Fungal Genetics and Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Regular Articles

Alcohols inhibit translation to regulate morphogenesis in *C. albicans*

Nkechi E. Egbe, Caroline M. Paget, Hui Wang, Mark P. Ashe *

Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, United Kingdom

ARTICLE INFO

Article history:
Received 4 December 2014
Revised 13 March 2015
Accepted 28 March 2015
Available online xxxx

Keywords:
Candida albicans
Protein synthesis
Filamentous growth
Eukaryotic initiation factor 2B (eIF2B)

ABSTRACT

Many molecules are secreted into the growth media by microorganisms to modulate the metabolic and physiological processes of the organism. For instance, alcohols like butanol, ethanol and isoamyl alcohol are produced by the human pathogenic fungus, *Candida albicans* and induce morphological differentiation. Here we show that these same alcohols cause a rapid inhibition of protein synthesis. More specifically, the alcohols target translation initiation, a complex stage of the gene expression process. Using molecular techniques, we have identified the likely translational target of these alcohols in *C. albicans* as the eukaryotic translation initiation factor 2B (eIF2B). eIF2B is the guanine nucleotide exchange factor for eIF2, which supports the exchange reaction where eIF2.GDP is converted to eIF2.GTP. Even minimal regulation at this step will lead to alterations in the levels of specific proteins that may allow the exigencies of the fungus to be realised. Indeed, similar to the effects of alcohols, a minimal inhibition of protein synthesis with cycloheximide also causes an induction of filamentous growth. These results suggest a molecular basis for the effect of various alcohols on morphological differentiation in *C. albicans*.

1. Introduction

Candida albicans is a major fungal pathogen of humans, causing life threatening septicaemic infections, especially in immunocompromised individuals. In addition, *C. albicans* can cause frequent and recurrent infections that are difficult to treat in healthy individuals. *C. albicans* has a variety of properties that have been implicated in pathogenicity, including the ability to switch growth between a variety of morphological forms, such as yeast, pseudohyphal and hyphal forms. Such switches in morphology occur as a response to environmental cues, where external stimuli are communicated via different signal transduction pathways (Biswas et al., 2007; Brown and Gow, 1999; Liu, 2001). Pseudohyphae are morphologically distinguishable from hyphae; they also differ fundamentally in their cell cycle organisation and in mechanisms of polarised growth (reviewed in Sudbery (2011)).

A variety of yeasts secrete alcohols such as butanol and isoamyl alcohol during growth (Dickinson et al., 1998, 2003; Ghosh et al., 2008; Hazelwood et al., 2008; Martins et al., 2007; Webb and Ingraham, 1963). These alcohols are major components of fusel oil; a by-product of yeast fermentation, and hence they have been collectively termed fusel alcohols (Webb and Ingraham, 1963). The addition of fusel alcohols to a yeast culture has been shown to induce a range of specific morphological effects such as pseudohyphal growth. It has been suggested that these alcohols might signal nitrogen scarcity to elicit these effects (Dickinson, 1996). Consistent with this hypothesis, both non-pathogenic and pathogenic yeasts switch from the budding yeast form to the filamentous form when starved for nitrogen (Csank and Haynes, 2000; Gimeno et al., 1992).

The role of fusel alcohols and other alcohols in signalling to alter the growth and physiology of *Candida* species is starting to become more widely appreciated. Martins et al. (2007) detected Isoamyl alcohol, 2-phenethylethanol, 1-dodecanol in supernatants of planktonic and biofilm forms of *C. albicans* and *C. dubliniensis*, where these alcohols inhibited the morphological transition from yeast to filamentous form by over 50%. Aromatic alcohols i.e. phenethyl alcohol, tyrosol and tryptophol are produced by *C. albicans*, especially under nitrogen poor conditions (Chen and Fink, 2006; Chen et al., 2004; Ghosh et al., 2008; Lingappa et al., 1969; Martins et al., 2007). These aromatic amino alcohols induce pseudohyphal formation in *Saccharomyces cerevisiae*, with tyrosol also inducing germ-tube formation in *C. albicans* (Chen and Fink, 2006).

