 days on mCDB agar in darkness at different temperatures. AOH and AME are produced in the same amounts at 22, 25, and 28°C, but the production of both was strongly reduced at 30°C. Growth was not reduced at 30°C.
indicated changes in the concentrations of mycotoxins in lreA deletion strains grown in blue-light cultures in comparison to the wild type (data not shown). The increase of the production of AOH in blue light was reduced to only a small increase in the mutant, suggesting an activating function for LreA. In comparison, the wild type produced ATX-I only under blue-light conditions. Interestingly, the lreA mutant also produced this compound in the dark, suggesting a repressing function of LreA. To quantify these results, LC/MS analysis has been done with the same samples (Fig. 7A). The results for AOH in the wild type showed a 2.8-fold increase in blue light (Fig. 7B). In contrast, AOH in the lreA mutant was increased only slightly by a factor of 1.4 in blue light in relation to the dark. This residual blue-light induction suggests the presence of an alternative blue-light receptor, possibly the cryptochrome (Fig. 1).

Altertoxins I and II were not detectable in the dark in wild-type cultures. After blue-light exposure, ATX-I and ATX-II appeared. In the lreA mutant, the concentrations for ATX-I and -II were quite similar in blue light and darkness, with only a slight increase in blue light.

**LreA controls the expression of several polyketide synthases.** To test the influence of LreA on the expression level of genes involved in secondary metabolism, the expression of several polyketide synthases was measured in the wild-type strain in comparison to the lreA mutant in darkness and white light (Fig. 8). Among the 10 polyketide synthase-encoding genes, 2 were silent and only 8 were expressed (7). From those, we have chosen pksA,
because it is involved in melanin biosynthesis (29; R. Fetzner, M. Wenderoth, K. Seither, A. Herr, R. Fischer, submitted for publication), and \( pksJ \), because it is involved in alternariol biosynthesis (7). In addition, two genes of unknown function, \( pksC \) and \( pksD \), were chosen. \( pksA \) was upregulated 2-fold in the wild type under light conditions. This stimulation did not occur in the \( \Delta lreA \) mutant strain, suggesting an activating function of LreA. The levels of expression of \( pksC \) were similar in dark and light for the wild type but higher in the \( \Delta lreA \) mutant in light, suggesting an inhibiting effect of LreA on the expression of \( pksC \) in light (Fig. 8). In the wild type, the expression of \( pksD \) was slightly (1.3-fold) upregulated under light conditions. In the \( \Delta lreA \) strain, in contrast, the expression was upregulated about 2-fold in the dark as well as in light, which suggests an inhibiting effect of LreA independent of light. The expression of \( pksJ \) was 3.4-fold upregulated in light in comparison to the dark. In the deletion strain, the expression levels were similar in the two light situations. This indicates an inhibiting effect of LreA in darkness. This fits to the data obtained for alternariol formation, where the production is stimulated in the dark in the absence of LreA (Fig. 7B). In summary, our data suggest different roles of LreA at the promoters of different \( pks \) genes.

