Fungal Genetics and Biology 100 (2017) 13–21

Contents lists available at ScienceDirect

Fungal Genetics and Biology

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

Regular Articles

The *Aspergillus nidulans* Pbp1 homolog is required for normal sexual development and secondary metabolism

Alexandra A. Soukup [a], Gregory J. Fischer [a], Jerry Luo [b], Nancy P. Keller [b,c,*]

[a] Department of Genetics, University of Wisconsin-Madison, WI, United States
[b] Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, WI, United States
[c] Department of Bacteriology, University of Wisconsin-Madison, WI, United States

ARTICLE INFO

Article history:
Received 19 October 2016
Revised 2 January 2017
Accepted 8 January 2017
Available online 9 January 2017

Keywords:
Stress response
RNA granule
Secondary metabolism
Sexual development

ABSTRACT

P bodies and stress granules are RNA-containing structures governing mRNA degradation and translational arrest, respectively. *Saccharomyces cerevisiae* Pbp1 protein localizes to stress granules and promotes their formation and is involved in proper polyadenylation, suppression of RNA-DNA hybrids, and preventing aberrant rDNA recombination. A genetic screen for *Aspergillus nidulans* mutants aberrant in secondary metabolism identified the Pbp1 homolog, PbpA. Using Dcp1 (mRNA decapping) as a marker for P-body formation and FabM (Pab1, poly-A binding protein) to track stress granule accumulation, we examine the dynamics of RNA granule formation in *A. nidulans* cells lacking *pub1*, *edc3*, and *pbpA*. Although PbpA acts as a functional homolog of yeast PBP1, PbpA had little impact on either P-body or stress granule formation in *A. nidulans* in contrast to Pub1 and Edc3. However, we find that PbpA is critical for sexual development and its loss increases the production of some secondary metabolites including the carcinogen sterigmatocystin.

1. Introduction

Regulation of mRNA levels are critical processes by which cells may rapidly respond to flux in inter- or extracellular conditions. This regulation is achieved by such means as mRNA localization, mRNA stability, and mRNA translation. Recent evidence indicates that these processes can be co-regulated by segregation of non-translating mRNA molecules and their associated proteins into distinct structures within the cytoplasm, termed mRNP granules (Buchan, 2014). These mRNP granules include both P-bodies and stress granules, which perform separate but overlapping functions, and share a number of constituents (Kedersha and Anderson, 2009). Formation of mRNP granules is interdependent, as that P-body formation is required for normal stress granule assembly (Buchan et al., 2008). mRNA trafficking and sequestration in fungi have been associated with such diverse processes as stress response (Keller, 2015; Ren et al., 2016; Wang et al., 2015), polar growth (Becht et al., 2006; Inglis et al., 2013; Tey et al., 2005), nutrient acquisition (Morozov et al., 2010b), and morphological switching (Göhre et al., 2013).

While both P-bodies and stress granules contribute to the regulation of translation, P-bodies are more commonly associated with mRNA decay, and are defined by an enrichment of proteins involved in this process. In yeast, these include decapping enzymes (Dcp1/2), activators of decapping (Edc3 and Lsm1-7), and an exonuclease (Xrn1). P-bodies are normally present in low numbers, but can increase after translation inhibition or exposure to certain environmental stresses (reviewed in (Parker and Sheth, 2007). In *Aspergillus nidulans*, P-bodies components have been linked to response to nitrogen sufficiency by facilitating turnover of the transcription factor AreA (Morozov et al., 2010a, 2010b). Deletion of the P-body component Edc3 in this system led to severe depletion of P-bodies (as monitored by Dcp1-GFP) and a defect in the rate of both global and targeted RNA turnover, although no significant growth phenotype was noted.