In *S. cerevisiae* a number of examples exist where nutritional alterations induce filamentous growth and also affect protein synthesis. Hence, nitrogen limitation affects translation initiation and also induces pseudohyphal growth in diploids (Gancedo, 2001; Gimeno et al., 1992; Ibrahimo et al., 2006). Moreover, the effects of various alcohols as well as glucose depletion have been shown to modulate both protein synthesis and filamentous growth in *S. cerevisiae* (Ashe et al., 2000, 2001; Lorenz et al., 2000).

* Corresponding author. Tel.: +44 (0)161 306 4164.
E-mail address: mark.p.ashe@manchester.ac.uk (M.P. Ashe).

http://dx.doi.org/10.1016/j.fgb.2015.03.008
1087-1845/

Translation initiation is the predominant phase where protein synthesis is regulated. The process of translation initiation requires interaction of the initiator methionyl tRNA with eukaryotic initiation factor 2 (eIF2) bound to GTP to form a ternary complex. This is then recruited to the small ribosomal subunit (40S) to form the 43S complex. Following the binding of the 43S complex to the mRNA and start codon recognition, the GTP on eIF2 is hydrolysed and released as eIF2-GDP (Hershey and Merrick, 2000). The initial formation of the ternary complex represents a prominent point of translational regulation across all eukaryotes. For instance, in *S. cerevisiae* activation of the eIF2α kinase, Gcn2p, during amino acid starvation leads to the phosphorylation of the α subunit of eIF2. This phosphorylated form binds tightly and sequesters eIF2B; the guanine nucleotide exchange factor that is responsible for recycling eIF2-GDP to eIF2-GTP. Hence, the inhibition of eIF2B results in less eIF2-GTP, less ternary complex and reduced levels of global protein synthesis (Hinnebusch, 2005; Pavitt, 2005). Even though regulation of eIF2B results in a global regulation of translation, specific mRNAs such as *GCN4* are activated in a mechanism requiring four upstream open reading frames (uORFs) (Hinnebusch, 2005). *GCN4* encodes a transcription factor that regulates the expression of a host of genes involved in the regulation of nitrogen resources in cells (Natarajan et al., 2001). Therefore Gcn4p up-regulation reverses the starvation by promoting amino acid biosynthesis as part of the general control pathway. *C. albicans* also harbours a single eIF2α kinase, Gcn2p (Tournu et al., 2005). As in *S. cerevisiae*, amino acid starvation inhibits global translation via this kinase while activating translation of *GCN4*, yet in this case there are just three uORFs involved in the regulation (Sundaram and Grant, 2014b).

In this current study, we have assessed the translational and morphological responses of *C. albicans* to the effects of various alcohols. We confirm previous analyses showing that fusel alcohols and ethanol induce a switch from vegetative growth to pseudohyphal growth. We show that the alcohols also rapidly inhibit translation initiation and induce the translation of the *GCN4* mRNA in both the wild type and the *gcn2Δ* strains. Therefore, Gcn2p is not involved in this pathway of translation regulation in *C. albicans* and a pathway that is independent of the eIF2α kinases must regulate translation to induce *GCN4* expression. Given the known effects of fusel alcohols targeting eIF2B in *S. cerevisiae*, these results are entirely consistent with the presence of a similar mechanism in *C. albicans* and opens up the possibility that such a regulation could be involved in morphogenetic alterations and pathogenicity. Indeed we show that minimal inhibition of translation with cycloheximide leads to a similar degree of filamentation as alcohol treatment.

