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A mutation in the converter subdomain of *Aspergillus nidulans* MyoB blocks constriction of the actomyosin ring in cytokinesis

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ABSTRACT

We have identified a mutant allele of the *Aspergillus nidulans* homologue of myosin II (*myoB*; AN4706), which prevents normal septum formation. This is the first reported myosin II mutation in a filamentous fungus. Strains expressing the *myoB*^{G843D} allele produce mainly aberrant septa at 30 °C and are completely aseptate at temperatures above 37 °C. Conidium formation is greatly reduced at 30 °C and progressively impaired with increasing temperature. Sequencing of the *myoB*^{G843D} allele identified a point mutation predicted to result in a glycine-to-aspartate amino acid substitution at residue 843 in the myosin II converter domain. This residue is conserved in all fungal, plant, and animal myosin sequences that we have examined. The mutation does not prevent localization of the *myoB*^{G843D} gene product to contractile rings, but it does block ring constriction. MyoB^{G843D} rings at sites of abortive septation disassemble after an extended period and dissipate into the cytoplasm. During contractile ring formation, both wild type and mutant MyoB::GFP colocalize with actin – an association that begins at the pre-ring "string" stage. Down-regulation of wild-type *myoB* expression under control of the *alcA* promoter blocks septation but does not prevent actin from aggregating at putative septation sites – the actin rings, however, do not fully coalesce. Both septation and targeting of MyoB are blocked by disruption of filamentous actin using latrunculin B. We propose a model in which myosin assembly at septation sites depends upon the presence of F-actin, but assembly of the actin component of contractile rings depends upon normal levels of myosin only for the final stages of ring compaction.

1. Introduction

Cytokinesis in the fungi shares many features with cytokinesis in animals (Balasubramanian et al., 2004). In both kingdoms, cell division involves the marking of future division sites and the construction at those sites of a cortical contractile actomyosin ring (CAR), followed by invagination or ingrowth of the plasma membrane and the full or partial isolation of separate cells, or compartments in the case of fungal hyphae (Barr and Gruneberg, 2007). The process in fungi differs from that in animals principally by including centripetal wall construction in the wake of membrane invagination (Mouriño-Pérez, 2013), resulting in crosswalls termed septa. In the unicellular yeasts septal walls partially dissolve, and daughter cells become completely separated as independent individuals, while in the filamentous fungi septa remain intact, and adjacent cell compartments maintain functional communication via persistent central septal pores (Moore and McAlear, 1962; Mouriño-Pérez, 2013). The persistent septa in hyphal fungi serve a number of functions, including damage control (Collinge and Markham, 1985) and providing sufficient separation to allow adjacent compartments to be in different stages of the cell cycle or to pursue different developmental paths (Bleichrodt et al., 2012; Lai et al., 2012; Shen et al., 2014).

The mechanisms of septum formation are complex and still far from fully understood. The process is best characterized in fission yeast (*Schizosaccharomyces pombe*), where over 130 cytokinesis genes have been identified (Pollard and Wu, 2010). Homologues of many of these same proteins are involved in cytokinesis in the filamentous fungi, though their exact functions often differ from the roles they play in yeasts (Seiler and Justa-Schuch, 2010). In both filamentous fungi and yeasts, cytokinesis and the nuclear cycle are coordinated via specific kinase cascades (Simanis, 2003). However, where in yeasts cytokinesis and mitosis are closely coordinated, and each nuclear division triggers a

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corresponding septation (Wolfe and Gould, 2005), independent positional signals operating in the filamentous fungi may override the linkage between mitosis and cytokinesis, resulting in a much looser connection between the two processes (Clutterbuck, 1970; Seiler and Justa-Schuch, 2010).

The work reported here deals with the principal structural and motive components of the CAR: actin and class II (“conventional”) myosin. The actin cytoskeleton in filamentous fungi plays a variety of roles, which have been well reviewed in Berepiki et al. (2011). Previous work has shown that an actin ring is an early marker of the site of future cytokinesis (Momany and Hamer, 1997), and septum formation can be blocked by inhibitors that prevent formation of actin filaments (Harris et al., 1994; Torralba et al., 1998). The cytokinetic actin ring forms in a series of stages, as demonstrated by recent work in *Neurospora crassa* by Berepiki et al. (2010) and Delgado-Álvarez et al. (2014) using the *in vivo* reporter Lifeact. The earliest cytologically recognizable stage of actin ring formation consists of a cytoplasmic “tangle” of filaments, which gradually coalesces into the classic peripheral (cortical) ring.

The myosin superfamily contains over 20 different structural classes (Mooseker and Foth, 2008). Each myosin molecule consists of one or two heavy chains containing a conserved *N*-terminal “head” or “motor” domain, followed by a neck region which binds calmodulin or calmodulin-related light chains, and a class-specific *C*-terminal tail domain (Landsverk and Epstein, 2005). The motor domain, which is responsible both for actin binding and ATP hydrolysis, is further subdivided into four subdomains, the smallest of which, the converter, forms a flexible hinge that undergoes a ca. 65-degree rotation during every power stroke (Llinas et al., 2009). The converter and the directly attached neck region together constitute a “lever arm”, which amplifies the small shifts in head conformation that occur with each ATP hydrolysis cycle.