Stress granules are much less common under standard cellular conditions, and normally form under strong inhibition of translation, as induced by translational inhibitors or other environmental stimuli (Kedersha et al., 2005). mRNA arrest in these stress granules can lead to reintroduction of the mRNAs back into the translating pools at a later time. Although the exact composition of stress granules can vary depending on their cause of formation and species, in *S. cerevisiae* protein components can include Poly-A binding proteins (Pab1 and Pub1), elongation factors (eIF4G, eIF4E), proteins involved in RNA destabilization (Ngr1), and Pbp1, a Poly-A binding protein (PAB1) binding protein (Buchan et al., 2008; Hoyle et al., 2007). Removal of both *pub1* and *pbp1* have

* Corresponding author at: 1550 Linden Drive, Madison, WI 53706, United States.
E-mail address: npkeller@wisc.edu (N.P. Keller).

http://dx.doi.org/10.1016/j.fgb.2017.01.004
1087-1845/

been shown to limit stress granule formation in *S. cerevisiae* (Buchan et al., 2008), while the essential gene PAB1 is often used as a marker (Buchan et al., 2008; Kozubowski et al., 2011). In *A. oryzae*, various stresses led to accumulation of the PAB1 homolog (Huang et al., 2013). Presumed limitation of stress granule formation by deletion of the *pub1* homolog led to greatly increased sensitivity to stress, suggesting that the integrity of these granules may be critical to respond to adverse conditions (Huang et al., 2013).

Numerous previous studies have shown a link between stress and fungal secondary metabolism (Keller, 2015; Ren et al., 2016; Wang et al., 2015). We have reported on the applicability of using a genetic suppressor screen to look for genes involved in secondary metabolism including RsmA, a bZIP transcription factor linking both secondary metabolism and sexual development with the stress response in *A. nidulans* (Shaaban et al., 2010; Yin et al., 2012). From this same screen, we now present our identification of an *A. nidulans* homolog of the *S. cerevisiae* Pab1 binding protein Pbp1 as also playing an unexpected role in secondary metabolism and sexual development. In both yeast and mammalians cells, Ppb1 and its mammalian homolog Ataxin-2 have been shown to be involved in assembly of stress granules (Buchan et al., 2008), with mutations in *ATXN2*, the gene encoding Ataxin-2, associated with the neurodegenerative disease spinocerebellar ataxia type 2 (Stevanin et al., 2000). Deletion of *PBP1* in *S. cerevisiae* leads to significant decreases in stress granule formation under glucose deprivation, while P-bodies are unaffected. In line with its role in stress granule assembly, *PBP1* has also been implicated in resistance to various stresses, including caffeine, cycloheximide, hydroxyurea (Kapitzky et al., 2010), and recovery from ethanol stress (Kato et al., 2011), as well as regulation of poly-A tail length (Mangus et al., 1998, 2004), and cell growth (Kimura and Irie, 2013). Here we show that *AN1325*, here called *pbpA*, is a homolog of yeast *PBP1* and is required for normal sexual sporulation and has a repressive effect on secondary metabolism but has no major role in either P-body or stress granule biology in *A. nidulans*.

2. Materials and methods

2.1. Sequence analysis

Genomic DNA sequence and translation of *AN1325.4* (*pbpA*) gene was obtained from the Aspergillus Genome Database (www.aspgd.org). *fabM* (*AN4000* (Marhoul and Adams, 1996)), *edc3* and *dcp1* (*AN6893* and *AN7746* (Morozov et al., 2010b), and *pub1* (*AN10164* (Huang et al., 2013)) have been previously described.

Alignment was performed using the NCBI's Conserved Domain Detection tools (Marchler-Bauer et al., 2015) and COBALT (Papadopoulos and Agarwala, 2007) with the following Genbank accession sequences: *Homo sapiens* ATXN2: AAI14547; *Aspergillus nidulans*: XP_658929; *Saccharomyces cerevisiae*: CAA97204; *Schizosaccharomyces pombe*: CAB57927; *Candida albicans*: XP_717736; and *Cryptococcus neoformans*: XP_571007. Percent identity was calculated using Clustal Omega (Sievers et al., 2011). Amino acid alignments are included in Fig. S1.