2. Materials and methods

2.1. Media and growth conditions

The *Candida* strains used in this study are listed in Table 1. The CAI4 background is used throughout (Fonzi and Irwin, 1993), however experiments carried out comparing the CAI4 and CAF2-1 strains showed that the two strains responded similarly to various alcohol treatments in terms of growth and filament formation (data not shown). Therefore, as CAI4 is the parental strain for the *gcn2Δ* mutant, this strain was selected for use in this study. The strains were grown and maintained on rich yeast extract-Peptone-Dextrose (YPD) media (2% (w/v), glucose; 2% (w/v) bactopeptone; and 1% (w/v), yeast extract) or YPD agar solid media (YPD with 2% agar). Unless otherwise stated, alcohols (butanol, isoamyl alcohol and ethanol) were added for 15 min at the particular concentrations stated. Alcohol tolerance was assessed by growing strains in YPD to OD_{600} 0.1. Butanol (0.5%, 1% and 2%), isoamyl alcohol (0.1%, 0.25%, 0.5% and 1%) and ethanol (2%, 4%, 6% and 8%) were added and growth was assessed hourly.

Table 1
Yeast strains used in this study.

Strain name	Genotype	Source
CAI4	*ura3::λimm434/ura3::λimm434*	Fonzi and Irwin (1993)
gcn2Δ	*ura3::λimm434/ura3::λimm434 gcn2::hisG/gcn2::hisG*	Tournu et al. (2005)
yMK2313	*ura3::λimm434/ura3::λimm434 GCD1-GFP::NAT/GCD1*	This study
efg1Δ	*ura3::λimm434/ura3::λimm434 efg1::hisG/efg1::hisG-URA3-hisG*	Lo et al. (1997)
cph1Δ	*ura3::λimm434/ura3::λimm434 cph1::hisG/cph1::hisG-URA3-hisG*	Liu et al. (1994)

2.2. Morphogenesis assays

Strains were grown at 30 °C, washed in water to remove trace media constituents and re-inoculated into fresh YPD medium supplemented with alcohols to OD_{600} 0.1 then incubated at 37 °C. As positive and negative controls, cells were re-inoculated into 10% serum medium or YPD lacking alcohol, respectively. At various times during incubation (1 h, 2 h, 3 h, 4 h), the morphology of the cells was monitored using a Nikon Eclipse E600 and Axiocam MRm camera. Images were acquired using Axiovision 4.5 software. The proportion of cells forming germ tubes or pseudohyphae were counted using a haemacytometer. Each count was repeated three times and the mean of the three counts recorded. Colony morphology on solid media was assessed by plating on YPD agar or YPD agar supplemented with the indicated alcohol, 10% serum or cycloheximide concentrations ranging from 10 μg/ml to 200 μg/ml. Micro-colonies were photographed using a Nikon Eclipse E600 and Axiocam MRm camera and the images were acquired using Axiovision 4.5 software.

2.3. Polysome analysis

C. albicans strains were grown to an OD_{600} of 0.7 and treated with butanol, isoamyl alcohol and ethanol as described above. Extracts were prepared in 1 mg/ml cycloheximide and these were layered onto 15–50% sucrose gradients. The gradients were sedimented via centrifugation at 40,000 rpm using a SW41 Beckman rotor for 2.5 h (Fullerton, CA) and the A_{254} was measured continuously to give the traces shown in the figures (Ashe et al., 2000).

2.4. [^{35}S] methionine incorporation assay

C. albicans strains were grown to OD_{600} of 0.7 in synthetic complete dextrose (SCD) medium lacking methionine (Guthrie and Fink, 1991). The culture was split into two flasks and methionine was added to a final concentration of 60 ng/ml, of which 0.5 ng/ml was [^{35}S] methionine (cell-labelling grade 1175 Ci/mmol; New England Nuclear, Boston, MA). Alcohol at the indicated concentration was added to one flask and samples (1 ml) were taken and processed as described previously (Ashe et al., 2000). For each experiment, three biological replicates were assessed.

2.5. Western blot analysis of phosphoserine 51 eIF2α

50 ml OD_{600} 0.7 of culture grown in YPD was harvested in a clinical centrifuge at 5000 rpm for 5 min. The cells were lysed, and protein samples were prepared as described previously