In comparison to animals, fungal genomes encode comparatively few myosin classes (Berg et al., 2001), and each type is usually represented by just a single gene. The genomes of *Neurospora crassa* and *Aspergillus nidulans*, for instance, each contain just one gene encoding a myosin I, one encoding a myosin II, and one encoding a myosin V (Xiang and Plamann, 2003). Despite this lack of diversity, fungal myosins play essential roles in a wide range of functions, ranging from cytokinesis to endocytosis and vesicle motility (Steinberg, 2000). Deletion of myosin II genes in *A. nidulans* (Taheri-Talesh et al., 2012), *Fusarium solani* (Song et al., 2013), and *Penicillium marneffei* (Cánovas et al., 2011) effectively blocks both septation and conidiation. Type II myosin, like actin, goes through a stepwise process of coalescence at septation sites (Delgado-Álvarez et al., 2014; Taheri-Talesh et al., 2012), appearing first as cytoplasmic strings before condensing into a compact cortical ring. Though no reports have yet colocalized actin and myosin in the same hypha during all stages of CAR coalescence, it is reasonable to assume that the separately imaged string-like arrays of actin and myosin consist of complexes of both proteins.

In this research we report the discovery of a temperature-sensitive mutation in the converter subdomain of *Aspergillus nidulans myoB*, which encodes the organism’s sole type II myosin. The mutation, which we designate *myoB*^{G843D}, results in malformation of contractile actomyosin rings and blockage of ring constriction. To our knowledge this is the first myosin II mutation reported in a filamentous fungus. We also present evidence that the absence of normal levels of *myoB* function, whether caused through mutation or through suppressed expression of the wild type gene, does not prevent the localization of actin filaments to potential septation sites, though the F-actin arrays fail to fully coalesce into tight cortical rings and they are unable to constrict. Conversely, localization of MyoB to potential septation sites is completely blocked when actin filament formation is impaired with the F-actin inhibitor latrunculin B. We propose, therefore, a model for CAR coalescence in which myosin assembly at septation sites depends upon the presence of F-actin, but assembly of the F-actin component of the CAR depends upon normal levels of myosin only in the final stages of ring compaction.

2. Materials and methods

2.1. Strains, media and basic culture methods

Strains used in this study are listed in Table 1. Complete medium (CM) consisted of 1% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, 5% nitrate salts, 1% trace elements, 0.1% vitamin mix, 1.2 mM L-arginine, and 50 mg ml^{-1} ampicillin. Vitamin mix and nitrate salts are described in the appendix of Kafer (1977). Trace element solution is described in Hill and Kafer (2001). Minimal medium (MM) consisted of 1% glucose, 5% nitrate salts, 1% trace elements, 0.001% thiamine hydrochloride, 25 ng biotin ml^{-1}, and 50 mg ml^{-1} ampicillin. Where required, appropriate nutrient supplements were included in order to support growth of auxotrophs. For expression of genes regulated by the *A. nidulans alcA* promoter (*alcA*(p)), 1% glycerol was substituted for glucose in MM. Solid media contained 1.5% agar. Unless otherwise specified, all cultures were incubated at 30 °C.

2.2. Mutagenesis, screening, and Mendelian methods

Conidia of *A. nidulans* strain A28 were mutagenized to 50% mortality with 4-nitroquinoline-1-oxide as described by Harris et al. (1994). Survivors were first screened at both 30 °C and 42 °C for hypersensitivity to 10 mg ml^{-1} Calcofluor White (CFW; ‘Blankophor BBH’, a gift from Bayer Corporation) as described in Hill et al. (2006), followed separately by staining with CFW (Harris et al., 1994) to identify those strains also having septation defects. Septation-deficient strain RCH2 was selected for further study.

Mendelian analyses and strain construction followed standard genetic procedures for *A. nidulans* (Kafer, 1977; Kaminskyj, 2001). Crosses between septation-deficient strain RCH2 and strain A773 (GR5) demonstrated that CFW hypersensitivity and non-septation co-segregate in a 1:1 ratio, and the phenotype is recessive in the diploid state. Diploids were created between strain RCH2 and strains bearing previously published class II or class III *sep* mutations (*sepA1*, *sepD5*, *sepG1*, *sepH1*; Harris et al., 1994), demonstrating that the RCH2 mutation occurs at an independent locus.

2.3. Sequencing the mutant allele

To identify whether the *myoB* gene of strain RCH2 contains a mutation, genomic DNA (gDNA) from strain RCH2 was used as a template to amplify the coding region of AN4706 (plus 1000 bp upstream and 300 bp downstream) using Phusion® High-Fidelity DNA Polymerase (New England Biolabs). Primers for this and other PCR reactions are described in Supplementary Table 1. Products from two separate PCR reactions were then Sanger-sequenced using primers designed to give overlapping coverage of each base. In order to identify the genetic lesion, the consensus sequence was aligned to the *A. nidulans* strain A4 gDNA sequence in the AspGD database (Cerqueira et al., 2013; http://www.aspgd.org/) and to our own sequencing of the identical region of strain A28 (parent of strain RCH2) using ClustalW (European Bioinformatics Institute; http://www.ebi.ac.uk/clustalw/).

2.4. Cloning wild type AN4706 (myoB) and complementation of the mutant phenotype

The coding region of AN4706 (*myoB*) along with 909 base pairs of upstream sequence and 300 base pairs of downstream sequence