Available online at www.sciencedirect.com
ScienceDirect
Fungal Genetics and Biology 45 (2008) 103–116
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

Carbon source affects PKA-dependent polarity of *Neurospora crassa* in a CRE-1-dependent and independent manner

Carmit Ziv, Rena Gorovits, Oded Yarden *

Department of Plant Pathology and Microbiology, The Otto Warburg Minerva Center for Agricultural Biotechnology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Received 16 March 2007; accepted 22 May 2007
Available online 10 June 2007

Abstract

A defect in *mcb*, encoding the cAMP-dependent protein kinase A (PKA) regulatory subunit in *Neurospora crassa*, which confers an apolar growth phenotype, is accompanied by an increase in PKA activity levels. Both PKA and CRE-1 [a key carbon catabolite repression (CCR) regulator] mediate the cellular response to carbon-source availability. Inactivation of the *cre-1* gene resulted in reduced growth rate, abnormal hyphal morphology and altered CCR. Both PKA and CRE-1 affected morphology in a carbon-dependent manner, as fructose suppressed the apolar morphology of the *mcb* strain and enabled faster growth of the Δ*cre-1* mutant. An increase in *cre-1* transcript abundance was observed in *mcb* and a reduction in PKA activity levels was measured in Δ*cre-1*. CRE-1 is involved in determining PKA-dependent polarity, as an *mcb;*Δ*cre-1* strain displayed partial reestablishment of hyphal polarity. Taken together, our results demonstrate regulatory interactions between PKA and CRE-1 that affect cell polarity in a filamentous fungus.

Keywords: Polarity; Carbon catabolite repression; Conidial germination; Hyphae; Fructose; Aberrant splicing

1. Introduction

Filamentous fungi are defined by their ability to form highly polarized hyphae, which is a prerequisite for efficient colonization of growth niches and substrate utilization (reviewed by Harris, 2006). During vegetative growth, the establishment of polarity is an important initiation step for primary germ-tube emergence from the spore/conidium on the one hand, and branch emergence from existing hyphae on the other. Once established, polarity must be maintained during hyphal extension. Structural components of the cytoskeleton (especially actin) play a pivotal role in establishing and maintaining fungal polar growth (reviewed by Momany, 2002; Harris and Momany, 2004; Harris, 2006).

One of the key regulators of polarity in fungi as well as in higher eukaryotic cells is cAMP-dependent protein kinase A (PKA)[1] (Harris, 2006). In its inactive form, PKA is a tetramer composed of two regulatory subunits bound to two catalytic subunits. In response to signals that increase intracellular cAMP levels, cAMP binds to the regulatory subunit and triggers conformational changes that release the active catalytic subunit (Lengeler et al., 2000). PKA has been shown to control a number of developmental events, such as germination and growth polarity, in different fungi, including *Neurospora crassa* (Bruno et al., 1996), *Aspergillus niger* (Saudohar et al., 2002) and *A. fumigatus* (Zhao et al., 2006).

The *N. crassa mcb* temperature-sensitive (t.s.) mutant, which is defective in the gene encoding the regulatory subunit of PKA, shows complete loss of growth polarity during both conidial germination and hyphal elongation when incubated at restrictive temperatures (32 °C and above). In the *mcb* mutant, actin patches have been shown to be

* Corresponding author. Fax: +972 8 9468785.
E-mail address: Oded.Yarden@huji.ac.il (O. Yarden).

[1] *Abbreviations used:* CCR, carbon catabolite repression; PKA, cAMP-dependent protein kinase A.

1087-1845/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.fgb.2007.05.005

distributed uniformly throughout hyphae whose growth has become apolar or bulbous (Bruno et al., 1996), in contrast to their typical localization to hyphal tips (Tinsley et al., 1998). Thus, PKA has been suggested to be involved in the regulation of actin microfilament organization. Although the genetic nature of the *mcb* t.s. mutation has not been investigated, it was proposed that at restrictive temperatures the defective regulatory subunit is released from the catalytic subunit, resulting in increased PKA activity (Bruno et al., 1996).

More than four decades ago, morphogenesis was proposed to be dependent on the rate of intracellular catabolic and biosynthetic reactions (Brody and Tatum, 1966), and this has been recently supported by genetic studies demonstrating that primary metabolic processes are determinants of hyphal morphology (Osherov and May, 2001; Momany, 2002; Seiler and Plamann, 2003; Harris, 2006). The observation that mutations in both regulatory elements and metabolism-related and structural-element-encoding ('housekeeping') genes can result in phenotypically similar polarity defects suggests that metabolic input might regulate polar growth.