2.2. Culture conditions, southern, and northern analysis

All strains (Table S1) were propagated at 37 °C on glucose minimum medium (GMM) with appropriate supplements. Fungal DNA was isolated as previously described (Shimizu and Keller, 2001). DNA manipulations, Southern, and northern analysis were conducted according to standard procedures (Sambrook and Russell, 2001).

2.3. Yeast complementation assay

PCR was used to amplify either *ScPBP1* + 1 kb flanks or *pbpA* and *PBP1* flanks. Yeast recombineering was used to insert these fragments into the backbone of pGAD424 (Chien et al., 1991). These plasmids were transformed into BY4741 and BY4741 Δ*PBP1*. For assessment of the petite negative phenotype, overnight cultures were grown in YPD. Five-fold dilutions of these cultures were performed and plated on YPD and YPD + 40 μg/ml ethidium bromide (Hwang et al., 2007).

2.4. Construction of mutant pbpA strains

One kb flanks upstream and downstream of *pbpA* were amplified and fused to an *A. parasiticus pyrG–A. nidulans gpdA* fusion cassette from pJMP9 (Soukup et al., 2012b) using double joint PCR (Yu et al., 2004). The resulting knockout construct was transformed into RJMP1.1 as previously described (Szewczyk et al., 2006). Transformants were examined for targeted replacement of the native locus by PCR and Southern blotting (Fig. S2A and B), and confirmed by northern blot of the appropriate transcript (Fig. S2C). Prototrophic overexpression strains were obtained by crossing the transformants with RTMH207.13 or DVARI (Kim et al., 2002). Desired recombinants were confirmed by PCR screening.

For *pub1* deletion strains, 1 kb of flanking regions were amplified and fused to *A. parasiticus pyrG* from pJW24 (Calvo et al., 2004) using double joint PCR. The resulting knockout construct was transformed into TJMP1.1. Transformants were examined for targeted replacement of the native locus by PCR and Southern blotting. Prototrophic deletion strains were obtained by crossing transformants with RTMH207.13 or DVARI.

For complementation of *pbpA*, the coding region and 1 kb upstream and downstream flanks were amplified using PCR and cloned into the *Not*I sites of pJW53. The resulting knockout constructs was transformed into RJMP1.59 as previously described (Szewczyk et al., 2006). Transformants were examined for integration by PCR and Southern blotting (Fig. S2D). These were crossed to TAAS110.7 to produce prototrophic *pbpA* complements.

2.5. Northern analysis

Fifty milliliter cultures of liquid GMM were inoculated with 1×10^6 spores per ml and incubated at 250 rpm and 37 °C for 36 h under light. Mycelia were harvested, lyophilized overnight, and total RNA was extracted using Isol-RNA Lysis Reagent (5 Prime) according to manufacturer's recommendations. Subsequent northern analysis was done using radiolabeled probes for the corresponding transcript (primers are listed in Table S2).

2.6. Fluorescent strain construction

For initial C-terminal tagging of *pbpA*, *dcp1*, and *fabM*, 1 kb of the 3′ end of the gene and 1 kb downstream of the gene of interest were amplified and fused to either pXDRFP4 (RFP) or pFNO3 (GFP) (Yang et al., 2004) via double joint PCR (Yu et al., 2004). The resulting constructs was transformed into RJMP1.1 as previously described (Szewczyk et al., 2006). Transformants were examined for targeted integration at the native locus by PCR and Southern blotting (Fig. S4). To obtain the final florescent strains sequential crosses were performed between TAAS228.16 and PW1 to yield RAAS235.6. This was crossed to TAAS227.6 to yield RAAS236.1. RAAS236.1 (*fabM::gfp::pyrG; dcp1::rfp::pyrG; metG1; biA1*) was crossed to the appropriate transformants. Desired strains were confirmed via PCR. The fluorescent prototroph (RAAS237.2: