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Changes of global gene expression and secondary metabolite accumulation during light-dependent *Aspergillus nidulans* development

Özgür Bayram [a,1], Kirstin Feussner [b], Marc Dumkow [a], Cornelia Herrfurth [b], Ivo Feussner [b], Gerhard H. Braus [a,*]

[a] Department of Molecular Microbiology and Genetics, Georg-August-Universität, Grisebachstr. 8, D-37077 Göttingen, Germany
[b] Department of Plant Biochemistry, Georg-August-Universität, Justus-von-Liebig-Weg 11, D-37077 Göttingen, Germany

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ABSTRACT

Fungal development and secondary metabolite production are coordinated by regulatory complexes as the trimeric velvet complex. Light accelerates asexual but decreases sexual development of the filamentous fungus *Aspergillus nidulans*. Changes in gene expression and secondary metabolite accumulation in response to environmental stimuli have been the focus of many studies, but a comprehensive comparison during entire development is lacking. We compared snapshots of transcript and metabolite profiles during fungal development in dark or light. Overall 2.014 genes corresponding to 19% of the genome were differentially expressed when submerged vegetative hyphae were compared to surface development. Differentiation was preferentially asexual in light or preferentially sexual connected to delayed asexual development in dark. Light induces significantly gene expression within the first 24–48 h after the transfer to surfaces. Many light induced genes are also expressed in dark after a delay of up to two days, which might be required for preparation of enhanced sexual development. Darkness results in a massive transcriptional reprogramming causing a peak of lipid-derived fungal pheromone synthesis (*psi* factors) during early sexual development and the expression of genes for cell-wall degradation presumably to mobilize the energy for sexual differentiation. Accumulation of secondary metabolites like antitumoral terrequinone A or like emericellamide start under light conditions, whereas the mycotoxin sterigmatocystin or asperthecin and emodin appear under dark conditions during sexual development. Amino acid synthesis and pool rapidly drop after 72–96 h in dark. Subsequent initiation of apoptotic cell-death pathways in darkness happens significantly later than in light. This illustrates that fungal adaptation in differentiation and secondary metabolite production to light conditions requires the reprogramming of one fifth of the potential of its genome.

1. Introduction

Multicellular development of eukaryotic organisms is a complex process that requires coordinated orchestration of gene expression. Numerous environmental signals influence the developmental responses of most organisms. One global environmental signal that determines the way of life on earth is light where chlorophyll bearing organisms harvest the photon derived energy. In fungi, light can affect growth, the mode of reproduction or the sporulation rate (Bayram et al., 2010; Corrochano, 2011; Rodriguez-Romero et al., 2010). Most fungi are sessile with a saprobic and often plant associated life style. They decay organic materials and produce small chemicals also named secondary metabolites (SM) with bioactive potential (Brakhage, 2013; Keller et al., 2005). Genes coding for the synthesis of SM are frequently organized in gene clusters, which are often localized in subtelomeres of fungal chromosomes and are mostly silenced under normal laboratory conditions. SM gene clusters can be activated in response to environmental signals, in the presence of other organisms or during fungal development (Schroeckh et al., 2009).

The filamentous fungus *Aspergillus nidulans* is widely used as a model system for eukaryotic genetics and SM. This mold reacts to light by adapting its reproduction style. Germination of asexual or sexual spores on appropriate substrates leads to vegetative hyphae, which are initially incompetent to react to environmental triggers (Etxebeste et al., 2010). When developmental competence is established, the fungus can receive and respond to

* Corresponding author.
E-mail address: gbraus@gwdg.de (G.H. Braus).
[1] Present address: Department of Biology, Maynooth University, National University of Ireland, Co., Kildare, Ireland.

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environmental signals such as light, CO_2 or pH. Growth under light predominantly leads to conidiophores carrying asexual spores also called conidia, which are released into the air. In the absence of a light signal, asexual development is delayed and the fungus undergoes primarily the sexual cycle. This results in the establishment of sexual fruiting bodies named cleistothecia containing meiotically formed ascospores. The cleistothecia are the overwintering structures of the fungus in the soil. The sharp response to the light signal is a consequence of millions years of evolutionary adaptation under illumination by the sun. Short term light exposure (30 min) results in a differential gene expression of 5% of the *A. nidulans* genome (Ruger-Herreros et al., 2011). The light signal is sensed by a variety of light receptors sensitive to daylight ranging from short (350 nm) to long (650 nm) wavelengths. The visible light (350–650 nm) is sensed by four types of light receptors: (I) phytochrome FphA (red light receptor), (II) white-collar homologs LreA/LreB (blue light receptors), (III) cryptochrome-photolyase like protein CryA (UVA and blue light), (IV) fungal opsin NopA (green light receptor) (Bayram et al., 2008a; Blumenstein et al., 2005; Purschwitz et al., 2008). Red and blue light receptors FphA/LreA&LreB physically interact with each other and control light responses. FphA is responsible for repression of cleistothecia formation under red light conditions, whereas blue light receptors LreA and LreB repress the cleistothecia formation under blue light illumination (Purschwitz et al., 2008). The exact molecular function of NopA is yet unknown.

A. nidulans produces up to 30 groups of SMs among which the polyketide mycotoxin sterigmatocystin (ST) of the aflatoxin family and the non-ribosomal peptide antibiotic penicillin are the most prominent examples. Many metabolites have been recently identified from *A. nidulans* including emericellin, emericellamide A–F, emodin, shamixanthone, benzaldehyde or orsellinic acid and its derivatives (Chiang et al., 2010; Giles et al., 2011; Marquez-Fernandez et al., 2007; Sanchez et al., 2010, 2011).

