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Light regulation of asexual development in the rice blast fungus, *Magnaporthe oryzae*

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Abstract

Light is a major environmental factor that influences many biological processes. We characterized the roles of light in asexual development (including the formation of aerial hyphae and conidiophore) in *Magnaporthe oryzae*, which is the causal agent of rice blast disease. Our data revealed a complex nature of light regulation in the asexual developments of *M. oryzae*. Asexual development of *M. oryzae* is suppressed by blue light in a light/dark cycling environment and asexual spore release is controlled by both blue and red light. We demonstrated that even very dim light, about 10 μmol m^{-2}, is sufficient to suppress spore-release behavior in *M. oryzae*. We also generated knockout strains of a blue light receptor, *mgwc-1*, the *M. oryzae* homolog of *white collar-1* in *Neurospora crassa*, and demonstrated blue-light-specific regulation in the asexual development and spore release in *M. oryzae*. Our findings in this agriculturally important pathogen, *M. oryzae*, broaden our understanding of the roles of light in fungal development.

Keywords: Light regulation; Asexual development; Filamentous fungi; *Magnaporthe oryzae*; *Magnaporthe grisea*; *Pyricularia oryzae*; Spore release; Conidiophore

1. Introduction

Environmental factors, both physical and nutritional, influence many stages of fungal development (Moore-Landecker, 1992; Kues and Liu, 2000; Romani et al., 2003; Schneper et al., 2004). Information of a microorganism's habitual environment as collected through several environment-sensing pathways is crucial for its survival. Microorganisms will respond to the ambient environment by making metabolic adjustments or morphological changes in order to cope with adverse environments and/or to take full advantage of favorable environments (Hill, 1976; Schreckenbach et al., 1981; Sipiczki et al., 1998; Belo et al., 2005).

Among the major environmental factors, ambient light conditions have been characterized for an extensive period of time in many different aspects of fungal development and behavior including phototropism (Harding and Melles, 1983), photoperiodism (Roenneberg and Merrow, 2001; Tan et al., 2004), carotene synthesis (De Fabo et al., 1976), sclerotia formation (Moore-Landecker and Shropshire, 1982), sexual spore discharge (Ingold and Dring, 1957; Ingold and Oso, 1969), circadian rhythm (Sargent and Briggs, 1967; Dunlap and Loros, 2004), asexual development (Mooney and Yager, 1990; Lauter and Russo, 1991; Flaherty and Dunkle, 2005), and sexual development (Esser, 1980; Innocenti et al., 1983). It is also well documented that different parts of the light spectrum modulate different aspects of fungal development and behavior (Brooke, 1969; Leach, 1972; Honda and Yunoki, 1975; Mooney and Yager, 1990). Fungi sense not only different qualities of light, but also different intensities of light

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(Schwerdtfeger and Linden, 2001, 2003), and require a certain amount of photons for light regulation (Innocenti et al., 1983; Crosthwaite et al., 1995; Froehlich et al., 2002). In *Neurospora crassa*, a major emphasis of light regulation has been focused on the effects of blue light (Ballario et al., 1996; Linden et al., 1997a,b; Schwerdtfeger and Linden, 2001; Froehlich et al., 2002; He et al., 2002; Schwerdtfeger and Linden, 2003). *Neurospora crassa* mutants that are defective in light transduction pathways were generated through forward genetic approaches (Paietta and Sargent, 1983; Degli-Innocenti and Russo, 1984; Linden et al., 1997a,b), and two blue-light receptors, White Collar-1 (WC-1) and VIVID (VVD), have been characterized at the molecular level (Froehlich et al., 2002; Schwerdtfeger and Linden, 2003). The advancement in our understanding of blue-light photoreceptors that Neurospora studies have provided has assisted in the identification and characterization of blue-light photoreceptors in other fungal systems (Ambra et al., 2004; Casas-Flores et al., 2004; Idnurm and Heitman, 2005; Lu et al., 2005).