**DISCUSSION**

*Alternaria alternata* is one of the most common fungal species and normally grows saprophytically. However, a number of different pathogenic variants have been described which produce certain plant-specific toxins. These variants cause necrotic disease on different plants (9, 30). Despite the wide distribution and the great economic impact, the number of molecular studies is rather limited. Only some genes have been analyzed by gene deletion; e.g.,
the role of peroxisomes in toxin formation has recently been addressed by deletion of a gene encoding a Pex6 homologue (31). In addition to methods for gene deletion by homologous replacement, high-throughput gene disruption methods have been established (20). Another possibility for gene inactivation is the use of RNA silencing, which has recently also been used in A. alternata (32). We encountered extreme difficulties in our efforts to obtain a clean lreA deletion strain. Apparently, heterokaryon formation is very common and a very stable situation. Clonal purification of strains is hindered by the fact that the asexual spores consist of several compartments, although the nuclear distribution in hyphae and during spore formation suggested earlier that spores are homokaryotic (33). We were unable to obtain homokaryotypic strains by plating spores but had to generate protoplasts instead, rather suggesting that spores are heterokaryotic (Fig. 2, bottom panel, inset). Another big problem in the analysis of strains and their phenotypes is the large variability of A. alternata. Sometimes the color of the mycelium changes from green to brown or even orange. This might be due to epigenetic modifications or instabilities of the genome. There are several studies that describe a high level of genetic diversity in A. alternata (34–36) despite the fact that such great variations are more common for fungi with sexual reproduction and that a sexual cycle for Alternaria spp. is not known. One indication for such genomic variabilities is the fact that A. alternata harbors a dispensable chromosome, on which the ACT toxin biosynthesis gene clusters are located (32). These properties of A. alternata may also explain some apparently contradictory results obtained in our study in comparison to work published earlier by Häggblom (8, 37). However, it has to be considered that the production of secondary metabolites is controlled by many parameters and is also linked to the primary metabolism. It has been reported recently that in A. nidulans, changes in the glucose concentration may have drastic effects on the light response (38). Häggblom performed drop culture experiments at a temperature of 20°C. We normally used 28°C because of the optimal growth of A. alternata at that temperature. Of course, we performed TLC experiments also with cultures grown at 20°C, but there was no difference from the results at 28°C. Häggblom also used another kind of light source in his blue-light experiments whose intensity (0.21 W/m²) was lower than that of the light source that we used and kept the dark controls wrapped in aluminum foil, which does often increase the water activity values. The water activity is one of the parameters that is able to influence mycotoxin production. For AOH, a maximum of production at an aw value of 0.98 has been shown in other studies (39, 40). Given the complexity of the regulation and the as yet rather limited molecular insights into the regulatory networks, it is currently difficult to develop guidelines for food or feed storage to prevent toxin formation by Alternaria species. Much more work is clearly necessary to fully understand the production of secondary metabolites.

Another very interesting result of this study is that the white-collar 1 homologue LreA has activating and repressing functions in A. alternata (Fig. 9). White-collar 1 has been studied very well in several fungi, and the molecular function is best known in the control of the circadian clock in N. crassa (41–44). Here, WC-1 forms a complex with WC-2 (WCC) which acts, for instance, as a positive regulator for the negative-feedback protein Frequency and the photoadaption protein Vivid (44, 45). It is light activated and directly binds to the promoters of light-regulated genes (46). We found that A. alternata LreA apparently fulfills a repressing function in the dark as well as an activating function in light in the control of secondary metabolism and sporulation. Interestingly, mycotoxin production is not coupled with sporulation, which was described for several mycotoxigenic genera, including Aspergillus (47), Claviceps, Penicillium, and Fusarium (26). In A. alternata, the highest toxin levels were obtained in blue light, where sporulation in contrast is strongly inhibited. The repressing function of a white-collar homologue has been reported before for A. nidulans, although the derepressing effect on conidiospore formation was very small (Purschwitz et al. [16]).

Although our results do not allow us to speculate further at the moment on a molecular model explaining how this repressing effect can be mediated and on the effect of light, the action as a repressor of the ATX-I and ATX-II biosyntheses is especially interesting because LreA might also repress other secondary metabolite biosyntheses. A complete profiling of secondary metabolites in an A. alternata wild type and the lreA mutant may reveal novel

FIG 9 Working model for the LreA-dependent regulation of mycotoxin formation. Expression for AOH and ATX genes is differently regulated by LreA. In the case of AOH, gene expression takes place also in the dark, positively regulated by an unknown factor (left, upper panel). In light, LreA appears to additionally stimulate the expression and the transcription level is increased (left, lower panel). It is unknown whether the effect of LreA is direct or indirect. In contrast, LreA appears to repress the expression of the ATX genes. A complex of LreA and LreB as a transcriptional regulator is proposed in analogy to the situation in N. crassa and A. nidulans.

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secondary metabolites which are normally not produced under laboratory conditions. Recent studies, especially in *A. nidulans*, revealed that the induction of sleeping gene clusters is a potent strategy for the discovery of novel, bioactive secondary metabolites (48–50).

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