A clear link between the PKA pathway and carbon-source sensing has been established in *N. crassa* (Li and Borkovich, 2006) and *Aspergillus* (Oliver et al., 2002), and has been studied in depth in *Saccharomyces cerevisiae* (Rolland et al., 2002). Addition of glucose to de-repressed cells of *S. cerevisiae* was shown to cause a rapid, transient, increase in cAMP levels (Beullens et al., 1988). The cAMP signal induces a protein-phosphorylation cascade which is mediated, in part, by the activation of PKA (reviewed by D'Souza and Heitman, 2001; Santangelo, 2006). When cAMP levels increase, the PKA catalytic subunits are rendered enzymatically active and can phosphorylate target substrates that include metabolic enzymes as well as transcription factors (D'Souza and Heitman, 2001). Among the PKA-regulated DNA-binding proteins are transcription factors that have been found to be involved in cell morphogenesis and filamentation (Pan and Heitman, 1999; Tebarth et al., 2003), as well as growth regulation, stress response and carbohydrate-store accumulation (Smith et al., 1998).

One of the most studied transcriptional regulation mechanisms in response to glucose is carbon catabolite repression (CCR). Even though they are sometimes considered synonymous, glucose repression is only one specific case of a general carbon repression phenomenon (Ruijter and Visser, 1997). In filamentous ascomycetes, glucose transcriptional repression is mediated by CreA, a zinc-finger protein related to Mig1p from *S. cerevisiae* (Ronne, 1995; Ruijter and Visser, 1997). The *creA* genes of several filamentous fungi have been cloned and sequenced (Dowzer and Kelly, 1991; Drysdale et al., 1993; Strauss et al., 1995; de la Serna et al., 1999; Vautard et al., 1999; Tudzynski et al., 2000). However, little is known about the biological activity of CreA in the various systems, mainly due to a lack of available mutants. Although the aforementioned CreA-related DNA-binding proteins exhibit a strong similarity to the yeast Mig1p, they are probably not fully functionally interchangeable between uni- and multicellular fungi as, for example, the *Sclerotinia sclerotiorum cre1* gene does not complement yeast Δ*mig1* mutants (Vautard et al., 1999). However, functional interchangeability among filamentous fungi has been demonstrated in several instances, including complementation of the *A. nidulans creA* loss-of-function mutant with the *S. sclerotiorum cre1* gene (Vautard et al., 1999), as well as with the *creA* gene from *Gibberella fujikuroi* or *Botrytis cinerea* (Tudzynski et al., 2000).

Functional regulation of the CreA/Cre1 transcription factor in filamentous fungi is not yet fully understood. In *A. nidulans*, *S. sclerotiorum* and *Hypocrea jecorina*, *creA*/*cre1* expression is regulated at the transcriptional level and has been shown to be repressed in the presence of glucose as a result of auto-regulation (Ilmen et al., 1996; Strauss et al., 1999; Vautard-Mey and Fevre, 2003). In contrast, expression of *G. fujikuroi* and *B. cinerea creA* was demonstrated to be continuously high in the presence of all carbon sources tested (Tudzynski et al., 2000). However, the transcript-abundance pattern of the *creA*/*cre1* gene did not correlate with the repressing activity of the protein (Strauss et al., 1999; Vautard-Mey et al., 1999; Tudzynski et al., 2000). Thus, a post-translational mechanism is probably also involved in the regulation of Cre-mediated repression activity. In fact, CreA/Cre1 has been shown to be capable of undergoing several modes of post-translational modification, including phosphorylation (Vautard-Mey and Fevre, 2000; Cziferszky et al., 2002) and ubiquitination (Lockington and Kelly, 2001; Boase and Kelly, 2004), which were found to affect transcription factor abundance and activity.

A long history of genetic and biochemical studies of metabolism in *N. crassa* has shown that many genes are subject to CCR. However, the molecular basis of CCR in this fungus has yet to be elucidated. Some processes known to be regulated by CCR in *N. crassa* are repressed to a similar extent by glucose and fructose, while others are subjected exclusively to glucose repression. This suggests that there are at least two forms of CCR in *N. crassa* and that one of these is glucose-specific (Ebbole, 1998). For example, several extracellular hydrolytic enzymes are repressed by glucose (Ebbole, 1998). Moreover, fructose uptake, which is completely distinct from glucose uptake, is repressed by glucose (Schneider and Wiley, 1971a,b; Rand and Tatum, 1980).

These findings prompted us to further investigate the differential effects of glucose and fructose on polarity during vegetative growth and to examine the possible involvement of the CRE-1 transcription factor, in addition to PKA, in the regulation of polarity.

In this paper, we show that fructose can suppress the apolar morphological consequence of elevated PKA levels occurring in the *mcb* strain (whose genetic defect is also analyzed). Our results demonstrate a regulatory role for fructose in determining PKA-dependent polarity, thereby