Fungal development and production of SMs are coordinated by the trimeric velvet complex, composed of VelB-VeA-LaeA, that activates the sexual development and ST production in the absence of light (Bayram et al., 2008b)). Light inhibits formation of the heterotrimeric complex by preventing nuclear accumulation of the VeA bridging component. The VeA-VelB velvet domain heterodimer acts as fungal kingdom specific transcription factor structurally similar to mammalian NF-kappaB; LaeA is a S-adenosyl methionine (SAM) dependent methyltransferase required for the activation of SM gene clusters (Ahmed et al., 2013; Bok and Keller, 2004; Sarikaya Bayram et al., 2010). VipC and VapB represent additional methyltransferases, which are part of a novel VapA-VipC-VapB membrane complex which controls an accurate light response and affects secondary metabolism at the epigenetic level (Sarikaya-Bayram et al., 2014).

Other triggers of morphogenesis in *A. nidulans* include lipid derived hormones *p*recocious *s*exual *i*nducer, (*psi* factors), which act similar to local prostaglandin hormones of mammals or jasmonates in plants. The fungal oxylipins are involved in the control of the ratio of asexual and sexual sporulation (Tsitsigiannis and Keller, 2006; Tsitsigiannis et al., 2005). *ppoA* and *ppoC* encode two dioxygenases that are involved in the biosynthesis of two antagonistic *psi* factors. The gene product of *ppoA* converts the fatty acids to their 8-monohydroperoxy and 5,8-dihydroxy derivatives and is required for sexual development, whereas the gene product of *ppoC* promotes asexual development (Brodhun et al., 2009; Tsitsigiannis and Keller, 2006; Tsitsigiannis et al., 2004a). Deletion of either gene results in a shift towards opposite developmental program.

Reactive oxygen species (ROS) are internal signals that are required for accurate morphogenesis. There are several enzymes involved in the generation and detoxification of ROS molecules. Various metabolic reactions as well as special enzymes can produce ROS. For example NADPH-oxidase (NoxA) generating superoxide molecules from hydrogen peroxide is required for sexual differentiation in *A. nidulans* as well as other fungi (Dirschnabel et al., 2014; Lara-Ortiz et al., 2003). The exact developmental functions of ROS scavenging and oxidative response enzymes including catalases or superoxide dismutases remain to be shown.

Cellular amino acid levels are essential for fungal development (Hoffmann et al., 2001). Any perturbation in the cellular amino acid pool causes developmental deficiencies such as small microcleistothecia that are devoid of sexual ascospores. Deletion or overexpression of cross pathway control, *cpcA* encoding the central transcription factor for amino acid biosynthesis leads to growth defects during amino acid starvation (Eckert et al., 1999; Hoffmann et al., 2001).

In this study, we addressed the global changes in gene expression and metabolite accumulation in response to light during fungal morphogenesis. A detailed transcriptome and non-targeted metabolome analysis as well as the analysis of *psi* factors of the early and late stages of development resulted in comprehensive insights into the developmental responses of *A. nidulans*. Fungal development requires primary metabolism during vegetative growth, which ensures constant energy supply for the later developmental stages. Asexual and sexual sporulation of the fungus consumes the biomass and energy accumulated during vegetative growth. Starvation during asexual and sexual development triggers the formation of secondary metabolites that protect the next generation of fungal life forms.

2. Materials and methods

2.1. A. nidulans strains, growth media and culturing conditions

A. nidulans FGSC A4 Glasgow wild type strain (Fungal Genetics Stock Center (University of Missouri, Kansas City, MO, USA) was used as single strain for all performed experiments in this work. FGSC A4 possesses no auxotrophic markers and bears the wild type *veA* gene required for the development and SM production. The wild type strain FGSC A4 was grown in glucose minimal medium (GMM) (0.52 g l^{-1} KCl, 0.52 g l^{-1} $MgSO_4$, 1.52 g l^{-1} KH_2PO_4, 0.1% trace element solution, pH 6.5) containing 1% glucose as carbon source. Vegetative mycelia were obtained from submerged liquid cultures inoculated with 10^6 spores/ml and grown on rotary shaker for 20 h at 37 °C. They were washed (0.96% NaCl), filtered through miracloth and shifted to solid GMM to developmentally synchronize the cultures. Cultures were induced 24 h (A24) and 48 h (A48) for asexual development under white fluorescent light (90 μW m^2) containing wavelengths ranging from blue to red light (400–700 nm). Cultures induced for sexual development were cultivated for 24 h (S24), 48 h (S48), 72 h (S72) and 96 h (S96) in the darkness. For sexual cultures, plates were wrapped with parafilm to limit oxygenation and further induce the sexual development.

2.2. Microscopic analysis

A. nidulans colonies, hyphae, asexual and sexual structures were taken with a Kappa PS30 digital camera (Kappa opto-electronics, Germany) used in combination with a Zeiss Axiolab (Zeiss AG, Germany) light microscope or an Olympus SZX12 binocular (Olympus GmbH, Germany).

2.3. Transcriptome analysis

2.3.1. Sequence analysis

Sequences from *A. nidulans* were retrieved from the National Center For Biotechnology Information (NCBI, gi: 40747330), from