In this report, we characterized light-regulated phenotypes in the filamentous fungus *Magnaporthe oryzae*, which is the causal agent of rice blast disease. The pathogenic interaction between host and pathogen goes through several distinctive stages, which are often referred to as the disease cycle. The naturally occurring disease cycle of rice blast begins with the infected plant material. The presence of a pathogenic microbe and a susceptible host in the same place is not sufficient for disease development; rather it requires a favorable environment for disease development. When the environment is favorable, the fungus in the previously infected tissue produces a specialized structure, a conidiophore, which produces three-cell conidia (Hashioka, 1963). This developmental process has been studied previously by Dr. Leung and his colleagues, who have generated mutants that are defective in conidiophore development and who have elucidated the epistatic relationship that exists between the different genes in conidiophore development (Leung and Shi, 1994; Shi and Leung, 1994, 1995; Shi et al., 1998). Regarding the role of ambient light conditions for asexual development in *M. oryzae*, it was reported that reduced light intensity enhanced hyphal extension (Imura, 1938, 1941). There are also reports that secondary infection occurs in the field through asexual spores released at night, with the airborne asexual spores in the field peaking between midnight and 7 a.m. or right before sunrise, and then disappearing at sunrise (Barksdale and Asai, 1961; Kim et al., 1990). In these studies, the change in the amount of airborne spores in the field was explained by humidity rather than light conditions (Hashioka, 1948). Although one could infer the spore-release behavior of the fungus by counting trapped spores in the air, the complete mechanism of asexual spore release in *M. oryzae* is still not well understood (Barksdale and Asai, 1961). The effect of wind was proposed as a primary means of spore dispersal in *M. oryzae* (Ono and Suzuki, 1959). Although it was a dominant view that asexual spores are the primary means of secondary infection in the field because of the repeated infection cycle in one season (Leung and Shi, 1994), it was recently reported that *M. oryzae* can also infect rice through the root system of the host plant (Sesma and Osbourn, 2004). The spores that land on a plant surface germinate form an appressorium, which produces an infection peg that invades the plant tissue (Howard and Valent, 1996).

In this report, we chose to focus on the effect of light on asexual development (including the formation of aerial hyphae and conidiophore) and spore release, which are the first two initial steps in the secondary infection in the field. We tested our hypothesis that ambient light conditions regulate the asexual development of *M. oryzae*. Our goals were to identify and characterize light-dependent phenotypes in *M. oryzae* in different qualities and quantities of light. Our data revealed a complex nature of light regulation in the agriculturally important fungus, *M. oryzae*.

It should be noted that, until recently, this fungus was better known in the literature as *Pyricularia grisea*, the anamorph of *M. grisea*. However, recent work by Dr. Kohn's group suggested that the fungus should be more appropriately named *M. oryzae* instead of *M. grisea* (Couch and Kohn, 2002; Couch et al., 2005). Due to this, we will use the new name throughout this manuscript.

2. Materials and methods

2.1. Strains and growth conditions

Magnaporthe oryzae strain Guy 11 was received from Daniel Ebbole (Texas A&M University, TX). Two *mgwc-1* knockout mutants, KOM1-02 and KOM1-14, showed the same defective phenotypes in blue-light sensing. Thus, we have presented only the phenotypes of one strain, KOM1-02, in this report. We observed variation in fungal behavior in different media conditions. Therefore, to minimize the variation between experiments, the fungus was maintained, and experiments were performed using 10% V8 juice media (pH 6.3, agar 1.5 g/l). For better visibility of the aerial hyphae development in the traditional and open race tubes (described below), we prepared the media using V8 juice supernatant in which the juice was centrifuged for 10 min at 2600 rpm (GS-6 Centrifuge, Beckman) and the pellet discarded. We used three different chambers for the different light experiments. Model E-36L (Percival) was used for white light and constant dark conditions, E-30LED (Percival) was used for different wavelengths of light (blue, 470 nm; red, 670 nm; far-red: 735 nm), and PGW-30 (Percival) was used for measuring the threshold of fluence for spore-release behavior. The chambers were maintained at 60% relative humidity and at 24 °C. Approximate fluence rates for individual constant light conditions at the position where we placed plates or race tubes were as follows: white light, 400 $\mu mol\ m^{-2}\ s^{-1}$; blue light, 20 $\mu mol\ m^{-2}\ s^{-1}$; red light, 142 $\mu mol\ m^{-2}\ s^{-1}$; and far-red